Comprehensive Gynecology and Obstetrics

Shigeru Saito
Editor

Preeclampsia

Basic, Genomic, and Clinical

Springer

Comprehensive Gynecology and Obstetrics

Series Editors

Ikuo Konishi
National Kyoto Medical Center
Kyoto
Japan

Hidetaka Katabuchi
Department of Obstetrics and Gynecology
Kumamoto University
Kumamoto
Japan

This series presents the current and future perspectives of medical science in gynecology and obstetrics. The authors fully describe the current understanding of a disease including clinical features, imaging, pathology, and molecular biology, and also include the historical aspects and theories for exploring the etiology of the disease. Also, recent developments in diagnostic strategy, medical treatment, surgery, radiotherapy, prevention, and better health-care methods are clearly shown. Thus, each volume in the series focuses on the scientific basis for the pathogenesis of a disease and provides clinical applications that make it possible to offer personalized treatment for each patient.Over the past 20 years, physicians have been working to develop a standard treatment and publish clinical guidelines for a disease based on epidemiological evidence, mainly through the use of randomized clinical trials and meta-analyses. Recently, however, comprehensive genomic and genetic analyses have revealed the differences and variations in biological characteristics even among patients with the same diagnosis and have been focusing on personalized therapy. Now all physicians and patients are entering a new world of "precision medicine" through the use of genomic evidence.We are confident that readers will greatly benefit from the contents of the series with its purview of the exciting and promising future of gynecology and obstetrics.

More information about this series at http://www.springernature.com/series/13621

Shigeru Saito
Editor

Preeclampsia

Basic, Genomic, and Clinical

Springer

Editor
Shigeru Saito
Department of Obstetrics and Gynecology
University of Toyama
Toyama
Japan

ISSN 2364-1932 ISSN 2364-219X (electronic)
Comprehensive Gynecology and Obstetrics
ISBN 978-981-10-5890-5 ISBN 978-981-10-5891-2 (eBook)
https://doi.org/10.1007/978-981-10-5891-2

Library of Congress Control Number: 2018933025

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

The frequency of preeclampsia is 2–8% of pregnancies. Preeclampsia causes the highest risk of poor outcome for both the mother and the baby. More than 70,000 maternal deaths and 500,000 neonatal deaths are caused by preeclampsia each year. Therefore, we must understand the pathophysiology of preeclampsia and establish prevention methods, new therapies, or both.

For this book, we have invited 17 international contributors—among the best in the field—to discuss the risk factors of preeclampsia, genetic background in preeclampsia, pathological findings in preeclampsia, the pathophysiology of preeclampsia, the prediction of preeclampsia, new therapy for preeclampsia and the future risk for cardiovascular events in preeclamptic cases.

In all sections of the book, recent advanced data have been introduced, and I believe that readers will see the whole picture of this difficult and refractory disease, preeclampsia. Recent advances in medical science have achieved great developments for the management of preeclampsia from the new point of view of pathophysiology. In this book, several new hypotheses of that pathophysiology are introduced, and these new concepts may surprise readers. Medical science is making steady progress, thus readers will be able to understand the new findings as well as plan new prevention methods and therapy in the near future.

I sincerely hope that this book becomes a useful resource not only for basic scientists but also for clinicians. To younger generations who have just begun their research, this book is very useful for understanding preeclampsia from its basic scientific background to clinical management.

I express my gratitude to all the contributors and to Ms. Machi Sugimoto at Springer Japan for her kind cooperation for the publication of this book.

Toyama, Japan

Shigeru Saito, M.D., Ph.D.

Contents

Part I

Risk Factors for Preeclampsia

Risk Factors for Preeclampsia

1

Arihiro Shiozaki and Shigeru Saito

Abstract

Several factors are known to increase the risk of preeclampsia. Preeclampsia generally occurs in the first pregnancy. Primipaternity as well as limited sperm exposure; pregnancy after oocyte donation, donor insemination, and embryo donation; multifetal pregnancy; and hydatidiform mole have also been identified as risk factors for preeclampsia. The protective effects of a previous preeclamptic pregnancy are reduced in women who have changed partners. Preeclampsia is more frequent in women with an advanced age, obesity, insulin resistance, pre-existing hypertension and/or renal disease, pre-existing diabetes or gestational hypertension, a family history of preeclampsia, and maternal susceptibility genes. In contrast, cigarette smoking and maternal physical activity reduce the risk of preeclampsia. Further research is needed in order to expand epidemiological evidence for the prevention of early-onset severe preeclampsia. The development of forceful and targeted interventions prior to conception is required to prevent the development of preeclampsia.

Keywords

Preeclampsia • Risk Factor • Nulliparity • Body Mass Index • Oocyte Donation

A. Shiozaki, M.D., Ph.D. • S. Saito, M.D., Ph.D. (✉)
Department of Obstetrics and Gynecology, University of Toyama, Toyama, Toyama, Japan
e-mail: s30saito@med.u-toyama.ac.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_1

1.1 Introduction

Preeclampsia is a disorder associated with pregnancy, affects 4.6% (95% confidence interval (CI), 2.7–8.2) of all pregnancies, and remains a leading cause of maternal and perinatal morbidity and mortality worldwide [1]. In the severe form of preeclampsia, which generally occurs in midpregnancy, a significant increase is observed in perinatal mortality and morbidity. The pathogenesis of preeclampsia has been unclear for many years, and the precise underlying mechanisms have not yet been elucidated. However, several risk factors for preeclampsia have been identified.

1.2 Prevalence

The prevalence of preeclampsia is summarized in Table 1.1 [2–31]. In a systematic review, the prevalence of preeclampsia was reported to be 4.6% (95% CI, 2.7–8.2) across regions [1] ranging from 1.0% in the Eastern Mediterranean (EMRO) region to 5.6% in the African (AFRO) region.

1.3 Risk Factors

Several risk factors for preeclampsia have been identified. Risk factors have been classified into pregnancy-specific factors, pre-existing maternal conditions, and external environmental factors. The risk factors for preeclampsia described above are listed in Table 1.2. We herein discuss each risk factor shown below.

1.3.1 Pregnancy-Specific Factors

1.3.1.1 First Pregnancy (Nulliparity)

Preeclampsia is generally regarded as a disease of the first pregnancy [32]. A previous normal pregnancy is associated with a markedly decreased frequency of preeclampsia [33]. Therefore, nulliparity is a significant risk factor for preeclampsia. The pooled unadjusted relative risk (RR) of preeclampsia among women with nulliparity was previously reported to be 2.1 (95% CI, 1.9–2.4) [34]. This finding supports immunological maladaptation as a cause of preeclampsia [35].

Table 1.1 Prevalence of preeclampsia

Region/country	Prevalence [%, (year)]	Authors	Reference
Africa			
Algeria	7.8 (2012–2013)	Kichou B et al.	[2]
Congo	6–13 (2003–2007)	Elongi Moyene JP et al.	[3]
Ethiopia	2.2 (2009)	Wagnew M et al.	[4]
	5.58 (2013)		
Kenya	3.7 (N/A)	Bansal YP	[5]
Nigeria	6.2 (N/A)	Udenze IC et al.	[6]
Uganda	4.1 (2011–2013)	Kiondo P et al.	[7]
North America			
Canada	2.64 (1989)	Auger N et al.	[8]
	5.06 (2012)		
United States of America	2.5 (1987)	Wallis et al.	[9]
	3.2 (2004)		
Latin America			
Brazil	6.74 (N/A)	Rezende KB et al.	[10]
Mexico	7.6 (N/A)	Canto-Cetina T et al.	[11]
	3.88 (2010)	Hernández B et al.	[12]
Peru	4.6 (2013)[a]	Wynn A et al.	[13]
Asia			
Cambodia	2.8 (1993–2006)	Cripe SM et al.	[14]
China	1.8 (2010–2011)	Li X et al.	[15]
India	3.4 (2010–2014)	Eswarappa M et al.	[16]
	4.9 (2010–2011)	Aabidha PM et al.	[17]
	13.4 (2012–2013)	Gupta A et al.	[18]
Japan	2.7 (2001–2005)	Shiozaki A et al.	[19]
	2.3 (2014–2015)	Yamada T et al.	[20]
Philippines	6.8 (1985–2001)[b]	Rao AK et al.	[21]
Thailand	12.6 (1982)	Quillan JP et al.	[22]
	15 (2002–2012)	Hanprasertpong T et al.	[23]
Vietnam	2.3 (1993–2006)	Cripe SM et al.	[14]
Europe			
Denmark	2.58 (1997–2002)	Pedersen M et al.	[24]
Finland	4.16 (2006–2010)	Metsälä J et al	[25]
Germany	2.31 (2006)	Schneider S et al.	[26]
Norway	2.35 (3 studies)[c]	Alsnes IV et al.	[27]
Sweden	3.9 (1992–2012)	Cnattingius S et al.	[28]
Switzerland	2.31 (2008–2011)	Purde MT et al.	[29]
United Kingdom	4.85 (1976–2005)	Bhattacharya S et al.	[30]
International cohort			
SCOPE	4.9 (2004–2011)	Kenny LC et al.	[31]

[a]Hypertension a or preeclampsia
[b]Women delivering at the University of California, San Francisco
[c]HUNT1 study + HUNT2 study + Hunt3 study

Table 1.2 Risk factors for preeclampsia

Risk factors	Category	RR or OR (95% CI)	Pooled unadjusted RR (95% CI)	Authors	Year	Reference
Pregnancy-specific factors						
First pregnancy (nulliparity)	Immunological	2.91 (1.28–6.61)	2.1 (1.9–2.4)	Bartsch et al.	2016	[34]
New paternity	Immunological	8.6 (3.1–23.5)		Tubbergen et al.	1999	[38]
Limited sperm exposure	Immunological	3.10 (1.59–6.73)		Wang et al.	2002	[43]
Interval between pregnancies	Immunological	1.12 (1.11–1.13)		Skjaerven et al.	2002	[47]
Oocyte donation	Immunological	2.11 (1.42–3.15) [singleton pregnancy]		Storgaard et al.	2017	[49]
		3.31 (1.61–6.80) [multiple pregnancy]		Storgaard et al.	2017	[49]
		6.60 (4.55–9.57)		Pecks et al.	2011	[50]
Multiple pregnancy	Immunological	2.93 (2.04–4.21)	2.9 (2.6–3.1)	Bartsch et al.	2016	[34]
Pre-existing maternal conditions						
Older age	Inflammatory	1.68 (1.23–2.29) [primiparas]		Duckitt, et al.	2005	[71]
		1.96 (1.34–2.87) [multiparas]		Duckitt, et al.	2005	[71]
(>35 years old)			1.2 (1.1–1.3)	Bartsch et al.	2016	[34]
(>40 years old)			1.5 (1.2–2.0)	Bartsch et al.	2016	[34]
(25–29 years old)		1.28 (1.08–1.53)		Funai et al.	2005	[63]
(30–34 years old)		1.77 (1.43–2.19)		Funai et al.	2005	[63]
(35–39 years old)		2.43 (1.89–3.13)		Funai et al.	2005	[63]
(40+ years old)		4.84 (3.61–6.49)		Funai et al.	2005	[63]

Higher body mass index or obesity	Inflammatory					
(BMI ≥ 26.1)		2.47(1.66–3.67)		Duckitt, et al.	2005	[71]
(BMI > 25)			2.1 (2.0–2.2)	Bartsch et al.	2016	[34]
(BMI > 30)			2.8 (2.6–3.1)	Bartsch et al.	2016	[34]
(BMI = 26.1–29.0)		1.57 (1.49–1.64)		Conde-Agudelo et al.	2000	[52]
(BMI > 29.0)		2.81 (2.69–2.94)		Conde-Agudelo et al.	2000	[52]
Personal or family history of preeclampsia						
Personal history	Genetic	7.19 (5.85–8.83)		Duckitt, et al.	2005	[71]
			8.4 (7.1–9.9)	Bartsch et al.	2016	[34]
Family history	Genetic	2.90 (1.70–4.93)		Duckitt, et al.	2005	[71]
Pre-existing maternal conditions	Inflammatory					
Pre-existing hypertension (chronic hypertension)		1.99 (1.78–2.22)		Conde-Agudelo et al.	2000	[52]
(BP ≥ 130/80 mmHg)		1.38–2.37		Duckitt, et al.	2005	[71]
			5.1 (4.0–6.5)	Bartsch et al.	2016	[34]
Pre-existing diabetes (insulin-dependent DM)		3.56 (2.54–4.99)		Duckitt, et al.	2005	[71]
			3.7 (3.1–4.3)	Bartsch et al.	2016	[34]
Pre-existing renal disease		10.36 (6.28–17.09)		Zhang et al.	2015	[82]
			1.8 (1.5–2.1)	Bartsch et al.	2016	[34]
		17.98 (3.50–92.52)		McDonald et al.	2009	[83]
Antiphospholipid antibody syndrome (APS)		9.72 (4.34–21.75)		Duckitt et al.	2005	[71]
			2.8 (1.8–4.3)	Bartsch et al.	2016	[34]
(Without severe features)		1.56 (1.22–1.95)		Saccone et al.	2017	[87]
(With severe features)		1.66 (1.19–2.49)		Saccone et al.	2017	[87]
Systemic lupus erythematosus (SLE)		7.8 (4.8–12.9)		Simard et al.	2017	[90]
Infections						

(continued)

Table 1.2 (continued)

Risk factors	Category	RR or OR (95% CI)	Pooled unadjusted RR (95% CI)	Authors	Year	Reference
(Urinary tract infection)		1.57 (1.45–1.70)		Conde-Agudelo et al.	2000	[52]
		1.22 (1.03–1.45)		Minassian et al.	2013	[93]
(Periodontal disease)		1.76 (1.43–2.18)		Conde-Agudelo et al.	2000	[52]
Factors for preventing preeclampsia						
Smoking	Anti-inflammatory					
(All cohort studies)		0.68 (0.67–0.69)		Conde-Agudelo et al.	1999	[98]
(Case-control studies)		0.67 (0.57–0.81)		Conde-Agudelo et al.	1999	[98]

1.3.1.2 New Paternity (Primipaternity Hypothesis)

Robillard et al. [36] reported that the incidence of hypertensive disorders of pregnancy was 11.9% among primigravidae, 4.7% among same-paternity multigravidae, and 24.0% among new-paternity multigravidae, that the length of sexual cohabitation before conception for primigravidae and multigravidae was inversely related to the incidence of HDP, and that similar findings were observed after adjustments for race, education, maternal age, marital status, and the number of pregnancies. The protective effect of multiparity on preeclampsia is negated by a change of partner. Feeney and Scott [37] reviewed 34, 201 multigravid deliveries and identified 47 patients who had severe preeclampsia after previous normotensive, non-albuminuric pregnancies. In 13 of these patients the affected pregnancy was apparently by a new father compared with 3 matched controls ($p < 0.01$). Tubbergen et al. [38] examined the impact of a change in paternity on the incidence of preeclampsia in multiparous women. They found that the prevalence of new paternity was significantly higher ($p < 0.0001$) for preeclampsia than for controls, with an odds ratio (OR) of 8.6 (95% CI, 3.1–23.5). Saftlas et al. [39] also investigated whether nulliparous women with a prior abortion who changed partners also lost the protective effect of the prior pregnancy. The risk of preeclampsia was nearly 50% less in women with a history of miscarriage or fetal loss who conceived again with the same partner (adjusted OR, 0.54; 95% CI, 0.31–0.97). In contrast, they found that women with a fetal loss history who conceived with a new partner had the same risk of preeclampsia as women without a history of miscarriage or fetal loss (adjusted OR, 1.03; 95% CI, 0.72–1.47). These epidemiological findings support inadequate tolerance induction via regulatory T (Treg) cells inducing preeclampsia [35]. In a second pregnancy with the same partner, paternal antigen-specific Treg cells quickly expand at the early stage of pregnancy in mice [40]. This expansion of paternal antigen-specific Treg cells plays important roles in the maintenance of pregnancy, resulting in a reduced risk of preeclampsia.

1.3.1.3 Limited Sperm Exposure

The risk of preeclampsia is increased among women who have limited exposure to their partner's sperm. Klonoff-Cohen et al. [41] found that the risk of preeclampsia increased 2.37-fold (95% CI, 1.01–5.58) for users of contraceptives that prevent exposure to sperm in a case-control study. Robillard et al. [42] speculated that an extended duration of sexual cohabitation before conception may protect against hypertensive disorders of pregnancy. Wang et al. [43] demonstrated that the risk of preeclampsia was threefold higher in women never exposed to their partner's sperm, namely, those treated with ICSI performed with surgically obtained sperm, than in those exposed to their partner's sperm cells and seminal fluid, that is, those treated with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with ejaculated sperm (OR, 3.10; 95% CI, 1.59–6.73). These findings suggested that long-term seminal fluid exposure reduced the risk of preeclampsia. Robertson et al. and our colleagues showed that seminal plasma priming plays an important role in the induction of paternal antigen-specific Treg cells [44–46].

1.3.1.4 Interval between Pregnancies

Using data from the Medical Birth Registry of Norway, a population-based registry (551,478 women who had 2 or more singleton deliveries and 209,423 women who had 3 or more singleton deliveries), preeclampsia occurred during 3.9% of first pregnancies, 1.7% of second pregnancies, and 1.8% of third pregnancies when a woman had the same partner [47]. After adjustments for the presence or absence of a change of partner, maternal age, and year of delivery, the OR for preeclampsia for each 1-year increase in the interbirth interval was 1.12 (95% CI, 1.11–1.13). This finding is explained by the life span of paternal antigen-specific Treg cells [35]. When more than 10 years have passed since the last delivery, the number of paternal antigen-specific Treg cells may decrease, resulting in a high-risk pregnancy complicated by preeclampsia.

1.3.1.5 Assisted Reproductive Technology

Advances in assisted reproductive technology have introduced several challenges that increase the risk of preeclampsia. Women who are older than 40 years of age, are infertile prior to their first pregnancy, are obese with polycystic ovary syndrome, or who become pregnant by donated gametes, oocyte donation (OD), or even embryo transfer are more likely to develop preeclampsia [48]. Regarding OD pregnancies versus conventional IVF/ICSI pregnancies, the risk of preeclampsia was adjusted OR (AOR) 2.11 (95% CI, 1.42–3.15) in a singleton pregnancy and AOR 3.31 (95% CI, 1.61–6.80) in a multiple pregnancy [49]. Therefore, single-embryo transfer is recommended in OD cycles to avoid the additional increase in risk from multiplicity. In a recent analysis, Pecks et al. revealed that OD increased the risk that a recipient will develop pregnancy-induced hypertension [50]. In another recent analysis, Masoudian et al. [51] showed that the risk of preeclampsia was higher in OD pregnancies than in other methods of assisted reproductive technology (OR, 2.54; 95% CI, 1.98–3.24; $p < 0.0001$) or natural conception (OR, 4.34; 95% CI, 3.10–6.06; $p < 0.0001$). In IVF pregnancies in which seminal fluid is not transferred with semen, the risk of preeclampsia may become high. Additionally, OD pregnancies increase the risk of preeclampsia because fetuses are complete allografts to the maternal host; the egg is derived from a third party and sperm is derived from the husband. Therefore, more strict tolerance is required than that in conventional pregnancy during which the fetus is a semi-allograft to the maternal host.

1.3.1.6 Multiple Pregnancy

Preeclampsia is more common in women who are carrying twins, triplets, or other multiples.

Multifetal pregnancy has been associated with an increased risk of preeclampsia (RR 2.10, 95% CI, 1.90–2.32) [52]. The reported incidence of preeclampsia ranges between 8 and 20% for twin gestation and between 12 and 34% for triplets [48]. The pooled unadjusted RR in women with a multifetal pregnancy was 2.9 (95% CI, 2.6–3.1) [34]. In a retrospective, case-control study, Mastrobattista et al. [53] found that the rate of severe preeclampsia was significantly higher in the triplet group (12 out of 53 (22.6%)) than in the twin group (3 out of 53 (5.7%)) (OR, 4.9; 95% CI, 1.2–23.5; $p = 0.02$), whereas the overall rate of preeclampsia was not. In twin pregnancies,

chorionicity did not affect the incidence of preeclampsia (OR, 1.19; 95% CI, 0.61–2.3; $p = 0.6$) [54]. In a logistic regression analysis performed to control for confounding factors including maternal age, gestational age at delivery, assisted reproduction, and male sex, the dizygotic state was associated with an OR of 1.4 (95% CI, 0.5–3.9) for developing preeclampsia in nulliparous women and 1.2 in multiparous women (95% CI, 0.3–5.0) [54]. Shiozaki et al. [55] also showed that the incidence of preeclampsia was the same between dichorionic and monochorionic pregnancies in a large cohort study. Therefore, neither the chorionicity nor zygosity of pregnancies alters the increased risk of preeclampsia. These findings imply that the incidence of preeclampsia in twin pregnancies is doubled because of twofold fetal antigens, not because of HLA mismatch between the mother and fetuses.

1.3.1.7 Hydatidiform Mole

Hydatidiform mole (HM) is a pathological pregnancy characterized by a hyperplastic trophoblast with little or no fetal development. Women with HM exhibit symptoms resembling preeclampsia. The fetuses are allografts for their mothers because complete HMs are generally diploid and androgenic in origin, with either a 46 XX or 46 XY karyotype [56]. Enlarged vesicles in the molar placenta may contain high levels of anti-angiogenic factors, leading to an angiogenic imbalance in the mother, and higher levels of anti-angiogenic factors may play a role in the development of preeclampsia [57, 58]. Koga et al. [59] found that serum soluble fms-like tyrosine kinase concentrations were significantly higher in the molar group than in the control and preclinical preeclampsia groups. Clinicians need to be aware that a pregnant woman complicated with HM may have preeclampsia-like syndrome before 20 weeks of gestation [58].

1.3.1.8 Fetal Gender

After a study on fetal gender by Toivanen and Hirvonen [60], increasing evidence indicates sex-specific interplay between the mother, placenta, and fetus. They found a ratio of 1.24:1 in 1061 women with hypertensive disorders in pregnancy. Shiozaki et al. [55] showed that a female fetal gender was a risk factor for preeclampsia in Japan. In singleton pregnancies, pregnant women carrying female fetuses had a significantly higher incidence of preeclampsia than those carrying male fetuses. In MD twin pregnancies, the incidence of preeclampsia was significantly higher in mothers carrying female–female fetuses than in those carrying male–male fetuses, and a marked difference was observed in primiparous cases. In DD twin pregnancies, the incidence of preeclampsia was significantly higher in mothers with female–female fetuses than in those with male–male fetuses, while those with male–female fetuses had intermediate values. The incidence of preeclampsia in MD twin pregnancies was similar to that in DD twin pregnancies with male–male fetuses or female–female fetuses. The Global Pregnancy Collaboration team also revealed that sexual dimorphic differences exist in the occurrence of preeclampsia, with preterm preeclampsia being more prevalent among pregnancies with a female fetus than with a male fetus, whereas no significant differences were noted with respect to term preeclampsia [61]. Preeclamptic women, who are underweight or normal weight and who are expecting a female fetus, may deliver their

babies slightly earlier due to early-onset type severe preeclampsia. However, the reasons why a female fetus is a risk factor for preeclampsia currently remain unclear. Vatten et al. found that the sex ratio in preeclampsia displayed a pattern that was strongly dependent on the length of gestation: female babies were more frequent in preeclampsia with preterm delivery, whereas preeclampsia with term delivery was dominated by male offspring in Norway [62]. In countries in which early-onset preeclampsia is more frequent in underweight women, such as Japan, female babies may become a risk factor for preeclampsia. In contrast, in countries in which late-onset preeclampsia is more common in overweight women, such as Western countries, male babies may be a risk factor for preeclampsia.

1.3.2 Pre-existing Maternal Conditions

1.3.2.1 Older Age

The risk of preeclampsia is higher for older pregnant women. Funai et al. [63] reported that the risk of preeclampsia increased in an approximately linear manner with maternal age and that this pattern was similar in nulliparae and multiparae. Pooled unadjusted RR in women with a maternal age >35 and >40 years were 1.2 (95% CI, 1.1–1.3) and 1.5 (95% CI, 1.2–2.0), respectively [34]. In a large cohort study, advanced maternal age was identified as a risk factor for gestational hypertension and preeclampsia [19]. Women of an advanced age are more likely to develop atherosclerosis, which affects small arteries, such as those in the kidneys and uterus, leading to hypertension. Therefore, older pregnant women may easily develop preeclampsia. Recent studies demonstrated placental senescence in the placenta of preeclampsia cases [64, 65]. Therefore, maternal age may correlate with placental senescence.

1.3.2.2 Higher Body Mass Index or Obesity

Obesity is a definite risk factor for preeclampsia [48]. Almost all observational research demonstrated a strong positive association between maternal prepregnancy body mass index (BMI) and the risk of preeclampsia. The risk of preeclampsia markedly increases from a BMI of 15–30 (kg/m^2).

Pooled unadjusted RR in women with prepregnancy BMI >25 and BMI >30 were 2.1 (95% CI, 2.0–2.2) and 2.8 (95% CI, 2.6–3.1), respectively [34]. RR estimates with women with a normal prepregnancy BMI (19.8–26.0) were 1.57 (95% CI, 1.49–1.64) and 2.81 (95% CI, 2.69–2.94), respectively, for overweight women (prepregnancy BMI = 26.1–29.0) and obese women (prepregnancy BMI > 29.0) [52]. In Japan, prepregnancy BMI was significantly higher in women with gestational hypertension (23.6) and preeclampsia (22.7) than in normotensive women (21.1) ($p < 0.001$, $p < 0.001$, respectively) [19]. Higher BMI is linked to the genetic tendency for hypertension, diabetes mellitus, and insulin resistance and also to the effects of obesity on chronic inflammatory conditions. Obesity adversely affects the maternal environment by creating conditions that increase the risk of gestational diabetes (insulin resistance), hypertensive disorders of pregnancy, fetal growth abnormalities, and congenital anomalies. In a Chilean study, prepregnancy obesity was positively associated with increased insulin resistance (OR, 18; 95%

CI, 5.2–62.7), metabolic syndrome (OR, 3.3; 95% CI, 1.3–8.3), and hyperglycemia (OR, 3; 95% CI, 1.1–8.6) in premenopausal women 10 years postpartum [66]. Th1 immunity has been reported in obese pregnant women [67]. Furthermore, the administration of Th1 cells to pregnant mice induced preeclampsia-like symptoms [68, 69], suggesting that Th1-type immunity induces preeclampsia. Obesity is one of the major risk factors for preeclampsia and may be modifiable. Body weight control efforts before and also during pregnancy may help to reduce the risk of preeclampsia as well as maternal chronic disease in the future.

1.3.2.3 Personal or Family History of Preeclampsia

Personal History

Preeclampsia in a previous pregnancy is a strong predictor of preeclampsia in a subsequent pregnancy. Several studies suggested the risk of developing preeclampsia again was approximately 20%, but may be changed to between 5% and 80% depending on when women had preeclampsia previously and its severity. The pooled unadjusted RR of preeclampsia among women with preeclampsia in a previous pregnancy was 8.4 (95% CI, 7.1–9.9) [34]. If a woman had preeclampsia in a previous pregnancy, a healthcare provider needs to carefully monitor her during pregnancy for any signs or symptoms. Many researchers have already identified preeclampsia susceptibility genes in their study of the effects of maternal susceptibility genes on the development of preeclampsia and identification of high-risk mothers (Table 1.3). In a Japanese study [70], mothers in the lowest quartile for

Table 1.3 Genes associated with preeclampsia

Official symbol	Official full name	Also known as	Chromosome location	Authors	Year
ACVR2A	Activin A receptor type 2A	ACVR2; ACTRII	2q22.3-q23.1	Moses EK et al.	2006
				Williamson RD et al.	2015
ERAP2	Endoplasmic reticulum aminopeptidase 2	LRAP; L-rap	5q15	Johnson MP et al.	2009
				Vanhille DL et al.	2013
GAS6	Growth arrest-specific 6	AXSF; AXLLG	13q34	Ozakpinar OB et al.	2016
				Ye L et al.	2017
SIGLEC6	Sialic acid-binding Ig-like lectin 6	CD327; CD33L; OBBP1; CD33L1; CD33L2; CDW327	19q13.41	Winn VD et al.	2009
				Rumer KK et al.	2013
STOX1	Storkhead box 1	C10orf24	10q22.1	Van Dilk M et al.	2005
				Berends AL et al.	2007
TAC3	Tachykinin 3	NKB; HH10; NKNB; PRO1155; ZNEUROK1	12q13.3	Page NM et al.	2006
TGFB1	Transforming growth factor beta 1	CED; LAP; DPD1; TGFB; TGFbeta	19q13.2	Deepthi G et al.	2015

height (<155 cm, approximately 5 ft. 1 in.) were at a higher risk of preeclampsia (RR, 1.35; 95% CI, 1.25–1.45).

Family History

Women whose mothers, sisters, grandmothers, or aunts were preeclamptic are more likely to develop preeclampsia. The RR of preeclampsia in women with a family history of preeclampsia was previously reported to be 2.90 (95% CI, 1.70–4.93) [71]. Boyd et al. found that previous early-, intermediate-, or late-onset preeclampsia increased the risk of recurrent preeclampsia with the same timing of onset by 25.2-fold (95% CI, 21.8, 29.1), 19.7-fold (95% CI, 17.0–22.8), and 10.3-fold (95% CI, 9.85–10.9), respectively, that of having no such history [72]. They also speculated that early-onset preeclampsia has the largest genetic component even though genetic factors play a role in preeclampsia regardless of the timing of its onset. These findings suggest that future genetic studies need to consider the timing of the onset of preeclampsia.

Paternal Factor (So-Called Dangerous Father)

A population-based study by Lei et al. [73]. showed that the risk of developing preeclampsia was 1.8 (95% CI, 1.2–2.6) in a woman who became pregnant by a man who had already become the father of a child by another woman who developed preeclampsia. Another population study by Esplin et al. [74] found that if a male partner was born of a pregnancy associated with preeclampsia, the risk of his partner developing preeclampsia was 2.1 (95% CI, 1.0–4.3). Dekker et al. provided an overview on how and to what extent paternal factors play a role in the causation of preeclampsia [75]. They focused on the paternal contribution to preeclampsia and summarized that seminal cytokine levels and their effects on maternal immune deviations, specific paternal HLA characteristics, and specific paternal single nucleotide polymorphisms (SNPs), particularly those in paternally expressed genes affecting placentation, contributed to preeclampsia.

1.3.2.4 Ethnicity or Race

African-Americans are known to be at a higher risk of preeclampsia than Caucasians, while Hispanics have a lower risk than non-Hispanics. Yang et al. [76] reported a number of relationships suggesting that midtrimester angiogenic and anti-angiogenic factors are associated with early-onset preeclampsia, and also while low levels of placental growth factor and high levels of soluble endoglin were strongly associated with early-onset preeclampsia across racial–ethnic groups, these associations were stronger in Caucasians and Hispanics than in African-Americans (AOR in Caucasians and Hispanics were >4.4 times the AOR in African-Americans). They concluded that racial–ethnic differences may exist.

1.3.2.5 Pre-existing Hypertension (Chronic Hypertension)

Approximately 2–3% of pregnant women already have high blood pressure when they become pregnant.

Approximately 25% of pregnant women with pre-existing hypertension develop superimposed preeclampsia [50]. However, this rate is only 15% in those who have mild hypertension before conception or early in pregnancy, whereas it approaches 50% in those with severe prepregnancy hypertension [50]. A systematic review and meta-analysis of large cohort studies showed that, in women with chronic hypertension, the pooled RR of preeclampsia was 5.1 (95% CI, 4.0–6.5) [34]. Women with chronic hypertension with superimposed preeclampsia are at a higher risk of giving birth to an infant who is small for gestational age and placental abruption than those with chronic hypertension without superimposed preeclampsia [77].

1.3.2.6 Pre-existing Diabetes Mellitus (Insulin-Dependent Diabetes Mellitus)

The prevalence of preeclampsia in women with type 1 diabetes is 17%. Clinical predictors associated with an increased prevalence of preeclampsia are diabetic nephropathy (OR, 3.7–23.5), microalbuminuria (OR, 3.8–11.7), diabetic retinopathy (OR, 1.9–2.9), and pre-existing hypertension (OR, 3.8–17.1) as well as high blood pressure within the normotensive range [78]. The risk for preeclampsia among women with pre-existing diabetes mellitus depends on the duration of diabetes mellitus as well as the presence of vascular complications and maternal glucose control during pregnancy. The incidence of preeclampsia increases in a manner that parallels the severity of diabetes mellitus by White's classification. Diabetes mellitus is associated with increased oxidative stress caused by hyperglycemia, reactive oxygen species production, and inflammatory signals. The levels of stress-induced proteins (heat shock protein 70 and heme oxygenase-1) in the first trimester placenta were previously shown to be higher in women with diabetes mellitus type 1 than in normal women [79]. Elevated stress in the early placentas of these women may contribute to the development of preeclampsia. A previous study reported a relationship between an increased risk of preeclampsia and loose glucose control (fasting blood glucose above 7 mmol/L) [80].

1.3.2.7 Pre-existing Renal Disease

A retrospective observational study ($N = 80$) revealed that the incidences of preeclampsia ($p = 0.001$) and moderate to severe anemia ($p = 0.001$) were significantly higher in late-stage than in early-stage disease [81]. A systematic review and meta-analysis of large cohort studies showed that the pooled RR of preeclampsia was 1.8 in women with chronic kidney disease (95% CI, 1.5–2.1) [34]. A systematic review and meta-analysis of published cohort studies and case-control studies ($N = 506{,}340$) found that women with chronic kidney disease had greater odds of preeclampsia (10.36; 95% CI, 6.28–17.09) [82]. In a population-based retrospective cohort study ($N = 1954$ preeclamptic women), McDonald et al. [83] demonstrated that the risk of recurrent severe de novo preeclampsia was increased in women with pre-existing renal disease (adjusted OR, 17.98; 95% CI, 3.50–92.52).

In an experiment using nonhuman primates, uteroplacental ischemia caused by uterine artery ligation resulted in preeclampsia (increased blood pressure, proteinuria, endotheliosis on renal biopsy, and elevated soluble fms-like tyrosine kinase 1). PlGF was significantly reduced after ischemia, and a treatment with recombinant human PlGF reduced systolic pressure and proteinuria [84]. Aggravated renal function in women with pre-existing renal disease during pregnancy may cause uteroplacental ischemia, leading to a reduced level of PlGF, and, ultimately, preeclampsia.

1.3.2.8 Antiphospholipid Antibody Syndrome

Antiphospholipid antibody syndrome (APS) is associated with thrombotic events as well as obstetric morbidities in patients who are persistently positive for antiphospholipid (aPL) antibodies. APS is also the most frequently acquired risk factor for a treatable cause of recurrent pregnancy loss. Women with aPL antibodies are at a greater risk of conditions associated with ischemic placental dysfunction, such as miscarriage, intrauterine fetal death, preeclampsia, preterm birth, and fetal growth restriction. In an experimental murine model of APS induced by the passive transfer of human aPL antibodies, Salmon and Girardi showed that complement activation played an essential and causative role in obstetrical APS and that blocking the activation of the complement cascade rescued pregnancies [85]. The use of low-dose aspirin and unfractionated or low-molecular-weight heparin, as well as intravenous immunoglobulin, has now markedly improved pregnancy outcomes in obstetric APS. Statins have recently been linked to improved pregnancy outcomes in mouse models of APS and preeclampsia, possibly due to their protective effects on the endothelium. Lefkou et al. indicated that pravastatin improves pregnancy outcomes in women with refractory obstetric APS when administered at the onset of preeclampsia and/or fetal growth restrictions until the end of pregnancy [86]. A systematic review and meta-analysis of large cohort studies showed that the pooled RR of preeclampsia was 2.8 in women with APS (95% CI, 1.8–4.3) [34]. Women with multiple antibody (anticardiolipin antibodies, anti-β_2 glycoprotein-I, and/or lupus anticoagulant)-positive test results were at a higher risk of preeclampsia without (54.5% vs. 34.8%; AOR, 1.56; 95% CI, 1.22–1.95) and with severe features (22.7% vs. 13.8%; AOR, 1.66; 95% CI, 1.19–2.49) than those with only one antiphospholipid antibody-positive test result [87]. Pooled OR for the association of anticardiolipin antibodies with preeclampsia was 2.86 (95% CI, 1.37–5.98). Pooled OR for anticardiolipin antibodies and severe preeclampsia was 11.15 (95% CI, 2.66–46.75) [88].

1.3.2.9 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that is associated with preeclampsia. Since SLE is mainly a T helper cell type 2 (Th2)-mediated disease, a predominance of the Th2 response may be expected during pregnancy, making SLE exacerbated. However, lower levels of estrogens, progesterone, and Th2 cytokines were found in the third trimester of pregnancy in SLE patients than in healthy pregnant women. A correlation has been reported between active lupus nephritis and both the onset of maternal hypertension during

pregnancy and rate of preterm delivery. A history of lupus nephritis has also been associated with preeclampsia [89]. Among 742 births to women with SLE and 10,484 births to non-SLE women, SLE was associated with an increased risk of early-onset preeclampsia (RR, 7.8; 95% CI, 4.8–12.9) [90].

1.3.2.10 Infections

Ponzetto et al. [91] showed higher seropositivity for *Helicobacter pylori* infection in 47 mothers with PE (51.1%) than in 47 women with an uneventful pregnancy (31.9%). This difference was greater when considering positivity for cytotoxin-associated antigen A-positive strains of HP (80.9 and 14.9%, respectively). Two years later, Conde-Agudelo et al. [92] found that women with urinary tract infection during pregnancy were 57% (95% CI, 45–70) more likely to develop preeclampsia than those without urinary tract infection. In women with urinary tract infection, the OR for preeclampsia was 1.57 (95% CI, 1.45–1.70). They also reported that women with evidence of periodontal disease during pregnancy had a 76% (95% CI, 43–118) higher risk of preeclampsia than women without periodontal disease. In women with periodontal infection, the OR for preeclampsia was 1.76 (95% CI, 1.43–2.18). However, there were no relationships between preeclampsia and the presence of antibodies to *Chlamydia pneumoniae, H. pylori,* cytomegalovirus, treated and non-treated HIV infection, and malaria. Individual studies also did not find a relationship among preeclampsia and herpes simplex virus type 2, bacterial vaginosis, and *Mycoplasma hominis*. In a recent matched nested case-control study, after adjusting for maternal age, pre-existing hypertension, diabetes, renal disease, and multiple pregnancies, the odds of preeclampsia were increased in women prescribed antibiotic drugs (AOR, 1.28; 95% CI, 1.14–1.44) and in those with urinary tract infection (AOR, 1.22; 95% CI, 1.03–1.45) [93]. Thereafter, in a meta-analysis using 3 observational cohort studies and 8 case-control studies, including 11,566 preeclampsia patients in an Asian population, a negative correlation was observed between chronic hepatitis B infection and preeclampsia (OR, 0.77; 95% CI, 0.65–0.90; $p = 0.002$) [94]. Flanagan et al. showed that exposure to malarial antigens in utero resulted in the expansion of malaria-specific FOXP3(+) regulatory T cells and more generalized Foxp3(+) CD4(+) regulatory T cells in chronic and resolved placental malaria, alongside increased Th1 pro-inflammatory responses (IFN-gamma, TNF-alpha, and IFN-gamma/IL-10) in resolved placental malaria infection only [95]. Persistent infections without inflammation may induce the activation of regulatory T cells, which prevents the development of preeclampsia.

1.3.3 Environmental Factors

1.3.3.1 High Altitude

Residing at a high altitude (higher than 2700 m above sea level) is the only environmental factor that has been linked to an increase in the prevalence of preeclampsia. Moore et al. reported that pregnancy-induced hypertension occurred in 12%, 4%, and 3% of pregnancies at 3100 m, 2410 m, and 1600 m, respectively [96]. In

their study, blood pressure was generally higher in all pregnant women at a high altitude. Moreover, in a small group of women with pregnancy-induced hypertension, arterial oxygen saturation inversely correlated with blood pressure. Moore and colleagues [97] subsequently compared pro- and anti-inflammatory cytokines between women with early- and late-onset PE at high altitudes and investigated the relationships among these variables and compromised UA BF and fetal growth. They concluded that pro-inflammatory cytokines (IL-6 and IL-8) adversely influenced disease severity and contributed to the heavy toll exerted by PE in high-altitude regions.

1.3.3.2 Income

Obstetricians have believed for many years that the family income of preeclamptic women was low because the mortality rate of eclampsia inversely correlated with the average family income and maternal deaths from eclampsia increased in areas of poverty. This finding was contrary to expectation. The incidence of preeclampsia varied according to family income. When grouped by income, the highest incidences of preeclampsia were found in upper middle-income countries, whereas eclampsia appeared to be more frequent in lower middle-income countries [1].

1.4 Factors for Preventing Preeclampsia

1.4.1 Smoking

Several studies demonstrated that the incidence of preeclampsia was lower in cigarette smokers than in non-smokers. A systematic review of 28 cohort studies reported an inverse association between cigarette smoking during pregnancy and the incidence of preeclampsia (typical RR, 0.68; 95% CI, 0.67–0.69), and the findings obtained in case-control studies were similar (typical OR, 0.68; 95% CI, 0.57–0.81) [98]. In another systematic review and meta-analysis of prospective studies, Wei et al. [99] showed an inverse correlation between smoking during pregnancy and the incidence of preeclampsia (RR, 0.67; 95% CI, 0.60–0.75), with strong heterogeneity ($I^2 = 91.7\%$, $p < 0.001$). Although the precise mechanisms responsible for the relationship between smoking during pregnancy and the incidence of preeclampsia have not yet been fully elucidated, an experimental study revealed that carbon monoxide, one of the main toxic chemicals of smoking, lowers soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) production in endothelial cells and placental cultures through the monooxygenase-1/carbon monoxide pathway [100]. In human studies, Levine et al. found that anti-angiogenic factors such as sFlt1 and sEng may play an important role in the pathogenesis of preeclampsia [101, 102]. Jeyabalan et al. [103] also suggested that non-smokers have a higher circulating level of sFlt1 during pregnancy, while smokers have lower maternal sFlt1 concentrations during pregnancy. These reduced levels of sFlt1 in smokers may result in a decrease in the incidence of preeclampsia.

Nicotine has therapeutic potential for preeclampsia. Mimura et al. found that nicotine significantly facilitated endothelial migration and tube formation, restored soluble fms-like tyrosine kinase 1 and/or soluble endoglin-reduced endothelial functions, and stimulated placental growth factor production [104]. Yamada-Nomoto et al. revealed that, in a murine endometriosis model, an alpha-7 nicotinic acetylcholine receptor (nAChR) agonist significantly suppressed lipopolysaccharide-induced interleukin-1beta expression [105]. Based on these findings, nicotine may activate anti-inflammatory functions, and an unknown substance with similar functions to an alpha-7 nAChR agonist has potential as a new candidate for the treatment of preeclampsia.

1.4.2 Summer Births

Algert et al. reported that pregnancy hypertension rates, by month of conception, were the lowest in autumn (7.3%) and highest in spring (8.9%) [106]. They also revealed that higher sunlight intensity before delivery, but not around conception, was associated with decreased pregnancy hypertension and suspected that increased sunlight around conception may decrease the rate of early-onset preeclampsia ($p = 0.09$). De-Regil et al. found that women who received vitamin D with calcium had a lower risk of preeclampsia than those not receiving any intervention (RR 0.51; 95% CI, 0.32–0.80) [107]. In summer, increasing sunlight levels may result in the synthesis of high levels of vitamin D and may also increase ambient temperature, leading to vasodilatation.

1.4.3 Maternal Physical Activity

The effects of maternal physical activity on preeclampsia are conflicting. Aune et al. [108] showed that the risk of preeclampsia was reduced with increases in physical activity levels before pregnancy and during early pregnancy. However, the findings of a meta-analysis showed that, in 17 trials, including 5075 pregnant women, the incidence of preeclampsia was similar in both groups (2.3% vs. 2.8%; RR 0.79, 95% CI, 0.45–1.38; 6 studies, 2230 participants) [109].

Conclusion

We herein presented extensive epidemiological findings on preeclampsia. Preeclampsia may be regarded as a familial condition in which many faulty immune responses are triggered by a particular mother or male partner or particular materials in an age of plenty. Most modifiable risk factors for preeclampsia are acquired factors associated with maternal inflammation and infection. In contrast, most unmodifiable risk factors are congenital factors associated with genetics and ethnicity. A large amount of research worldwide is aimed at the elucidation of these complex relationships between preeclampsia and unmodifiable risk factors including ethnicity and gene susceptibility. It is essential to facilitate cooperation and collaboration among researchers in order to reduce the incidence of preeclampsia.

References

1. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013;170:1–7.
2. Kichou B, Henine N, Kichou L, Benbouabdellah M. Epidemiology of pre-eclampsia in Tizi-ouzou city (Algeria). Ann Cardiol Angeiol. 2015;64(3):164–8.
3. Elongi Moyene JP, Scheers H, Tandu-Umba B, Haufroid V, Buassa-Bu-Tsumbu B, Verdonck F, Spitz B, Nemery B. Preeclampsia and toxic metals: a case-control study in Kinshasa, DR Congo. Environ Health. 2016;15:48.
4. Wagnew M, Dessalegn M, Worku A, Nyagero J. Trends of preeclampsia/eclampsia and maternal and neonatal outcomes among women delivering in Addis Ababa selected government hospitals, Ethiopia: a retrospective cross-sectional study. Pan Afr Med J. 2016;25(Suppl 2):12.
5. Bansal YP. Pre-eclampsia/eclampsia: a profile from Pumwani Maternity Hospital, Nairobi. Kenya East Afr Med J. 1985;62(10):691–8.
6. Udenze IC, Arikawe AP, Makwe CC. Early pregnancy plasminogen activator inhibitor-1 levels in Nigerian women and its relationship with preeclampsia. Niger J Clin Pract. 2017;20(5):517–22.
7. Kiondo P, Wamuyu-Maina G, Wandabwa J, Bimenya GS, Tumwesigye NM, Okong P. The effects of vitamin C supplementation on pre-eclampsia in Mulago Hospital, Kampala, Uganda: a randomized placebo controlled clinical trial. BMC Pregnancy Childbirth. 2014;14:283.
8. Auger N, Luo ZC, Nuyt AM, Kaufman JS, Naimi AI, Platt RW, Fraser WD. Secular trends in preeclampsia incidence and outcomes in a large Canada database: a longitudinal study over 24 years. Can J Cardiol. 2016;32(8):987.e15–23.
9. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987–2004. Am J Hypertens. 2008;21(5):521–6.
10. Rezende KB, Bornia RG, Esteves AP, Cunha AJ, Amim Junior J. Preeclampsia: prevalence and perinatal repercussions in a University Hospital in Rio de Janeiro, Brazil. Pregnancy Hypertens. 2016;6(4):253–5.
11. Canto-Cetina T, Coral-Vázquez RM, Rojano-Mejía D, Godoy SP, Coronel A, Canto P. Higher prepregnancy body mass index is a risk factor for developing preeclampsia in Maya-Mestizo women: a cohort study. Ethn Health. 2017:1–9.
12. Hernández B, Ortiz-Panozo E, Pérez-Cuevas R. Facility-based care for delivery and management of complications related to pregnancy and childbirth in Mexico. Salud Publica Mex. 2012;54(5):496–505.
13. Wynn A, Cabeza J, Adachi K, Needleman J, Garcia PJ, Klausner JD. Frequency of maternal and newborn birth outcomes, Lima, Peru, 2013. PLoS One. 2015;10(3):e0116102.
14. Cripe SM, O'Brien W, Gelaye B, Williams MA. Perinatal outcomes of Southeast Asians with pregnancies complicated by gestational diabetes mellitus or preeclampsia. J Immigr Minor Health. 2012;14(5):747–53.
15. Li X, Tan H, Huang X, Zhou S, Hu S, Wang X, Xu X, Liu Q, Wen SW. Similarities and differences between the risk factors for gestational hypertension and preeclampsia: a population based cohort study in south China. Pregnancy Hypertens. 2016;6(1):66–71.
16. Eswarappa M, Rakesh M, Sonika P, Snigdha K, Midhun M, Kaushik K, Chennabasappa GK, Sujeeth B. Spectrum of renal injury in pregnancy-induced hypertension: experience from a single center in India. Saudi J Kidney Dis Transpl. 2017;28(2):279–84.
17. Aabidha PM, Cherian AG, Paul E, Helan J. Maternal and fetal outcome in pre-eclampsia in a secondary care hospital in South India. J Family Med Prim Care. 2015;4(2):257–60.
18. Gupta A, Kant S, Pandav CS, Gupta SK, Rai SK, Misra P. Dietary calcium intake, serum calcium level, and their association with preeclampsia in rural North India. Indian J Community Med. 2016;41(3):223–7.

19. Shiozaki A, Matsuda Y, Satoh S, Saito S. Comparison of risk factors for gestational hypertension and preeclampsia in Japanese singleton pregnancies. J Obstet Gynaecol Res. 2013;39(2):492–9.
20. Yamada T, Obata-Yasuoka M, Hamada H, Baba Y, Ohkuchi A, Yasuda S, Kawabata K, Minakawa S, Hirai C, Kusaka H, Murabayashi N, Inde Y, Nagura M, Umazume T, Itakura A, Maeda M, Sagawa N, Ohno Y, Kataoka S, Fujimori K, Kudo Y, Ikeda T, Nakai A, Minakami H. Isolated gestational proteinuria preceding the diagnosis of preeclampsia—an observational study. Acta Obstet Gynecol Scand. 2016;95(9):1048–54.
21. Rao AK, Cheng YW, Caughey AB. Perinatal complications among different Asian-American subgroups. Am J Obstet Gynecol. 2006;194(5):e39–41.
22. Quillan JP, Kwong A, Passmore P. An epidemiological investigation of pre-eclampsia and elevated blood pressure among Kampuchean refugee women at Sakaeo Holding Center, Thailand. J Trop Med Hyg. 1983;86(5):185–91.
23. Hanprasertpong T, Hanprasertpong J. Pregnancy outcomes in Southeast Asian migrant workers at Southern Thailand. J Obstet Gynaecol. 2015;35(6):565–9.
24. Pedersen M, Halldorsson TI, Olsen SF, Hjortebjerg D, Ketzel M, Grandström C, Raaschou-Nielsen O, Sørensen M. Impact of road traffic pollution on pre-eclampsia and pregnancy-induced hypertensive disorders. Epidemiology. 2017;28(1):99–106.
25. Metsälä J, Stach-Lempinen B, Gissler M, Eriksson JG, Koivusalo S. Risk of pregnancy complications in relation to maternal prepregnancy body mass index: population-based study from Finland 2006–10. Paediatr Perinat Epidemiol. 2016;30(1):28–37.
26. Schneider S, Freerksen N, Röhrig S, Hoeft B, Maul H. Gestational diabetes and preeclampsia—similar risk factor profiles? Early Hum Dev. 2012;88(3):179–84.
27. Alsnes IV, Vatten LJ, Fraser A, Bjørngaard JH, Rich-Edwards J, Romundstad PR, Åsvold BO. Hypertension in pregnancy and offspring cardiovascular risk in young adulthood: prospective and sibling studies in the HUNT study (Nord-Trøndelag Health Study) in Norway. Hypertension. 2017;69(4):591–8.
28. Cnattingius S, Wikström AK, Stephansson O, Johansson K. The impact of small for gestational age births in early and late preeclamptic pregnancies for preeclampsia recurrence: a cohort study of successive pregnancies in Sweden. Paediatr Perinat Epidemiol. 2016;30(6):563–70.
29. Purde MT, Baumann M, Wiedemann U, Nydegger UE, Risch L, Surbek D, Risch M. Incidence of preeclampsia in pregnant Swiss women. Swiss Med Wkly. 2015;145:w14175.
30. Bhattacharya S, Campbell DM, Liston WA, Bhattacharya S. Effect of body mass index on pregnancy outcomes in nulliparous women delivering singleton babies. BMC Public Health. 2007;7:168.
31. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, McCowan LM, Simpson NA, Dekker GA, Roberts CT, Rodems K, Noland B, Raymundo M, Walker JJ, North RA. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014;64(3):644–52.
32. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005;365:785–99.
33. Dekker G, Sibai B. Primary, secondary, and tertiary prevention of preeclampsia. Lancet. 2001;357:209–15.
34. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ. 2016;353:i1753.
35. Saito S, Sakai M, Sasaki Y, Nakashima A, Shiozaki A. Inadequate tolerance induction may induce pre-eclampsia. J Reprod Immunol. 2007 Dec;76(1–2):30–9.
36. Robillard PY, Dekker G, Chaouaut G, Husley TC, Saftlas A. Epidemiological studies on primipaternity and immunology in preeclampsia—a statement after twelve years after workshops. J Reprod Immunol. 2011;89:104–17.
37. Feeney JG, Scott JS. Pre-eclampsia and changed paternity. Eur J Obstet Gynecol Reprod Biol. 1980;11:35–8.

38. Tubbergen P, Lachmeijer AM, Althuisius SM, Vlak ME, van Geijn HP, Dekker GA. Change in paternity: a risk factor for preeclampsia in multiparous women? J Reprod Immunol. 1999;45:81–8.
39. Saftlas AF, Levine RJ, Klebanoff MA, Martz KL, Ewell MG, Morris CD, et al. Abortion. changed paternity, and risk of preeclampsia in nulliparous women. Am J Epidemiol. 2003;157:1108–14.
40. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature. 2012;490(7418):102–6.
41. Klonoff-Cohen HS, Savitz DA, Cefalo RC, McCann MF. An epidemiologic study of contraception and preeclampsia. JAMA. 1989;262(22):3143–7.
42. Robillard PY, Hulsey TC, Périanin J, Janky E, Miri EH, Papiernik E. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. Lancet. 1994;344(8928):973–5.
43. Wang JX, Knottnerus AM, Schuit G, Norman RJ, Chan A, Dekker GA. Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia. Lancet. 2002;359:673–4.
44. Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlström AC, Care AS. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol Reprod. 2009;80(5):1036–45.
45. Robertson SA, Guerin LR, Moldenhauer LM, Hayball JD. Activating T regulatory cells for tolerance in early pregnancy—the contribution of seminal fluid. J Reprod Immunol. 2009;83(1–2):109–16.
46. Shima T, Inada K, Nakashima A, Ushijima A, Ito M, Yoshino O, Saito S. Paternal antigen-specific proliferating regulatory T cells are increased in uterine-draining lymph nodes just before implantation and in pregnant uterus just after implantation by seminal plasma-priming in allogeneic mouse pregnancy. J Reprod Immunol. 2015;108:72–82.
47. Skjaerven R, Wilcox AJ, Lie RT. The interval between pregnancies and the risk of preeclampsia. N Engl J Med. 2002;346:33–8.
48. Barton JR, Sibai BM. Prediction and prevention of recurrent preeclampsia. Obstet Gynecol. 2008;112(2 Pt 1):359–72.
49. Storgaard M, Loft A, Bergh C, Wennerholm UB, Söderström-Anttila V, Romundstad LB, et al. Obstetric and neonatal complications in pregnancies conceived after oocyte donation: a systematic review and meta-analysis. BJOG. 2017;124:561–72.
50. Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-induced hypertension: a meta-analysis and case series. Dtsch Arztebl Int. 2011;108(3):23–31.
51. Masoudian P, Nasr A, de Nanassy J, Fung-Kee-Fung K, Bainbridge SA, El Demellawy D. Oocyte donation pregnancies and the risk of preeclampsia or gestational hypertension: a systematic review and metaanalysis. Am J Obstet Gynecol. 2016;214(3):328–39.
52. Conde-Agudelo A, Belizán JM. Risk factors for pre-eclampsia in a large cohort of Latin American and Caribbean women. BJOG. 2000;107:75–83.
53. Mastrobattista JM, Skupski DW, Monga M, Blanco JD, August P. The rate of severe preeclampsia is increased in triplet as compared to twin gestations. Am J Perinatol. 1997;14:263–5.
54. Maxwell CV, Lieberman E, Norton M, Cohen A, Seely EW, Lee-Parritz A. Relationship of twin zygosity and risk of preeclampsia. Am J Obstet Gynecol. 2001;185:819–21.
55. Shiozaki A, Matsuda Y, Satoh S, Saito S. Impact of fetal sex in pregnancy-induced hypertension and preeclampsia in Japan. J Reprod Immunol. 2011;89:133–9.
56. Kajii T, Ohama K. Androgenetic origin of hydatidiform mole. Nature. 1977;268(5621):633–4.
57. Zhao M, Yin Y, Guo F, Wang J, Wang K, Chen Q. Placental expression of VEGF is increased in pregnancies with hydatidiform mole: possible association with developing very early onset preeclampsia. Early Hum Dev. 2013;89(8):583.
58. Yoneda N, Shiozaki A, Miura K, Yonezawa R, Takemura K, Yoneda S, et al. A triploid partial mole placenta from paternal isodisomy with a diploid fetus derived from one sperm and one oocyte may have caused angiogenic imbalance leading to preeclampsia-like symptoms at 19 weeks of gestation. Placenta. 2013;34:631–4.

59. Koga K, Osuga Y, Tajima T, Hirota Y, Igarashi T, Fujii T, Yano T, Taketani Y. Elevated serum soluble fms-like tyrosine kinase 1 (sFlt1) level in women with hydatidiform mole. Fertil Steril. 2010;94(1):305–8.
60. Toivanen P, Hirvonen T. Sex ratio of newborn: preponderance of males in toxemia of pregnancy. Science. 1970;170:187–8.
61. Global Pregnancy Collaboration, Schalekamp-Timmermans S, Arends LR, Alsaker E, Chappell L, Hansson S, Harsem NK, Jälmby M, Jeyabalan A, Laivuori H, Lawlor DA, Macdonald-Wallis C, Magnus P, Myers J, Olsen J, Poston L, Redman CW, Staff AC, Villa P, Roberts JM, Steegers EA. Fetal sex-specific differences in gestational age at delivery in pre-eclampsia: a meta-analysis. Int J Epidemiol. 2017;46(2):632–42. pii: dyw178.
62. Vatten LJ, Skjaerven R. Offspring sex and pregnancy outcome by length of gestation. Early Hum Dev. 2004;76(1):47–54.
63. Funai EF, Paltiel OB, Malaspina D, Friedlander Y, Deutsch L, Harlap S. Risk factors for preeclampsia in nulliparous and parous women: the Jerusalem perinatal study. Paediatr Perinat Epidemiol. 2005;19(1):59–68.
64. Cox LS, Redman C. The role of cellular senescence in ageing of the placenta. Placenta. 2017;52:139–45.
65. Roberts JM, Hansson SR, Vaiman D, Redman CWG, Global Pregnancy Collaboration. Global Pregnancy Collaboration symposium on placental health: summary and recommendations. Placenta. 2017;52:116–21.
66. Garmendia ML, Zamudio C, Araya M, Kain J. Association between prepregnancy obesity and metabolic risk in Chilean premenopausal women 10 y postpartum. Nutrition. 2017;38:20–7.
67. Molvarec A, Shiozaki A, Ito M, Toldi G, Stenczer B, Szarka A, Nakashima A, Vásárhelyi B, Rigó J Jr, Saito S. Increased prevalence of peripheral blood granulysin-producing cytotoxic T lymphocytes in preeclampsia. J Reprod Immunol. 2011;91(1–2):56–63.
68. Hayakawa S, Fujikawa T, Fukuoka H, Chisima F, Karasaki-Suzuki M, Ohkoshi E, Ohi H, Kiyoshi Fujii T, Tochigi M, Satoh K, Shimizu T, Nishinarita S, Nemoto N, Sakurai I. Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. J Reprod Immunol. 2000;47(2):121–38. Erratum in: J Reprod Immunol 2001; 49(1): 95.
69. Zenclussen AC, Fest S, Joachim R, Klapp BF, Arck PC. Introducing a mouse model for preeclampsia: adoptive transfer of activated Th1 cells leads to pre-eclampsia-like symptoms exclusively in pregnant mice. Eur J Immunol. 2004;34(2):377–87.
70. Ogawa K, Morisaki N, Saito S, Sato S, Fujiwara T, Sago H. Association of shorter height with increased risk of ischaemic placental disease. Paediatr Perinat Epidemiol. 2017;31(3):198–205.
71. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ. 2005;330:565.
72. Boyd HA, Tahir H, Wohlfahrt J, Melbye M. Associations of personal and family pre-eclampsia history with the risk of early-, intermediate- and late-onset preeclampsia. Am J Epidemiol. 2013;178(11):1611–9.
73. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. Fetal and maternal contributions to risk of preeclampsia: a population based study. BMJ. 1998;316: 1343–7.
74. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, et al. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med. 2001;344: 867–72.
75. Dekker G, Robillard PY, Roberts C. The etiology of preeclampsia: the role of the father. J Reprod Immunol. 2011;89(2):126–32.
76. Yang J, Pearl M, DeLorenze GN, Romero R, Dong Z, Jelliffe-Pawlowski L, et al. Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. Am J Obstet Gynecol. 2016;215:359.e1–9.
77. Seely EW, Ecker J. Chronic hypertension in pregnancy. N Engl J Med. 2011;365:439–46.

78. Vestgaard M, Sommer MC, Ringholm L, Damm P, Mathiesen ER. Prediction of preeclampsia in type 1 diabetes in early pregnancy by clinical predictors: a systematic review. J Matern Fetal Neonatal Med. 2017:1–7. https://doi.org/10.1080/14767058.2017.1331429.
79. Gauster M, Majali-Martinez A, Maninger S, Gutschi E, Greimel PH, Ivanisevic M, Djelmis J, Desoye G, Hiden U. Maternal type 1 diabetes activates stress response in early placenta. Placenta. 2017;50:110–6.
80. Middleton P, Crowther CA, Simmonds L. Different intensities of glycaemic control for pregnant women with pre-existing diabetes. Cochrane Database Syst Rev. 2016;5:CD008540.
81. Bharti J, Vatsa R, Singhal S, Roy KK, Kumar S, Perumal V, Meena J. Pregnancy with chronic kidney disease: maternal and fetal outcome. Eur J Obstet Gynecol Reprod Biol. 2016;204:83–7.
82. Zhang JJ, Ma XX, Hao L, Liu LJ, Lv JC, Zhang H. A systematic review and meta-analysis of outcomes of pregnancy in CKD and CKD outcomes in pregnancy. Clin J Am Soc Nephrol. 2015;10:1964–78.
83. McDonald SD, Best C, Lam K. The recurrence risk of severe de novo pre-eclampsia in singleton pregnancies: a population-based cohort. BJOG. 2009;116:1578–84.
84. Makris A, Yeung KR, Lim SM, Sunderland N, Heffernan S, Thompson JF, Iliopoulos J, Killingsworth MC, Yong J, Xu B, Ogle RF, Thadhani R, Karumanchi SA, Hennessy A. Placental growth factor reduces blood pressure in a uteroplacental ischemia model of preeclampsia in nonhuman primates. Hypertension. 2016;67(6):1263–72.
85. Salmon JE, Girardi G. Antiphospholipid antibodies and pregnancy loss: a disorder of inflammation. J Reprod Immunol. 2008;77(1):51–6.
86. Lefkou E, Mamopoulos A, Dagklis T, Vosnakis C, Rousso D, Girardi G. Pravastatin improves pregnancy outcomes in obstetric antiphospholipid syndrome refractory to antithrombotic therapy. J Clin Invest. 2016;126(8):2933–40.
87. Saccone G, Berghella V, Maruotti GM, Ghi T, Rizzo G, Simonazzi G, et al.; PREGNANTS (PREGNancy in Women with ANTiphospholipid Syndrome) Working Group. Antiphospholipid antibody profile based obstetric outcomes of primary antiphospholipid syndrome: the PREGNANTS study. Am J Obstet Gynecol. 2017;216(5):525.e1–12. pii: S0002–9378(17)30148–5. https://doi.org/10.1016/j.ajog.2017.01.026.
88. do Prado AD, Piovesan DM, Staub HL, Horta BL. Association of anticardiolipin antibodies with preeclampsia: a systematic review and meta-analysis. Obstet Gynecol. 2010;116(6):1433–43.
89. de Jesus GR, Mendoza-Pinto C, de Jesus NR, Dos Santos FC, Klumb EM, Carrasco MG, Levy RA. Understanding and managing pregnancy in patients with lupus. Autoimmune Dis. 2015;2015:943490.
90. Simard JF, Arkema EV, Nguyen C, Svenungsson E, Wikström AK, Palmsten K, Salmon JE. Early-onset preeclampsia in lupus pregnancy. Paediatr Perinat Epidemiol. 2017;31:29–36.
91. Ponzetto A, Cardaropoli S, Piccoli E, Rolfo A, Gennero L, Kanduc D, et al. Pre-eclampsia is associated with Helicobacter pylori seropositivity in Italy. J Hypertens. 2006;24:2445–9.
92. Conde-Agudelo A, Villar J, Lindheimer M. Maternal infection and risk of preeclampsia: systematic review and metaanalysis. Am J Obstet Gynecol. 2008;198:7–22.
93. Minassian C, Thomas SL, Williams DJ, Campbell O, Smeeth L. Acute maternal infection and risk of pre-eclampsia: a population-based case-control study. PLoS One. 2013;8(9):e73047.
94. Huang QT, Chen JH, Zhong M, Hang LL, Wei SS, Yu YH. Chronic hepatitis B infection is associated with decreased risk of preeclampsia: a meta-analysis of observational studies. Cell Physiol Biochem. 2016;38:1860–8.
95. Flanagan KL, Halliday A, Burl S, Landgraf K, Jagne YJ, Noho-Konteh F, Townend J, Miles DJ, van der Sande M, Whittle H, Rowland-Jones S. The effect of placental malaria infection on cord blood and maternal immunoregulatory responses at birth. Eur J Immunol. 2010;40(4):1062–72.
96. Moore LG, Hershey DW, Jahnigen D, Bowes W. The incidence of pregnancy-induced hypertension is increased among Colorado residents at high altitude. Am J Obstet Gynecol. 1982;144:423–9.

97. Dávila RD, Julian CG, Vaughn A, Browne VA, Toledo-Jaldin L, Wilson MJ, et al. Role of cytokines in altitude-associated preeclampsia. Pregnancy Hypertens. 2012;2(1):65–70.
98. Conde-Agudelo A, Althabe F, Belizán JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gynecol. 1999;181:1026–35.
99. Wei J, Liu CX, Gong TT, Wu QJ, Wu L. Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies. Oncotarget. 2015;6:43667–78. https://doi.org/10.18632/oncotarget.6190.
100. Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, et al. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation. 2007;115:1789–97.
101. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350:672–83.
102. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355:992–1005.
103. Jeyabalan A, Powers RW, Durica AR, Harger GF, Roberts JM, Ness RB. Cigarette smoke exposure and angiogenic factors in pregnancy and preeclampsia. Am J Hypertens. 2008;21:943–7.
104. Mimura K, Tomimatsu T, Sharentuya N, Tskitishvili E, Kinugasa-Taniguchi Y, Kanagawa T, Kimura T. Nicotine restores endothelial dysfunction caused by excess sFlt1 and sEng in an in vitro model of preeclamptic vascular endothelium: a possible therapeutic role of nicotinic acetylcholine receptor (nAChR) agonists for preeclampsia. Am J Obstet Gynecol. 2010;202(5):464.e1–6.
105. Yamada-Nomoto K, Yoshino O, Akiyama I, Ushijima A, Ono Y, Shima T, Nakashima A, Hayashi S, Kadowaki M, Osuga Y, Saito S. Alpha-7 nicotinic acetylcholine receptor (nAChR) agonist inhibits the development of endometriosis by regulating inflammation. Am J Reprod Immunol. 2016;76(6):491–8.
106. Algert CS, Roberts CL, Shand AW, et al. Seasonal variation in pregnancy hypertension is correlated with sunlight intensity. Am J Obstet Gynecol. 2010;203:215.e1–5.
107. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev. 2016;1:CD008873.
108. Aune D, Saugstad OD, Henriksen T, Tonstad S. Physical activity and the risk of preeclampsia: a systematic review and meta-analysis. Epidemiology. 2014;25(3):331–43.
109. Magro-Malosso ER, Saccone G, Di Tommaso M, Roman A, Berghella V. Exercise during pregnancy and risk of gestational hypertensive disorders: a systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2017;96(8):921–931.

Part II

Genetic Background in Preeclampsia

Genetic Background of Preeclampsia

2

Junichi Sugawara, Yuji Oe, and Maiko Wagata

Abstract

Preeclampsia is a multifactorial disease caused by complex interactions of genetic and environmental components. Accumulating evidence suggests that genetic contributions to the pathogenesis of the disease are composed of maternal, fetal, and paternal factors. In this chapter, genetic research into preeclampsia was reviewed from diversified perspectives including recent approaches of genome-wide association studies and immunological genetic analysis of human leucocyte antigens. In the last few decades, extensive research by basic, clinical, and epidemiological fields revealed a variety of causative genetic factors, including maternal activin receptor type 2 gene, inhibin beta B gene, paternal glutathione S-transferase P1-1 gene, and fetal catechol-O-methyltransferase gene. However, major problems in genetic research include a relatively small number of cases, the diversity of genetic racial background, and unstandardized diagnostic criteria for the disease. On the other hand, recent innovative progression of system biology may enable us to discover unknown mechanisms through the use of very large genetic databases. To uncover causal relationship between genetic and environmental factors in preeclampsia, large-scale association studies with familial pedigree information should be undertaken by collaborative global networks.

J. Sugawara, M.D., Ph.D. (✉)
Division of Feto-Maternal Medical Science, Department of Community Medical Support, Tohoku Medical Megabank Organization, Tohoku University, 2-1 Seiryocho, Sendai, 9808573 Miyagi, Japan

Department of Obstetrics and Gynecology, Tohoku University Graduate School of Medicine, 2-1 Seiryocho, Sendai, Miyagi, Japan
e-mail: jsugawara@med.tohoku.ac.jp

Y. Oe, M.D., Ph.D. • M. Wagata, M.D.
Division of Feto-Maternal Medical Science, Department of Community Medical Support, Tohoku Medical Megabank Organization, Tohoku University, 2-1 Seiryocho, Sendai, 9808573 Miyagi, Japan

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_2

Keywords
Multifactorial disease • Gene environmental interaction • Human genetics • Genome-wide association study • Single nucleotide polymorphism

2.1 Introduction

Preeclampsia has been recognized as a multifactorial disease with great phenotypic diversity, and it is caused by a complex interplay between genetic and environmental factors [1]. It has been hypothesized that multifactorial diseases are caused by qualitative and quantitative changes stemming from variants of associated gene products as well as environmental and incidental factors. Theoretically, the clinical phenotype is expressed when the total effects of those factors exceed the threshold [2].

A genetic component of preeclampsia was suggested by the observed clustering of cases in affected families in the nineteenth century [3].

Recently, familial aggregation and twin studies have revealed that preeclampsia has a higher relative risk compared to controls, and accumulating evidence strongly suggests that genetic analysis is a promising approach for this multifactorial disease [4–7].

Although large research efforts have been devoted to the analysis of individual genes, no universally reliable genetic variants have been identified. Major difficulties of this approach can be ascribed to small influences of individual genetic loci, complex interactions of environmental exposure, and polygenic susceptibility [8, 9]. In addition, inconsistency of clinical diagnosis and ethnic variations within study populations may influence those analytical approaches.

Improvements in bioinformatic technologies now enable us to conduct genome-wide association studies (GWAS). GWAS is an unbiased approach to the identification of candidate genes and the quantification of the associated risks by the assessment of single nucleotide polymorphisms (SNP) [10]. GWAS has identified genetic variants associated with common diseases, including essential hypertension, coronary artery disease, and type 2 diabetes [11]; however, few studies of this nature have been conducted in the field of preeclampsia [12, 13].

Taken altogether, it is quite certain that genetic factors play most important roles in the pathogenesis of this multifactorial disease. This chapter describes the current state of the genetic analysis of preeclampsia and discusses the future directions of such research.

2.2 Overview of Genetic Factors in the Pathogenesis of Preeclampsia

To date, the importance of genetic factors in preeclampsia has been widely accepted as a result of various epidemiologic studies. Maternal, paternal, fetal, and couple genetic effects in combination with a variety of environmental factors are strongly

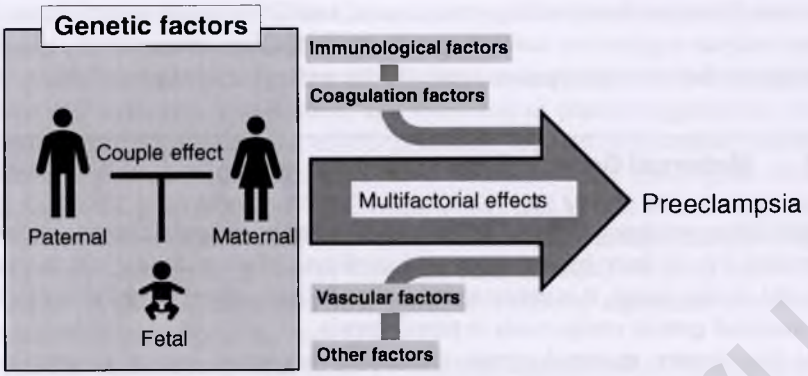


Fig. 2.1 Genetic components and onset of preeclampsia. Maternal, paternal, fetal, and couple genetic components play an important role in the pathogenesis of preeclampsia. Genetic factors and other factors coordinately cause poor placentation, which in turn leads to systemic endothelial dysfunction and onset of the disease

related to the onset of this complex disease (Fig. 2.1). Familial aggregation is an important characteristic of multifactorial diseases. The association of family history of preeclampsia and incidence rate has been examined. Specifically, the incidence in pregnant women with affected mothers is approximately 20–40% and 37% with affected sisters [14]. Furthermore, it was reported that the relative risk in pregnant women with familial history of preeclampsia is 2.9 (95%, CI 1.7–4.9) [15].

In monozygotic sib-pairs, whose genetic backgrounds are theoretically matched completely, the incidence rate of preeclampsia in one of the twins with affected sisters is 33.6 (95%, CI 7.8–145.0) [6]. On the other hand, it was reported that no preeclamptic patients were observed in monozygotic twins [16, 17], although the sample sizes of those studies were relatively small. Taken together, in twin studies, the incidence rate of preeclampsia might be different, and pathogenic effects of other factors in addition to genetic predispositions play important roles in the onset of this complex disease.

Recent reports suggested that the etiology of preeclampsia and hypertension has common genetic backgrounds. Pregnant women having mothers affected with chronic hypertension exhibited higher odds ratio, i.e., 1.9 (95%, CI 1.1–3.2), and women with affected fathers showed even higher odds, i.e., 2.6 (95%, CI 1.2–5.5). Furthermore, those with an affected mother and sister had a 4.7 odds ratio (95%, CI 1.9–11.6) [18].

Genetic interactions between mother, father, and fetus have been reported in many studies. Fathers having partners with a past history of preeclampsia exhibited odds ratio of 1.8 (95%, CI 1.2–2.6) in pregnancy with the other partner [19]. In another study, males and females having mothers with preeclampsia showed statistically higher odds in later pregnancy as follows: male 2.1 (95%, CI 1.0–4.3)and female 3.3 (95%, CI 1.5–7.5) [20]. With regard to the concordance rate of preeclampsia in sib-pairs, one cohort study investigating the contribution rates of various factors suggested that the variance of susceptibility to preeclampsia was as follows: 35% was attributable to maternal genetic effects, 20% to fetal genetic effects, 13% to couple effects,

less than 1% to the shared sibling environment, and 32% to unmeasured factors [5]. These analyses suggest that maternal genetic predispositions and paternally inherited fetal genetic factors might be associated with the pathogenesis of preeclampsia.

2.3 Maternal Genetic Factors

Accumulating evidence suggest that women who had preeclamptic pregnancy are at increased risk of later hypertension and cardiovascular morbidity and mortality [21–26]. In this aspect, it is necessary to pursue definite contributing factors including maternal genetic components in preeclampsia.

In this chapter, maternal genetic factors in association with preeclampsia are discussed in terms of meta-analysis studies. Noteworthy meta-analysis of maternal genes of preeclampsia is shown in Table 2.1.

2.3.1 Vasoactive-Related Gene Polymorphism

Recently, maternal genetic polymorphisms related to preeclampsia have been reported, with a focus on the renin-angiotensin system. M235T (T704C, rs699) variants in exon 2 of the angiotensinogen gene have been suggested to correlate with elevated circulating angiotensinogen levels. Meta-analysis of preeclamptic patients, including 31 studies involving 2555 patients and 6114 controls, suggested that M235T variants exhibited a significant association with the incidence of preeclampsia, especially in Caucasian and Asian ethnicities. On the other hand, T174M showed no correlations [27]. With regard to insertion/deletion mutation (rs1799752) in intron 16 of the angiotensin-converting enzyme (*ACE*) gene, D-type mutations have been reported to be associated with atherosclerosis, ischemic heart diseases, and elevated circulating ACE levels. In a meta-analysis that included 30 studies with 3523 preeclampsia cases and 4817 controls, those with insertion/deletion mutations showed a statistically significant difference in the onset of the disease [28]. The

Table 2.1 Noteworthy meta-analysis of maternal genes of preeclampsia

Gene symbol	Genotype		Sample size		No. of studies	Ref.
			case	control		
AGT	rs699	M235T (T704C)	2555	6114	31	[21]
ACE	rs1799752	Ins/del	3523	4817	30	[22]
NOS3	rs1799983	G894T	3503	6843	30	[26]
	rs2070744	T796C	2232	3068	15	
VEGF	rs3025039	C936T	805	1033	11	[28]
	rs2010963	G634T	441	485		
PAI 1	rs1799889	675 4G/5G	1297	1791	11	[32]
F5	rs6025	G1691A	3131	4036	23	[34]
F2	rs1799963	G20210A	1063	1946	37	[34]
MTHFR	rs1801133	C677T	7398	11,320	54	[37]
IL10	rs1800871	C819T	1861	3632	11	[41]
	rs1800872	C592A				

A1166C (rs5186) mutation in the AT1 receptor gene exhibited no association with the incidence of preeclampsia [29].

Endothelial nitric oxide synthase (eNOS)plays an important role in endothelium-derived NO synthesis, vasodilation, and inhibition of platelet aggregation. In an animal model of preeclampsia, eNOS knockouts resulted in decreased placental perfusion and hypoxia [30]. Representative mutations in the eNOS gene (*NOS3*) such as G894T (rs1799983), T-796C (rs2070744), and VNTR 4b/a are reportedly associated with essential hypertension and ischemic cardiovascular diseases [31]. In a meta-analysis, G894T (30 studies with 3503 cases and 6843 controls) and T-796C (15 studies with 2232 cases and 3068 controls) exhibited significant correlations with the onset risk of preeclampsia [32].

Vascular endothelial growth factor (VEGF), an essential factor for angiogenesis and vasculogenesis, plays important roles in placental development. Representative gene mutations of VEGF such as C936T (rs3025039), G-634C (rs2010963), C-2578A (rs1570360), G-1154A (rs699947), and C936T were associated with decreased levels of circulating VEGF in healthy controls [33]. In meta-analyses, C936T (805 preeclampsia cases and 1033 controls) and G-634T (441 cases and 485 controls) were suggested to be associated with the onset of preeclampsia [34].

2.3.2 Coagulation-Related Genes

Plasminogen activator inhibitor 1 (PAI1) suppresses the activation of plasminogen to plasmin through inhibition of tissue plasminogen activator, and it is well known that PAI1 downregulates the fibrinolytic system and, subsequently, upregulates the coagulation pathway and inflammatory reactions [35, 36]. In the transcription activation region of the PAI1 gene, about 675 4G/5G variants (rs1799889) have been reported, and the 4G variant is known to stimulate transcriptional activation of the PAI1 gene [37]. In a meta-analysis of preeclamptic patients, investigating 11 studies involving 1297 PE cases and 1791 controls, the 4G allele contributes to an increased risk of preeclampsia [38].

Gene mutations related to the coagulation pathway have been suggested to be associated with the onset of the disease. The Leiden mutation of factor V (G1691A, rs6025), which is observed in Caucasians, resulted in an increased risk of thromboembolism through inactivation of factor V and the fibrinolytic systems [39]. In a meta-analysis of 23 studies involving 3131 cases and 4036 controls, factor V Leiden increased the risk of preeclampsia 1.6-fold and 2.45-fold in severe cases [40]. Gene mutation G20210A in prothrombin elevates the risk of thromboembolism. A meta-analysis including 12 studies involving a total of 1063 cases and 1946 controls showed that the A allele was associated with preeclampsia 1.8-fold more frequently and threefold more often in severe cases [40]. In HuGE review [41], it was suggested that gene mutations related to coagulation pathways contribute to the pathogenesis of severe preeclampsia. Taken together, it is suggested that coagulation pathways play a major role in the progression of preeclampsia.

The C677T variant (rs1801133) in the methylenetetrahydrofolate reductase (*MTHFR*) gene was associated with hyperhomocysteinemia caused by inactivation

of MTHFR. It was suggested that this elevated the risk of thromboembolism, atherosclerosis, and cardiovascular disease [42]. In a meta-analysis of *MTHFR* variants including 7398 preeclampsia cases and 11,230 controls, TT variants showed odds ratio of 1.37 compared to CC or CT variants. Importantly, this study clearly demonstrated that higher risk was observed especially in Asian and Caucasians [43].

2.3.3 Cytokine-Related Genes

In preeclamptic patients, elevated levels of circulating inflammatory cytokines were observed, and it was suggested that inflammatory pathways were a key to clarifying the pathogenesis of the disease [44, 45]. However, a recent study demonstrated no associations between variants of TNF-α (G308A, rs1800629), IL-6 (G174C, rs76144090), and IL-10 (A-1082G, rs1800896) and the risk of preeclampsia [46]. A meta-analysis involving 11 studies of IL-10 gene polymorphisms (C819T, C592A) exhibited an association with the risk of preeclampsia, although the total number of studies was relatively small [47].

2.3.4 Others

Matrix metalloproteinase 9 (MMP9) is highly expressed in placental tissues and plays an important role in placental development through uterine spiral artery remodeling [48, 49]. It was suggested that the C1562T variant in the transcription region affected transcriptional inactivation [50]. However, those mutations showed no association with the risk of preeclampsia [51].

2.4 Paternal/Fetal Genetic Factors

2.4.1 Paternal Genetic Factors

Accumulating epidemiological evidence suggests that paternal genetic factors are strongly associated with the risk of preeclampsia. It was suggested that higher paternal age and paternal obesity affected the risk of the disease [52]. Recent study suggested that SNP105Ile → Val of the glutathione S-transferase P1-1 (*GSTP1*-1) gene, a detoxification enzyme, resulted in a decreased capacity for anti-oxidative stress in the placenta. Interestingly, a study that focused on the variant clearly demonstrated that paternal and maternal contributions were observed in relation to increased risk of preeclampsia [53].

2.4.2 Fetal Genetic Factors

Fetal genetic components have received increasing attention in the pathogenesis of preeclampsia. Pregnant women carrying fetuses with Beckwith-Wiedemann syndrome (including those fetuses with *CDKN1C* mutations) showed an increased risk

of preeclampsia and gestational hypertension, and there was a lower risk in mothers with fetuses with trisomy 21 [54].

Catechol-O-methyltransferase (*COMT*) is a key enzyme in the metabolism of estrogens [55]. Two reports suggested that a deficiency in COMT was associated with preeclampsia [56, 57]. In normal pregnancy, circulating 2-methoxyestradiol (2-ME), which was generated by COMT from 17β-estradiol, increased more than 1000-fold compared with nonpregnant women [58]. In preeclampsia, lower levels of 2-ME were observed than in normal pregnancy, and plasma levels of 2-ME were significantly associated with severity of the disease [59]. COMT Val108/158Met SNP resulted in reduced thermostability and activity of the enzyme [60]. Recently, Pertegal M et al. [61] investigated 53 preeclampsia and 72 control families and genotyped maternal and fetal COMT polymorphisms and found that the odds ratio of the risk of preeclampsia for fetal COMT Met/Met was 3.22. To date, only a few studies have focused on fetal and paternal genetic components and the risk of preeclampsia. Extensive research should be conducted in this area.

2.5 HLA Polymorphisms

Maternal immunological adaptive responses for the fetus in preeclampsia have been studied, and it was postulated that the first pregnancy was a definitive risk factor for preeclampsia [62]. Primipaternity or partner specificity is an important risk factor for parous women, since a change of partner might increase the risks of preeclampsia [63–65].

It was postulated that specific human leukocyte antigen (HLA) class I and class II allele frequencies, maternal homozygosity, couple sharing, and maternal-fetal sharing could impact the risk of preeclampsia. Thus far, no universally accepted results have demonstrated an association due to heterogeneity in study designs. Maternal-fetal gene-gene interactions should be investigated under adequately planned studies [66].

Allogeneic extravillous trophoblasts (EVT) express a specific combination of three HLA class I molecules: HLA-C, HLA-E, and HLA-G [66–69]. EVT HLA-C is polymorphic and plays important roles in allorecognition of maternal immune cells. Uterine natural killer (NK) cells recognize allogeneic antigens through various receptors, including the killer immunoglobulin receptor (KIR) families [70]. Interestingly, Hiby SE et al. [71] clearly demonstrated that the combination of maternal KIR AA genotype, lacking most or all activating receptors, with a fetal HLA-C2 is associated with an increased risk of preeclampsia. In Japanese population, the frequency of KIR AA genotype is high at around 60%, whereas HLA-C2 genotype is low at 9% [72]. On the other hand, Caucasians have HLA-C2 genotype at 30–35% [73]. From these observations, Saito S et al. [74] investigated the frequency of preeclampsia in couples of Japanese women and Caucasian men; however, no significant increase in the frequency of preeclampsia was observed between couples of Japanese women and Caucasian men and the control group. Although this evidence has not been universally determined, it is postulated that activation of uterine NK cells causes poor trophoblast invasion through insufficiency of uterine artery remodeling.

HLA-G is a nonclassical HLA class I molecule. It is expressed only in EVTs at the maternal-fetal interface, and HLA-G is not polymorphic compared to other classical antigens. It exhibits little difference between mother and fetus [75–77]. It is assumed that HLA-G acts as a defensive factor against maternal lymphocytes and plays an important role for immunotolerance [78].

In preeclampsia, expression of HLA-G protein was decreased in EVTs compared to normal pregnancy [78], and plasma levels of soluble HLA-G1/G5 molecule were significantly decreased in the second trimester in pregnant women who later developed preeclampsia [79]. Taken altogether, it is suggested that aberrant expression of HLA-G may be a key causative factor for the onset and progression of preeclampsia.

The 14-bp deletion/insertion polymorphism in the 3′-untranslated region (3′-UTR) of the *HLA-G* gene (rs66554220) was intensively investigated to clarify the aberrant expression of HLA-G [80–82]. Alternatively spliced *HLA-G* mRNA isoforms, including a 14-bp polymorphism in the 3′-UTR end, are expressed at a significantly lower level than the corresponding *HLA-G* mRNA isoforms with the 14-bp deletion [80]. Several studies investigated allelic and genotypic frequencies of 14-bp deletion/insertion polymorphism in the 3′-UTR of *HLA-G* in family triads of mother, father, and offspring [81, 82]. These studies suggested that the genotype of the *HLA-G* 14-bp deletion/insertion polymorphism is significantly associated with preeclampsia in combination with mother- or father-offspring, and its distribution differs between the timing of onset and severity of the disease [81, 82]. In a meta-analysis of the *HLA-G* 14-bp deletion/insertion polymorphism in the family triad, there were no significant associations between preeclampsia and the polymorphism in any of the family triad members. However, in a subgroup analysis, a significant association between the polymorphism and preeclampsia was detected in offspring from primipara (OR = 1.66–1.95, $P = 0.04$) and European Caucasian pregnancies (OR = 1.37–2.03, $P = 0.02$–0.03) [83], although the number of studies was insufficient to determine associations between the increased risk of the *HLA-G* 14-bp deletion/insertion and the development of preeclampsia.

2.6 Maternal and Fetal Genotypes

Here, we will further discuss maternal and fetal gene-gene interactions. Vefring H et al. [84] investigated the combined genetic effects of maternal angiotensinogen (*AGT*) and fetal renin (*REN*) haplotypes on the increased risks of preeclampsia in 99 affected family triads including mother, father, and newborns. They examined combinations of maternal *AGT* haplotypes, rs2148582 (C/t), rs2478545 (c/T), and rs943580 (A/g), and fetal *REN* haplotypes, rs5705 (A/c), rs1464816 (G/t), and rs3795575 (C/t), for their impact on the risk of preeclampsia. There was a significant protective effect on the risk of preeclampsia among maternal heterozygous *AGT* haplotype C-T-A and those homozygous for fetal *REN* haplotype A-G-C; however, no interactions of maternal *AGT* and fetal *REN* haplotype were detected [84].

A large-scale association study was conducted to evaluate 775 SNPs in 190 selected genes in mother-offspring on the risk of preeclampsia [85], and there was a significant association between risks of preeclampsia and maternal COL1A1

(rs17639446) and IL1A (rs3783550) and PLAUR (rs4251923) for the offspring genotype. However, after adjusting for multiple testing using the false discovery rate, there was no statistically significant evidence of association for any of the candidate loci evaluated [85].

2.7 Genome-Wide Association Studies

Representative genome-wide association studies are summarized in Table 2.2.

Genome-wide linkage analysis of preeclampsia pedigrees revealed several maternal susceptibility loci, including that on chromosome 2 [86–90]. Recently, a variance component-based linkage approach determined a susceptibility quantitative trait locus for preeclampsia on chromosome 2q22, and an objective prioritization strategy assigned the highest priority to the activin receptor type 2 gene (*ACVR2A*) [91]. In a large case-control cohort with 1139 preeclampsia patients and 2269 controls, associations between candidate genes at the 2q22 susceptibility locus, including *ACVR2A*, and the risk of preeclampsia were investigated [92]. As a result, four SNPs of the *ACVR2A* gene (rs1424941, rs1014064, rs2161983, and rs3768687) were shown to exhibit significant associations. *ACVR2A* encodes the activin A type 2 receptor, and potential ligands for this receptor include activin A, activin B, and inhibin A, but not TGF-β [93, 94]. Altered expression of *ACVR2A* may affect activin A action with resultant trophoblast invasion and placental dysfunction, although the precise biological mechanisms remain unclear [91].

A genome-wide association study was performed in 538 preeclampsia cases and 540 controls of Caucasians in Australia. The analysis included genotypes corresponding to about 650,000 SNPs [12]. There was a strong association for a risk locus on chromosome 2q14.2 defined by significant genetic association of the inhibin, beta B (*INHBB*) gene (rs7579169, rs12711941, and rs7576192). However, it was suggested that those SNPs might not be functionally important by bioinformatic approach and there were no replicate significant associations in Norwegian or Finnish case-control cohorts [12].

Table 2.2 Representative genome-wide association studies of preeclampsia

Gene		Sample size			
Gene symbol	Genotype (rs number)	Case	Control	Ethnicity	Ref.
ACVR2A	rs1424941 rs1014064 rs2161983 rs3768687	1139	2269	Norwegian	[83]
INHBB	rs7579169 rs12711941 rs7576192	538	540	Caucasian	[12]
PSG11	15 kb deletion	177	116	Caucasian	[86]
FGF14	rs11617740	21	1049	Afro-Caribbean	[13]
MYCBP2	rs7322722	50	1207	European ancestry	
LZTS1	rs17412740	62	661	Hispanic	

Another GWAS was conducted to identify potential preeclampsia-related SNPs and copy number variations (CNVs) in 177 cases and 116 controls [95]. Interestingly, there were rare deletions enriched in preeclampsia cases, including a 15 kb deletion in 19q13.31 that encompasses the *PSG11* gene, although no statistically significant SNPs were associated with cases. Pregnancy-specific glycoproteins (PSGs) are mainly secreted by placental syncytiotrophoblasts and belong to the immunoglobulin superfamily. They induce monocytic secretion of anti-inflammatory cytokines that contribute to the maintenance of a successful pregnancy [96].

More recently, a GWAS using a case-control design was performed in Afro-Caribbean (n = 21 cases, 1049 controls), European ancestry (50 cases, 1207 controls), and Hispanic ethnic groups (62 cases, 661controls) using public genotype data from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study [13, 97]. This study identified several SNP candidates, *FGF14* (rs11617740), *MYCBP2* (rs7322722), and *LZTS1* (rs17412740), for each of the HAPO ethnic groups, although none of these showed a significant association with the risk of preeclampsia.

Further large replication and functional studies are needed to confirm SNPs or CNVs that are associated with the risk of preeclampsia using specially designed ethnic SNP arrays.

2.8 Future Directions

Over the last decade, extensive research has been conducted to better understand the genetic components of the pathophysiology of preeclampsia. However, no universally accepted genetic factors can explain the onset and progression of this multifactorial disease. Recent innovations in sequencing technologies and bioinformatic approaches should enable us to browse comprehensive [98] and disease-specific [99] large databases. New genetic approaches are needed: large-scale cohorts to account for racial differences, case-control studies with multiple familial generations, and universal standardized definitions with higher phenotypic resolutions. Furthermore, a global consortium for preeclampsia research would be a promising approach to uncover the causes of this enigmatic disease.

References

1. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10:466–80.
2. Waken RJ, de Las Fuentes L, Rao DC. A review of the genetics of hypertension with a focus on gene-environment interactions. Curr Hypertens Rep. 2017;19:23. https://doi.org/10.1007/s11906-017-0718-1.
3. Ward K, Lindheimer MD. Genetic factors in the etiology of preeclampsia/eclampsia. In: Chesley's hypertensive disorders in pregnancy. London: Elsevier; 2990. p. 51–72.
4. Ros HS, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genet. 2000;91:256–60.
5. Cnattingius S, Reilly M, Pawitan Y, Lichtenstein P. Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study. Am J Med Genet A. 2004;130:365–71.

6. Nilsson E, Salonen Ros H, Cnattingius S, Lichtenstein P. The importance of genetic and environmental effects for pre-eclampsia and gestational hypertension: a family study. BJOG. 2004;111:200–6.
7. Boyd HA, Tahir H, Wohlfahrt J, Melbye M. Associations of personal and family preeclampsia history with the risk of early-, intermediate-and late-onset preeclampsia. Am J Epidemiol. 2013;178:1611–9.
8. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet. 2001;357: 53–6.
9. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet. 2003;33:177–82.
10. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461:747–53.
11. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447:661–78.
12. Johnson MP, Brennecke SP, East CE, Göring HH, Kent JW Jr, Dyer TD, et al. Genome-wide association scan identifies a risk locus for preeclampsia on 2q14, near the inhibin, beta B gene. PLoS One. 2012;7:e33666.
13. Zhao L, Bracken MB, DeWan AT. Genome-wide association study of pre-eclampsia detects novel maternal single nucleotide polymorphisms and copy-number variants in subsets of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study cohort. Ann Hum Genet. 2013;77:277–87.
14. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol. 1986;93:898–908.
15. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ. 2005;330:565.
16. Thornton JG, Macdonald AM. Twin mothers, pregnancy hypertension and pre-eclampsia. Br J Obstet Gynaecol. 1999;106:570–5.
17. Treloar SA, Cooper DW, Brennecke SP, Grehan MM, Martin NG. An Australian twin study of the genetic basis of preeclampsia and eclampsia. Am J Obstet Gynecol. 2001;184:374–81.
18. Qiu C, Williams MA, Leisenring WM, Sorensen TK, Frederick IO, Dempsey JC, et al. Family history of hypertension and type 2 diabetes in relation to preeclampsia risk. Hypertension. 2003;41:408–13.
19. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: population based study. BMJ. 1998;316:1343–7.
20. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, et al. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med. 2001;344:867–72.
21. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension. 2009;53:944–51.
22. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: common antecedents? Circulation. 2010;122:579–84.
23. Skjaerven R, Wilcox AJ, Klungsøyr K, Irgens LM, Vikse BE, Vatten LJ, et al. Cardiovascular mortality after pre-eclampsia in one child mothers: prospective, population based cohort study. BMJ. 2012;345:e7677.
24. Cirillo PM, Cohn BA. Pregnancy complications and cardiovascular disease death: 50-year follow-up of the Child Health and Development Studies pregnancy cohort. Circulation. 2015;132:1234–42.
25. Cain MA, Salemi JL, Tanner JP, Kirby RS, Salihu HM, Louis JM. Pregnancy as a window to future health: maternal placental syndromes and short-term cardiovascular outcomes. Am J Obstet Gynecol. 2016;215:484.e1–484.e14.
26. Bokslag A, Teunissen PW, Franssen C, van Kesteren F, Kamp O, Ganzevoort W, et al. Effect of early-onset preeclampsia on cardiovascular risk in the fifth decade of life. Am J Obstet Gynecol. 2017;216:523. e1–523.e7.

27. Lin R, Lei Y, Yuan Z, Ju H, Li D. Angiotensinogen gene M235T and T174M polymorphisms and susceptibility of pre-eclampsia: a meta-analysis. Ann Hum Genet. 2012;76:377–86.
28. Chen Z, Xu F, Wei Y, Liu F, Qi H. Angiotensin converting enzyme insertion/deletion polymorphism and risk of pregnancy hypertensive disorders: a meta-analysis. J Renin-Angiotensin-Aldosterone Syst. 2012;13:184–95.
29. Zhao L, Dewan AT, Bracken MB. Association of maternal AGTR1 polymorphisms and preeclampsia: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2012;25:2676–80.
30. Kulandavelu S, Whiteley KJ, Qu D, Mu J, Bainbridge SA, Adamson SL. Endothelial nitric oxide synthase deficiency reduces uterine blood flow, spiral artery elongation, and placental oxygenation in pregnant mice. Hypertension. 2012;60:231–8.
31. Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, et al. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. Hypertension. 1998;32:3–8.
32. Zeng F, Zhu S, Wong MC, Yang Z, Tang J, Li K, et al. Associations between nitric oxide synthase 3 gene polymorphisms and preeclampsia risk: a meta-analysis. Sci Rep. 2016;6:23407.
33. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res. 2000;37:443–8.
34. Cheng D, Hao Y, Zhou W, Ma Y. Vascular endothelial growth factor +936C/T, -634G/C, -2578C/A, and -1154G/A polymorphisms with risk of preeclampsia: a meta-analysis. PLoS One. 2013;8:e78173.
35. Loskutoff DJ, Curriden SA. The fibrinolytic system of the vessel wall and its role in the control of thrombosis. Ann N Y Acad Sci. 1990;598:238–47.
36. Collen D. The plasminogen (fibrinolytic) system. Thromb Haemost. 1999;82:259–70.
37. Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A. 1995;92:1851–5.
38. Zhao L, Bracken MB, Dewan AT, Chen S. Association between the SERPINE1 (PAI-1) 4G/5G insertion/deletion promoter polymorphism (rs1799889) and pre-eclampsia: a systematic review and meta-analysis. Mol Hum Reprod. 2013;19:136–43.
39. Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. Haemostasis. 1994;24:139–51.
40. Wang X, Bai T, Liu S, Pan H, Wang B. Association between thrombophilia gene polymorphisms and preeclampsia: a meta-analysis. PLoS One. 2014;9:e100789.
41. Fong FM, Sahemey MK, Hamedi G, Eyitayo R, Yates D, Kuan V, et al. Maternal genotype and severe preeclampsia: a HuGE review. Am J Epidemiol. 2014;180:335–45.
42. Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutr Rev. 1996;54:1–30.
43. Wu X, Yang K, Tang X, Sa Y, Zhou R, Liu J, et al. Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for preeclampsia: a meta-analysis. J Assist Reprod Genet. 2015;32:797–805.
44. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592–4.
45. Moffett A, Hiby SE. How does the maternal immune system contribute to the development of pre-eclampsia? Placenta. 2007;28:S51–6.
46. Xie C, Yao MZ, Liu JB, Xiong LK. A meta-analysis of tumor necrosis factor-alpha, interleukin-6, and interleukin-10 in preeclampsia. Cytokine. 2011;56:550–9.
47. Yang W, Zhu Z, Wang J, Ye W, Ding Y. Evaluation of association of maternal IL-10 polymorphisms with risk of preeclampsia by a meta-analysis. J Cell Mol Med. 2014;18:2466–77.
48. Shimonovitz S, Hurwitz A, Dushnik M, Anteby E, Geva-Eldar T, Yagel S. Developmental regulation of the expression of 72 and 92 kd type IV collagenases in human trophoblasts: a possible mechanism for control of trophoblast invasion. Am J Obstet Gynecol. 1994;171:832–8.

49. Merchant SJ, Davidge ST. The role of matrix metalloproteinases in vascular function: implications for normal pregnancy and pre-eclampsia. Br J Gynaecol. 2004;111:931–9.
50. Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation. 1999;13:1788–94.
51. Gong LL, Liu H, Liu LH. Lack of association between matrix metalloproteinase-9 gene-1562C/T polymorphism and preeclampsia: a meta-analysis. Hypertens Pregnancy. 2014;33:389–94.
52. Dekker G, Robillard PY, Roberts C. The etiology of preeclampsia: the role of the father. J Reprod Immunol. 2011;89:126–32.
53. Zusterzeel PL, te Morsche R, Raijmakers MT, Roes EM, Peters WH, Steegers EA. Paternal contribution to the risk for pre-eclampsia. J Med Genet. 2002;39:44–5.
54. Petry CJ, Beardsall K, Dunger DB. The potential impact of the fetal genotype on maternal blood pressure during pregnancy. J Hypertens. 2014;32:1553–61.
55. Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab. 2002;3:321–49.
56. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453:1117–21.
57. Hernandez M, Hernandez I, Rodriguez F, Pertegal M, Bonacasa B, Salom MG, et al. Endothelial dysfunction in gestational hypertension induced by catechol-O-methyltransferase inhibition. Exp Physiol. 2013;98:856–66.
58. Berg D, Sonsalla R, Kuss E. Concentrations of 2-methoxyoestrogens in human serum measured by a heterologous immunoassay with an 125I-labelled ligand. Acta Endocrinol. 1983;103:282–8.
59. Pertegal M, Fenoy FJ, Bonacasa B, Mendiola J, Delgado JL, Hernández M, et al. 2-methoxyestradiol plasma levels are associated with clinical severity indices and biomarkers of preeclampsia. Reprod Sci. 2015;22:198–206.
60. Männistö PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev. 1999;51:593–628.
61. Pertegal M, Fenoy FJ, Hernández M, Mendiola J, Delgado JL, Bonacasa B, et al. Fetal Val108/158Met catechol-O-methyltransferase (COMT) polymorphism and placental COMT activity are associated with the development of preeclampsia. Fertil Steril. 2016;105:134–43. e1–3.
62. Billington WD. The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar. J Reprod Immunol. 2003;60:1–11.
63. Feeny JG, Scott JS. Pre-eclampsia and changed paternity. Eur J Obstet Reprod Biol. 1980;11:35–8.
64. Robillard PY, Husley TC, Alexander GR, Keenan A, de Caunes F, Papiernik E. Paternity patterns and risk of pre-eclampsia in the last pregnancy in multiparae. J Reprod Immunol. 1993;24:1–12.
65. Li DK, Wi S. Changing paternity and the risk of preeclampsia/eclampsia in the subsequent pregnancy. Am J Epidemiol. 2000;151:57–62.
66. Saftlas AF, Beydoun H, Triche E. Immunogenetic determinants of preeclampsia and related pregnancy disorders: a systematic review. Obstet Gynecol. 2005;106:162–72.
67. King A, Allan DSJ, Bowen JM, Powis SJ, Joseph S, Verma S, et al. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. Eur J Immunol. 2000;30:1623–31.
68. King A, Burrows TD, Hiby SE, Joseph S, Verma S, Lim PB, et al. Surface expression of HLA-C antigen by human extravillous trophoblast. Placenta. 2000;21:376–87.5.
69. Hiby SE, King A, Sharkey A, Loke YW. Molecular studies of trophoblast HLA-G polymorphism, isoforms, imprinting and expression in pre-implantation embryo. Tissue Antigens. 1999;53:1–13.
70. Vilches C, Parham P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. Annu Rev Immunol. 2002;20:217–51.

71. Hiby SE, Walker JJ, O'shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. J Exp Med. 2004;200:957–65.
72. Yawata M, Yawata N, McQueen KL, Cheng NW, Guethlein LA, Rajalingam R, et al. Predominance of group A KIR haplotypes in Japanese associated with diverse NK cell repertoires of KIR expression. Immunogenetics. 2002;54:543–50.
73. Williams F, Meenagh A, Patterson C, Middleton D. Molecular diversity of the HLA-C gene identified in a caucasian population. Hum Immunol. 2002;63:602–13.
74. Saito S, Takeda Y, Sakai M, Nakabayahi M, Hayakawa S. The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men. J Reprod Immunol. 2006;70:93–8.
75. Trowsdale J, Travers JP, Bodmer WF, Patillo RA. Expression of HLA-A, -B, -C and b2m antigen in human choriocarcinoma cell lines. J Exp Med. 1980;152:11s–17s.
76. Ellis SA, Palmer MS, McMicael AJ. Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA class I molecule. J Immunol. 1990;144:731–5.
77. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. Science. 1990;248:220–3.
78. Hara N, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y. Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in preeclampsia: immunohistological demonstration with anti-HLA-G specific antibody "87G" and anti-cytokeratin antibody "CAM5.2". Am J Reprod Immunol. 1996;36:349–58.
79. Steinborn A, Varkonyi T, Scharf A, Bahlmann F, Klee A, Sohn C. Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy. Am J Reprod Immunol. 2007;57:277–86.
80. Hviid TV, Hylenius S, Rørbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics. 2003;55:63–79.
81. Hylenius S, Andersen AM, Melbye M, Hviid TV. Association between HLA-G genotype and risk of pre-eclampsia: a case-control study using family triads. Mol Hum Reprod. 2004;10:237–46.
82. Zhang Z, Li Y, Zhang LL, Jia LT, Yang XQ. Association of 14 bp insertion/deletion polymorphism of the HLA-G gene in father with severe preeclampsia in Chinese. Tissue Antigens. 2012;80:158–64.
83. Pabalan N, Jarjanazi H, Sun C, Iversen AC. Meta-analysis of the human leukocyte antigen-G (HLA-G) 14 bp insertion/deletion polymorphism as a risk factor for preeclampsia. Tissue Antigens. 2015;86:186–94.
84. Vefring H, Wee L, Jugessur A, Gjessing H, Nilsen S, Lie R. Maternal angiotensinogen (AGT) haplotypes, fetal renin (REN) haplotypes and risk of preeclampsia; estimation of gene-gene interaction from family-triad data. BMC Med Genet. 2010;11:90.
85. Goddard KA, Tromp G, Romero R, Olson JM, Lu Q, Xu Z, et al. Candidate-gene association study of mothers with pre-eclampsia, and their infants, analyzing 775 SNPs in 190 genes. Hum Hered. 2007;63:1–16.
86. Harrison GA, Humphrey KE, Jones N, Badenhop R, Guo G, Elakis G, et al. A genome wide linkage study of preeclampsia/eclampsia reveals evidence for a candidate region on 4q. Am J Hum Genet. 1997;60:1158–67.
87. Arngrimsson R, Sigurard ttir S, Frigge ML, Bjarnadóttir RI, Jónsson T, Stefánsson H, et al. A genome-wide scan reveals a maternal susceptibility locus for pre-eclampsia on chromosome 2p13. Hum Mol Genet. 1999;8:1799–805.
88. Moses EK, Lade JA, Guo G, Wilton AN, Grehan M, Freed K, et al. A genome scan in families from Australia and New Zealand confirms the presence of a maternal susceptibility locus for pre-eclampsia, on chromosome 2. Am J Hum Genet. 2000;67:1581–5.
89. Lachmeijer AM, Arngrimsson R, Bastiaans EJ, Frigge ML, Pals G, Sigurdardóttir S, et al. A genome-wide scan for preeclampsia in the Netherlands. Eur J Hum Genet. 2001;9:758–64.

90. Laivuori H, Lahermo P, Ollikainen V, Widen E, Häivä-Mällinen L, Sundström H, et al. Susceptibility loci for preeclampsia on chromosomes 2p25 and 9p13 in Finnish families. Am J Hum Genet. 2003;72:168–77.
91. Moses EK, Fitzpatrick E, Freed KA, Dyer TD, Forrest S, Elliott K, et al. Objective prioritization of positional candidate genes at a quantitative trait locus for pre-eclampsia on 2q22. Mol Hum Reprod. 2006;12:505–12.
92. Roten L, Johnson M, Forsmo S, Fitzpatrick E, Dyer T, Brennecke S, et al. Association between the candidate susceptibility gene ACVR2A on chromosome 2q22 and preeclampsia in a large Norwegian population-based study (the HUNT study). Eur J Hum Genet. 2009;17:250–7.
93. Mathews LS, Vale WW. Expression cloning of an activin receptor, a predicted transmembrane serine kinase. Cell. 1991;65:973–82.
94. Donaldson CJ, Mathews LS, Vale WW. Molecular cloning and binding properties of the human type II activin receptor. Biochem Biophys Res Commun. 1992;184:310–6.
95. Zhao L, Triche E, Walsh K, Bracken M, Saftlas A, Hoh J, et al. Genome-wide association study identifies a maternal copy-number deletion in PSG11 enriched among preeclampsia patients. BMC Pregnancy Childbirth. 2012;12:61.
96. Snyder S, Wessner D, Wessells J, Waterhouse R, Wahl L, Zimmermann W, Dveksler G. Pregnancy-specific glycoproteins function as immunomodulators by inducing secretion of IL-10. IL-6 and TGF-beta1 by human monocytes. Am J Reprod Immunol. 2001;45:205–16.
97. HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study. Int J Gynaecol Obstet. 2002;78:69–77.
98. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen. ClinGen—the clinical genome resource. N Engl J Med. 2015;372:2235–42.
99. Uzun A, Triche EW, Schuster J, Dewan AT, Padbury JF. dbPEC: a comprehensive literature-based database for preeclampsia related genes and phenotypes. Database (Oxford). 2016;2016. pii: baw006. doi: https://doi.org/10.1093/database/baw006.

Part III

Pathological Findings in Preeclampsia

Trophoblast Invasion: Remodelling of Spiral Arteries and Beyond

3

Berthold Huppertz

Abstract

Despite massive scientific and clinical efforts, the etiology of preeclampsia remains a mystery. Quite a variety of different hypotheses has been developed and this is ongoing, but none of them has been able to explain the symptoms of the different preeclampsia subtypes satisfactorily. Here, the hypothesis of shallow trophoblast invasion and its relation to preeclampsia will be discussed. New pathways of trophoblast invasion will be described, including those into spiral arteries (endoarterial trophoblast), into uterine veins (endovenous trophoblast), into uterine glands (endoglandular trophoblast), and into uterine lymph vessels (endolymphatic trophoblast). Moreover, failure of trophoblast invasion will be related to oxygenation of the placenta and subsequently to preeclampsia.

The data presented in this chapter will clarify that (1) there is no direct relation between shallow invasion and preeclampsia; (2) there is no proof of placental hypoxia, either in IUGR and/or preeclampsia; and (3) new routes of scientific self-conception need to be established to enable new and out-of-the-box thinking.

Keywords

Invasion • Oxygen • IUGR • Preeclampsia • Trophoblast

B. Huppertz, Ph.D.
Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Medical University of Graz, Graz, Austria
e-mail: berthold.huppertz@medunigraz.at

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_3

3.1 Introduction

Although the recent decades have seen an enormous effort to decipher the etiology of preeclampsia, today preeclampsia remains what it has been since decades: the pathology of hypotheses.

A few items are distinct and clear: It needs the placenta but not the fetus to develop preeclampsia. Women with a molar pregnancy without a fetus may still develop preeclampsia. As soon as the placenta is born, the clinical symptoms of the woman disappear. Based on the manifold factors that increase the risk to develop preeclampsia, it is assumed that multifactorial events are decisive for the development of preeclampsia. It is obvious that such events may occur in the placenta, while the maternal predisposition also needs to be taken into account. The interplay between the two, placenta and mother, decides whether the woman will develop preeclampsia and which peculiarities the syndrome will have: early or late, mild or severe, and with or without IUGR.

Due to the fact that a number of scientists and clinicians still believe that alterations in the invasive behavior of extravillous trophoblast are directly causative for preeclampsia, this chapter will first focus on the new insights into trophoblast invasion of the last years and will then show the missing links between trophoblast invasion and the etiology of preeclampsia.

3.2 Extravillous Trophoblast

So far, current knowledge is that very early during placental development, mononuclear trophoblasts come into contact with maternal tissues for the first time only around day 15 p.c. (post conception) or early in week 5 p.m. (post menstruation). Due to the fact that these trophoblasts are found outside the villous core structures of the placenta, these cells are termed extravillous trophoblasts. A general feature of all extravillous trophoblasts is the expression of the major histocompatibility antigen G (human leukocyte antigen G, HLA-G), which distinguishes these cells from villous trophoblasts [1]. HLA-G is especially used for immunohistochemical staining of tissues as unique marker to identify extravillous trophoblasts, e.g., in the placental bed.

During the lacunar phase of placental development, trophoblast cells percolate the syncytiotrophoblast and form trophoblastic cell columns at the site of contact between maternal tissues and syncytiotrophoblast. Within the cell columns, a gradient develops from the basement membrane of the anchoring villus toward the decidual tissues of the maternal uterus. Trophoblast cells in direct contact to the basement membrane show proliferative activity and generate daughter cells in the second row. The daughter cells stop proliferation and start to differentiate toward the extravillous phenotype. Due to the proliferation pressure of the cells at the basement membrane, the daughter cells are pushed forward toward maternal tissues without being invasive during their transition from a villous to an extravillous phenotype.

Finally, the cells reach the lower end of the cell columns and start their invasive journey through maternal tissues. These first invasive cells within the group of extravillous trophoblast are termed interstitial trophoblasts as these cells invade

through the interstitium of the uterus. Interstitial trophoblasts show a specific repertoire of matrix-degrading proteases (matrix metalloproteases, MMPs), integrins, as well as matrix proteins secreted into the surrounding microenvironment.

Although these features are very similar to those of invading tumor cells, invading trophoblasts show a number of peculiarities that distinguish them from tumor cells. One of the main distinguishing features is the loss of their proliferative activity during invasion. Even if these invading trophoblasts are shed into the maternal bloodstream, metastases cannot be developed due to the absence of proliferation of these cells. This feature also defines the depth of invasion of trophoblasts. This depth is directly correlated to the life span of a single cell and cannot be transferred to a daughter cell that develops during invasion as in tumor cell invasion.

Invasion of extravillous trophoblasts is not restricted to early placental development but is a continuous process until the end of pregnancy. This becomes especially obvious at the margin of the growing placenta, which shows invasion of trophoblasts also late in gestation.

The part of the uterus below the placenta and invaded by extravillous trophoblasts is termed placental bed. The part of the decidua that is present in the placental bed and hence is invaded by extravillous trophoblasts is called decidua basalis. The part of the decidua basalis that is delivered with the placenta is called basal plate.

Trophoblast invasion serves separate purposes and is implemented by different populations of extravillous trophoblasts (Fig. 3.1):

1. *Interstitial Trophoblast*: Origin of all other populations of extravillous trophoblast. Starting from trophoblastic cell columns, this subtype invades into the interstitium of the decidua basalis and the inner third of the myometrium of the placental bed. Its main functions are being the source for all other subtypes as well as adhering and fixing the placenta to the uterine wall.
2. *Endoglandular Trophoblast*: Erosion and opening of uterine glands to allow histiotrophic nutrition of the embryo during the first trimester of pregnancy.
3. *Endovascular Trophoblast*: So far, this term was only used for those trophoblasts penetrating the walls of spiral arteries and ending in the lumen of such arteries. Recently, it has become clear that also uterine veins are invaded by trophoblasts, and hence the nomenclature needs to be redefined [2]. Now the term endovascular trophoblast comprises trophoblasts penetrating into spiral arteries (endoarterial trophoblast) as well as trophoblasts penetrating into uterine veins (endovenous trophoblast).
   (a) *Endoarterial Trophoblast*: Erosion, opening, and transformation of uterine spiral arteries to ensure hemotrophic nutrition of the fetus after the first trimester of pregnancy.
   (b) *Endovenous Trophoblast*: Erosion and opening of uterine veins to guarantee the backflow of maternal blood from the intervillous space of the placenta into the maternal vascular system.
4. *Endolymphatic Trophoblast*: By now also invasion of trophoblasts into uterine lymph vessels has been described [3, 4]. The function of this type of invasion is yet unknown.

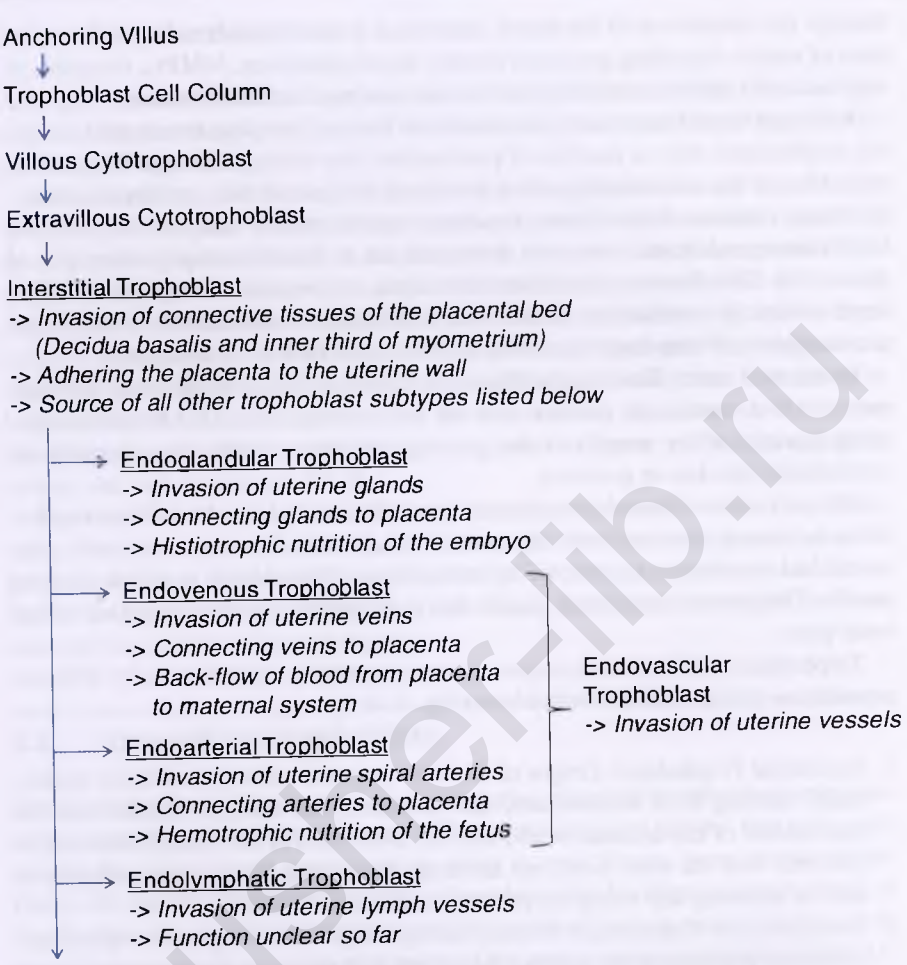


Fig. 3.1 Subtypes of extravillous trophoblast. This figure shows the pathway of the development of the subtypes of extravillous trophoblast. It all starts with the generation of cell columns at the tips of anchoring villi. This is the major source of all extravillous trophoblasts invading into uterine tissues. As soon as extravillous trophoblasts detach from these columns, they are referred to as interstitial trophoblasts which give rise to all subsequent subtypes of extravillous trophoblast

3.2.1 Interstitial Trophoblast

This type of extravillous trophoblasts invades the connective tissues of the decidua basalis and migrates down into the myometrium of the placental bed (Fig. 3.2). Here it seems as if the smooth muscle layers lead to slowdown of invasion until the trophoblasts stop invasion within the inner third of the myometrium. Hence, in normal cases interstitial trophoblasts do not reach to the perimetrium of the uterus.

On their way through the uterine connective tissues, interstitial trophoblasts secrete a specific composition of matrix proteins, called matrix-type fibrinoid [5].

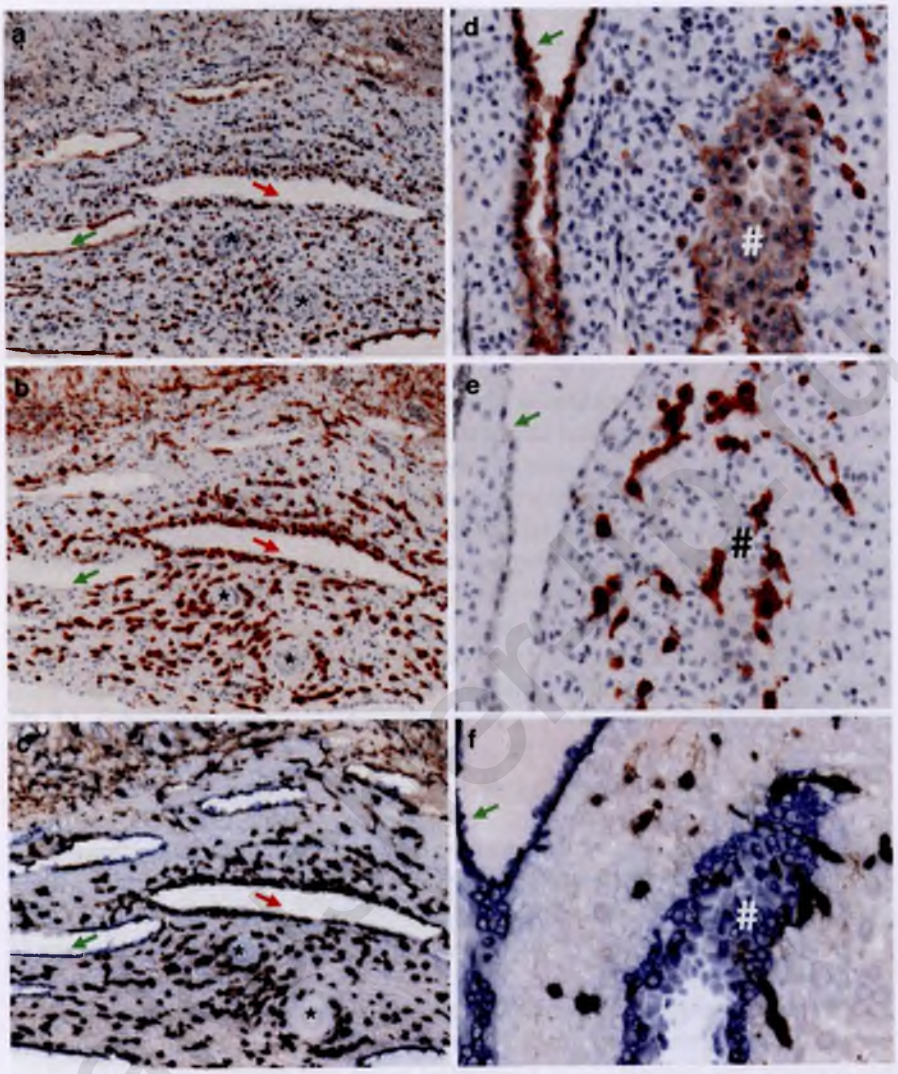


Fig. 3.2 Interstitial and endoglandular trophoblast. The images show the comparison between staining of extravillous trophoblasts with an antibody against cytokeratin 7 (**a, d**) and an antibody against HLA-G (**b, e**). (**c, f**) show double staining using both antibodies. Staining for cytokeratin 7 and HLA-G reveals staining of interstitial trophoblasts in the connective tissues of the decidua basalis as well as staining of trophoblasts invading into luminal structures. However, the antibody against cytokeratin 7 additionally stains glandular epithelial cells (green arrows). Both antibodies do not stain endothelial cells of a uterine vessel (red arrows). The hash in (**d–f**) shows the difficulties arising from staining for cytokeratin 7. The cytokeratin stain (**d**) suggests invasion into a spiral artery with plugging of this vessel. Similar images have been published over the last centuries claiming invasion into spiral arteries. The HLA-G staining reveals that the cells "plugging the vessel" are indeed no extravillous trophoblasts (**e**) but rather glandular epithelial cells. The double stain (**f**) clearly identifies this structure as a uterine gland invaded by endoglandular trophoblasts. The asterisks in (**a–c**) depict two cross sections of a non-invaded spiral artery. Original magnification: (**a–c**) ×100; (**d–f**) ×200. Images courtesy of Dr. Gerit Moser, Medical University of Graz, Austria

Specific components of this matrix such as oncofetal fibronectin have been called the glue to fix the placenta to the uterine wall [6].

The population of interstitial trophoblast is morphologically and functionally extremely heterogeneous, and hence, multiple phenotypes can be distinguished. These phenotypes differ regarding invasive behavior, secretion of matrix proteins, and expression of integrins and MMPs [7]. So far the molecular basis for these differences is not known; however, first approaches to explain different trophoblast phenotypes have been made in the mouse [8].

3.2.2 Endoglandular Trophoblast

In the last few years, the picture of trophoblast invasion has dramatically changed. Until then there was interstitial trophoblast and endovascular trophoblast invading into spiral arteries. All other routes of invasion have been denied. Today, the picture has become much more complex with new populations of trophoblasts invading into luminal structures of the placental bed [9, 10]. One of these populations is endoglandular trophoblast that—starting as interstitial trophoblast—reaches and erodes uterine glands very early during pregnancy (Fig. 3.2).

Already 15 years ago, Burton et al. [11] have described that secretion products of uterine glands reach the intervillous space rather than the uterine cavity during the first trimester of pregnancy. However, the mechanisms of how such glands are opened toward the placenta were unknown [11, 12]. Only with the detection of extravillous trophoblasts that do not simply bypass glands but actively invade these luminal structures, it became clear how histiotrophic nutrition during the first trimester of pregnancy is guaranteed [9]. This nutritional supply of the embryo is important since at that time of pregnancy there is no supply of maternal blood toward the placenta. Infiltration of the uterine epithelium and substitution of these epithelial cells by endoglandular trophoblast enable the opening of the glandular structures toward the intervillous space of the placenta [9].

Very recent studies on the topic have broadened the picture even more. Detailed investigation of archival material of very early specimens of human implantation has revealed that endoglandular trophoblast is the first population of extravillous trophoblast that reaches luminal structures in the uterus [13].

3.2.3 Endovascular Trophoblast

For more than half a century, the hypothesis remained that the only luminal structures in the uterus that is invaded by extravillous trophoblasts are spiral arteries. Invasion into uterine veins has always been excluded. At the same time, it has been ignored that maternal blood flowing into the placenta needs to be drained back into the maternal vascular system—and thus opening of uterine veins

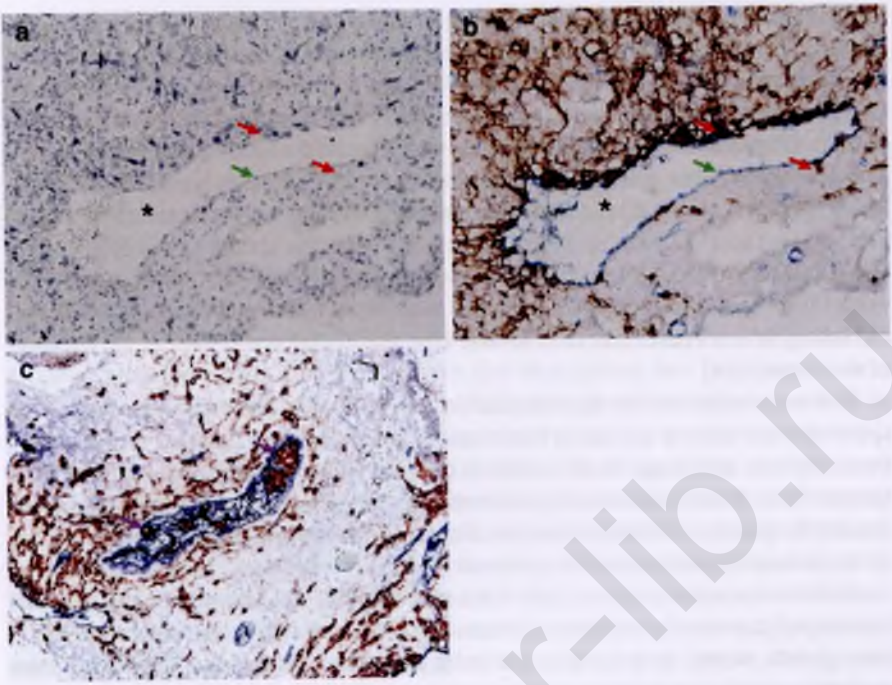


Fig. 3.3 Endovascular trophoblast combining endovenous and endoarterial trophoblast. (**a**) shows staining using an antibody against desmin as a marker of smooth muscle cells in the media of the vessel. No staining can be revealed using this antibody, even on the non-invaded side of the vessel. (**b**) shows double staining using an antibody against HLA-G as marker for extravillous trophoblast and an antibody against von Willebrand factor (vWF) as marker for endothelial cells. The endothelial lining of the veins stains positive for vWF and is mostly intact on the non-invaded side (green arrows in **a** and **b**), while endovenous trophoblast invading the wall of the vein can be identified by staining for HLA-G (**b**) or by their larger nuclei (**a**). (**c**) shows double staining using antibodies against HLA-G and vWF. Endoarterial trophoblasts can be seen in the lumen of this spiral artery (purple arrows). Original magnification: (**a**–**b**) ×200; (**c**) ×100. Images courtesy of Dr. Gerit Moser, Medical University of Graz, Austria

toward the intervillous space of the placenta is essential [2]. Hence, the discovery of trophoblast invasion into uterine veins did not come completely unexpected (Fig. 3.3).

This new discovery causes the need for a redefinition of the term "endovascular trophoblast." So far, this term described those extravillous trophoblasts invading into spiral arteries only [14]. Today, this term needs to be used in a broader sense (Fig. 3.1), including all trophoblast cells invading into vascular structures (arteries and veins) [2]. Those cells invading into arteries are referred to as endoarterial trophoblasts, and those cells invading into veins are referred to as endovenous trophoblasts [13].

3.2.3.1 Endoarterial Trophoblast

Starting as interstitial trophoblasts, a specific subpopulation of extravillous trophoblasts reaches the spiral arteries within the placental bed. They migrate through the vessel wall including the endothelial lining and finally reach the lumen of these arteries (Fig. 3.3c). Here it needs to be clarified that invasion into spiral arteries takes place from the interstitium through the vessel wall to the lumen (intravasation). Since these arteries need to be opened toward the intervillous space, there is no direct route of invasion from the lumen to the interstitium (extravasation) [14]. Of course, once endoarterial trophoblasts have reached the vessel lumen, it is tempting to speculate that such cells crawl along the endothelial lining of the vessel and then invade into the vessel wall again at another site of the vessel [14].

The main tasks of the endoarterial trophoblasts are not only opening of the spiral arteries toward the intervillous space of the placenta but also transforming these vessels into large bore conduits that have lost vasomotor control of the mother. This transformation is performed at the very end of the opened vessel toward the placenta in the decidua basalis, while further down in the myometrium, no such transformation can be detected. Invasion into spiral arteries is the only trophoblast invasion pathway into luminal structures that combines erosion and opening of the vessels with transformation of the walls of the structure. Invasion into glands, veins, and lymphatics only involves erosion and opening of the structures.

Transformation of spiral arteries can be divided into three phases:

1. Maternal factors induce first alterations of the spiral arteries very early during pregnancy, long before any extravillous trophoblast has reached the vessel [15]. The arteries start to dilate due to reduced organization of vascular smooth muscle cells and altered morphology of endothelial cells. The cell type responsible for these early alterations has not been definitively identified yet, but it may well be maternal uterine immune cells being involved in this scenario. Such cells accumulate in the decidua in close vicinity to spiral arteries and hence could well play a major role in the early phases of transforming spiral arteries to the needs of the growing baby.
2. It seems as if the disorganization of the vessel wall enables or at least eases invasion of trophoblasts from the connective tissues into this luminal structure. During the early phase of invasion into spiral arteries, endoarterial trophoblasts that have reached the lumen of the vessel form plugs within the lumen hindering maternal blood from flowing into the placenta [16]. Hence, between the opening of arteries toward the placenta at around mid first trimester and disintegration of the plugs at around the beginning of the second trimester, there is only a flow of blood plasma into the placenta.

   It is important to note that there is indeed a fluid flow throughout the intervillous space during the first trimester without the inclusion of maternal blood cells. Besides blood plasma, also secretion products of the uterine glands add to the fluids flowing through the placenta that will then be drained back into the maternal system via opened uterine veins.

3. Subsequently, endoarterial trophoblasts invade into deeper portions of the vessels, either by first interstitially invading the decidua and myometrium and then invading into the vessel walls or by crawling down the lining of endothelial cells within the vessels and then invading back into the wall of the artery. This invasion into various sites of the vessel wall leads to further disorganization of the wall and in combination with reduced activity of smooth muscle cells, and loss of elastic fibers results in further dilation of the vessels.

3.2.3.2 Endovenous Trophoblast

Invasion of extravillous trophoblasts into uterine veins has first been described in 2017 by Moser et al. [2]. Shortly after this first description, two more papers were published confirming this data [3, 4]. As already described above, there needs to be a mechanism on how maternal blood plasma (and glandular secretion products) is drained back into the maternal vascular system. So far, this point has not been considered. The new concept of trophoblast invasion into uterine veins by endovenous trophoblasts broadens the already existing concept of trophoblast invasion into spiral arteries and finally clarifies the whole transfer of fluids during pregnancy from the maternal circulation into the placenta and back into the maternal circulation.

While endoarterial invasion into spiral arteries combines erosion, opening, and transformation of the vessel wall plus plugging of the vessel lumen, endovenous invasion into uterine veins—similar to endoglandular invasion into uterine glands—only comprises erosion and opening of the veins to the intervillous space of the placenta. Endovenous trophoblasts can be found in close vicinity to uterine veins, replacing venous endothelial cells and also as single cells within the lumen of the veins (Fig. 3.3a, b). However, larger groups of cells within the venous lumen or even complete plugs of endovenous trophoblasts have not been reported yet. Functionally, plugging of veins would not make sense as the backflow into the maternal system needs to be established very early during placental development.

3.2.4 Endolymphatic Trophoblast

Very recently, also invasion into lymphatic structures within the placental bed has been described [3, 4]. Today, any functional aspect of this route of invasion has not been identified. It may simply be explained by the fact that extravillous trophoblasts are more invasive than they have been described so far. Hence, these cells may simply invade any luminal structure throughout their invasive pathway.

3.3 General Considerations on Trophoblast Invasion

The invasive potential of extravillous trophoblasts needs to be and is indeed optimally regulated. So far, only very few deteriorations of extravillous trophoblast have been described. Choriocarcinomas develop from villous rather than extravillous trophoblast.

3.3.1 Deep Invasion

The biological mechanisms leading to excessive trophoblast invasion have not been detected yet. At the same time, it has become clear that the maternal tissues play an important role in determining the depth of trophoblast invasion. Alterations of the placental bed may have dramatic effects on the depth of trophoblast invasion. Scars within the myometrium (e.g., after a cesarean section in a preceding pregnancy) are filled with connective tissue rather than smooth muscle cells. As trophoblast invasion is reduced and stopped within the inner third of the myometrium, it can be speculated that the presence of smooth muscle cells (and the secretion of respective but so far unknown factors) results in a stop of trophoblast invasion. A pathway of connective tissues within a scar may open a route for trophoblasts to invade much deeper than in the presence of smooth muscle cells [17].

Typical forms of excessive trophoblast invasion can be grouped as follows:

1. Placenta accreta: The decidua basalis is missing partly or completely. Hence, the placenta is tightly linked to the uterus and cannot easily be removed.
2. Placenta increta: In such cases trophoblast invasion may extend deep into the myometrium.
3. Placenta percreta: Extravillous trophoblasts invade through the whole wall of the uterus and may even infiltrate the bladder wall (with a placenta lying at the anterior side of the uterus).

All of the above scenarios are associated with poor placental detachment and maternal bleeding. Reasons for the increased incidence of excessive trophoblast invasion are (1) the increased rate of cesarean sections in a preceding pregnancy [18] as well as (2) endoscopic surgeries because of an Asherman syndrome [19]. It has become obvious that the muscle layers within the myometrium stop invasion. In all of the above cases, scar tissues filled with connective tissues rather than muscle cells allow trophoblasts to penetrate much deeper than in the presence of a fully developed muscle layer.

3.3.2 Shallow Invasion in the First Trimester

By contrast, insufficient depth of trophoblast invasion may result in a deficient placental attachment to the uterine wall and hence to premature detachment of the placenta either prior to delivery or during labor. A complete absence of trophoblast invasion into spiral arteries (absence of endoarterial trophoblast) has been noted in cases of spontaneous abortion in the late first trimester with chromosomally normal fetuses [20]. Interestingly, spontaneous abortions occurring early during the first trimester do not show any alterations in trophoblast invasion [21].

Insufficiency of endoarterial trophoblasts to plug all eroded spiral arteries that have been opened toward the intervillous space of the placenta may result in preterm flow of maternal blood cells into local sites of the placenta. This flow of

maternal blood into specific regions of the placenta already during the first trimester of pregnancy (e.g., around week 8) will have deleterious effects on the villous tissues at these sites. This oxygen-rich blood leads to a reduced proliferation rate of the villous trophoblast and reduced placental angiogenesis within the villi. This disruption of placental development may lead to abortion or in less severe cases to growth restriction of the fetus (IUGR) with or without preeclampsia of the mother [22, 23]. The untimely and premature inflow of maternal blood cells into the placenta can be traced using transvaginal ultrasound [22].

These clinically extremely important alterations show how important it is to gain deep knowledge about trophoblast invasion.

3.4 Preeclampsia and Shallow Invasion

Is there any direct relation between shallow invasion of trophoblast and preeclampsia?

Worldwide most of all preeclampsia cases (>80%) occur at the time of delivery (>37 weeks) or preterm (34–37 weeks) and are described as late-onset preeclampsia cases. Most of these preeclampsia cases are associated with normally grown babies or babies with higher birth weight than normal. Only about 15% of these cases show growth restriction of the baby [24].

Only a small subset of preeclampsia cases occurs prior to 34 weeks, referred to as early-onset preeclampsia [25]. In clinical routine, these cases are the most important ones as delivery of the baby (as in the late-onset cases) is not easily done due to complications for the fetus delivered preterm. Also, most of these cases do not only present with clinical symptoms of the mother (preeclampsia) but additionally with clinical symptoms of the fetus (IUGR). A recent cohort study in Sweden showed that about 70% of the early-onset preeclampsia cases are associated with IUGR of the babies [24].

This group of early-onset preeclampsia cases is characterized by fetal symptoms including alterations of uterine artery blood flow (such as increased peripheral resistance within these arteries as identified by Doppler ultrasound) as well as alterations of blood flow within the umbilical arteries (such as an increased systole/diastole (S/D) ratio in preserved (PEDF), absent (AEDF), or even reversed end diastolic flow (PEDF)). Alterations of blood flow through the uterine arteries have been associated with incomplete or shallow invasion of extravillous trophoblast.

Interestingly, the above listed features of early-onset preeclampsia are not specific for preeclampsia but rather are specific for fetal growth restriction (IUGR) [26, 27]. Doppler ultrasound assessment of the uterine arteries has been used to predict preeclampsia and/or IUGR. The ultrasound assessment of blood flow through uterine arteries at 11+0 to 13+6 weeks revealed a detection rate for early-onset preeclampsia of 40% at a 10% false positive rate [26]. A similar study resulted in a sensitivity for all preeclampsia cases (early and late onset) of 21%. The sensitivity of predicting early-onset preeclampsia rose to 33.3% (similar to the 40% of the above study). Interestingly, the sensitivity for detecting early-onset IUGR was 100% [27].

Putting this data into a general context reveals the following:

- Preeclampsia is associated with IUGR in about 20% of all cases, mostly in early-onset preeclampsia.
- The major clinical symptoms of early-onset preeclampsia are not specific for and thus do not seem to be directly related to preeclampsia.
- The major features (abnormal uterine as well as umbilical blood flow) are directly related with and can be used as predictors for IUGR.
- Hence, shallow trophoblast invasion as a cause for the alterations in uterine artery blood flow is associated with IUGR rather than preeclampsia.

3.4.1 Placental Blood Flow

As described above, during the first trimester of pregnancy, maternal blood plasma and secretion products of the uterine glands seep through the intervillous space of the placenta. This results in an oxygen partial pressure of below 20 mmHg since only physically dissolved oxygen within the plasma is transported to the placenta [28]. This oxygen concentration is normoxic for this organ at this time of pregnancy. This is not hypoxia or "physiological hypoxia." This is simply a normoxic low oxygen concentration needed for the development of placenta and embryo at that time of pregnancy. Only with the onset of maternal blood flow through the placenta at the beginning of the second trimester that the intraplacental oxygen partial pressure rises to about 60 mmHg [28, 29]. This steep increase in oxygen concentrations within the placenta is followed by a steady slow decline until the end of pregnancy, when oxygen partial pressures are still in the range of 30–50 mmHg [30–32].

At the same time, it needs to be taken into account that invasion of extravillous trophoblasts takes place within the placental bed, i.e., the decidua basalis and the myometrium. These tissues within the uterine wall are normally oxygenated by the mother throughout pregnancy with about 90 mmHg oxygen in the arterial branch of circulation and about 40 mmHg in the venous branch [28]. Within these tissues, oxygen concentrations do not change during pregnancy, and hence, trophoblast invasion occurs in a normally oxygenated environment—independent on the depth and extent of trophoblast invasion [33]. Thus, shallow trophoblast invasion may alter the flow of maternal blood to the placenta, but it cannot alter oxygenation within the placental bed.

Later in gestation, the volume of blood flowing into the placenta increases from 450 mL/min at week 28 to nearly 1000 mL/min in week 38 [34]. At the same time, blood flow velocities in the uterine arteries remain relatively constant during the second half of pregnancy with values of 33–50 cm/s [35]. This seemingly discrepancy can be explained with the invasion of more arteries that add their blood to the placental flow and with an increased diameter of those arteries that have already been invaded.

Erosion, opening, and transformation of spiral arteries mostly take place at the very end of the arteries close to the placenta [14, 36]. This results in a funnel-shaped opening of the arteries toward the intervillous space. This shape of the opening is

critical for the flow of maternal blood through the placenta. The funnel shape of the opening reduces blood flow from the arteries into the intervillous space to about 10 cm/s [37]. This slow inflow of blood into the placenta keeps the fragile villous structures intact and allows distribution of maternal blood through the narrow passages between the villous trees.

3.4.2 Preeclampsia and Placental Hypoxia

3.4.2.1 Effect of Shallow Trophoblast Invasion

Shallow trophoblast invasion into the walls of spiral arteries and hence a missing transformation of the opening into a funnel lead to an increased velocity of the blood flowing into the intervillous space. Calculations of this flow have revealed that the flow may reach a speed of 1–2 m/s, which is 10–20 times faster than in normal pregnancy [37]. This, of course, has a dramatic effect on the morphology and functionality of the placenta. Faster blood flow through the placenta (1) damages the villous surface and thus leads to more fibrin depositions, (2) pushes the chorionic plate higher and hence makes the placenta thicker, (3) results in detachment of anchoring villi and thus further reduces the number of invading trophoblasts, and (4) alters the architecture of the villous trees. In total fetal supply is impaired that may result in growth restriction of the baby.

But does this missing transformation have an effect on placental oxygenation?

3.4.2.2 Placental Perfusion and Oxygenation

Interestingly, there are hundreds of publications that use malperfusion and hypoxia of the placenta as basis for their studies. At the same time, to date there is not a single measurement providing the proof for placental hypoxia. Looking at in vivo measurements or surrogates for in vivo data, only a few studies have been published so far. Measuring the pO_2 in blood samples of the uterine veins during cesarean section and prior to delivery of the baby as surrogate for the placental pO_2 shows that in cases with IUGR (with and without preeclampsia) the mean value of the placental pO_2 is 1.3 times higher than in normal cases (delivery between weeks 24 and 36) [32]. Measuring the concentration of oxygenated hemoglobin as surrogate for the tissue pO_2 of the placenta reveals that the tissue oxygenation index in normal pregnancy is about 71% [30, 38]. In cases with IUGR, this index increases to about 78%, and in cases with early-onset IUGR with preeclampsia, this index rises to about 80% [30, 38].

Taken together, there is no data that proofs placental hypoxia in relation to shallow trophoblast invasion. This is true for IUGR and especially so for preeclampsia. By contrast, all data published so far reveal higher oxygenation levels within the placenta in cases with IUGR (with and without preeclampsia). Also, one has to remember that only less than 20% of all preeclampsia cases are associated with shallow trophoblast invasion (those with IUGR). In more than 80% of all preeclampsia cases, shallow invasion cannot at all be the cause for the etiology of the syndrome [39].

Conclusions

The very close interplay between the fetus, placenta, and mother during pregnancy allows a powerful crosstalk between the mother and fetus throughout gestation. This is a disadvantage for science as the identification of causes and effects, biomarkers, and predictive measures is extremely difficult as number and origin of parameters are not easily identifiable. This has resulted in a number of misleading hypotheses and assumptions that are based on wrong interpretation of the interaction between mother, placenta, and fetus.

To promote a better understanding of the etiology of pregnancy pathologies such as preeclampsia, at least the following two items should be taken into account:

1. An open-minded scientific community is important to renew hypotheses based on current knowledge. Only accepting already existing hypotheses and points of view hinders scientific progress and knowledge increase.
2. Researchers and scientists working on pregnancy pathologies and using samples from pregnant women and placenta biomarkers for preeclampsia are down to high-quality samples and data. This has not been looked at during the last decades, and hence respective data on low-quality samples have been published. Hence, harmonized standards need to be developed to collect and store high-quality samples and their data at multiple sites with comparable quality to better guarantee the high-quality studies on such samples and data. This of course has to include a transparent government structure to access such samples.

References

1. McMaster MT, Librach CL, Zhou Y, Lim KH, Janatpour MJ, DeMars R, Kovats S, Damsky C, Fisher SJ. Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. J Immunol Baltim. 1995;154:3771–8.
2. Moser G, Weiss G, Sundl M, Gauster M, Siwetz M, Lang-Olip I, Huppertz B. Extravillous trophoblasts invade more than uterine arteries: evidence for the invasion of uterine veins. Histochem Cell Biol. 2017;147:353–66.
3. He N, van Iperen L, de Jong D, Szuhai K, Helmerhorst FM, van der Westerlaken LAJ, Chuva de Sousa Lopes SM. Human extravillous trophoblasts penetrate decidual veins and lymphatics before remodeling spiral arteries during early pregnancy. PLoS One. 2017;12:e0169849.
4. Windsperger K, Dekan S, Pils S, Golletz C, Kunihs V, Fiala C, Kristiansen G, Knöfler M, Pollheimer J. Extravillous trophoblast invasion of venous as well as lymphatic vessels is altered in idiopathic, recurrent, spontaneous abortions. Hum Reprod. 2017;28:1–10. https://doi.org/10.1093/humrep/dex058.
5. Kaufmann P, Huppertz B, Frank HG. The fibrinoids of the human placenta: origin, composition and functional relevance. Ann Anat. 1996;178:485–501.
6. Feinberg RF, Kliman HJ, Lockwood CJ. Is oncofetal fibronectin a trophoblast glue for human implantation? Am J Pathol. 1991;138:537–43.
7. Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. Cell Tissue Res. 1998;291:133–48.

8. Simmons DG, Fortier AL, Cross JC. Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta. Dev Biol. 2007;304:567–78.
9. Moser G, Gauster M, Orendi K, Glasner A, Theuerkauf R, Huppertz B. Endoglandular trophoblast, an alternative route of trophoblast invasion? Analysis with novel confrontation co-culture models. Hum Reprod. 2010;25:1127–36.
10. Moser G, Weiss G, Gauster M, Sundl M, Huppertz B. Evidence from the very beginning: endoglandular trophoblasts penetrate and replace uterine glands in situ and in vitro. Hum Reprod. 2015;30:2747–57.
11. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J Clin Endocrinol Metab. 2002;87(6):2954–9.
12. Burton GJ, Jauniaux E, Charnock-Jones DS. Human early placental development: potential roles of the endometrial glands. Placenta. 2007;28(Suppl A):S64–9.
13. Moser G, Huppertz B. Implantation and extravillous trophoblast invasion: from rare archival specimens to modern biobanking. Placenta. 2017;56:19–26. https://doi.org/10.1016/j.placenta.2017.02.007. pii: S0143-4004(17)30150-9.
14. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69:1–7.
15. Craven CM, Morgan T, Ward K. Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. Placenta. 1998;19:241–52.
16. Weiss G, Sundl M, Glasner A, Huppertz B, Moser G. The trophoblast plug during early pregnancy: a deeper insight. Histochem Cell Biol. 2016;146:749–56.
17. Jauniaux E, Collins SL, Jurkovic D, Burton GJ. Accreta placentation: a systematic review of prenatal ultrasound imaging and grading of villous invasiveness. Am J Obstet Gynecol. 2016;215:712–21.
18. Silver RM, Landon MB, Rouse DJ, Leveno KJ, Spong CY, Thom EA, Moawad AH, Caritis SN, Harper M, Wapner RJ, Sorokin Y, Miodovnik M, Carpenter M, Peaceman AM, O'Sullivan MJ, Sibai B, Langer O, Thorp JM, Ramin SM, Mercer BM. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units NetworkMaternal morbidity associated with multiple repeat cesarean deliveries. Obstet Gynecol. 2006;107:1226–32.
19. Fernandez H, Al-Najjar F, Chauveaud-Lambling A, Frydman R, Gervaise A. Fertility after treatment of Asherman's syndrome stage 3 and 4. J Minim Invasive Gynecol. 2006;13:398–402.
20. Ball E, Bulmer JN, Ayis S, Lyall F, Robson SC. Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. J Pathol. 2006;208:535–42.
21. Ball E, Robson SC, Ayis S, Lyall F, Bulmer JN. Early embryonic demise: no evidence of abnormal spiral artery transformation or trophoblast invasion. J Pathol. 2006;208:528–34.
22. Jauniaux E, Greenwold N, Hempstock J, Burton GJ. Comparison of ultrasonographic and Doppler mapping of the intervillous circulation in normal and abnormal early pregnancies. Fertil Steril. 2003;79:100–6.
23. Kadyrov M, Schmitz C, Black S, Kaufmann P, Huppertz B. Pre-eclampsia and maternal anaemia display reduced apoptosis and opposite invasive phenotypes of extravillous trophoblast. Placenta. 2003;24:540–8.
24. Cnattingius S, Wikström AK, Stephansson O, Johansson K. The impact of small for gestational age births in early and late preeclamptic pregnancies for preeclampsia recurrence: a cohort study of successive pregnancies in Sweden. Paediatr Perinat Epidemiol. 2016;30:563–70.
25. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy. 2003;22:143–8.
26. Nicolaides KH, Bindra R, Turan OM, Chefetz I, Sammar M, Meiri H, Tal J, Cuckle HS. A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound. Ultrasound Obstet Gynecol. 2006;27:13–7.
27. Pilalis A, Souka AP, Antsaklis P, Basayiannis K, Benardis P, Haidopoulos D, Papantoniou N, Mesogitis S, Antsaklis A. Screening for pre-eclampsia and small for gestational age fetuses at the 11–14 weeks scan by uterine artery Dopplers. Acta Obstet Gynecol Scand. 2007;86:530–4.

28. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of maternal arterial blood flow and placental oxidative stress; a possible factor in human early pregnancy failure. Am J Pathol. 2000;157:2111–22.
29. Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol. 1992;80:283–5.
30. Kakogawa J, Sumimoto K, Kawamura T, Minoura S, Kanayama N. Noninvasive monitoring of placental oxygenation by near-infrared spectroscopy. Am J Perinatol. 2010;27:463–8.
31. Schaaps JP, Tsatsaris V, Goffin F, Brichant JF, Delbecque K, Tebache M, Collignon L, Retz MC, Foidart JM. Shunting the intervillous space: new concepts in human uteroplacental vascularization. Am J Obstet Gynecol. 2005;192:323–32.
32. Sibley CP, Pardi G, Cetin I, Todros T, Piccoli E, Kaufmann P, Huppertz B, Bulfamante G, Cribiu FM, Ayuk P, Glazier J, Radaelli T. Pathogenesis of intrauterine growth restriction (IUGR)-conclusions derived from a European Union Biomed 2 Concerted Action project 'Importance of Oxygen Supply in Intrauterine Growth Restricted Pregnancies'—a workshop report. Placenta. 2002;23(Suppl A):S75–9.
33. Huppertz B, Gauster M, Orendi K, König J, Moser G. Oxygen as modulator of trophoblast invasion. J Anat. 2009;215:14–20.
34. Konje JC, Kaufmann P, Bell SC, Taylor DJ. A longitudinal study of quantitative uterine blood flow with the use of color power angiography in appropriate for gestational age pregnancies. Am J Obstet Gynecol. 2001;185:608–13.
35. Bahlmann F, Fittschen M, Reinhard I, Wellek S, Steiner E. Reference values for blood flow velocity in the uterine artery in normal pregnancies from 18 weeks to 42 weeks of gestation calculated by automatic Doppler waveform analysis. Ultraschall Med. 2012;33:258–64.
36. Pijnenborg R, Vercruysse L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. Placenta. 2006;27:939–58.
37. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta. 2009;30:473–82.
38. Kawamura T, Kakogawa J, Takeuchi Y, Takani S, Kimura S, Nishiguchi T, Sugimura M, Sumimoto K, Kanayama N. Measurement of placental oxygenation by transabdominal near-infrared spectroscopy. Am J Perinatol. 2007;24:161–6.
39. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension. 2008;51:970–5.

Part IV

Pathophysiology of Preeclampsia

Immunological Maladaptation

4

Shigeru Saito, Tomoko Shima, and Akitoshi Nakashima

Abstract

Numerous risk factors for preeclampsia have been proposed. Immunological maladaptation is one of the known risk factors, because epidemiological evidence supports a relationship between preeclampsia and inadequate tolerance. Regulatory T (Treg) cells play a central role in the induction and maintenance of tolerance. Treg cells are decreased in number and show impaired function in preeclampsia, while Th1 cells and cytotoxic T cells are increased, suggesting disruption of the tolerance system. Chronic inflammation is observed in preeclampsia, which could be explained by an impaired function of Treg cells and an increase number of M1 macrophages, Th1 cells, and Th17 cells. In addition, production of agonistic autoantibodies targeting the angiotensin II type 1 receptor (AT1-AA) occurs in preeclampsia due to the decrease of Treg cells and increase of activated B cells. Moreover, the antiangiogenic factor sEng is increased in preeclampsia, and it plays an important role in induction of Th17 cell differentiation and inhibition of Treg cell differentiation by inhibiting TGF-β. These changes of the immune system seem to be important in the pathophysiology of preeclampsia. Recent studies have shown that immunotherapy can be effective in animal models of preeclampsia, including infusion of Treg or treatment with IL-10, anti-TNF antibody, CTLA-4 agonistic antibody, rituximab, and PDL1-Fc.

Keywords

Immunological maladaptation • Regulatory T cell • Preeclampsia • Th17 • Tolerance

S. Saito, M.D., Ph.D. (✉) • T. Shima, M.D., Ph.D. • A. Nakashima, M.D., Ph.D.
Department of Obstetrics and Gynecology, Graduate School of Medicine and Pharmaceutical Science for Research, University of Toyama, Toyama, Japan
e-mail: s30saito@med.u-toyama.ac.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_4

4.1 Introduction

A fetus is a semi-allograft to the maternal host, so pregnancy only continues because immune tolerance prevents maternal rejection of the fetus. Various subsets of maternal immune cells such as NK cells, macrophages, T cells, dendritic cells (DC), γδT cells, and NK T cells are present at the feto-maternal interface. Recent data have suggested that preeclampsia is due to breakdown of this maternal tolerance system [1–6] (Fig. 4.1). Tolerance is induced by regulatory T (Treg) cells, while the immunoregulatory role of Treg cells is disturbed by chronic inflammation, Th1 type immunity, and Th17 type immunity [1–4]. Importantly, these immune states are present in preeclampsia, while Treg cells are decreased in preeclampsia [7–10] (Fig. 4.1). Breakdown of maternal-fetal tolerance due to such abnormalities could induce insufficient placentation, shallow trophoblast invasion, and insufficient vascular remodeling that result in the development of preeclampsia. Furthermore, there is much epidemiological evidence supporting a relationship between impairment of tolerance and preeclampsia [1, 2, 11–15]. Moreover, it was recently reported that some new immunomodulatory therapies improved symptoms of preeclampsia in various animal models [16–20]. Therefore,

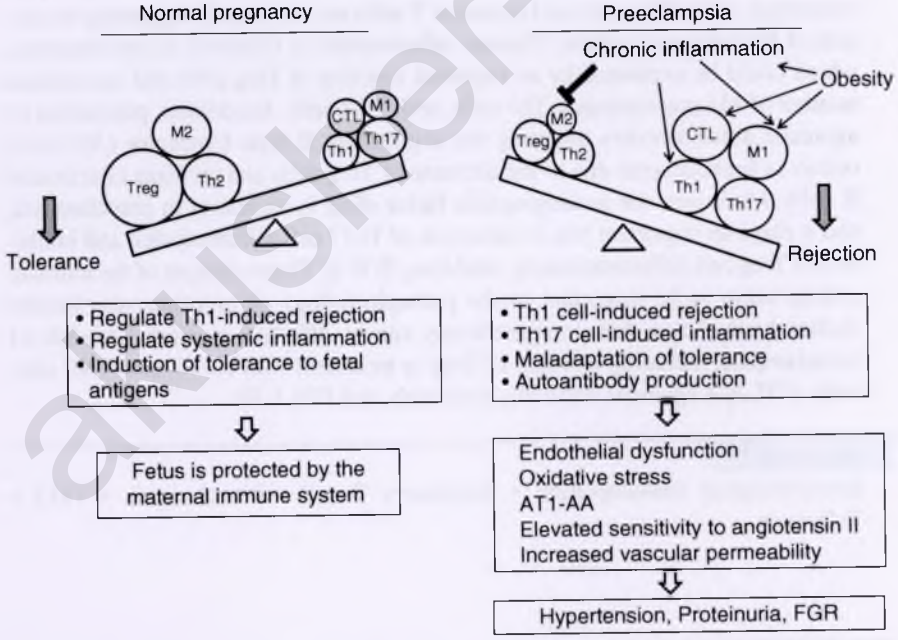


Fig. 4.1 The Th1/Th2/Th17/Treg balance in normal pregnancy and preeclampsia. In normal pregnancy, the predominance of Treg cells, M2 macrophages, and Th2-type immunity prevents fetal rejection and excessive inflammation. In preeclampsia, there is a shift to predominance of Th1, Th17, cytotoxic T cells, and M1 macrophages, resulting in suppression of tolerance and induction of inflammation

it is very important to understand the immunopathophysiology of preeclampsia and these new findings that may lead to novel treatment for this condition. This chapter reviews the pathophysiology of preeclampsia from the immunological perspective.

4.2 Role of the Immune System

The immune system is classified into innate and adaptive immune systems (Fig. 4.2). Antigen-presenting cells, such as dendritic cells (DC) and macrophages (MΦ), take up trophoblast debris or fetal cells and present fetal antigens to maternal T cells and B cells. The activated T cells then differentiate into Th1 cells involved in cellular immunity or fetal rejection, Th2 cells involved in humoral immunity, regulatory T cells involved in induction and maintenance of tolerance, and Th17 cells involved in inflammation or fetal rejection (Fig. 4.3). During normal pregnancy, the Th1/Th2 balance shifts to a Th2 predominant state, but Th1 dominance occurs in preeclampsia [21, 22] (Fig. 4.1). $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells play a central role in the induction and maintenance of tolerance [23]. A decrease of Treg cells induces implantation failure and fetal loss in mice [24, 25], and the percentage of Treg cells among $CD4^+$T cells in the decidua is significantly lower in miscarriage associated with normal fetal chromosomes than in normal pregnancy [26, 27],

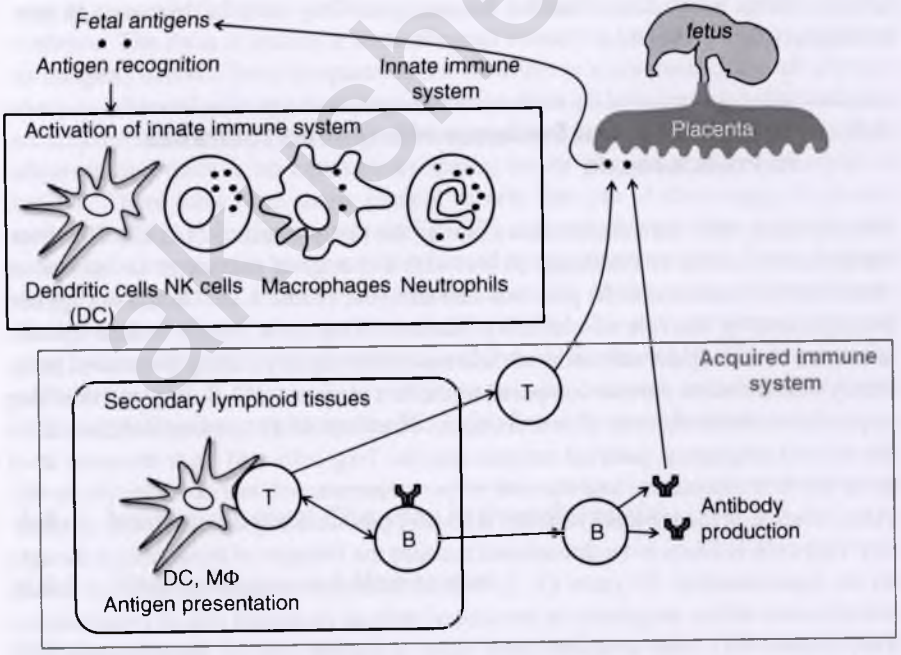


Fig. 4.2 Innate and acquired immune systems

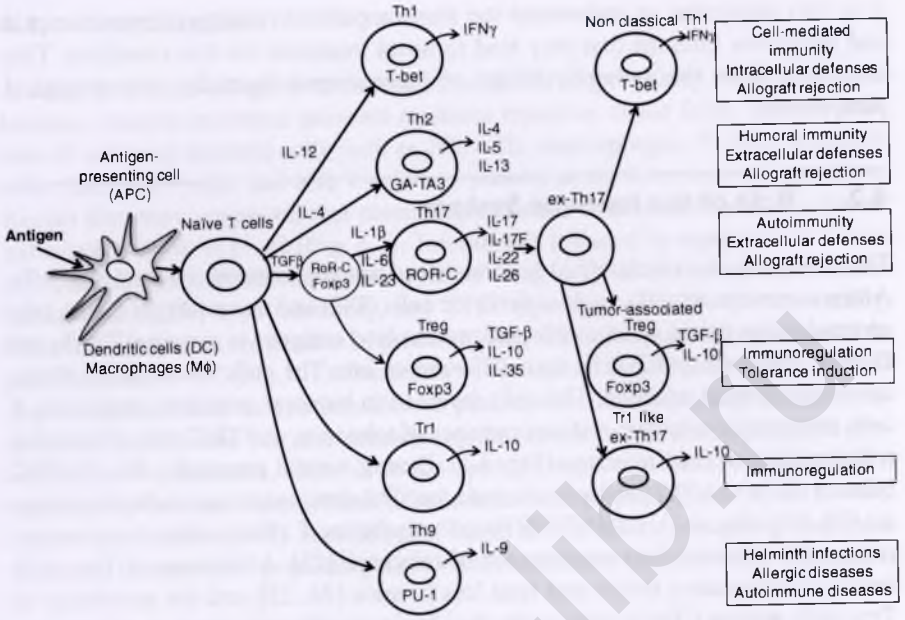


Fig. 4.3 Differentiation and functions of Th1, Th2, Th17, Treg, Tr1, and Th9 cells

suggesting that a decrease of Treg cells could lead to failure of pregnancy. Indeed, several studies have shown that the frequency of Treg cells is decreased in preeclampsia [7–10] (Fig. 4.1).

4.3 Epidemiological Evidence of Impaired Tolerance in Preeclampsia

Preeclampsia most commonly occurs during the first pregnancy [11, 12]. However, the protective effect of multiparty is lost with a change of partner or an interval of more than 10 years since the previous delivery [14] (Table 4.1). These findings can be explained by the role of immunity. Memory Treg cells, which induce specific tolerance to fetal (paternal) antigens, increase more rapidly during the second pregnancy with the same partner compared to the first pregnancy [1, 2, 28], and this Treg expansion reduces the risk of preeclampsia. However, if the partner is different for the second pregnancy, paternal antigen-specific Treg cells will be at the same level as in the first pregnancy, and the risk of preeclampsia will not decrease [1, 2, 12]. Also, when it is longer than 10 years since the previous delivery, the pool of memory Treg cells is likely to be diminished because the lifespan of these cells is thought to be approximately 10 years [1, 2, 14]. In addition, a short duration of sexual cohabitation before pregnancy is associated with an increased risk of preeclampsia [13] (Table 4.1), and condom users have a higher risk of preeclampsia [15] (Table 4.1). These findings suggest that long-term exposure to sperm or seminal fluid might induce specific tolerance of paternal antigens. Robertson and our group

Table 4.1 Epidemiological evidence supporting a relationship between preeclampsia and impaired tolerance

Epidemiological findings	Immunological findings
• Primigravidity • Protective effect of multiparity is lost with a change of partner • More than 10 years after the previous delivery	Insufficient induction of paternal antigen-specific Treg cells
• Short duration of sexual cohabitation • Condom users	Long-term seminal fluid exposure induces paternal antigen-specific Treg cells
• Donor sperm pregnancy	Lack of exposure to seminal fluid
• Donor egg pregnancy	Fetus is a complete allograft All MHC antigens are non-self
• Donor embryo pregnancy	Lack of exposure to seminal fluid Fetus is a complete allograft

have shown that priming with seminal plasma induces proliferation of paternal antigen-specific Treg before implantation [29, 30]. Accordingly, seminal plasma priming may play an important role in the induction and expansion of paternal antigen-specific Treg cells, resulting in a lower risk of preeclampsia.

It should also be noted that preeclampsia shows a very high frequency in pregnancies involving donor gametes [31–33] (Table 4.1). Salha et al. reported preeclampsia in 12.1% and 11.1% of pregnancies achieved with donor sperm or donor eggs, respectively, increasing to 25.0% for donor embryos [31]. Maternal exposure to sperm is deficient when pregnancy is achieved with donor sperm or donor embryos. The fetus is usually a semi-allograft inside the maternal host with 50% of its antigens derived from the partner, but the fetus is a complete allograft if pregnancy is achieved with egg or embryo donation since all histocompatibility antigens are derived from a third party. When a donor embryo is used, the fetus is a complete allograft, and there is no exposure to sperm, so the frequency of preeclampsia is highest. There have been many clinical reports that use of donor eggs is associated with a high risk of preeclampsia [31–33], and remodeling of the spiral arteries seems to be impaired in pregnancies achieved by egg donation [34]. These findings strongly suggest that immune maladaptation is one of the causes of preeclampsia. It has been found that adoptive transfer of Th1-like cells to normal pregnant mice induces hypertension, proteinuria, and liver dysfunction [16, 17]. Importantly, adoptive transfer of these cells to nonpregnant mice does not induce such changes [16], suggesting that pregnancy itself is a risk factor for preeclampsia.

4.4 Immunological Changes in Preeclampsia

4.4.1 Macrophages and Monocytes

Enhancement of innate immunity with increased activation of macrophages and monocytes is observed in normal pregnancy [3–5, 35]. These cells contribute to protecting the fetus from microorganisms. Macrophages also play

an important role in placental development by promoting extravillous trophoblast invasion and remodeling of spiral arteries. Monocytes are classified into three categories, which are $CD14^{hi}CD16^{-}CCR2^{hi}$ $CX3CR1^{lo}$ classical monocytes, $CD14^{hi}CD16^{+}CCR2^{+}CCR5^{hi}$ intermediate monocytes, and $CD14^{lo}CD16^{+}CCR2^{-}CX3CR1^{hi}CCR5^{+}$ nonclassical monocytes [5, 36]. In nonpregnant women, classical, intermediate, and nonclassical cells account for 90%, 5%, and 5% of all monocytes, respectively. The main function of classical monocytes, intermediate monocytes, and nonclassical monocytes is phagocytosis, pro-inflammatory responses, and patrolling, respectively [5]. Changes of these monocyte subsets occur during pregnancy, with an increase of activate intermediate monocytes and a decrease of classical monocytes, indicating that pregnancy is a pro-inflammatory state [5, 35, 36]. In the tissues, monocytes undergo differentiation into $CD11c^{+}CD206^{-}CD163^{-}$ M1 macrophages involved in inflammation through production of IL-12 and TNFα, as well as $CD11c^{+}CD206^{+}CD163^{+}$ M2 macrophages involved in tissue remodeling and anti-inflammatory responses through production of IL-10. Decidual macrophages show significantly higher production of IL-10 than peripheral blood monocytes, suggesting that M2 macrophages are dominant at the feto-maternal interface [37–40] (Fig. 4.1). Recent data have shown that decidual macrophages can be classified as $CD14^{+}CD11c^{lo}$DC-$SIGN^{+}CD206^{+}CD163^{+}$neuropilin^{+}ICAM3^{-}HLA-G^{+}ILT4^{+}CCR7^{+}-M2 macrophages involved in immune tolerance and $CD14^{+}$ $CD11c^{hi}$ DC-$SIGN^{-}$ $CD206^{-}$ $CD163^{-}$neuropilin^{-}ICAM3^{+}-M1 macrophages involved in inflammation [5, 40, 41]. IL-10 plays an important role in upregulation of the expression of tolerogenic factors such as HLA-G and ILT4 in macrophages [40–43]. Decidual macrophages show low expression of the co-stimulatory molecule CD86 and high expression of indoleamine 2, 3-dioxygenase (IDO), which induces tolerance [44]. In preeclampsia, there is an increase of $CD83^{+}$DC-$SIGN^{+}CD14^{+}$ monocytes, but these cells show lower expression of HLA-G and ILT compared to cells from healthy pregnant women, suggesting that tolerogenic activity is reduced and M1 macrophages are increased in preeclampsia [4, 45, 46] (Fig. 4.1). Activation of macrophages has been reported in preeclampsia, and these activated macrophages inhibit trophoblast invasion and spiral artery remodeling *in vitro* [47, 48]. Increased production of pro-inflammatory cytokines is also reported in preeclampsia [2, 35]. Furthermore, M1 macrophages are increased in the spiral arteries of women with preeclampsia [49–51], suggesting that these macrophages may be involved in the development of acute atherosis as well as arteriosclerosis [52, 53]. These changes of macrophage populations play a role in the pathogenesis of preeclampsia.

4.4.2 Dendritic Cells (DCs)

DC only account for about 1% of mononuclear cells in the decidua [54, 55], but play an important role in the activation of T cells and B cells by presenting various antigens, and are also involved in the induction of antigen-specific Treg cells. The former type of antigen-presenting DC is very important for stimulation of

the immune response, while the latter type of tolerogenic DC [56] plays a role in immunoregulation.

In mice, selective depletion of CD11c$^+$DC induces failure of implantation, decidualization, and vascularization in both allogeneic and syngeneic pregnancy, suggesting that uterine DCs play a central and essential role in the implantation process independently of their influence on immunological tolerance [57]. On the other hand, in a mouse model with Treg cell depletion, failure of implantation is only observed in allogeneic pregnancy, but not in syngeneic pregnancy [24, 25]. Thus, Treg cells have a role in preventing fetal rejection by maternal immunocompetent cells, and DCs are also involved in the induction of tolerance to fetal antigens. Decidual DCs are derived from myeloid cells, and reduced IL-12 secretion by DCs is involved in regulation of fetal rejection [54]. Decidual DCs promote the differentiation of CD4$^+$ T cells into Th2 cells, thus preventing Th1-mediated fetal rejection [54]. Decidual DCs express IDO, an enzyme involved in catabolism of tryptophan, and have an important role in inducing tolerance during pregnancy [44]. Production of IFNγ by Th1 cells and NK cells, as well as CTLA-4 expression on Treg cells, leads to the upregulation of IDO expression by DC and macrophages, resulting in induction of tolerance [44]. GM-CSF is also a regulator of T cell activation in uterine DCs [58].

The characteristics of DCs show various changes in preeclampsia. First, there is marked upregulation of GM-CSF production by decidual cells [59, 60]. IL-1β and TNFα have been shown to enhance GM-CSF production by cultured decidual cells [58]. Preeclampsia-like symptoms develop when pregnant mice are injected with Th1 cells [16, 17], and it is interesting that both decidual DCs and macrophages are increased in this mouse model, along with elevated production of GM-CSF [59]. It is well known that an increase of decidual DCs occurs in preeclampsia [45, 59, 61]. Preeclampsia is associated with systemic inflammation, and key pro-inflammatory cytokines (e.g., IL-1β) enhance the production of various chemokines that recruit DC, including CCL2, CCL4, CCL5, CCL7, and CCL20 [61]. In women with preeclampsia, circulating DCs show increased expression of TLR3, TLR4, and TLR9, and these cells constitutively produce high levels of IL-6, TNFα, and IL-12. However, TLR activation does not induce a further increase of cytokine production, suggesting dysregulation of DC function in preeclampsia [60]. Furthermore, there is low expression of B7-H1 (PD-L1), which is involved in immunoregulation, on DCs in preeclampsia, suggesting downregulation of DC tolerogenic activity in this condition [62].

Decidual DCs play an important role in induction of Treg cells during pregnancy. Contact between DC-SIGN$^+$APCs (DCs and macrophages) and Treg cells is observed in normal pregnancy, and there is a strong correlation between the number of DC-SIGN$^+$APCs and Treg cells in healthy pregnancy, suggesting that such cell-cell contact is important in the induction of Treg cells [4, 45]. In preeclampsia, there is a significant increase of DC-SIGN$^+$APCs compared with healthy pregnancy, but contact between these cells and Treg cells is rare, suggesting the disruption of cross talk between DCs and Treg cells. Indeed, preeclamptic DCs show impairment of the ability to induce Treg cells from naïve CD4$^+$ T cells *in vitro* [45].

4.4.3 NK Cells

NK cells, especially $CD16^{-}CD56^{bright}$ NK cells, are a major component of decidual immunocompetent cells and are involved in secretion of cytokines that induce accumulation of EVT, as well as participating in remodeling of the spiral arteries [63–67]. Macrophages accumulate around the spiral arteries at 7–8 weeks of gestation, followed by the accumulation of NK cells at 8–10 weeks of gestation [68, 69]. NK cells produce matrix metalloproteinase (MMP)-2, MMP-9, urinary plasminogen activator (uPA), and urinary plasminogen activator receptor (uPAR), which promote the separation of vascular smooth muscle cells [69, 70]. NK cells subsequently induce the apoptosis of endothelial cells [70, 71], and finally the vessel lumen is completely replaced by EVT at 12–16 weeks of gestation. NK cells produce chemotactic factors such as IL-8 and IP-10 that are important for accumulation of EVT around the spiral arteries [64, 65, 72]. It is difficult to evaluate NK cell function during early pregnancy in women who develop preeclampsia after 20 weeks of gestation. Cartwright et al. investigated NK cell function in women with abortion and suspected poor spiral artery remodeling according to uterine artery Doppler ultrasound [73–75]. They found that a high uterine artery Doppler resistance index (UA-RI) predicts an increased risk of preeclampsia and fetal growth restriction (FGR) associated with poor vascular remodeling. They also showed that cultured decidual NK cells from pregnant women with a normal UA-RI were more effective at promoting trophoblast migration and invasion than cells from women with a high UA-RI [75]. Moreover, decidual NK cells from high UA-RI pregnancies were less effective at inducing apoptosis of vascular endothelial cells *in vitro* [74].

Villous trophoblasts do not show surface expression of any MHC class I or MHC class II antigens, but EVT express polymorphic HLA-C antigens from MHC class I. Feto-maternal HLA-C mismatch induces T cell activation in the decidua [76]. NK cells express receptors for HLA-C antigens. KIR is a receptor for HLA-C and is classified into the category of inhibitory and activating receptors. The combination of maternal homozygosity for the KIR A haplotype (KIR-AA) on NK cells and the fetal HLA-C2 phenotype increases the risk of preeclampsia [74]. Interestingly, expression of KIR B (an activating receptor for HLA-C2) is associated with protection against preeclampsia. It was recently reported that activation of $KIRsDS1^{+}$ decidual NK cells by HLA-C2 stimulates the production of GM-CSF, which enhances trophoblast migration, suggesting that GM-CSF production by NK cells may be beneficial for placentation [77]. Therefore, Moffett et al. speculated that activation of maternal uterine NK cells and increased production of GM-CSF are necessary for normal placentation. However, it was reported that GM-CSF production by decidual stromal cells is increased in preeclampsia [59], and an epidemiological study did not support their hypothesis. Japanese have the highest prevalence of the KIR-AA haplotype and lowest prevalence of HLA-C2 [78], while Caucasian show a moderate prevalence of both KIR-AA and HLA-C2. If Moffett's hypothesis is correct, the frequency of preeclampsia should be increased in Japanese women with Caucasian partners, but our data showed that the frequency of preeclampsia in these couples was similar to that in Japanese

women with Japanese partners [79]. Accordingly, the origins of preeclampsia are not so simple, although some of women may develop it due to the combination of maternal KIR and fetal HLA-C genes.

4.4.4 Th1/Th2/Th17/Treg Balance

$CD4^+$T cells are classified into Th1 cells producing IFNγ and TNFα that involved in cellular immunity and rejection; Th2 cells producing IL-4, IL-6, and IL-13 that are involved in antibody production; Th17 cells producing IL-17, IL-21, and IL-22 that are involved in autoimmunity or inflammation; and Treg cells producing TGF-β, IL-10, and IL-35 that are involved in immunoregulation or induction of tolerance [80] (Fig. 4.3).

In women with preeclampsia, the immune system shows Th1 predominance, and Th1 cytokine levels are closely correlated with the severity of symptoms [21, 22]. Adoptive transfer of Th1 cells to pregnant mice induces hypertension and proteinuria [16, 17], suggesting that Th1 cells are involved in the pathogenesis of preeclampsia. $CD4^+CD25^+CD127^{-low}Foxp3^+$Treg cells play a central role in the induction and maintenance of tolerance, while depletion of Treg cells induces failure of implantation and fetal resorption in allogeneic mice, but not syngeneic mice [24, 25, 81]. We previously reported that preeclampsia was associated with a decrease of Treg cells in the peripheral blood and decidua [7] (Fig. 4.1), and many other studies have also shown a decrease of Treg cells in preeclampsia [8–10]. A recent study showed that $CD4^+Foxp3^+$T cells can be classified into $CD4^+CD45RA^+Foxp3^+$ naïve Treg cells with weak immunoregulatory activity, $CD4^+CD45RA^-Foxp3^{++}$effector Treg cells with strong immunoregulatory activity, and $CD4^+CD45RA^-Foxp3^+$effector T cells that produce IL-2 and IFNγ and are not involved in immunoregulation [82]. Both effector Treg cells and naïve Treg cells are decreased in preeclampsia, but effector T cells that induce rejection are increased, suggesting maladaptation of tolerance [9].

Next, the mechanisms underlying the decrease of Treg cells in preeclampsia are discussed. The first mechanism is an imbalance between differentiation of Treg cells and Th17 cells [8, 83] (Fig. 4.4). Naïve T cells differentiate into Th17 cells that induce inflammation and Treg cells that induce tolerance. In the presence of inflammatory cytokines such as IL-1β and IL-6 and a low TGF-β concentration, naïve $CD4^+$ T cells differentiate into Th17 cells. Inflammatory cytokine production is upregulated in preeclampsia, and soluble endoglin (sENG) is increased, inhibiting TGF-β activity. As a result, Treg cells are decreased and Th17 cells are increased in preeclampsia [8]. With regard to the second mechanism, decidual DCs induce Treg cells from naïve T cells in normal pregnancy, resulting in an increase of Treg cells, but impairment of decidual APCs that induce Treg cells is observed in preeclampsia [45]. The third mechanism is increased susceptibility of Treg cells to apoptotic signals in preeclampsia. Expression of anti-apoptotic Bcl-2 is downregulated, and expression of pro-apoptotic BAX is upregulated in the Treg cells of women with preeclampsia, suggesting that Treg cells are more susceptible to apoptosis [84].

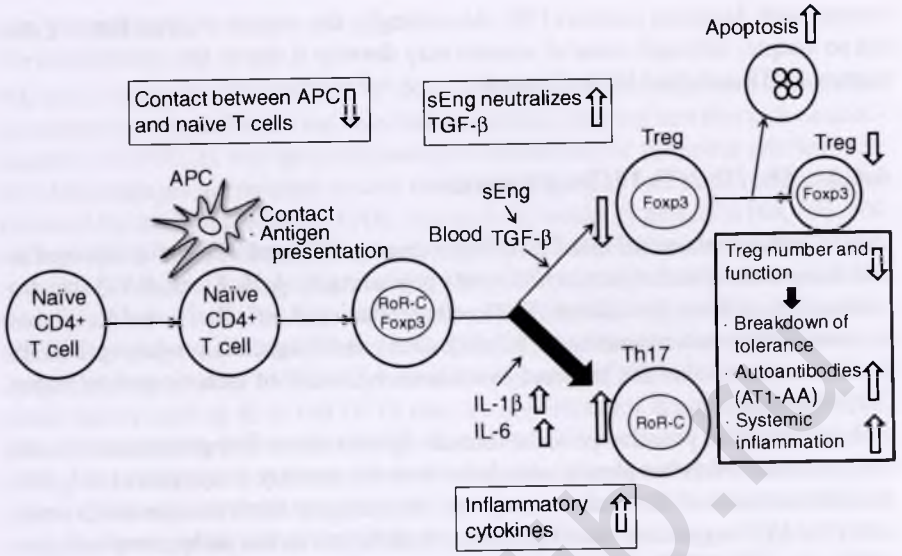


Fig. 4.4 Th17/Treg balance in preeclampsia. In preeclampsia, increased production of sEng, IL-1β, and IL-6 induces Th17 cells and suppresses Treg cells. Apoptosis of Treg cells increases, and these cells show functional impairment, resulting in breakdown of tolerance and induction of AT1-AA and inflammation

Not only is the number of Treg cells reduced in preeclampsia, but also Treg cell functions are suppressed due to the influence of chronic inflammation. It is well known that chronic inflammation reduces Treg cell activity [85], and chronic inflammation occurs in preeclampsia [3, 35] (Fig. 4.1).

4.5 The Immune-Mediated Pathophysiology of Preeclampsia

The functional impairment and reduced number of Treg cells induces an uncontrolled systemic pro-inflammatory response in preeclampsia. Inflammation induces leucocyte migration into vessel walls, leading to oxidative stress, endothelial dysfunction, and vasoconstriction [86]. IL-6 and TNFα induce endothelial dysfunction, while IL-17 promotes oxidative stress and hypersensitivity to angiotensin II [87, 88]. These pro-inflammatory effects lead to increased production of the vasoconstrictor endothelin-1 (ET-1) and decreased production of the vasodilator nitric oxide, resulting in the development of hypertension [88]. Endothelial damage also leads to proteinuria and edema.

Another mechanism is related to Treg cell regulation of autoantibody production. Since both the number and function of Treg cells are suppressed in preeclampsia [7, 8, 89, 90], production of autoantibodies can occur more easily, such as autoantibodies activating the angiotensin II type I receptor (AT1-AA) [91] (Fig. 4.4, Table 4.2). The renin-angiotensin system (RAS) plays an important role in preeclampsia.

Although RAS activation occurs in normal pregnancy, vascular responsiveness to angiotensin II is reduced. In contrast, the levels of renin, angiotensin II, and angiotensinogen are lower in preeclampsia than normal pregnancy, but vascular responsiveness to angiotensin II is characteristically elevated. It was recently reported that AT1-AA recognizes a seven-amino acid sequence of the second extracellular loop of the angiotensin II type I receptor and has an agonistic effect [91, 92]. Elevation of AT1-AA in preeclampsia has been reported by many authors. Placental ischemia is an important stimulus for AT1-AA production, so both immunological maladaptation and hypoxia induce AT1-AA [88, 93]. Transfusion of purified AT1-AA IgG into pregnant mice and rats induces preeclampsia-like symptoms, suggesting that AT1-AA is important in the pathogenesis of preeclampsia.

Antiangiogenic factors such as sFlt-1 and sEng are associated with preeclampsia, and these factors are useful markers for predicting its onset [94, 95] (Table 4.2). sFlt-1 inhibits the activity of VEGF and PlGF, so the sFlt-1/VEGF or sFlt-1/PIGF balance is important with regard to angiogenesis. VEGF is produced by a wide variety of cells, including macrophages, T cells, NK cells, osteoblasts, smooth muscle cells, fibroblasts, endothelial cells, cardiomyocytes, skeletal muscle cells, keratinocytes, and placental cells. Interestingly, production of VEGF by T cells and NK cells is downregulated in preeclampsia [96]. Soluble endoglin (sEng) is a TGF-β inhibitor and inhibits vascularization. TGF-β is an important cytokine that promotes differentiation of naïve $CD4^+$T cells into Treg cells, so an increase of sEng in

Table 4.2 Risk factors for preeclampsia and correlations with immunological maladaptation

Risk factors for preeclampsia and correlations with immunological maladaptation

Risk factors		Immunological maladaptation
·Poor placentation → placental ischemia	→	T cell activation, Th17⬆, Treg⬇, M1Mϕ⬆ NK cell cytotoxicity⬆, , AT1-AA⬆
·Anti-angiogenic factors (sFlt-1⬆, sEng⬆, PlGF⬇)	TGF-β pathway inhibition →	, Treg⬇, Th17⬆
·Systemic inflammation (Trophoblast debris)	→	M1Mϕ⬆, M2Mϕ⬇, Treg ⬇, Th17 ⬆
·AT1-AA (Angiotensin II type 1 receptor agonistic autoantibody)	←	Treg⬇, B cell activation⬆, CD4 cell activation⬆
·Obesity	→	Cytotoxic T⬆, NK cell cytotoxicity⬆ M1Mϕ⬆
·Genetic factors	←	Polymorphism of complement, Foxp3, IL-17, and IL-27 genes
·Primigravidity, >10 yearsafter the previous delivery	→	Inadequate tolerance
·Short duration of sexual cohabitation, condom users		
·Donor gametes (sperm, egg, or embryo)		
·Twinpregnancy → excessive exposure to fetal antigens		
·History of preeclampsia	←	Altered immune response and excessive inflammation
·Autoimmune diseases (SLE, antiphospholipid syndrome)	↔	Autoantibodies such as AT1-AA and antiphospholipid antibodies , Treg⬇
	↔	Inflammatory cytokines⬆
·Fetal sex (female)	↔	?
·Aging	↔	?

preeclampsia could reduce the number of Treg cells, resulting in maladaptation of tolerance [83] (Fig. 4.4).

sEng also inhibits autophagy [97]. Autophagy plays a role in the regulation of inflammasomes and thus in regulation of inflammation [98]. Indeed, activation of NLRP3 inflammasomes has been reported in monocytes from women with preeclampsia, and uric acid (which increases in preeclampsia) activates inflammasomes [99]. Autophagy is important for removal of inflammasomes to regulate inflammation [98]. Therefore, an increase of sEng in preeclampsia may inhibit autophagy in immunocompetent cells, resulting in systemic inflammation. As autophagy is also important for EVT invasion and vascular remodeling, impaired autophagy could be involved in problems with placentation [97]. Hypoxia induces production of inflammatory cytokines and endothelial cell dysfunction. All of these findings indicate that the immune system plays an important role in the pathogenesis of preeclampsia.

4.6 Immunological Findings in Animal Models of Preeclampsia

Several animal models of preeclampsia have been reported (Table 4.3), and these models all feature hypertension, proteinuria, and fetal growth restriction (FGR). Various immunological changes are recognized, such as alteration of Th1 type immunity, an increase of Th17 cells, a decrease of Treg cells, elevation of inflammatory cytokine production, and elevation of AT1-AA (Table 4.1). These immunological changes are similar to those in women with preeclampsia (Table 4.3).

Table 4.3 Immunological findings in animal models of preeclampsia

Immunological findings in animal models of preeclampsia

Animal model	Immunological findings
Th1 cell infusion mouse model (J. Reprod. Immunol. 2000, 47, 121–138, Eur. J. Immunol. 2004, 34, 377–387)	Th1⬆, decidual MΦ⬆, decidual DC⬆, decidual GM-CSF⬆
L-NG-nitroargininemethyl ester (L-NAME)-induced rat model (Scientific Rep. 2016, 6, 27683)	Treg⬇, Th17⬆
Female rats transgenic for human angiotensinogen mated with male rats transgenic for human renin (Hypertension 2015, 65, 1298–1306)	Treg⬇
CD40L-expressing embryo transfer mouse model (Hypertension Res. 2016, 39, 407–414)	Th1⬆, Th17➡, Th2➡, sFlt-1⬆, sEng⬆
Reduced uterine perfusion pressure (RUPP) rat model (Hypertension 2011, 57, 949–955)	CD4+T⬆, Th17⬆, Treg⬇
Infusion of CD4+ T cells from RUPP rats into normal pregnant rats (Hypertension 2011, 57, 949–955)	TNFα⬆, sFlt-1⬆

Models based on infusion of Th1 cells have shown that these immune cells can directly induce preeclampsia [16, 17]. In a rat model induced with an NO inhibitor (L-NAME), Treg cells are decreased and Th17 cells are increased [18], suggesting that NO inhibitor treatment directly alters the immunological environment to a Th17-dominant state and suppresses Treg cells.

Female rats transgenic for human angiotensinogen develop hypertension and proteinuria after being mated with male rats transgenic for human renin, and Treg cells are reduced in this model, suggesting a correlation between the RAS system and impaired tolerance [19] (Table 4.3).

A mouse model involving CD40L-expressing embryo transfer develops preeclampsia-like symptoms, suggesting that activation of T cells could induce preeclampsia during pregnancy [100] (Table 4.3). Th1 cells are increased in this model, while Th2 and Th17 cells are unchanged, suggesting that Th1 cells are more important in the pathogenesis of preeclampsia.

A rat model of reduced uterine perfusion pressure (RUPP) is the most representative animal model of preeclampsia. Small clips are placed on both uterine artery arcades on day 14 of gestation to reduce uterine blood flow by ~40% and cause placental ischemia. The immunological changes in this RUPP model are similar to those in human preeclampsia. Th1/Th2/Th17/Treg cells show a paradigm shift to Th1 and Th17 dominance with reduction of Treg cells, and AT1-AA is also detected in this model along with increased production of inflammatory cytokines [6] (Table 4.3). Furthermore, infusion of $CD4^+$T cells from RUPP rats into normal pregnant rats leads to hypertension and proteinuria with elevated levels of cytokines and AT1-AA [99, 101, 102], suggesting that $CD4^+$ T cells have an important role in the pathogenesis of preeclampsia, at least in part.

4.7 Challenges in Treating Preeclampsia with Immune Cells or Immunomodulators

Preeclampsia is the leading cause of maternal and perinatal mortality and morbidity, but effective treatment has still not been established. Some authors have suggested that immunotherapy might be a promising new approach to the treatment of preeclampsia.

Infusion of Treg cells derived from normal pregnant rats reduced the blood pressure, AT1-AA level, placental levels of ROS and ET-1, and serum levels of TNFα and IL-17, but fetal weight did not increase [103] (Table 4.4). In RUPP model rats, infusion of IL-10 reduced blood pressure, AT1-AA, serum TNFα and IL-17, and placental ROS and ET-1, but fetal weight also did not increase [104] (Table 4.4). TNFα blocking antibodies and CTLA-4 agonistic antibodies are well-known treatments for rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). These antibody therapies are effective for controlling symptoms (Table 4.4). In addition, the safety of TNFα blocking antibodies for pregnant women and fetuses has been established, so it may be possible to use these antibodies to treat preeclampsia although clinical trials have not yet started. Rituximab treatment (B cell depletion) is also effective in the

Table 4.4 Challenges in treating preeclampsia with immune cells or immunomodulators

Challenges in treating preeclampsia with immune cells or immunomodulators

Treatment	Model	Effects
Treg infusion (Am J Physiol Regul Inter Comp Physiol.2015, 309, R884–891)	RUPP rats	BP⬇, Fetal weight ➡, AT1-AA⬇ Placental ROS and ET-1⬇, serum TNFα and IL-17⬇ serum IL-6 and IL-10 ➡
IL-10 infusion (Hypertensions Pregnancy 2015, 34, 291–306)	RUPP rats	BP⬇, Fetal weight➡, AT1-AA⬇, serum TNFα and IL-17⬇, Placental ROS and ET-1⬇
TNFα inhibitor Ab (Hypertension 2008, 52, 1161–1167)	RUPP rats	BP⬇, Fetal weight➡, serum TNFα⬇ tissue ET1⬇
Rituximab (B cell depletion) (Hypertension 2011, 57, 865–871)	RUPP rats	BP⬇, Fetal weight ?, AT1-AA⬇ tissue ET1⬇
Abatacept (Fusion protein composed of Fc region of IgG1 and CTLA-4) (J. Hypertension 2012, 2, 1000116)	RUPP rats	BP⬇, Fetal weight➡, AT1-AA⬇ Plasma TNFα⬇, placental sFlt-1⬇, CD4+T⬇
CTLA-4agonistic Ab (Hypertension 2015, 65, 1298–1306)	Human angiotensinogen transgenicfemale rats mated with human renin transgenic male rats	BP➡, Proteinuria➡, Fetal weight⬆, Treg⬆, AT-1-AA⬇
PDL1-Fc (PD1 pathway activator) (Scientific Rep. 2016, 6, 27683)	L-NAME-induced rat model	BP⬇, Proteinuria⬇, Treg⬆, Th17⬇, fetal weight⬆ placental weight⬆

RUPP model [105], suggesting that autoantibodies such as AT1-AA are important in the pathogenesis of preeclampsia (Table 4.4). However, B cell depletion might induce severe infection during pregnancy, so this therapy does not seem to be safe. CTLA-4 agonistic antibody and PDL1-Fc augment immunoregulation by Treg cells are also effective against symptoms of preeclampsia in animal models [18, 19, 106]. These therapies might act by regulating Th1 cells, Th17 cells, and inflammation, but safety for pregnant women and fetuses has not been established.

Thus, immunotherapy has been shown to be effective in animal models, but clinical studies need to be performed to evaluate the therapeutic effect in early-onset preeclampsia.

Conclusion

There is abundant evidence that immunological maladaptation is one of the risk factors for preeclampsia (Table 4.2). Poor placentation in preeclampsia is explained by dysfunction of monocytes and NK cells and/or inhibition of autophagy by the antiangiogenic factor sEng, resulting in impairment of EVT invasion and vascular remodeling. Inhibition of autophagy by sEng also induces inflammation because inflammasomes are normally digested by autophagosomes. Trophoblast debris accumulate under hypoxic conditions, and these debris induce an inflammatory response mediated by immunocompetent cells. Obesity is also a risk factor for preeclampsia. In obese women, the M1/M2 balance is shifted to an M1 predominant state. We previously reported a positive correlation

between body mass index (BMI) and the levels of cytotoxic T cells or cytotoxic NK cells [107] (Table 4.2), while cytotoxic T cells have been shown to increase in preeclampsia [108]. Risk factors such as primigravidity and a period longer than 10 years since the previous delivery are explained by insufficient expansion of paternal antigen-specific Treg cells during pregnancy. Seminal plasma priming plays an important role in induction of paternal antigen-specific Treg cells. Therefore, inadequate establishment of tolerance in women with a short duration of sexual cohabitation or condom use could increase the risk of preeclampsia (Table 4.1). In donor gamete pregnancies, lack of seminal plasma priming and/or the presence of a complete fetal allograft might impair tolerance and result in preeclampsia (Table 4.1). Genetic factors such as polymorphism of complement, Foxp3, IL-17, and IL-27 might explain why a history of preeclampsia is a strong risk factor for further occurrence of preeclampsia (Table 4.2). Autoimmune disease is also a risk factor for preeclampsia (Table 4.2), suggesting that various immune abnormalities may promote its development.

These findings suggest that immunological maladaptation causes the development of preeclampsia rather than being a consequence of preeclampsia. Therefore, immunomodulatory therapy might eventually become a curative treatment for preeclampsia (Table 4.4), and further progress with such therapy is expected in the future.

References

1. Saito S, Sakai M, Sasaki Y, et al. Inadequate tolerance induction may induce preeclampsia. J Reprod Immunol. 2007;76:30–9.
2. Saito S, Shiozaki A, Nakashima A, et al. The role of the immune system in preeclampsia. Mol Asp Med. 2007;28:192–209.
3. Redman CW, Sargent IL. Immunology of preeclampsia. Am J Reprod Immunol. 2010; 63:534–43.
4. Hsu P, Nanan RK. Innate and adaptive immune interactions at the fetal-maternal interface in healthy human pregnancy and pre-eclampsia. Front Immunol. 2014;5:125.
5. Tang MX, Hu XH, Liu ZZ, et al. What are the roles of macrophages and monocytes in human pregnancy? J Reprod Immunol. 2015;112:73–80.
6. LaMarca B, Cornelius DC, Harmon AC, et al. Identifying immune mechanisms mediating the hypertension during preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2016;311:R1–9.
7. Sasaki Y, Darmochwal-Kolarz D, Suzuki D, et al. Proportion of peripheral blood and decidual $CD4^+$ $CD25^{bright}$ regulatory T cells in pre-eclampsia. Clin Exp Immunol. 2007;149:139–45.
8. Santner-Nanan B, Peek MJ, Khanam R, et al. Systemic increase in the ratio between $Foxp3^+$ and IL-17-producing $CD4^+$ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183:7023–30.
9. Toldi G, Vásárhelyi ZE, Rigó J Jr, et al. Prevalence of regulatory T-Cell subtypes in preeclampsia. Am J Reprod Immunol. 2015;74:110–5.
10. Rahimzadeh M, Norouzian M, Arabpour F, et al. Regulatory T-cells and preeclampsia: an overview of literature. Expert Rev Clin Immunol. 2016;12:209–27.
11. Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol. 1998;179:1359–75.

12. Robillard PY, Hulsey TC, Alexander GR, et al. Paternity patterns and risk of preeclampsia in the last pregnancy in multiparae. J Reprod Immunol. 1993;24:1–12.
13. Robillard PY, Hulsey TC, Périanin J, et al. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. Lancet. 1994;344:973–5.
14. Skjaerven R, Wilcox AJ, Lie RT. The interval between pregnancies and the risk of preeclampsia. N Engl J Med. 2002;346:33–8.
15. Klonoff-Cohen HS, Savitz DA, Cefalo RC, McCann MF. An epidemiologic study of contraception and preeclampsia. JAMA. 1989;262:3143–7.
16. Hayakawa S, Fujikawa T, Fukuoka H, et al. Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. J Reprod Immunol. 2000;47:121–38.
17. Zenclussen AC, Fest S, Joachim R, et al. Introducing a mouse model for pre-eclampsia: adoptive transfer of activated Th1 cells leads to pre-eclampsia-like symptoms exclusively in pregnant mice. Eur J Immunol. 2004;34:377–87.
18. Tian M, Zhang Y, Liu Z, et al. The PD-1/PD-L1 inhibitory pathway is altered in pre-eclampsia and regulates T cell responses in pre-eclamptic rats. Sci Rep. 2016;6:27683.
19. Przybyl L, Ibrahim T, Haase N, et al. Regulatory T cells ameliorate intrauterine growth retardation in a transgenic rat model for preeclampsia. Hypertension. 2015;65:1298–306.
20. Li J, LaMarca B, Reckelhoff JF. A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model. Am J Physiol Heart Circ Physiol. 2012;303:H1–8.
21. Saito S, Sakai M. Th1/Th2 balance in preeclampsia. J Reprod Immunol. 2003;59:161–73.
22. Saito S, Sakai M, Sasaki Y, et al. Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin Exp Immunol. 1999;117:550–5.
23. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531–62.
24. Zenclussen AC, Gerlof K, Zenclussen ML, et al. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol. 2005;166:811–22.
25. Shima T, Sasaki Y, Itoh M, et al. Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice. J Reprod Immunol. 2010;85:121–9.
26. Inada K, Shima T, Nakashima A, et al. Characterization of regulatory T cells in decidua of miscarriage cases with abnormal or normal fetal chromosomal content. J Reprod Immunol. 2013;97:104–11.
27. Inada K, Shima T, Ito M, et al. Helios-positive functional regulatory T cells are decreased in decidua of miscarriage cases with normal fetal chromosomal content. J Reprod Immunol. 2015;107:10–9.
28. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature. 2012;490:102–6.
29. Robertson SA, Guerin LR, Bromfield JJ, et al. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol Reprod. 2009;80:1036–45.
30. Shima T, Inada K, Nakashima A, et al. Paternal antigen-specific proliferating regulatory T cells are increased in uterine-draining lymph nodes just before implantation and in pregnant uterus just after implantation by seminal plasma-priming in allogeneic mouse pregnancy. J Reprod Immunol. 2015;108:72–82.
31. Salha O, Sharma V, Dada T, et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. Hum Reprod. 1999;14:2268–73.
32. Masoudian P, Nasr A, de Nanassy J, et al. Oocyte donation pregnancies and the risk of preeclampsia or gestational hypertension: a systematic review and meta-analysis. Am J Obstet Gynecol. 2016;214:328–39.

33. Saito S, Nakabayashi Y, Nakashima A. A new era in reproductive medicine: consequences of third-party oocyte donation for maternal and fetal health. Semin Immunopathol. 2016;38:687–97.
34. Nakabayashi Y, Nakashima A, Yoshino O, et al. Impairment of the accumulation of decidual T cells, NK cells, and monocytes, and the poor vascular remodeling of spiral arteries, were observed in oocyte donation cases, regardless of the presence or absence of preeclampsia. J Reprod Immunol. 2016;114:65–74.
35. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999;180:499–506.
36. Melgert BN, Spaans F, Borghuis T, et al. Pregnancy and preeclampsia affect monocyte subsets in humans and rats. PLoS One. 2012;7:e45229.
37. Gustafsson C, Mjösberg J, Matussek A, et al. Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype. PLoS One. 2008;3:e2078.
38. Kämmerer U, Eggert AO, Kapp M, et al. Unique appearance of proliferating antigen-presenting cells expressing DC-SIGN (CD209) in the decidua of early human pregnancy. Am J Pathol. 2003;162:887–96.
39. Laskarin G, Cupurdija K, Tokmadzic VS, et al. The presence of functional mannose receptor on macrophages at the maternal-fetal interface. Hum Reprod. 2005;20:1057–66.
40. Svensson J, Jenmalm MC, Matussek A, et al. Macrophages at the fetal-maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. J Immunol. 2011;187:3671–82.
41. Houser BL, Tilburgs T, Hill J, et al. Two unique human decidual macrophage populations. J Immunol. 2011;186:2633–42.
42. Hsu P, Santner-Nanan B, Joung S, et al. Expansion of $CD4^+$ $HLA\text{-}G^+$ T Cell in human pregnancy is impaired in pre-eclampsia. Am J Reprod Immunol. 2014;71:217–28.
43. Moreau P, Adrian-Cabestre F, Menier C, et al. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol. 1999;11:803–11.
44. Miwa N, Hayakawa S, Miyazaki S, et al. IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-gamma increase in normal pregnancy but decrease in spontaneous abortion. Mol Hum Reprod. 2005;11:865–70.
45. Hsu P, Santner-Nanan B, Dahlstrom JE, et al. Altered decidual DC-SIGN+ antigen-presenting cells and impaired regulatory T-cell induction in preeclampsia. Am J Pathol. 2012;181:2149–60.
46. Hennessy A, Pilmore HL, Simmons LA, et al. A deficiency of placental IL-10 in preeclampsia. J Immunol. 1999;163:3491–5.
47. Renaud SJ, Macdonald-Goodfellow SK, Graham CH. Coordinated regulation of human trophoblast invasiveness by macrophages and interleukin 10. Biol Reprod. 2007;76:448–54.
48. Spaans F, Melgert BN, Chiang C, et al. Extracellular ATP decreases trophoblast invasion, spiral artery remodeling and immune cells in the mesometrial triangle in pregnant rats. Placenta. 2014;35:587–95.
49. Schonkeren D, van der Hoorn ML, Khedoe P, et al. Differential distribution and phenotype of decidual macrophages in preeclamptic versus control pregnancies. Am J Pathol. 2011;178:709–17.
50. Prins JR, Faas MM, Melgert BN, et al. Altered expression of immune-associated genes in first-trimester human decidua of pregnancies later complicated with hypertension or foetal growth restriction. Placenta. 2012;33:453–5.
51. Medeiros LT, Peraçoli JC, Bannwart-Castro CF, et al. Monocytes from pregnant women with pre-eclampsia are polarized to a M1 phenotype. Am J Reprod Immunol. 2014;72:5–13.
52. Katabuchi H, Yih S, Ohba T, et al. Characterization of macrophages in the decidual atherotic spiral artery with special reference to the cytology of foam cells. Med Electron Microsc. 2003;36:253–62.
53. Staff AC, Johnsen GM, Dechend R, et al. Preeclampsia and uteroplacental acute atherosis: immune and inflammatory factors. J Reprod Immunol. 2014;101–102:120–6.

54. Miyazaki S, Tsuda H, Sakai M, et al. Predominance of Th2-promoting dendritic cells in early human pregnancy decidua. J Leukoc Biol. 2003;74:514–22.
55. Gardner L, Moffett A. Dendritic cells in the human decidua. Biol Reprod. 2003;69: 1438–46.
56. Raker VK, Domogalla MP, Steinbrink K. Tolerogenic dendritic cells for regulatory T cell induction in man. Front Immunol. 2015;6:569.
57. Plaks V, Birnberg T, Berkutzki T, et al. Uterine DCs are crucial for decidua formation during embryo implantation in mice. J Clin Invest. 2008;118:3954–65.
58. Moldenhauer LM, Keenihan SN, Hayball JD, et al. GM-CSF is an essential regulator of T cell activation competence in uterine dendritic cells during early pregnancy in mice. J Immunol. 2010;185:7085–96.
59. Huang SJ, Zenclussen AC, Chen CP, et al. The implication of aberrant GM-CSF expression in decidual cells in the pathogenesis of preeclampsia. Am J Pathol. 2010;177:2472–82.
60. Panda B, Panda A, Ueda I, et al. Dendritic cells in the circulation of women with preeclampsia demonstrate a pro-inflammatory bias secondary to dysregulation of TLR receptors. J Reprod Immunol. 2012;94:210–5.
61. Huang SJ, Chen CP, Schatz F, et al. Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua. J Pathol. 2008;214:328–36.
62. Darmochwal-Kolarz D, Kludka-Sternik M, Kolarz B, et al. The expression of B7-H1 and B7-H4 co-stimulatory molecules on myeloid and plasmacytoid dendritic cells in pre-eclampsia and normal pregnancy. J Reprod Immunol. 2013;99:33–8.
63. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol. 2002;2:656–63.
64. Hanna J, Goldman-Wohl D, Hamani Y, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med. 2006;12:1065–74.
65. Saito S, Nishikawa K, Morii T, et al. Cytokine production by CD16-CD56^{bright} natural killer cells in the human early pregnancy decidua. Int Immunol. 1993;5:559–63.
66. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension. 2013;62:1046–54.
67. Naruse K, Innes BA, Bulmer JN, et al. Secretion of cytokines by villous cytotrophoblast and extravillous trophoblast in the first trimester of human pregnancy. J Reprod Immunol. 2010;86:148–50.
68. Smith SD, Dunk CE, Aplin JD. Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. Am J Pathol. 2009;174:1959–71.
69. Lash GE, Otun HA, Innes BA, et al. Regulation of extravillous trophoblast invasion by uterine natural killer cell is dependent on gestational age. Hum Reprod. 2010;25:1137–45.
70. Naruse K, Lash GE, Innes BA, et al. Localization of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitors for MMPs (TIMPs) in uterine natural killer cells in early human pregnancy. Hum Reprod. 2009;24:553–61.
71. Harris LK, Keogh RJ, Wareing M, et al. Invasive trophoblasts stimulate vascular smooth muscle cell apoptosis by a fas ligand-dependent mechanism. Am J Pathol. 2006;169:1863–74.
72. Red-Horse K, Rivera J, Schanz A, et al. Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an in vivo model of human placentation. J Clin Invest. 2006;116:2643–52.
73. Cartwright JE, James-Allan L, Buckley RJ, et al. The role of decidual NK cells in pregnancies with impaired vascular remodelling. J Reprod Immunol. 2017;119:81–4.
74. Fraser R, Whitley GS, Thilaganathan B, et al. Decidual natural killer cells regulate vessel stability: implications for impaired spiral artery remodelling. J Reprod Immunol. 2015;110:54–60.
75. Wallace AE, Whitley GS, Thilaganathan B, et al. Decidual natural killer cell receptor expression is altered in pregnancies with impaired vascular remodeling and a higher risk of preeclampsia. J Leukoc Biol. 2015;97:79–86.
76. Tilburgs T, Scherjon SA, van der Mast BJ, et al. Fetal-maternal HLA-C mismatch is associated with decidual T cell activation and induction of functional T regulatory cells. J Reprod Immunol. 2009;82:148–57.

77. Xiong S, Sharkey AM, Kennedy PR, et al. Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. J Clin Invest. 2013;123:4264–72.
78. Hiby SE, Walker JJ, O'shaughnessy KM, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. J Exp Med. 2004;200:957–65.
79. Saito S, Takeda Y, Sakai M, et al. The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men. J Reprod Immunol. 2006;70:93–8.
80. Saito S, Nakashima A, Shima T, et al. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63:601–10.
81. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5:266–71.
82. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009;30:899–911.
83. Saito S. Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia. Immunol Cell Biol. 2010;88:615–7.
84. Darmochwal-Kolarz D, Saito S, Tabarkiewicz J, et al. Apoptosis signaling is altered in $CD4^+CD25^+FoxP3^+$ T regulatory lymphocytes in pre-eclampsia. Int J Mol Sci. 2012;13:6548–60.
85. van Maren WW, Jacobs JF, de Vries IJ, et al. Toll-like receptor signalling on Tregs: to suppress or not to suppress? Immunology. 2008;124:445–52.
86. Noris M, Perico N, Remuzzi G. Mechanisms of disease: pre-eclampsia. Nat Clin Pract Nephrol. 2005;1:98–114.
87. Madhur MS, Lob HE, McCann LA, et al. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. Hypertension. 2010;55:500–7.
88. Dhillion P, Wallace K, Herse F, et al. IL-17-mediated oxidative stress is an important stimulator of AT1-AA and hypertension during pregnancy. Am J Physiol Regul Integr Comp Physiol. 2012;303:R353–8.
89. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, et al. The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. J Reprod Immunol. 2012;93:75–81.
90. Steinborn A, Schmitt E, Kisielewicz A, et al. Pregnancy-associated diseases are characterized by the composition of the systemic regulatory T cell (Treg) pool with distinct subsets of Tregs. Clin Exp Immunol. 2012;167:84–98.
91. Brewer J, Liu R, Lu Y, et al. Endothelin-1, oxidative stress, and endogenous angiotensin II: mechanisms of angiotensin II type I receptor autoantibody-enhanced renal and blood pressure response during pregnancy. Hypertension. 2013;62:886–92.
92. Wallukat G, Homuth V, Fischer T, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest. 1999;103:945–52.
93. LaMarca B, Wallukat G, Llinas M, et al. Autoantibodies to the angiotensin type I receptor in response to placental ischemia and tumor necrosis factor alpha in pregnant rats. Hypertension. 2008;52:1168–72.
94. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355:992–1005.
95. Zeisler H, Llurba E, Chantraine F, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med. 2016;374:13–22.
96. Molvarec A, Ito M, Shima T, et al. Decreased proportion of peripheral blood vascular endothelial growth factor-expressing T and natural killer cells in preeclampsia. Am J Obstet Gynecol. 2010;203:567.e1–8.
97. Nakashima A, Yamanaka-Tatematsu M, Fujita N, et al. Impaired autophagy by soluble endoglin, under physiological hypoxia in early pregnant period, is involved in poor placentation in preeclampsia. Autophagy. 2013;9:303–16.
98. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2008;456:264–8.
99. Wallace K, Richards S, Dhillon P, et al. CD4+ T-helper cells stimulated in response to placental ischemia mediate hypertension during pregnancy. Hypertension. 2011;57:949–55.

100. Matsubara K, Matsubara Y, Mori M, et al. Immune activation during the implantation phase causes preeclampsia-like symptoms via the CD40-CD40 ligand pathway in pregnant mice. Hypertens Res. 2016;39:407–14.
101. Wallace K, Novotny S, Heath J, et al. Hypertension in response to CD4(+) T cells from reduced uterine perfusion pregnant rats is associated with activation of the endothelin-1 system. Am J Physiol Regul Integr Comp Physiol. 2012;303:R144–9.
102. Novotny SR, Wallace K, Heath J, et al. Activating autoantibodies to the angiotensin II type I receptor play an important role in mediating hypertension in response to adoptive transfer of CD4+ T lymphocytes from placental ischemic rats. Am J Physiol Regul Integr Comp Physiol. 2012;302:R1197–201.
103. Cornelius DC, Amaral LM, Harmon A, et al. An increased population of regulatory T cells improves the pathophysiology of placental ischemia in a rat model of preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2015;309:R884–91.
104. Harmon A, Cornelius D, Amaral L, et al. IL-10 supplementation increases Tregs and decreases hypertension in the RUPP rat model of preeclampsia. Hypertens Pregnancy. 2015;34:291–306.
105. LaMarca B, Wallace K, Herse F, et al. Hypertension in response to placental ischemia during pregnancy: role of B lymphocytes. Hypertension. 2011;57:865–71.
106. Novotny S, Wallace K, Herse F, et al. CD4+ T Cells play a critical role in mediating hypertension in response to placental ischemia. J Hypertens (Los Angel). 2013;2:14873.
107. Molvarec A, Shiozaki A, Ito M, et al. Increased prevalence of peripheral blood granulysin-producing cytotoxic T lymphocytes in preeclampsia. J Reprod Immunol. 2011;91:56–63.
108. de Groot CJ, van der Mast BJ, Visser W, et al. Preeclampsia is associated with increased cytotoxic T-cell capacity to paternal antigens. Am J Obstet Gynecol. 2010;203:496.e1–6.

Glucose Intolerance and Insulin Resistance: Relevance in Preeclampsia

5

Keizo Kanasaki

Abstract

The major symptoms of preeclampsia are de novo hypertension and proteinuria during pregnancy, and the clinical diagnosis of preeclampsia has classically been based on these symptoms. However, glucose tolerance defects and insulin resistance are prominent features of preeclampsia but have not been considered significant phenotypes, particularly when establishing animal models of preeclampsia. This review seeks to evaluate the pathomechanical significance of glucose tolerance defects and insulin resistance in the biology of preeclampsia.

Keywords

Insulin resistance • Gestational hypertension • Estradiol • PPARγ • XOMT

5.1 Introduction

Preeclampsia is a hypertensive disorder that occurs during pregnancy. Preeclampsia affects approximately 5–8% of pregnancies worldwide, but its etiology has not yet been elucidated. The major symptoms of preeclampsia are de novo hypertension and proteinuria during pregnancy, and the clinical diagnosis is based on these symptoms. Moreover, several attempts to establish animal models of preeclampsia have focused on the onset of hypertension with proteinuria. However,

K. Kanasaki, M.D., Ph.D.
Department of Diabetology and Endocrinology, Kanazawa Medical University, Uchinada, Ishikawa, Japan

Division of Anticipatory Molecular Food Science and Technology, Medical Research Institute, Kanazawa Medical University, Uchinada, Ishikawa, Japan
e-mail: kkanasak@kanazawa-med.ac.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_5

metabolic defects are also characteristics of preeclampsia [1, 2], but no animal models of preeclampsia focus on this potential important symptom of preeclampsia. Normal pregnancy has been recognized as an insulin-resistant state. The levels of insulin reach twofold higher compared with the nonpregnant state. In the third trimester of pregnancy, the levels of insulin resistance appear to peak; after delivery, such insulin resistance disappears, and the glucose tolerance is restored to the prepregnancy levels. The mechanisms of insulin resistance during pregnancy have not been fully uncovered.

During early normal pregnancy, glucose tolerance is slightly ameliorated [3–5]. This amelioration of glucose tolerance has been attributed to increased maternal estrogen and progesterone, by which pancreatic ß-cells can proliferate and exhibit hyperplasia [6]. This is the reason for the rapid induction of insulin levels in early pregnancy, in response to insulin resistance. In the second to third trimesters, the continuous increase in the feto-placental factors may cause a reduction in maternal insulin sensitivity and the induction of insulin resistance. Such insulin resistance stimulates the mother's cells to use fuel sources other than glucose, such as free fatty acids; subsequently, the glucose supply to the fetus is increased [7]. In the normal pregnant condition, the level of fetal blood glucose is 10–20% less than that of maternal blood. Such a disparity in the glucose levels between the maternal and fetal blood allows the transport of glucose through the placenta to the fetal blood via a simple diffusion process and facilitated transport. Consequently, the fetus can utilize glucose as the main fuel required for development, as the energy for cellular metabolism or as the source for the synthesis of proteins, lipids, and glycogen.

During pregnancy, the insulin resistance of the entire body is increased to approximately three times that of the nonpregnant state. The insulin resistance is differentiated as occurring at the pre-receptor level (such as insulin antibodies), the receptor level (reduced number of cell surface receptors), or the post-receptor level (intracellular insulin signaling defects). The insulin resistance in pregnancy is well characterized as a post-receptor defect by which insulin cannot mobilize GLUT4 into the cell surface. This may be due to increased plasma levels of one or more of the pregnancy-associated hormones such as estrogen, progesterone, and human placental lactogen [8].

To evaluate insulin sensitivity directly, a euglycemic, hyperinsulinemic clamp study would be suitable. To utilize such a clamp study for the precise monitoring of insulin resistance with glucose tolerance, this method is most likely useful in the experimental setting. Instead, measurements of the maternal serum levels of glucose, insulin, and C-peptide taken at fasting and 1 h after oral glucose administration can also be utilized to assess insulin sensitivity in clinics. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study [9], in which pregnant women with a 75-g oral glucose test were evaluated for pregnancy outcomes, clearly indicated a continuous association between glucose levels and adverse pregnancy outcomes such as preeclampsia and cesarean sections. There was no specific threshold above which the frequency of such adverse outcomes was common; rather, increased glucose levels were associated with the prevalence adverse pregnancy outcomes in a linear fashion.

Indeed, we have no absolute answer regarding which organs are responsible for such increased insulin resistance. Pregnancy has been shown to decrease the metabolic index of glucose by 60% in maternal red postural muscles. Fasting induces similar modifications in nonpregnant rabbits and exaggerates the changes observed in fed pregnant animals [10]. In rabbits on day 30 of gestation, the muscle glucose uptake was not significantly induced at a plasma insulin concentration of 700 μU/mL, whereas it was stimulated by 30–40% in nonpregnant and in 24-day pregnant rabbits. At a similar plasma insulin concentration, the endogenous glucose production rate was decreased by 85% in both nonpregnant and 24-day pregnant rabbits, whereas in 30-day pregnant rabbits, it was suppressed by only 30% [11]. Leturque et al. reported the glucose tolerance in the basal condition and during a euglycemic, hyperinsulinemic (400 μU/mL) clamp in virgin and 19-day pregnant rats. They found that the fetal glucose utilization rate (22 mg/min/kg) was very high and was not stimulated by physiologic maternal hyperinsulinemia [12]. By contrast, the placental glucose utilization rate (29 mg/min/kg) was increased by up to 30% in the hyperinsulinemia condition [12]. The conceptus glucose utilization rate represented 23% of the total maternal glucose utilization rate in the basal state [12]. In the basal condition, the glucose utilization rates were not altered by pregnancy in the brain, skeletal muscles, and white adipose tissue compared with the virgin rat; during the hyperinsulinemic clamp, the glucose utilization rates in the extensor digitorum longus, epitrochlearis, and white adipose tissue were 30–70% lower in pregnant compared with virgin rats [12]. The insulin sensitivity of glucose metabolism in all the tissues tested other than the brain was 50% lower in pregnant than in virgin rats [12].

Human placental lactogen (HPL) has been reported as one of the responsible molecules by which insulin sensitivity is reduced during pregnancy because HPL is synthesized in the placenta and increases toward the end of pregnancy [7]. Recently, the role of several cytokines and elevated lipid levels during pregnancy has shown to be associated with longitudinal alterations of insulin sensitivity in nonpregnant [13] and pregnant women [14, 15]. The potential involvement of TNF-α in defective insulin signaling in skeletal muscle has also been shown in the mechanisms of insulin resistance [16, 17].

5.2 Preeclampsia and the Glucose Tolerance Defect

The association of glucose tolerance defects and insulin resistance with preeclampsia onset has been established. A cohort study recruiting 3637 patients without gestational diabetes who carried singleton fetuses revealed that glucose intolerance in women without overt gestational diabetes appeared to be associated with an increased incidence of preeclampsia and other complications during gestation [18]. A prospective study demonstrated that hyperinsulinemia at 20 weeks of gestation was significantly associated with preeclampsia in African-Americans [19]. The glucose levels at 1 h were positively correlated with the likelihood of preeclampsia even within the normal range after a 50-g oral glucose tolerance test [20]. In 2002, Wolf et al. performed a

prospective nested control study to analyze the association between first-trimester insulin resistance and subsequent preeclampsia in nulliparous pregnant women [21].

However, whether such insulin resistance in preeclampsia plays a causal role in the pathogenesis of preeclampsia is still under debate. From the perspective of preeclampsia risk, insulin resistance is most likely significant. However, some cofounders make it difficult to evaluate the role of insulin resistance in preeclampsia. In particular, the presence of obesity, which is a known risk factor for both insulin resistance and preeclampsia, has not been considered in most studies. Whether insulin resistance contributes to the onset of preeclampsia in obese pregnant women is also not established because obesity is associated with other risks that may be relevant in the pathomechanism of preeclampsia, such as systemic low-level inflammation, adipokine alteration, and endothelial dysfunction.

In 2006, Parretti et al. reported an important finding regarding the link between insulin resistance and preeclampsia onset [22]. They recruited 829 cases of first-trimester pregnant nulliparous females with singleton pregnancies, the absence of chronic hypertension, pregestational body mass index (BMI) between 19 and 25 kg/m^2, and the absence of gestational diabetes mellitus (GDM). They performed a 75-g OGTT at two points (early pregnancy [16–20 weeks of gestation] and late pregnancy [26–30 weeks of gestation]). By utilizing fasting glucose (G_0: mmol/L) and insulin (I_0: μU/mL) values, they estimated the insulin sensitivity by IS_{HOMA} (according to Matthews [23]: $G_0 \times I_0/22.5$) or IS_{QUICKI} (Katz [24]: $1/[\log(I_0) + \log(G_0)]$. Dynamic insulin sensitivity was monitored by OGIS during OGTT, a model-derived measurement of glucose clearance (mL/min per m^2). Higher levels of IS_{HOMA} and lower levels of IS_{QUICKI} and OGIS revealed insulin resistance. Of 829 pregnant women, 53 (6.4%) subsequently developed insulin resistance and were positively associated with the 75th percentile of IS_{HOMA}, with a sensitivity of 79% in the early period and 83% in the late period and a specificity of 97% in both insulin resistance evaluations. Moreover, the IS_{QUICKI} 25th percentile was correlated with preeclampsia, with a sensitivity of 85% in the early period and 88% in the late period of the evaluation and a specificity of 97%. Preeclampsia is also associated with an OGIS below the 25th percentile. By excluding the pregestational obese population, this Parretti report [22] clearly demonstrated the significant link between insulin resistance and risk of preeclampsia. Following Parretti's report, utilizing this IS_{HOMA} and IS_{QUICKI} in the middle trimester of pregnancy in 1187 women, Hauth et al. found that IS_{HOMA} at the 75th percentile was significantly higher among the 85 nulliparous women who subsequently developed preeclampsia, compared with women who remained normotensive (40.5% vs. 24.8%; adjusted odds ratio, 1.9; 95% confidence interval, 1.1–3.2) [25]. The IS_{QUICKI} results were similar to the IS_{HOMA} results (40.5% vs. 25%; adjusted odds ratio, 1.9; 95% confidence interval, 1.1–3.2) [25]. Parretti's reports however did not aim to differentiate specifically the role of insulin resistance in either early- or late-onset preeclampsia [22]. With regard to this, D'Anna et al. demonstrated a small nested control study in 72 pregnant women (36 women with preeclampsia of which 20 late onset and 16 early onset were compared with 36 uncomplicated pregnancies who delivered at term) and found the first-trimester mean HOMA-IR

value was significantly higher in the late-onset preeclampsia when compared to that of the early-onset preeclampsia (2.5 ± 1.3 vs. 1.3 ± 0.3; $P = 0.02$) [26].

These data revealed that before the onset of preeclampsia in the first to middle trimester of the gestational period, insulin resistance monitoring by a simple measurement of the fasting glucose and insulin can provide a significant predictor of preeclampsia.

Although pregnancy is associated with an increased pancreatic β-cell mass and increased insulin levels during the gestational period, certain pregnant women are unable to induce the insulin production required for the degree of insulin resistance and consequently become hyperglycemic, developing gestational diabetes mellitus (GDM) [8]. Hypertensive disorders are increased two- to three-fold in pregnancies complicated with diabetes. Women with pregestational diabetes and GDM have been shown to display an increased risk of preeclampsia (10–50 and 10–30%, respectively). Montoro et al. [27] analyzed whether the magnitude of insulin resistance is associated with preeclampsia in GDM women. They recruited 150 normotensive women with GDM who underwent OGTTs, intravenous glucose tolerance tests (IVGTTs), and glucose clamp studies in the early third trimester and 89 15-month postpartum women. Among 150 pregnant GDM women with normal blood pressures, 29 women (19%) subsequently developed preeclampsia (21 with preeclampsia and 8 with severe preeclampsia) [27]. There was no difference in the age, weight indexes, or glycemic parameters between the nonpreeclamptic and preeclamptic women at entry. Women who developed preeclampsia were significantly taller (61.5 vs. 60.1 in.), were more often nulliparous (38 vs. 16%), and displayed a higher SBP (112 vs. 103 mmHg) and DBP (64 vs. 59 mmHg) at entry. However, there was no significant difference between the groups in any evaluation of the OGTT glucose levels, insulin sensitivity index, glucose effectiveness, acute response to glucose, or disposition index. During the euglycemic clamps, again, there was no difference in the basal and steady-state levels of glucose, insulin, FFA, glucose clearance, hepatic glucose output, glucagon, and C-peptide. At 15 months postpartum, the blood pressure remained significantly higher in women who experienced preeclampsia (19 of 89 cases), compared with women without preeclampsia (70 cases); the mean blood pressure levels in both groups were within the normal ranges. Furthermore, no differences in any glycemic or insulin resistance parameters were observed in the postpartum analysis [27]. These data suggested that in pregnant women with GDM (the subgroup that has been shown to have an elevated risk of preeclampsia), there was no association between insulin resistance and the onset of preeclampsia.

5.3 Preeclampsia Presents a Risk for Future Diabetes Development

Preeclampsia has shown to be associated with an increased incidence of future diabetes as a vascular risk factor. One can hypothesize that several common pathogenic pathways underlie the interaction between GDM, preeclampsia, and gestational diabetes, leading to an increased risk of diabetes because each of these conditions has

been shown to be associated with insulin resistance [22, 26, 28–30]. Several studies have also demonstrated that women with a history of preeclampsia and gestational hypertension exhibit higher levels of insulin resistance years after delivery, even after controlling for body mass index and excluding women with previous GDM [31, 32]. In addition, women with a history of preeclampsia or gestational hypertension display characteristics of the metabolic syndrome years after delivery, a syndrome associated with insulin resistance [33–35]. In women with either preeclampsia or gestational hypertension, the presence of endothelial dysfunction and chronic vascular inflammation has been described during the gestational and post-gestational periods [36–39], which is a similar finding preceding the development of hyperglycemia in patients who are at risk for type 2 diabetes [39].

Four previous studies analyzed the future risk of type 2 diabetes in women with a history of preeclampsia and/or gestational hypertension. In 2007, Callaway et al. reported that women enrolled in the Mater-University of Queensland Study of Pregnancy between 1981 and 1984 who had either preeclampsia or gestational hypertension at baseline were 1.76 times more likely to develop diabetes 21 years after delivery, even after adjusting for all potentially explanatory variables [40]. In a Danish cohort of women with preeclampsia or gestational hypertension, Lykke et al. reported that the risk of subsequent type 2 diabetes mellitus was increased 3.12-fold (range, 2.63–3.70) after gestational hypertension and 3.68-fold (range, 3.04–4.46) after severe preeclampsia over a median of 14.6 years [41]. Engeland et al. reported a registry study of women with preeclampsia in Norway, which revealed that 5 years after pregnancy, approximately 2% of women with preeclampsia received antidiabetic drugs, compared with 0.5% of those without preeclampsia. The registry data set was large; however, the follow-up interval was short, only 3.7 years, and the diabetes diagnosis was based on the prescription of antidiabetic drugs, which potentially underestimated the true incidence of diabetes [42]. Most recently Feig et al. [43] reported a population-based, retrospective cohort study for 1,010,068 pregnant women who delivered in Canada. In this cohort, they categorized the pregnant women into five groups (having preeclampsia alone (n = 22,933), gestational hypertension alone (n = 27,605), gestational diabetes alone (n = 30,852), gestational diabetes with preeclampsia (n = 1476), gestational diabetes with gestational hypertension (n = 2100), or none of these conditions (n = 925,102)). The follow-up was completed in 16.5 years. The incidence rate of diabetes per 1000 person-years was 6.47 for women with preeclampsia and 5.26 for gestational hypertension compared with 2.81 in women with none of these conditions. In the multivariable analysis, either preeclampsia alone (hazard ratio [HR] = 2.08; 95% CI 1.97–2.19) or gestational hypertension alone (HR = 1.95; 95% CI 1.83–2.07) was a risk factor for the future development of diabetes. Gestational diabetes increased the risk of postpartum diabetes (HR = 12.77; 95% CI 12.44–13.10); the copresence of either preeclampsia or gestational hypertension with gestational diabetes further elevated this risk (HR = 15.75, 95% CI 14.52–17.07, and HR = 18.49, 95% CI 17.12–19.96, respectively). In this cohort, the potential effects of obesity were not analyzed [43].

There may be several limitations of the abovementioned studies. Nonetheless, these investigations demonstrated the possibility that (1) hypertension and/or

endothelial damage during pregnancy can lead to the future development of diabetes or (2) preeclampsia may be a shared genetic or molecular mechanistic background in its pathogenesis with diabetes.

5.4 Perspective

As described above, insulin resistance and glucose tolerance defects are associated with preeclampsia onset; furthermore, a history of preeclampsia has been associated with a future metabolic risk for cardiovascular diseases, including type 2 diabetes [44–46]. Such a metabolic risk for cardiovascular disease in women with a preeclampsia history has often been explained by the hypothesis that vascular insults during pregnancy in preeclamptic women cause endothelial injuries that lead to metabolic defects [44–46]; the possibility of either a shared genetic background or molecular mechanism has not yet been investigated.

We have shown that a deficiency in catechol-*o*-methyltransferase (COMT), the enzyme responsible for the metabolism of catechols, such as catecholamines and catechol estrogens, is relevant to the onset of preeclampsia. Estradiol is metabolized into hydroxyestradiol by the aid of cytochrome P450 [1, 47] (Fig. 5.1). Hydroxyestradiol, which is a catechol estrogen, is the substrate for COMT, and COMT transmethylates hydroxyestradiol into 2-methoxyestradiol (2-ME) [1, 47] (Fig. 5.1). Regarding the physiological significance of 2-ME, COMT deficiency with associated 2-ME suppression leads to a preeclampsia-like phenotype in mice [48]. The human COMT

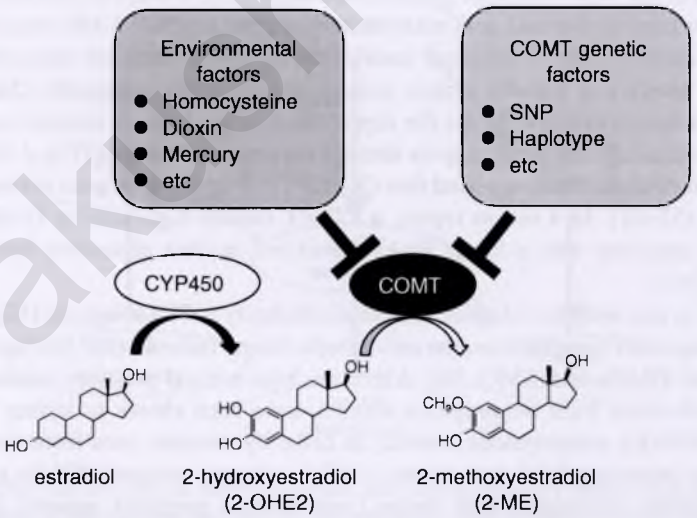


Fig. 5.1 The role of COMT in the synthesis of 2-ME. Estradiol is metabolized into 2-OHE2 by the cytochrome P450. 2-OHE2 is a catechol estrogen and is the substrate for COMT, and COMT transmethylates 2-OHE2 into 2-ME. COMT protein levels and activity are easily suppressed by genetic and environmental factors

gene exhibits functional SNPs, by which the protein stability of COMT is significantly diminished and the enzymatic activity is subsequently decreased (*COMT*$^{158Val\text{-}Met}$) [49]. Furthermore, COMT activity has shown to be easily suppressed by endogenous and exogenous inhibitors such as *S*-adenosyl-homocysteine, mercury, dioxin, and PCB (Fig. 5.1). The association between COMT and preeclampsia onset was first reported in 1988 by Barnea, when preeclamptic women exhibited significantly lower COMT activity in their placentas [50]. We have also shown that the COMT protein levels are lower in the severe form of preeclampsia [48].

To identify the physiological significance of COMT deficiency in the pathogenesis of preeclampsia, a *COMT* genetic knockout (*COMT*$^{-/-}$) mouse was analyzed. Indeed, pregnant *COMT−/−* mice developed a preeclampsia-like phenotype characterized by proteinuria, gestational hypertension, and a decidual vasculopathy-like alteration in the uteroplacental unit and an endotheliosis-like phenotype in the kidney. This phenotype in pregnant *COMT*$^{-/-}$ mice was associated with lower plasma levels of 2-ME and higher placental HIF-1α protein levels [48]. An intervention with 2-ME in pregnant *COMT*$^{-/-}$ mice ameliorated the preeclampsia-like phenotype associated with suppression of placental HIF-1α [48]. Taken together, the above data suggest that deficiencies in COMT and 2-ME play a significant role in the development of preeclampsia [48].

The precise molecular mechanisms by which 2-ME prevents the onset of preeclampsia remain unclear. 2-ME exhibits inhibitory effects on HIF-1α and sFlt1 [48], and there may be several diverse mechanisms by which 2-ME promotes vascular health. 2-ME prevents cerebral vasospasm [51]; furthermore, 2-ME has been shown to suppress vascular smooth muscle cell proliferation [52] and inhibits the synthesis of the vasoconstrictor protein endothelin [53]. 2-ME has also been shown to have renoprotective and anti-inflammatory properties [54]. 2-ME may also prevent the development of placental insufficiency [55], a condition that can deplete estradiol levels and thereby further worsen preeclampsia symptoms [56]. These reports indicated that apart from the suppression of the HIF-1α-induced pathways, 2-ME protects against preeclampsia through diverse mechanisms (Fig. 5.2).

Recent evidence has suggested that *COMT*$^{158Val\text{-}Met}$ may participate in obesity and diabetes [57–61]. In a recent report, a COMT rs4680 high-activity G-allele was found to associate with a lower HbA1c level and modest protection from type 2 diabetes [61].

2-ME is also known to display structural similarity to PPAR ligands [62], such as pioglitazone and rosiglitazone, the antidiabetic drugs. Indeed 2-ME has been shown to activate PPARγ in VSMCs [62, 63]. Sera from normal pregnant women, compared with those from nonpregnant women, have been shown to induce a nearly doubled PPARγ transcriptional activity in cells; by contrast, sera from severe preeclamptic women exhibit low serum 2-ME levels and reduced PPARγ transcriptional activity compared with those from normal pregnant women [64, 65]. Additionally, pregnant women with dominant negative mutations in *PPARγ* display preeclampsia [66].

This PPARγ activity of 2-ME is relevant to the protective role of 2-ME in the pathogenesis of preeclampsia. The mechanism of hypertension in preeclampsia has been shown to be the hypersensitivity to the pressor response against angiotensin II

(Ang II). We found that COMT deficiency, induced by either COMT inhibition or COMT siRNA induction in mice, was associated with hypersensitivity to the pressure response against low-dose Ang II (70 ng/kg/min); 2-ME ameliorated the low-dose Ang II-induced hypertension in the COMT-deficient mice [67]. In the mechanism of this anti-angiotensin-induced hypertension, we found that COMT deficiency and the resultant lack of 2-ME enhanced the angiotensin II receptor 1 (AT1R) levels in cultured smooth muscle cells and in the mouse aorta. Regarding the 2-ME-suppressed AT1R, PPARγ transcriptional activity of 2-ME was critical for the suppression of AT1R levels in both in vitro and ex vivo plasma in both male mice and pregnant mice. 2-ME levels are elevated toward the end of pregnancy [48, 68], thus demonstrating the essential role of 2-ME in the homeostasis of blood pressure by regulating the sensitivity to Ang II [67] (Fig. 5.2).

The significant role of COMT and 2-ME is not limited to blood pressure regulation. Recently, we discovered the essential role of COMT and 2-ME in the regulation of blood glucose levels in glucose tolerance [69]. First, we found that liver COMT protein levels were significantly suppressed in high-fat diet (HFD)-fed mice and pregnant mice, indicating that both of these conditions easily promoted the onset of liver COMT deficiency [69]. COMT suppression by either a COMT inhibitor or siRNA in HFD-fed mice and pregnant mice was associated with glucose tolerance defects; 2-ME rescued these glucose tolerance defects in diverse manners [69]. 2-ME ameliorated the insulin resistance associated with liver AMPK phosphorylation in long-term HFD and COMT inhibitor-treated normal-fed male mice or pregnant mice, partly associated with the PPARγ activity of 2-ME [70] (Fig. 5.2). However, in

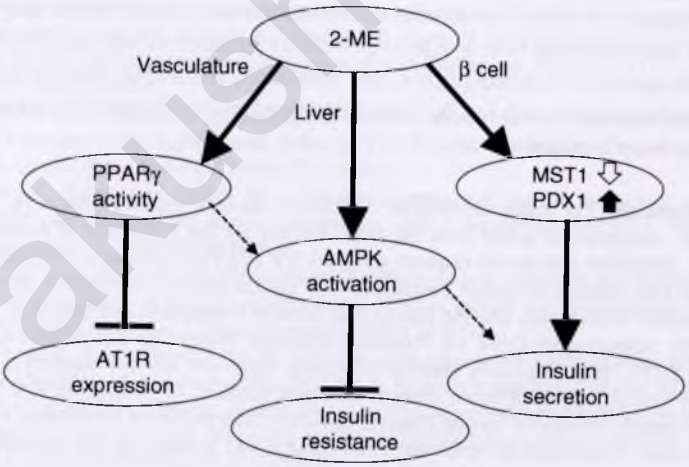


Fig. 5.2 The role of 2-ME on the hypertension and glucose tolerance defects in preeclampsia. 2-ME displays PPARγ activity, and such PPARγ activity of 2-ME is important for the suppression of AT1R on the VSMC. Also 2-ME activates AMPK, and such AMPK activation is relevant for the suppression of insulin resistance in the liver. Maybe PPARγ activity of 2-ME contributes in part for 2-ME-induced AMPK activation. In pancreatic β-cells, 2-ME also activates AMPK, but AMPK activation by 2-ME in β-cells is not sufficient for the induction of insulin secretion by 2-ME. Because 2-ME suppresses proapoptotic molecule MST1 and increased survival molecule PDX1 in β-cells, AMPK activator AICAR does not act same as 2-ME on these molecules

COMT inhibitor-treated HFD-fed mice, surprisingly, the glucose-lowering effects of 2-ME were associated with the stimulation of insulin secretion. Furthermore, the antidiabetic drug metformin restored the COMT protein levels in the HFD mice, and COMT inhibitor treatment abolished the glucose-lowering effects and AMPK phosphorylation by metformin in the HFD-fed mice [69]. Metformin stimulated insulin secretion in the HFD mice (2 weeks fed), and this induction of insulin by metformin was diminished by COMT inhibitor treatment [69]. These results suggest that the antidiabetic effect of metformin is associated with the restoration of COMT protein levels and the resultant accumulation of methoxy-catechols, such as 2-ME [69].

2-ME-induced liver AMPK activation may explain the anti-steatosis and anti-insulin resistance effects of 2-ME; the insulinogenic effects of 2-ME were not solely explained by the AMPK activation. In the insulinogenic mechanisms of 2-ME, we found that 2-ME stimulated insulin secretion associated with AMPK phosphorylation, proapoptotic MST-1 suppression, and increased PDX-1 levels in cultured β-cell line MIN-6 cells (Fig. 5.2). These findings reveal the relevance of COMT deficiency and its pathological significance in the onset of metabolic diseases such as metabolic syndrome, type 2 diabetes, and preeclampsia [69].

Conclusion

Considering the accumulating evidence from both the bedside and the bench, there are many shared phenotypes and molecular mechanisms between preeclampsia and metabolic defects such as insulin resistance, metabolic syndrome, and type 2 diabetes. Moreover, the interaction between genetic background and environmental factors may facilitate the onset of both preeclampsia and metabolic diseases. Among the several candidate genes, COMT SNPs may be relevant to understanding both syndromes. Further research would shed new light on the pathogenesis of preeclampsia and associated metabolic defects for a complete understanding and for the future development of therapeutic strategies for treating these syndromes.

Acknowledgments The author declares no conflicts of interests related to this work. This work was partially supported by grants from the Japan Society for the Promotion of Science for KK (23790381, 26460403) and several research grants for KK (the Daiichi-Sankyo Foundation of Life Science, the Ono Medical Research Foundation, the Takeda Science Foundation, Novo Nordisk Insulin Research Foundation, and the Banyu Life Science Foundation International). This work was partially supported by Grant for Promoted Research awarded to KK (S2014-4, S2015-3, S2016-3, S2017-1) from Kanazawa Medical University. This work was also partially supported by the Medical Research Encouragement Prize of the Japan Medical Association for KK. Boehringer Ingelheim (Japan), Mitsubishi Tanabe Pharma, and Ono Pharmaceutical contributed to establishing the Division of Anticipatory Molecular Food Science and Technology. KK is under a consultancy agreement with Boehringer Ingelheim.

References

1. Shenoy V, Kanasaki K, Kalluri R. Pre-eclampsia: connecting angiogenic and metabolic pathways. Trends Endocrinol Metab. 2010;21:529–36.

2. Kanasaki K, Kanasaki M. Angiogenic defects in preeclampsia: what is known, and how are such defects relevant to preeclampsia pathogenesis? Hypertens Res Pregnancy. 2014;1:9.
3. Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol. 1991;165:1667–72.
4. Catalano PM, et al. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. Am J Obstet Gynecol. 1992;167:913–9.
5. Catalano PM, et al. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. Am J Physiol. 1993;264:E60–7.
6. Rieck S, Kaestner KH. Expansion of beta-cell mass in response to pregnancy. Trends Endocrinol Metab. 2010;21:151–8. https://doi.org/10.1016/j.tem.2009.11.001.
7. Ryan EA, Enns L. Role of gestational hormones in the induction of insulin resistance. J Clin Endocrinol Metab. 1988;67:341–7. https://doi.org/10.1210/jcem-67-2-341.
8. Kuhl C. Aetiology of gestational diabetes. Baillieres Clin Obstet Gynaecol. 1991;5:279–92.
9. Yogev, et al. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: preeclampsia. Am J Obstet Gynecol. 2010;202:255.e1–7. https://doi.org/10.1016/j.ajog.2010.01.024.
10. Hauguel S, Leturque A, Gilbert M, Girard J. Effects of pregnancy and fasting on muscle glucose utilization in the rabbit. Am J Obstet Gynecol. 1988;158:1215–8.
11. Hauguel S, Gilbert M, Girard J. Pregnancy-induced insulin resistance in liver and skeletal muscles of the conscious rabbit. Am J Physiol. 1987;252:E165–9.
12. Leturque A, et al. Glucose utilization rates and insulin sensitivity in vivo in tissues of virgin and pregnant rats. Diabetes. 1986;35:172–7.
13. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A. 1994;91:4854–8.
14. Xiang AH, et al. Multiple metabolic defects during late pregnancy in women at high risk for type 2 diabetes. Diabetes. 1999;48:848–54.
15. Kirwan JP, et al. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes. 2002;51:2207–13.
16. Hotamisligil GS, et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996;271:665–8.
17. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest. 1996;97:1111–6. https://doi.org/10.1172/JCI118504.
18. Sermer M, et al. Impact of increasing carbohydrate intolerance on maternal-fetal outcomes in 3637 women without gestational diabetes. The Toronto Tri-Hospital Gestational Diabetes Project. Am J Obstet Gynecol. 1995;173:146–56.
19. Sowers JR, Saleh AA, Sokol RJ. Hyperinsulinemia and insulin resistance are associated with preeclampsia in African-Americans. Am J Hypertens. 1995;8:1–4. https://doi.org/10.1016/0895-7061(94)00166-9.
20. Joffe GM, et al. The relationship between abnormal glucose tolerance and hypertensive disorders of pregnancy in healthy nulliparous women. Calcium for Preeclampsia Prevention (CPEP) Study Group. Am J Obstet Gynecol. 1998;179:1032–7.
21. Wolf M, et al. First trimester insulin resistance and subsequent preeclampsia: a prospective study. J Clin Endocrinol Metab. 2002;87:1563–8. https://doi.org/10.1210/jcem.87.4.8405.
22. Parretti E, et al. Preeclampsia in lean normotensive normotolerant pregnant women can be predicted by simple insulin sensitivity indexes. Hypertension. 2006;47:449–53. https://doi.org/10.1161/01.HYP.0000205122.47333.7f.
23. Matthews DR, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
24. Kirwan JP, Huston-Presley L, Kalhan SC, Catalano PM. Clinically useful estimates of insulin sensitivity during pregnancy: validation studies in women with normal glucose tolerance and gestational diabetes mellitus. Diabetes Care. 2001;24:1602–7.
25. Hauth JC, et al. Maternal insulin resistance and preeclampsia. Am J Obstet Gynecol. 2011;204(327):e321–6. https://doi.org/10.1016/j.ajog.2011.02.024.

26. D'Anna R, et al. Adiponectin and insulin resistance in early- and late-onset pre-eclampsia. BJOG. 2006;113:1264–9. https://doi.org/10.1111/j.1471-0528.2006.01078.x.
27. Montoro MN, et al. Insulin resistance and preeclampsia in gestational diabetes mellitus. Diabetes Care. 2005;28:1995–2000.
28. Sierra-Laguado J, et al. Determination of insulin resistance using the homeostatic model assessment (HOMA) and its relation with the risk of developing pregnancy-induced hypertension. Am J Hypertens. 2007;20:437–42. https://doi.org/10.1016/j.amjhyper.2006.10.009.
29. Legro RS. Insulin resistance in women's health: why it matters and how to identify it. Curr Opin Obstet Gynecol. 2009;21:301–5. https://doi.org/10.1097/GCO.0b013e32832e07d5.
30. Ryan EA, et al. Defects in insulin secretion and action in women with a history of gestational diabetes. Diabetes. 1995;44:506–12.
31. Fuh MM, et al. Resistance to insulin-mediated glucose uptake and hyperinsulinemia in women who had preeclampsia during pregnancy. Am J Hypertens. 1995;8:768–71.
32. Soonthornpun K, Soonthornpun S, Wannaro P, Setasuban W, Thamprasit A. Insulin resistance in women with a history of severe pre-eclampsia. J Obstet Gynaecol Res. 2009;35:55–9. https://doi.org/10.1111/j.1447-0756.2008.00865.x.
33. Sattar N, Ramsay J, Crawford L, Cheyne H, Greer IA. Classic and novel risk factor parameters in women with a history of preeclampsia. Hypertension. 2003;42:39–42. https://doi.org/10.1161/01.HYP.0000074428.11168.EE.
34. Girouard J, Giguere Y, Moutquin JM, Forest JC. Previous hypertensive disease of pregnancy is associated with alterations of markers of insulin resistance. Hypertension. 2007;49:1056–62. https://doi.org/10.1161/HYPERTENSIONAHA.107.087528.
35. Smith GN, et al. A history of preeclampsia identifies women who have underlying cardiovascular risk factors. Am J Obstet Gynecol. 2009;200:58.e1–8. https://doi.org/10.1016/j.ajog.2008.06.035.
36. Agatisa PK, et al. Impairment of endothelial function in women with a history of preeclampsia: an indicator of cardiovascular risk. Am J Physiol Heart Circ Physiol. 2004;286:H1389–93. https://doi.org/10.1152/ajpheart.00298.2003.
37. Kvehaugen AS, et al. Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. Hypertension. 2011;58:63–9. https://doi.org/10.1161/HYPERTENSIONAHA.111.172387.
38. Hawfield A, Freedman BI. Pre-eclampsia: the pivotal role of the placenta in its pathophysiology and markers for early detection. Ther Adv Cardiovasc Dis. 2009;3:65–73. https://doi.org/10.1177/1753944708097114.
39. Caballero AE. Endothelial dysfunction, inflammation, and insulin resistance: a focus on subjects at risk for type 2 diabetes. Curr Diab Rep. 2004;4:237–46.
40. Callaway LK, et al. Diabetes mellitus in the 21 years after a pregnancy that was complicated by hypertension: findings from a prospective cohort study. Am J Obstet Gynecol. 2007;197(492):e491–7. https://doi.org/10.1016/j.ajog.2007.03.033.
41. Lykke JA, et al. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension. 2009;53:944–51. https://doi.org/10.1161/HYPERTENSIONAHA.109.130765.
42. Engeland A, et al. Risk of diabetes after gestational diabetes and preeclampsia. A registry-based study of 230,000 women in Norway. Eur J Epidemiol. 2011;26:157–63. https://doi.org/10.1007/s10654-010-9527-4.
43. Feig DS, et al. Preeclampsia as a risk factor for diabetes: a population-based cohort study. PLoS Med. 2013;10:e1001425. https://doi.org/10.1371/journal.pmed.1001425.
44. Facca TA, Kirsztajn GM, Sass N. Preeclampsia (marker of chronic kidney disease): from genesis to future risks. J Bras Nefrol. 2012;34:87–93.
45. Craici I, Wagner S, Garovic VD. Preeclampsia and future cardiovascular risk: formal risk factor or failed stress test? Ther Adv Cardiovasc Dis. 2008;2:249–59. https://doi.org/10.1177/1753944708094227.
46. Harskamp RE, Zeeman GG. Preeclampsia: at risk for remote cardiovascular disease. Am J Med Sci. 2007;334:291–5. https://doi.org/10.1097/MAJ.0b013e3180a6f094.

47. Kanasaki K, Kalluri R. The biology of preeclampsia. Kidney Int. 2009;76:831–7.
48. Kanasaki K, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453:1117–21. https://doi.org/10.1038/nature06951.
49. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry. 2006;60:141–51.
50. Barnea ER, MacLusky NJ, DeCherney AH, Naftolin F. Catechol-o-methyl transferase activity in the human term placenta. Am J Perinatol. 1988;5:121–7.
51. Yan J, et al. 2-methoxyestradiol reduces cerebral vasospasm after 48 hours of experimental subarachnoid hemorrhage in rats. Exp Neurol. 2006;202:348–56.
52. Barchiesi F, et al. 2-Methoxyestradiol, an estradiol metabolite, inhibits neointima formation and smooth muscle cell growth via double blockade of the cell cycle. Circ Res. 2006;99:266–74. https://doi.org/10.1161/01.RES.0000233318.85181.2e.
53. Dubey RK, Jackson EK, Keller PJ, Imthurn B, Rosselli M. Estradiol metabolites inhibit endothelin synthesis by an estrogen receptor-independent mechanism. Hypertension. 2001;37:640–4.
54. Dubey RK, Jackson EK. Potential vascular actions of 2-methoxyestradiol. Trends Endocrinol Metab. 2009;20:374–9. https://doi.org/10.1016/j.tem.2009.04.007.
55. Lee SB, et al. Preeclampsia: 2-methoxyestradiol induces cytotrophoblast invasion and vascular development specifically under hypoxic conditions. Am J Pathol. 2010;176:710–20.
56. Takanashi K, Honma T, Kashiwagi T, Honjo H, Yoshizawa I. Detection and measurement of urinary 2-hydroxyestradiol 17-sulfate, a potential placental antioxidant during pregnancy. Clin Chem. 2000;46:373–8.
57. Annerbrink K, et al. Catechol O-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. Metabolism. 2008;57:708–11.
58. Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab. 2002;3:321–49.
59. Need AC, Ahmadi KR, Spector TD, Goldstein DB. Obesity is associated with genetic variants that alter dopamine availability. Ann Hum Genet. 2006;70:293–303. https://doi.org/10.1111/j.1529-8817.2005.00228.x.
60. Tworoger SS, et al. The effect of CYP19 and COMT polymorphisms on exercise-induced fat loss in postmenopausal women. Obes Res. 2004;12:972–81.
61. Hall KT, et al. Catechol-O-methyltransferase association with hemoglobin A1c. Metabolism. 2016;65:961–7. https://doi.org/10.1016/j.metabol.2016.04.001.
62. Barchiesi F, et al. Candidate genes and mechanisms for 2-methoxyestradiol-mediated vasoprotection. Hypertension. 2010;56:964–72. https://doi.org/10.1161/HYPERTENSIONAHA.110.152298.
63. Matthews L, et al. Thiazolidinediones are partial agonists for the glucocorticoid receptor. Endocrinology. 2009;150:75–86. https://doi.org/10.1210/en.2008-0196.
64. Waite LL, et al. Placental peroxisome proliferator-activated receptor-gamma is up-regulated by pregnancy serum. J Clin Endocrinol Metab. 2000;85:3808–14. https://doi.org/10.1210/jcem.85.10.6847.
65. Waite LL, Louie RE, Taylor RN. Circulating activators of peroxisome proliferator-activated receptors are reduced in preeclamptic pregnancy. J Clin Endocrinol Metab. 2005;90:620–6. https://doi.org/10.1210/jc.2004-0849.
66. Barroso I, et al. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. Nature. 1999;402:880–3. https://doi.org/10.1038/47254.
67. Ueki N, Kanasaki K, Kanasaki M, Takeda S, Koya D. Catechol-O-methyltransferase deficiency leads to hypersensitivity of the pressor response against angiotensin II. Hypertension. 2017;69:1156–64. https://doi.org/10.1161/HYPERTENSIONAHA.117.09247.
68. Berg D, Sonsalla R, Kuss E. Concentrations of 2-methoxyoestrogens in human serum measured by a heterologous immunoassay with an 125I-labelled ligand. Acta Endocrinol (Copenh). 1983;103:282–8.

69. Kanasaki M, et al. Deficiency in catechol-o-methyltransferase is linked to a disruption of glucose homeostasis in mice. Sci Rep. 2017;7:7927.
70. Coletta DK, et al. Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo: a randomised trial. Diabetologia. 2009;52:723–32. https://doi.org/10.1007/s00125-008-1256-9.

Placental Adenosine Signaling in the Pathophysiology of Preeclampsia

6

Takayuki Iriyama and Yang Xia

Abstract

Impairment in placental development and function is known to be associated with the pathophysiology of preeclampsia. However, placenta-specific molecular basis leading to preeclampsia remains to be fully understood. Adenosine, an endogenous nucleotide, is a signaling molecule that is induced under various pathological conditions including hypoxia, energy depletion, and inflammation and contributes to various diseases. Recent report form Iriyama et al. revealed that a local increase of adenosine in the placenta is sufficient to trigger key features of preeclampsia by using mouse models, and adenosine was identified as one of pathogenic factors for preeclampsia. This chapter is to summarize current progress and the significance of adenosine signaling in different disease states and to detail the findings of enhanced placental adenosine signaling in the pathophysiology of preeclampsia.

Keywords

Adenosine • Placenta • Mouse model

T. Iriyama, M.D., Ph.D. (✉)
Faculty of Medicine, Department of Obstetrics and Gynecology, University of Tokyo, Tokyo, Japan
e-mail: iriyama-tky@umin.ac.jp

Y. Xia, M.D., Ph.D.
Department of Biochemistry and Molecular Biology, The University of Texas Health Science Center at Houston, Houston, TX, USA

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_6

6.1 Introduction

Preeclampsia (PE) is a life-threatening hypertensive complication of pregnancy and is a leading cause of maternal and neonatal morbidity and mortality [1, 2]. Despite intensive research efforts and several large clinical trials, current strategies for managing PE remain inadequate and are limited to symptomatic therapy or the termination of pregnancy. Thus, uncovering novel factors and signaling pathways that contribute to the pathogenesis of PE are needed for the establishment of mechanism-based preventative and therapeutic strategies to improve the prognosis of the disease.

Impairment in placental development and function is considered to contribute to the pathogenesis of PE [2, 3]. Considerable evidence indicates that dysregulation of cytoprotective pathways [4–6] and increase in anti-angiogenic growth factors [7, 8], complement activation [9], and autoantibodies [10] contribute to placental damage and the progression of the disease. However, the placenta-specific molecular basis responsible for placental impairment leading to PE has not been fully understood. Recently, we have identified the excess placental accumulation of adenosine, an endogenous nucleotide, and enhanced placental adenosine signaling as a novel pathogenic factor linking placental pathology to the development of PE [11]. In this chapter, we will review recent studies and advances in the understanding of pathophysiological mechanisms of PE by focusing the placental adenosine signaling.

6.2 Metabolism of Adenosine and Adenosine Signaling via Adenosine Receptors

Adenosine is an endogenous nucleotide that is ubiquitously induced in almost all cell types under physiological conditions and is further produced under various stresses such as hypoxia or energy-depleted conditions. Adenosine is considered a key signaling molecule that orchestrates the cellular response to hypoxia, energy depletion, and tissue damage by activation of G-protein-coupled receptors on multiple cell types [12, 13]. Under normal physiological states, basal extracellular adenosine levels are maintained in the nanomolar range [14]. However, under pathological conditions including hypoxia, ischemia, or inflammation, adenosine levels at the extracellular space increase up to the millimolar range [14, 15]. Extracellular adenosine levels are tightly regulated by multiple molecules (Fig. 6.1). ATP is released from damaged cells and subsequently dephosphorylated by ectonucleotidases, CD39, which converts ATP to ADP/AMP, and CD73, which converts AMP to adenosine. Synthesis of extracellular adenosine via sequential action of these two ectonucleotidases is the major source of extracellular adenosine production under hypoxia. Additionally, extracellular adenosine level is regulated by adenosine deaminase (ADA), an enzyme that converts adenosine to inosine, and

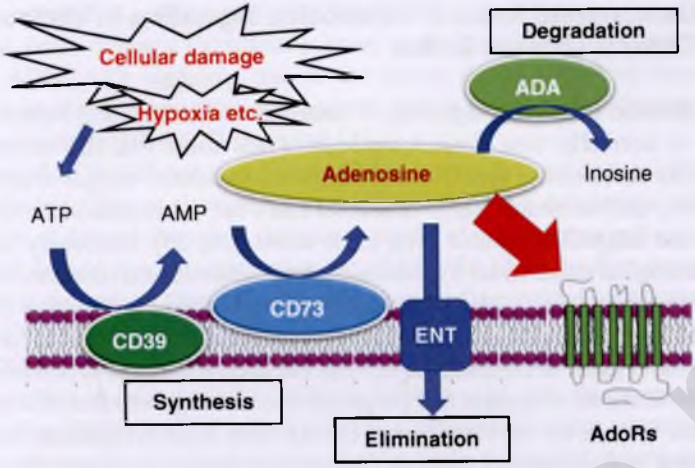


Fig. 6.1 Metabolism of extracellular adenosine signaling. Extracellular adenosine levels are tightly regulated by multiple factors involved in the synthesis from ATP by the sequential action of two ectonucleotidases (CD39 and CD73), degradation by adenosine deaminase (ADA), and cellular uptake by equilibrative nucleoside transporters (ENTs). Extracellular adenosine exerts its function through the activation of four G-protein-coupled cell surface adenosine receptors (AdoRs)

equilibrative nucleoside transporters (ENTs) that are responsible for the cellular uptake of adenosine.

Extracellular adenosine is known to exert its function on target cells through the activation of four G-protein-coupled cell surface adenosine receptors, ADORA1, ADORA2A, ADORA2B, and ADORA3 [12, 16]. Adenosine receptors are widely expressed in almost all cell types and tissues. All adenosine receptors contain a single polypeptide chain that is a structural motif of seven transmembrane helices leading to modulation of the intracellular levels of the second messenger cyclic AMP. Each adenosine receptor has a cellular- or tissue-specific distribution and distinct affinity for adenosine, and cellular response via adenosine receptors is predominantly dependent on the extracellular adenosine concentration. ADORA1, ADORA2A, and ADORA3 have a high affinity to adenosine and are activated with effective half-maximal concentration (EC_{50}) levels ranged in the nanomolar scale. Therefore, these three types of adenosine receptors are responsible for most physiological function of adenosine signaling since adenosine levels are generally controlled below 1 μM under normal condition. Among these four adenosine receptors, ADORA2B has the lowest affinity for adenosine, and the higher concentration of adenosine is required for the activation of ADORA2B (EC50 ~24 μM) [17–19]. However, in response to certain pathological conditions, especially hypoxia or chronic inflammation, adenosine concentrations can reach high enough levels to activate ADORA2B [20, 21].

6.3 Detrimental Roles of Adenosine Signaling in Various Chronic Disease States

Acutely elevated adenosine signaling is intended to be brief and beneficial. The response is normally time limited because of the short half-life of adenosine. Extracellular adenosine is upregulated in response to limited oxygen availability or acute injury, and adenosine plays beneficial roles for the maintenance of cellular function and adaptation against such acute stress [22, 23]. Especially, under the acute pathological states under hypoxia including ischemic heart disease, acute lung injury, and stroke, extracellular adenosine function of the vasodilatation [24], anti-vascular leakage [25], and anti-inflammatory response [26] is essential for the protection of the organs. In contrast, chronically elevated adenosine is detrimental and contributes to the development and progression of multiple chronic disease conditions (Table 6.1). In the development of chronic lung disease including pulmonary hypertension and pulmonary fibrosis, mouse and human evidence revealed that enhanced activation of ADORA2B contributes to the elevation of detrimental factors leading to pulmonary hypertension such as IL-6, matrix metalloproteinase, and endothelin-1 [27, 28]. Pharmacological approach antagonizing ADORA2B showed the therapeutic effects against the progression of air space stenosis, fibrosis, and pulmonary hypertension [27, 29]. Sickle cell disease (SCD) is an inherited red blood cell disorders characterized with sickle-shaped red blood cells and multiple health problems. Adenosine levels in the circulation are reportedly elevated in SCD patients, and adenosine signaling through ADORA2B is involved in the disease progression [21]. Animal study utilizing SCD model mouse revealed that suppression of adenosine by polyethylene glycol-modified ADA (PEG-ADA) or ADORA2B antagonist suppressed the disease phenotype. Additionally, in the pathophysiology of chronic kidney disease (CKD), chronic accumulation of renal adenosine is reported to contribute to the progression of CKD leading to renal fibrosis and

Table 6.1 Detrimental roles of adenosine receptors associated with chronic pathological states

Adenosine receptor	Disease	Detrimental actions	References
ADORA2B	Chronic lung disease	Pulmonary fibrosis, pulmonary hypertension	[27–29]
	Sickle cell disease	Erythrocyte sickling, multiorgan damage	[21]
	Chronic kidney disease	Renal fibrosis, hypertension	[30, 31]
	Priapism	Increased perfusion via the inhibition of phosphodiesterase5, penile fibrosis	[32]
ADORA2A	Scleroderma	Dermal fibrosis	[33]
	Hepatic dysfunction	Hepatic fibrosis leading to liver cirrhosis	[34]
	Stroke	Increased excitotoxicity, generation of oxidents	[35, 36]
	Parkinson's disease	Increased excitotoxicity, neuronal degeneration	[37]

hypertension via the activation of ADORA2B [30, 31]. Priapism is a condition in which the penis remains persistently erect in the absence of sexual excitation. Elevated ADORA2B signaling due to the excess accumulation of adenosine is reportedly involved in priapism, and animal studies indicated the therapeutic possibilities targeting ADORA2B signaling [32].

Not only ADORA2B but also ADORA2A is known to contribute to chronic disease conditions. Chan et al. reported that ADORA2A plays detrimental roles in the progression of dermal fibrosis in the mouse model of scleroderma induced by bleomycin [33]. In addition, chronic activation of ADORA2A in the liver is reported to contribute to the hepatic fibrosis and dysfunction leading to the development of liver cirrhosis [34]. Moreover, in the field of brain disease, pharmacological studies utilizing ADORA2A antagonists demonstrated that blockade of ADORA2A elicits the protective effect in animal models of brain ischemia [35, 36], neurogenerative disease including Parkinson's disease [37]. Thus, prolonged excess elevation of adenosine in various chronic disease is detrimental, and a number of animal and human studies support the therapeutic possibilities targeting adenosine signaling.

6.4 Evidence Associated with Adenosine Signaling and PE

6.4.1 Increased Levels of Adenosine in Maternal and Fetoplacental Circulation in PE Patients

Physiological roles of adenosine in normal pregnancy remain largely unknown. There have only been several human studies investigating the expression of adenosine and adenosine-related molecules during pregnancy. Yoneyama et al. from Japan measured the adenosine levels in maternal circulation throughout pregnancy and reported that circulatory adenosine levels in the third trimester are higher than those in the first and second trimester [38]. With respect to the association with PE, another report from the same group showed that adenosine concentration in plasma of PE patients increases as compared with normal pregnant women, and it was correlated with its disease severity [39]. Additionally, it is reported that increased circulating CD73 activity in plasma of PE patients may underlie the aberrated purine metabolism and adenosine elevation in PE patients [40], while mechanism leading to increased CD73 in the circulation remains unknown. The platelets are considered one of the major source of circulating adenosine. Therefore, it is indicated that increased platelet activation and aggregation in PE pathophysiology might be related to adenosine elevation in the maternal circulation [41]. Another explanation for the increased adenosine in maternal blood of PE patients is the potential chronic hypoxic state that results from impaired peripheral and organ perfusion [18, 42].

It is also reported that adenosine concentration in fetoplacental circulation is increased in PE patients. Espinoza et al. reported that the adenosine levels of PE patients in umbilical blood obtained by cord centesis increase as compared with normal pregnant women [43]. In addition, abnormal uterine artery Doppler flow

implicating decreased uteroplacental perfusion was correlated with the elevation of adenosine concentration. This report suggests the possibility that uteroplacental hypoxic and ischemic state underlying PE might cause the fetoplacental adenosine generation and could be related to its pathophysiology. However, there have been no reports that link fetoplacental adenosine concentration to maternal circulatory adenosine in paired samples both from mother and umbilical blood. Therefore, the direct relationship between fetoplacental and maternal adenosine remains unknown. In summary, as mentioned above, several studies had provided human evidence indicating the increased concentration of adenosine both in maternal and fetoplacental circulation; however, this phenomenon was considered not a causative factor for PE, but just a consequence due to PE pathophysiology.

6.4.2 Expression of Adenosine Receptors in the Placenta of PE Patients

Several reports have provided the evidence of placental expression of adenosine receptors. von Versen-Hoynck et al. reported that the expression of all four types of adenosine receptor increased in the placenta of PE patients both in mRNA and protein levels [44]. Adenosine receptors contain hypoxia response element in the promoter lesion and placental expression of ADORA2A, and ADORA2B is reportedly controlled by differential modulation of HIF-2α and HIF-1α, respectively [45]. It is speculated that increased expression of placental ADORA2A and ADORA2B may be related to the chronic hypoxic state of PE patient placenta. Evidence from these studies implied the possibility that the alteration of adenosine receptors might reflect the aberrated adenosine signaling leading to the impaired placental function in PE. However, the role of adenosine signaling in the pathophysiology of PE remained unknown and cannot be fully understood only by expression analysis and in vitro cell and organ culture systems.

6.5 Identification of Enhanced Placental Adenosine Signaling as a Novel Pathogenic Factor for PE

6.5.1 Generation of Genetically Engineered Pregnant Mouse with Placenta-Specific Elevation of Adenosine

As mentioned above, it is difficult to make it clear whether altered adenosine signaling contributes to the development and progression of PE or it is just a compensatory consequence of PE pathophysiology only by expression analysis of human samples and in vitro studies. Therefore, an in vivo animal studies were desperately needed to accurately and fully understand whether elevated adenosine signaling contributes to the pathogenesis of PE or not. We hypothesized that elevated placental adenosine may play a role in PE, and in order to fully address this question, we sought to generate pregnant animals specifically with elevated placental adenosine to determine the pathophysiological roles of elevated placental adenosine in PE.

In an effort to create the mice with elevated placental adenosine, we focused on ADA, an enzyme that degradates adenosine to inosine. In the state of ADA deficiency, adenosine is known to be excessively accumulated. However, global ADA-deficient mice were reported to be embryonic lethal because of liver degeneration. Therefore, to generate ADA-deficient mice, we had to prevent liver damage caused by adenosine accumulation because of ADA deficiency. To rescue ADA-deficient embryos, we genetically introduced ADA transgene expressed in the liver only during fetal period. This ADA transgene induces the degradation of adenosine accumulated in fetal lever, and adenosine levels were reduced. Thus, ADA-deficient mice were successfully born by this transgenic approach. However, since ADA-deficient females are infertile and adenosine is systemically elevated, in order to create pregnant mice in which adenosine is elevated only in placenta, we designed a mating strategy by crossing females heterozygous for ADA with ADA-deficient males (Fig. 6.2). In these dams, dams and half of placentas and fetuses are positive for ADA; therefore, adenosine is not increased. On the other hand, half of the placentas and fetuses are deficient for ADA; however, ADA-deficient fetuses are rescued by ADA transgene. Therefore, only half of the placentas are ADA-deficient and have elevated adenosine. We found that placental adenosine was significantly elevated in the ADA-negative placentas compared to the ADA-positive placentas on embryonic day 12.5 (E12.5) and remained elevated through E18.5. Thus, the dams in which adenosine is elevated only in placentas (dams with elevated placental adenosine) were successfully generated. In addition, the mating pair of females heterozygous for ADA vs. *wild type* (WT) males was utilized as a control. In this control, dams are the heterozygous for ADA, which is same as dams with elevated placental adenosine, but all the placentas are ADA-positive and adenosine is not elevated. The placentas in control dams with either $Ada^{+/-}$ or $Ada^{+/+}$ genotype contained similar levels of adenosine in the normal range on E18.5. The phenotype of dams with elevated placental adenosine was assessed by comparing with this control dams.

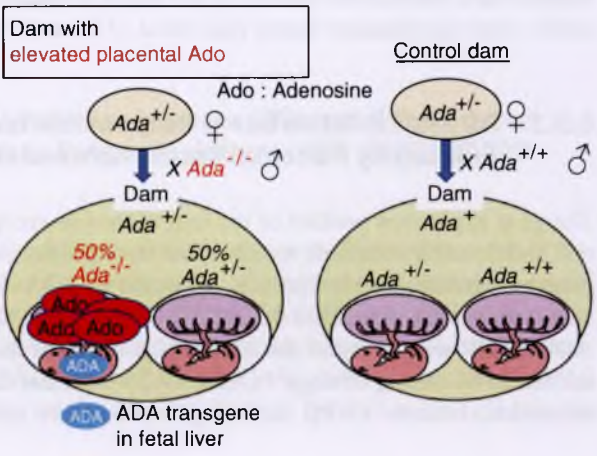


Fig. 6.2 Schema of mating strategy to generate dams with elevated placental adenosine and controls using ADA-deficient mice

6.5.2 Dams with Elevated Placental Adenosine Displayed Key Features of PE

Hallmark features of PE in dams with elevated placental adenosine and control dams were first assessed, and dams with elevated placental adenosine were found to display a significant increase in blood pressure late in pregnancy beginning on embryonic day 15.5 (E15.5) and remain significantly elevated through E17.5 as compared with the control dams. Elevated systolic blood pressure returned to normal by day 5 postpartum. Additionally, urinary protein (ratio of albumin/creatinine) was not significantly increased in the dams with elevated placental adenosine on E12.5, while it was significantly elevated on E18.5. By postpartum day 7, proteinuria was reduced to the levels similar to that of the nonpregnant state. Electron microscopic studies revealed that glomeruli in dams with elevated placental adenosine showed the typical pathologic change "glomerular endotheliosis" as seen in PE patients. It is interesting to note that elevated placental adenosine occurs on E12.5 prior to the maternal symptoms (i.e., hypertension and proteinuria) which develop around E15.5 and disappear postpartum. Thus, our studies provide strong in vivo evidence that elevated placental adenosine is a causative factor to induce maternal PE features.

In addition to maternal PE features, ADA-deficient placentas with elevated adenosine were smaller and weighed significantly less than ADA-positive placentas with normal levels of adenosine from dams with PE features. Likewise, the fetuses associated with ADA-deficient placentas with elevated placental adenosine were smaller and weighed significantly less than fetuses associated with ADA-positive placentas without morphological abnormalities.

Next, we examined placental vasculature using CD31 staining and found that ADA-negative placentas showed disorganized and impaired vasculature in the labyrinthine zone compared to ADA-positive placentas. Supporting this finding, ADA-deficient placentas with elevated adenosine contained significantly elevated *Flt-1* mRNA compared to ADA-positive placentas with normal levels of adenosine. The elevated *Flt-1* mRNA is likely to encode a potent anti-angiogenic factor, sFlt-1. Accordingly, maternal circulating sFlt-1 levels in dams with elevated placental adenosine were significantly higher than those of the control dams.

6.5.3 ADORA2B Activation Is Responsible for the Features of PE Induced by Placental Excess Accumulation of Adenosine

The gene expression profiles of the four adenosine receptors in placentas with normal and elevated adenosine revealed that among adenosine receptors, only *Adora2b* gene expression was significantly increased in ADA-deficient placentas with elevated adenosine compared to ADA-positive placentas. To assess the role of ADORA2B, we generated the ADORA2B-deficient dams with elevated placental adenosine by mating strategy. In ADORA2B-deficient dams with elevated placental adenosine, features of PE including hypertension and excess proteinuria were

almost completely recovered. Not only key features of PE, but also increased placental adenosine-induced phenotypes of fetal growth restriction, impairment of placental vasculature, and elevation of maternal circulating sFlt-1 were significantly suppressed by the genetic deletion of ADORA2B. Thus, we provided genetic evidence that excess placental adenosine coupled with enhanced ADOAR2B signaling is responsible for maternal PE features, impaired placentas, and subsequent fetal growth restriction.

6.5.4 Human Evidence of the Accumulated Placental Adenosine and Its Underlying Mechanisms Contributing to PE

As supporting mouse findings, adenosine levels were significantly elevated in the placentas of women with PE compared to placentas from normotensive (NT) pregnant women. Similarly, *ADORA2B* gene expression and protein level were found to be significantly elevated in the PE placentas compared to the NT placentas. In vitro experiments using human placental villous explants cultured from NT placentas revealed that ARORA2B activation directly induced *FLT-1* gene expression and sFlt-1 secretion.

In contrast to mouse placentas, ADA enzyme activity was extremely low in the normal human placentas without significant difference in enzyme activity between the placentas of normotensive pregnant women and those of PE patients. Thus, we concluded that reduction in ADA levels is not a major factor contributing to increased placental adenosine in PE patients. Expression profiling of purinergic molecules in the placentas revealed that mRNA encoding CD73, a key ectonucleotidase producing extracellular adenosine from AMP, was significantly elevated in placentas of PE patients. Additionally, the protein level and enzyme activity of CD73 were significantly increased in placentas of PE patients. In contrast, the mRNA levels encoding CD39 (an ectonucleotidase that converts ATP to AMP), ENT1, and ENT2 (adenosine transporters) showed no significant difference between NT and PE placentas. Thus, analysis of human placentas indicated that elevated CD73 is responsible for increased placental adenosine and subsequent disease development.

6.5.5 Elevated CD73 Underlies Increased Placental Adenosine and Contributes to Pathophysiology of PE via ADORA2B Activation

To test the hypothesis that elevated placental adenosine in PE patients is due to elevated CD73-mediated production of adenosine, an experimental mouse PE model induced by adoptive transfer of PE patient-derived IgG (PE-IgG) known to contain the pathogenic autoantibodies that activate the angiotensin II type 1 receptor agonistic autoantibody (AT1-AA) was conducted [10]. As with PE patients, placental CD73 activity and adenosine levels were significantly induced in PE-IgG-injected

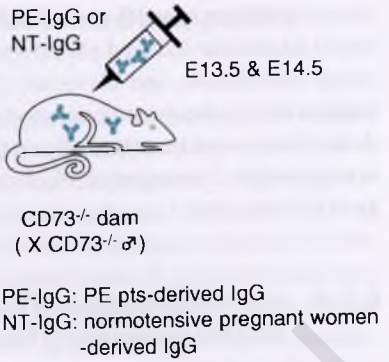


Fig. 6.3 Schema of AT_1-AA-induced PE mouse model. PE patient-derived IgG (PE-IgG) or normotensive pregnant women-derived IgG (NT-IgG) was injected into $CD73^{-/-}$ dams mated with $CD73^{-/-}$ males on E13.5 and E14.5

dams compared to normotensive pregnant women-derived IgG (NT-IgG)-injected dams. Similar to human studies, gene expression analysis revealed that mRNA and protein levels of CD73 and ADORA2B were significantly increased in the placentas of PE-IgG-injected dams.

Next, to determine whether the increased expression of CD73 was responsible for PE-IgG-induced placental adenosine production and the pathogenesis of PE, PE-IgG was injected into CD73-deficient dams (*Cd73*$^{-/-}$ females mated with *Cd73*$^{-/-}$ males) (Fig. 6.3). CD73 deficiency resulted in significantly reduced PE-IgG-induced production of adenosine in the placentas, indicating that elevated CD73 was required for the increase of placental adenosine induced by PE-IgG. Additionally, the key diagnostic features of PE, hypertension, and proteinuria were significantly attenuated in CD73-deficient dams injected with PE-IgG compared with PE-IgG-injected WT dams. In addition, the levels of maternal circulating sFlt-1 induced by PE-IgG were significantly suppressed in CD73-deficient as compared with those in PE-IgG-injected WT dams. Thus, these results revealed that elevated CD73 is essential for increased placental adenosine and that excess adenosine is responsible for disease development in the pathogenic autoantibody-induced animal model of PE.

Additionally, to determine whether elevated ADORA2B expression in the placentas has pathophysiologic significance, we injected PE-IgG into ADORA2B-deficient (*Adora2b*$^{-/-}$ females mated with *Adora2b*$^{-/-}$ males). All the preeclamptic features, including hypertension, proteinuria, and elevation of circulating sFlt-1, which were observed in PE-IgG-injected WT dams were significantly suppressed in ADORA2B-deficient dams. These results provide genetic evidence that elevated CD73-mediated chronically elevated adenosine in placentas exerts its detrimental effects in PE-IgG-injected pregnant mice thorough enhanced ADORA2B signaling.

6.6 Conclusion and Future Direction

The involvement of adenosine signaling in the pathogenesis of PE was unknown prior to our study [11]. Our findings support a novel but compelling concept of pathogenesis of PE: (1) elevated CD73 underlies increased placental adenosine. (2)

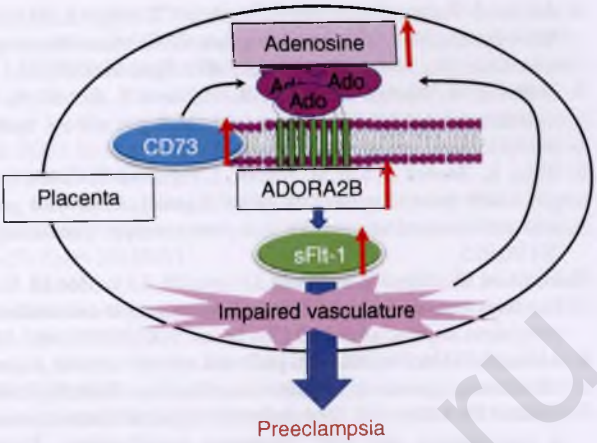


Fig. 6.4 Working model of enhanced placental adenosine signaling-induced PE pathophysiology: elevated placental adenosine coupled with enhanced ADORA2B signaling as a novel pathogenic factor for preeclampsia

Chronic excess placental adenosine preferentially signaling via elevated ADORA2B induces sFlt-1 production, impaired placentas, and small fetuses. Elevated circulating sFlt-1 from damaged placentas is considered a key mechanism that triggers maternal key PE features. Without interference, placental damage due to elevated sFlt-1-medialted impaired vasculature leads to further elevation of adenosine. As such, elevated CD73-mediated elevated placental adenosine, enhanced ADORA2B activation, and placental vasculature impairment function as a malicious cycle to promote the progression of maternal disease development by continuously inducing local placental accumulation of adenosine (Fig. 6.4). Overall, our findings reveal the pathogenic consequences of chronically elevated placental adenosine, the molecular basis for its elevation, and the specific signaling pathways leading to clinical features of PE.

These findings suggest multiple possible therapeutic options for the treatment of PE. Specifically, ADA enzyme therapy using PEG-ADA to reduce adenosine levels in the placenta might be a therapeutic strategy. PEG-ADA is a FDA-approved drug that has been successfully used to treat ADA-deficient patients for several decades. Another option is the use of ADORA2B-specific antagonist to inhibit the detrimental adenosine signaling in the placenta. Other possibilities suggested by our findings include pharmacological inhibition of CD73 activity to reduce the excess placental adenosine. It is our hope that the use of adenosine-based therapies to inhibit the progression of malicious cycle of enhanced placental adenosine signaling and prevent PE features will reduce the morbidity and mortality of the disease in the future.

References

1. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet. 2001; 357(9249):53–6. Epub 2001/02/24.
2. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010; 376(9741):631–44. Epub 2010/07/06.
3. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annu Rev Pathol. 2010;5:173–92. Epub 2010/01/19.

4. Ahmed A, Rahman M, Zhang X, Acevedo CH, Nijjar S, Rushton I, et al. Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation. Mol Med. 2000;6(5):391–409. Epub 2000/08/22.
5. Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, et al. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation. 2007;115(13):1789–97. Epub 2007/03/29.
6. Wang K, Ahmad S, Cai M, Rennie J, Fujisawa T, Crispi F, et al. Dysregulation of hydrogen sulfide producing enzyme cystathionine gamma-lyase contributes to maternal hypertension and placental abnormalities in preeclampsia. Circulation. 2013;127(25):2514–22. Epub 2013/05/25.
7. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111(5):649–58. Epub 2003/03/06.
8. Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ Res. 2004;95(9):884–91. Epub 2004/10/09.
9. Lynch AM, Salmon JE. Dysregulated complement activation as a common pathway of injury in preeclampsia and other pregnancy complications. Placenta. 2010;31(7):561–7. Epub 2010/04/30.
10. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, et al. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nat Med. 2008;14(8):855–62. Epub 2008/07/29.
11. Iriyama T, Sun K, Parchim NF, Li J, Zhao C, Song A, et al. Elevated placental adenosine signaling contributes to the pathogenesis of preeclampsia. Circulation. 2015;131(8):730–41. Epub 2014/12/30.
12. Chen JF, Eltzschig HK, Fredholm BB. Adenosine receptors as drug targets—what are the challenges? Nat Rev Drug Discov. 2013;12(4):265–86. Epub 2013/03/29.
13. Karmouty-Quintana H, Xia Y, Blackburn MR. Adenosine signaling during acute and chronic disease states. J Mol Med. 2013;91(2):173–81. Epub 2013/01/24.
14. Liu H, Xia Y. Beneficial and detrimental role of adenosine signaling in diseases and therapy. J Appl Physiol. 2015;119(10):1173–82. Epub 2015/09/01.
15. Zhang Y, Xia Y. Adenosine signaling in normal and sickle erythrocytes and beyond. Microbes Infect. 2012;14(10):863–73. Epub 2012/05/29.
16. Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. Pharmacol Rev. 2011;63(1):1–34. Epub 2011/02/10.
17. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev. 2001;53(4):527–52. Epub 2001/12/06.
18. Salsoso R, Farias M, Gutierrez J, Pardo F, Chiarello DI, Toledo F, et al. Adenosine and preeclampsia. Mol Aspects Med. 2017;55:126–39. Epub 2017/01/17.
19. Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. Biochem Pharmacol. 2001;61(4):443–8. Epub 2001/02/28.
20. Zhou Y, Schneider DJ, Morschl E, Song L, Pedroza M, Karmouty-Quintana H, et al. Distinct roles for the A2B adenosine receptor in acute and chronic stages of bleomycin-induced lung injury. J Immunol. 2011;186(2):1097–106. Epub 2010/12/15.
21. Zhang Y, Dai Y, Wen J, Zhang W, Grenz A, Sun H, et al. Detrimental effects of adenosine signaling in sickle cell disease. Nat Med. 2011;17(1):79–86. Epub 2010/12/21.
22. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature. 2001;414(6866):916–20. Epub 2002/01/10.
23. Sitkovsky M, Lukashev D. Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol. 2005;5(9):712–21. Epub 2005/08/20.

24. Shryock JC, Snowdy S, Baraldi PG, Cacciari B, Spalluto G, Monopoli A, et al. A2A-adenosine receptor reserve for coronary vasodilation. Circulation. 1998;98(7):711–8. Epub 1998/08/26.
25. Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK. A2B adenosine receptor dampens hypoxia-induced vascular leak. Blood. 2008;111(4):2024–35. Epub 2007/12/07.
26. Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, et al. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. J Immunol. 2007;178(12):8127–37. Epub 2007/06/06.
27. Karmouty-Quintana H, Zhong H, Acero L, Weng T, Melicoff E, West JD, et al. The A2B adenosine receptor modulates pulmonary hypertension associated with interstitial lung disease. FASEB J. 2012;26(6):2546–57. Epub 2012/03/15.
28. Sun CX, Zhong H, Mohsenin A, Morschl E, Chunn JL, Molina JG, et al. Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. J Clin Invest. 2006;116(8):2173–82. Epub 2006/07/15.
29. Karmouty-Quintana H, Weng T, Garcia-Morales LJ, Chen NY, Pedroza M, Zhong H, et al. Adenosine A2B receptor and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2013;49(6):1038–47. Epub 2013/07/17.
30. Dai Y, Zhang W, Wen J, Zhang Y, Kellems RE, Xia Y. A2B adenosine receptor-mediated induction of IL-6 promotes CKD. J Am Soc Nephrol. 2011;22(5):890–901. Epub 2011/04/23.
31. Zhang W, Zhang Y, Wang W, Dai Y, Ning C, Luo R, et al. Elevated ecto-5′-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. Circ Res. 2013;112(11):1466–78. Epub 2013/04/16.
32. Mi T, Abbasi S, Zhang H, Uray K, Chunn JL, Xia LW, et al. Excess adenosine in murine penile erectile tissues contributes to priapism via A2B adenosine receptor signaling. J Clin Invest. 2008;118(4):1491–501. Epub 2008/03/15.
33. Chan ES, Fernandez P, Merchant AA, Montesinos MC, Trzaska S, Desai A, et al. Adenosine A2A receptors in diffuse dermal fibrosis: pathogenic role in human dermal fibroblasts and in a murine model of scleroderma. Arthritis Rheum. 2006;54(8):2632–42. Epub 2006/07/28.
34. Chan ES, Montesinos MC, Fernandez P, Desai A, Delano DL, Yee H, et al. Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol. 2006;148(8):1144–55. Epub 2006/06/20.
35. Melani A, Gianfriddo M, Vannucchi MG, Cipriani S, Baraldi PG, Giovannini MG, et al. The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. Brain Res. 2006;1073–1074:470–80. Epub 2006/01/31.
36. Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D, et al. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J Neurosci. 1999;19(21):9192–200. Epub 1999/10/26.
37. Kalda A, Yu L, Oztas E, Chen JF. Novel neuroprotection by caffeine and adenosine A(2A) receptor antagonists in animal models of Parkinson's disease. J Neurol Sci. 2006;248(1–2):9–15. Epub 2006/06/30.
38. Yoneyama Y, Suzuki S, Sawa R, Takeuchi T, Kobayashi H, Takei R, et al. Changes in plasma adenosine concentrations during normal pregnancy. Gynecol Obstet Invest. 2000;50(3):145–8. Epub 2000/10/03.
39. Yoneyama Y, Suzuki S, Sawa R, Yoneyama K, Power GG, Araki T. Increased plasma adenosine concentrations and the severity of preeclampsia. Obstet Gynecol. 2002;100(6):1266–70. Epub 2002/12/07.
40. Yoneyama Y, Suzuki S, Sawa R, Otsubo Y, Miura A, Kuwabara Y, et al. Plasma 5′-nucleotidase activities and uric acid levels in women with pre-eclampsia. Gynecol Obstet Invest. 2002;54(3):168–71. Epub 2003/02/07.
41. Matsubara S, Sato I. Plasma adenosine levels and P-selectin expression on platelets in pre-eclampsia. Obstet Gynecol. 2001;98(2):354–5. Epub 2001/08/21.
42. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10(8):466–80. Epub 2014/07/09.

43. Espinoza J, Espinoza AF, Power GG. High fetal plasma adenosine concentration: a role for the fetus in preeclampsia? Am J Obstet Gynecol. 2011;205(5):485.e24–7. Epub 2011/08/23.
44. von Versen-Hoynck F, Rajakumar A, Bainbridge SA, Gallaher MJ, Roberts JM, Powers RW. Human placental adenosine receptor expression is elevated in preeclampsia and hypoxia increases expression of the A2A receptor. Placenta. 2009;30(5):434–42. Epub 2009/03/24.
45. St Hilaire C, Carroll SH, Chen H, Ravid K. Mechanisms of induction of adenosine receptor genes and its functional significance. J Cell Physiol. 2009;218(1):35–44. Epub 2008/09/04.

Obesity, Adipokines, and Lipokines

7

Katsuhiko Naruse

Abstract

Obesity during pregnancy is increasing in frequency worldwide. Similar to other complications, obesity is a strong risk factor for hypertensive disorders in pregnancy (HDP). The mechanisms of this vicious cycle, unclear up to the last century, have recently been elucidated in the field of internal medicine, where adipose tissue was found to be an important endocrine organ in the human body that releases several immunoactive molecules in high concentrations. In addition to circulating lipids and lipids subjected to oxidative stress, novel cytokines derived from adipocytes or other cells of the adipose tissue (adipokines and lipokines) enhance the pathological effects of HDP. Some adipokines behave in HDP in the same way as in hypertension or metabolic syndrome in nonpregnant subjects, but a few adipokines in pregnancy or HDP act differently from insulin-resistant syndromes. In addition, during normal pregnancy, insulin resistance is an important process for fetal growth, labor, and breastfeeding, and molecules produced by adipose tissue seem to act an immunologically critical role in HDP.

Keywords

Adipokine • Adipose tissue • Fatty acids • Lipokine • Obesity

7.1 Overview

Obesity is a common health problem found all over the world, being present not only in developed but also in low-income countries. It leads to hypertension, brain stroke, arthritis, cardiovascular diseases, diabetes mellitus, and other conditions.

K. Naruse, M.D., Ph.D.
St. Barnabas' Hospital, Osaka City, Japan
e-mail: naruse@naramed-u.ac.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_7

Pregnancy in the obese subject may easily lead to hypertensive disorders in pregnancy (HDP). In fact, obesity is a strong risk factor for HDP [1–3].

On the other hand, pregnancy is a state in which women experience a natural insulin resistance, even in a successful pregnancy that promotes fetal growth and is important for breastfeeding. Because of this homeostatic insulin-resistant condition, mediators of insulin resistance, most of them also related to inflammation, are strongly altered in almost all pregnant subjects and thus make the prediction of HDP based on these markers difficult.

Beginning at the end of the last century, biological techniques allow the in vitro culture of adipocytes, the main cell type that forms adipose tissue. Recently, cytokines produced predominantly by adipocytes were also identified and were named adipokines. The variety of adipokine functions revealed that adipose tissue not only provides energy storage but is also an endocrine organ of the human body. Additionally, well-known lipids of clinical relevance and oxidation were found to be mediators of inflammation, some of which are now called lipokines.

In this chapter, the pathophysiology and effects of obesity on HDP are described first, and then novel findings about adipokines and lipokines in HDP, which provide new insights for the understanding of pathology both in obese and nonobese subjects, are described.

7.2 Obesity

Obesity in females is increasing worldwide [3] and is considered a risk factor for preeclampsia from the beginning of the last century [4]. Even though a successful pregnancy requires increased estrogen and progressive insulin resistance for fetal growth and later breastfeeding, obese pregnant patients (Japanese criteria correspond to prepregnant body mass index (BMI) >25, although the criteria vary in each country) are known to be at a higher risk for HDP [5, 6], gestational diabetes mellitus, need of cesarean section at delivery (which leads to a higher rate of deep vein thrombosis), stillbirth, heavy-for-date infant, and neural tube defects [6, 7]. Additionally, reduced trophoblast mitochondrial respiration was recently detected in obese subjects, and some placental dysfunction may consequently occur [8]. In addition to weight gain during pregnancy, prepregnant BMI may be a useful criterion in obese pregnancy to target these complications [6].

Although HDP is known to occur more frequently in obese subjects, the underlying mechanisms of HDP induced by obesity have only recently been elucidated. Previous evidence showed only endothelial dysfunction in obese preeclamptic subjects. Additionally, a severe or early-onset preeclampsia occurred in some lean subjects, and obesity did not seem to contribute to the pathophysiology in these patients (Fig. 7.1). Recently, however, after functional characterization of adipokines and lipokines, obesity (and adipose tissue) was shown to influence the development of the disease through immune mechanisms that include inflammatory action, immune cell reaction, and vasoconstriction [1]. In other words, adipokines and lipokines link obesity and preeclampsia through systemic immune mechanism stages [9].

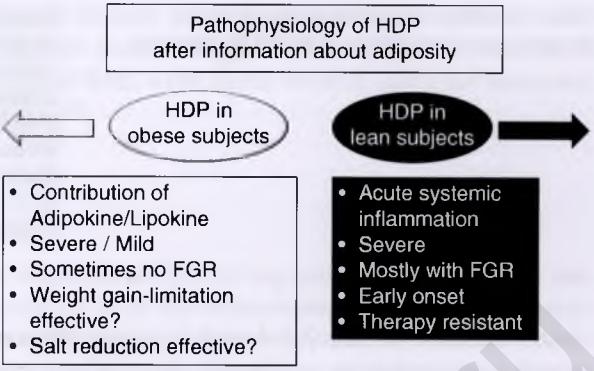


Fig. 7.1 Adiposity and HDP: two types of pathophysiology

Table 7.1 Criteria for adequate weight gain during pregnancy in Japan and in the USA

	Recommended body weight gain	Aim of body weight control
Japanese Society for the Study of Obesity (2011)	BMI <18.5: 9–12 kg BMI 18.5–25: 7–12 kg BMI >25: 5 kg (depends on patient)	Reduce obstetrical complications
Institute of Medicine (2009)	BMI <18.5: 12.7–18.1 kg BMI 18.5–25: 11.3–15.9 kg BMI 25–30: 6.8–11.3 kg BMI ≥30: 5.0–9.1 kg	Adequate birth weight (3000–4000 g in 39–40 w GA)

BMI body mass index, *GA* gestational age

Additionally, higher lipid concentration (mainly hypertriglyceridemia which damaging endothelium) [10] and oxidative stress on the lipids (regarded as a result of placental ischemia reperfusion injury) [11, 12] themselves are associated with onset of preeclampsia.

Some guidelines limit body weight gain in pregnancy (Table 7.1), but the aim of this limit is prevention of gestational diabetes, heavy-for-date infant, and shoulder dystocia. Weight gain limitation during pregnancy has not been associated with reduction of HDP, but recent Japanese study shows the possibility to reduce the incidence of hypertension in pregnancy by weight gain limitation (less than 12–13 kg) as well as prepregnant BMI control (less than 23 kg/m^2) [13].

7.3 Adipokine

Adipokine (or adipocytokine) is a cytokine produced by adipose tissue. Adipokine was initially thought to be released by only one kind of adipocyte, but recently it has been found to be a product of adipose tissue, which includes leukocytes, macrophages, fibroblasts, and extracellular matrix. Major adipokines mentioned in this

Table 7.2 Major adipokines related to normal pregnancy and hypertensive disorders in pregnancy

Tumor necrosis factor
Leptin
Adiponectin
Resistin
Plasminogen activator inhibitor
Monocyte chemoattractant protein-1
Visfatin
IL-6
Apelin

chapter are listed in Table 7.2. Adipokine alterations in pregnant subjects are mainly related to maternal body weight, but some differences were found between early- and late-onset preeclampsia [14]. Additionally, a large increase in the levels of some adipokines in HDP was also found in lean subjects [15].

7.3.1 Tumor Necrosis Factor

Tumor necrosis factor (TNF) is an adipokine produced by adipocytes that acts in two major pathophysiologies, inflammation and insulin resistance. TNF has a multifunctional role in the human body and is crucial in each functional change, including pregnancy. In early pregnancy, trophoblast invasion and programmed apoptosis are controlled by TNF through a protease or another pathway [16, 17]. In preeclampsia, the two major pathophysiologies mentioned above are key to the establishment of the disease; thus, research on TNF and its role in preeclampsia has continued since last century, especially in relation to endothelial dysfunction [18]. Detection of circulating TNF is quite difficult owing to its short half-life, but pathological aspects of the placenta [19] and increased circulating soluble TNF receptors [20] indicate the involvement of the molecule in preeclampsia. Recently, recombinant anti-TNF antibodies are being used to treat rheumatoid arthritis, multiple myeloma, and other diseases. Some of these antibodies are defined recently that can be safely used in pregnancy [21]. For HDP, animal study showed feasible effect of anti-TNF therapy on placental ischemia model [22], so further application on human disease is expected.

7.3.2 Leptin

Leptin is an adipokine associated with feeding behavior that is induced after food intake and suppresses appetite through action in the central nervous system. The increased amount of leptin in obese patient results from a higher number of adipocytes and from systemic leptin resistance. In pregnant women, the placenta is another source of leptin, the levels of which increase in the second and third trimester of pregnancy. Interestingly, leptin gene expression seems to be regulated by noncoding microRNAs [23]. Systemic increase of leptin levels in HDP is known, but its

pathological role in the disease remains unclear, except for a correlation with inflammatory factors [24]. Recent research suggests that leptin concentration is positively correlated with maternal BMI, a risk factor for HDP, but is not associated with HDP onset independently [25].

7.3.3 Adiponectin

Adiponectin is an important adipokine for the self-regulation of adipose tissue that increases insulin sensitivity and acts as an anti-inflammatory cytokine via TNF suppression. After adiponectin was discovered in this century, the importance of adipokines became clear, and research in adipose tissue increased dramatically. During pregnancy, adiponectin levels decrease reflecting homeostatic insulin resistance. On the other hand, alteration of adiponectin levels in HDP is still controversial. Severe HDP in lean subjects seem to be correlated with increased levels of adiponectin, which is regarded as a "paradox" and is not consistent with hypertension in non-pregnant subjects [15, 26]. The causes of this paradoxical increase are not known, but it has been suggested that it results from damage to adipocytes by other cytokines. Therefore, HDP may be not only a kind of metabolic syndrome but also an acute systemic inflammatory crisis. In HDP, increase in the homocysteine/adiponectin ratio [27], positive correlation of the molecule with brain natriuretic peptide (BNP) [28, 29], increased insulin resistance index (HOMA-IR) [30], and endothelial dysfunction [31] were also described.

7.3.4 Resistin

Resistin is an adipokine produced by adipose tissue. Although initially resistin was thought to be produced only by adipocytes, it was found to be also produced by other cells in adipose tissue such as macrophages. Resistin is generally regarded to be correlated with insulin resistance (although some studies found no correlation [32]) and inflammation similarly to TNF, and thus adipose tissue function is related to these pathophysiologies. During a normal pregnancy, resistin levels increase in the third trimester [33, 34]. The placenta is also a source of resistin and increases its concentration in the cord blood, which may support glucose transportation to the fetus [35]. Its role in preeclampsia remains unclear [34].

7.3.5 Plasminogen Activator Inhibitor

Plasminogen activator inhibitor (PAI) is a molecule that acts as a protease inhibitor in the blood and thus promotes coagulation. PAI is produced by vascular endothelium, liver, and adipose tissue. During normal pregnancy, PAI is also produced by the placenta and its levels increase in HDP, and thus PAI is regarded as a key molecule in the coagulation-related effects of HDP. Additionally, its function as a

protease inhibitor may play a key role in trophoblast invasion and placental maintenance, processes that may be affected by angiotensin II type 1 receptor agonistic autoantibodies (AT1-AA) [36]. Recently, polymorphisms in PAI-associated genes were shown to influence susceptibility to HDP [37].

7.3.6 Monocyte Chemoattractant Protein-1

Monocyte chemoattractant protein (MCP)-1 is a major chemokine distributed from adipose tissue and mainly produced by macrophages [38]. A major function of MCP-1 is to promote leukocyte migration for the anti-infection or inflammatory response, and therefore it is also regarded as a pro-inflammatory cytokine. However, in the insulin-resistant state, MCP-1 action results in vascular endothelial damage or atherosclerosis. During normal pregnancy, increased MCP-1 levels are observed in subjects with severe obesity [39] and with preeclampsia [39, 40]. On the other hand, MCP-1 levels decrease during a normal nonobese pregnancy and block the cascade from homeostatic insulin resistance to pathologic change [40], although an increase in levels of this chemokine in preeclampsia may remove this block and start an unfavorable cascade [40].

7.3.7 Visfatin

Visfatin (nicotinamide phosphoribosyltransferase (NAmPRTase or Nampt), or pre-B-cell colony-enhancing factor 1 (PBEF1)) was considered an adipokine produced by visceral adipose tissue, and its concentration positively correlated with insulin resistance. However, after retraction of some initial paper describing visfatin in an adipocyte study, it is currently not considered an adipokine. Recently, interest in this molecule is focused on its function in B-cell maturation and neutrophil apoptosis inhibition. Although it is expressed in the placenta, visfatin level alterations in normal pregnancy or HDP have not been described, except for a study describing an increase in visfatin levels in HDP [41]. Visfatin may be increased in the patients of fetal growth restriction only with HDP [42].

7.3.8 Interleukin-6

Interleukin (IL)-6 is a major cytokine in humoral immunity cascades. The major actions of IL-6 are inflammatory trigger and cell adhesion via MCP-1 level increase. IL-6 is produced by various kinds of cells, but it is sometimes regarded as an adipokine because it is also produced by adipocytes. During pregnancy, IL-6 is an essential cytokine for trophoblast invasion and immune tolerance, and its levels increase naturally in parallel with the physiological insulin-resistant state [43]. The systemic action of IL-6 during pregnancy is unclear and may be complex, but its increase in HDP has been reported [44]. The source of increased levels of IL-6 in HDP may result from either stimulated adipose tissue or a hypoxic placenta [44].

7.3.9 Apelin

Apelin is a bioactive peptide that acts as an endogenous ligand for a G-protein-coupled receptor. This peptide is secreted from several tissues such as vessels, heart, and brain as well as adipose tissue, and insulin stimulation increases secretion of this molecule [45]. In HDP, serum concentration of this peptide is significantly increased [46]. Interestingly, placental expression of the peptide increases [47], but apelin RNA levels decrease in preeclamptic subjects [48].

7.4 Lipokine

Lipokine is a hormone that controls lipid metabolism. Different from adipokines, some lipokines are lipids or lipid-binding proteins and are not restricted to adipocyte-derived factors. Certain types of lipids (including peroxidized lipid) are known to be related to pathological changes since the last century, such as the positive correlation between triglycerides and PAI [49] or between lipid peroxidation and DNA damage [50]. Recent innovative research into the biological activities or peroxidation of lipids described new roles of these molecules in metabolic syndromes and cardiovascular diseases. For example, lipid peroxidation within fat tissue known to generate by-products through free fatty acids that modulate adipogenic differentiation of precursor cells leading adipokine/lipokine generation [51].

Lipokine is synonymous to palmitoleate or palmitoleic acid (monounsaturated fatty acids, 16:1) and is considered a lipid hormone. In this section, however, several cytokines related to lipid metabolism (Table 7.3) are also discussed.

In research of HDP, lipid peroxidation has been studied mainly relating to systemic oxidative stress of the disease after placental implantation failure [12]. Recent challenge to use dextran sulfate cellulose apheresis on HDP patients for removal of soluble fms-like tyrosine kinase (sFlt)-1 is also known to reduce apolipoprotein or LDL-cholesterol [52, 53]. Of course, statin was also introduced in this field of study and reduced LDL-cholesterol as well as sFlt-1 [54]. These results suggesting that the lipids are also possible therapeutic targets in HDP.

7.4.1 Free Fatty Acids

Free fatty acids (FFA, also known as palmitate or NEFA, non-esterified fatty acid) are fatty acids circulating in the plasma not in their glycerol ester form (glycerides). FFA were found to be mediators of immune action, inflammation, and insulin resistance. FFA are mediators of the toll-like receptor (TLR)-4 and the NF-kappaB pathway of macrophages within adipose tissue, which leads to systemic

Table 7.3 Major lipokines related to normal pregnancy and hypertensive disorders in pregnancy

Free fatty acids
Palmitoleate
Retinol-binding protein 4
Adipocyte fatty acid-binding protein

inflammation especially in type 2 diabetes and cardiovascular disease [55]. In pregnant subjects, increased levels of FFA in gestational diabetes, preterm delivery, and normal pregnancy have been reported [56–58]. In HDP, significant increase in FFA levels was found [40, 58]. Even though FFA levels increase in normal pregnancy, decreased MCP-1 levels seem to block the vicious cycle of metabolic syndrome (see Sect. 7.3.6) [40]. However, MCP-1 levels increase in HDP and connect the insulin-resistant state by such lipokines to other pathological features in the endothelium, kidney, and placenta [40].

7.4.2 Palmitoleate (Palmitoleic Acid)

Palmitoleate is an unsaturated fatty acid abundantly present in human adipose tissue and liver [59]. It is known that some foods like fish or macadamia nuts contain a high amount of palmitoleates. In contrast to FFA, palmitoleate stimulates insulin sensitivity and metabolic responses in parallel with adipokines [59, 60]. Because oral intake is a main source of palmitoleate for modern humans, this lipokine has been recently proposed as a novel nutrition supplement to improve dietary habits and help prevent metabolic syndrome and cardiovascular diseases. Although extensive research has been conducted on oil supplementation (mostly fish oil or omega-3 fatty acids) in pregnancy or HDP, peripheral palmitoleate concentration in human pregnancy has not been determined. Of course, medical use of oral intake of lipokine has also not been reported.

7.4.3 Retinol-Binding Protein 4

Retinol-binding protein 4 (RBP4) binds to vitamin A (retinol) in human circulation and regulates insulin resistance and the immune response. RBP4 is produced by adipose tissue and is positively correlated with insulin resistance. The main pathways by which this molecule contributes to human insulin resistance are hepatic gluconeogenesis and insulin signaling in skeletal muscle [61]. Studies revealed that RBP4 levels increase in preeclampsia [62, 63]. Masuyama et al. showed that increased RBP4 levels in obese preeclamptic patients are correlated with insulin resistance index, HOMA-IR [63].

7.4.4 Adipocyte Fatty Acid-Binding Protein

Adipocyte fatty acid-binding protein (AFABP) is an intracellular protein that binds fatty acids but is also secreted in the circulation from mature adipocytes. AFABP levels are increased in the serum of obese subjects and are strongly related to the development of metabolic syndrome [64]. Altered levels of this molecule in preeclampsia were first determined by Fasshauer et al., which showed that AFABP levels are significantly increased in the disease [65]. Interestingly, serum creatinine

and maternal BMI are independent predictors of the increase in AFABP levels, showing that circulating lipokine levels are altered not only relative to adipose tissue function but also to renal clearance, which may be a key to a better understanding of the pathology of preeclampsia.

References

1. Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. Reproduction. 2010;140:373–85. https://doi.org/10.1530/REP-10-0074.
2. Takagi K, Yamasaki M, Nakamoto O, Saito S, Suzuki H. A review of best practice guide 2015 for care and treatment of hypertension in pregnancy. Hypertens Res Pregnancy. 2015;3:65–103. 10.14390/jsshp.3.105.
3. Mitchell S, Shaw D. The worldwide epidemic of female obesity. Best Pract Res Clin Obstet Gynaecol. 2015;29:289–99. https://doi.org/10.1016/j.bpobgyn.2014.10.002.
4. Mission JF, Marshall NE, Caughey AB. Obesity in pregnancy: a big problem and getting bigger. Obstet Gynecol Surv. 2013;68:389–99. https://doi.org/10.1097/OGX.0b013e31828738ce.
5. Morikawa M, Yamada T, Yamada T, Sato S, Cho K, Minakami H. Prevalence of hyperglycemia during pregnancy according to maternal age and pre-pregnancy body mass index in Japan, 2007-2009. Int J Gynaecol Obstet. 2012;118:198–201. https://doi.org/10.1016/j.ijgo.2012.04.019.
6. Enomoto K, Aoki S, Toma R, Fujiwara K, Sakamaki K, Hirahara F. Pregnancy outcomes based on pre-pregnancy body mass index in Japanese women. PLoS One. 2016;11:e0157081. https://doi.org/10.1371/journal.pone.0157081.
7. Santangeli L, Sattar N, Huda SS. Impact of maternal obesity on perinatal and childhood outcomes. Best Pract Res Clin Obstet Gynaecol. 2015;29:438–48. https://doi.org/10.1016/j.bpobgyn.2014.10.009.
8. Myatt L, Maloyan A. Obesity and placental function. Semin Reprod Med. 2016;34:42–9. https://doi.org/10.1055/s-0035-1570027.
9. Spradley FT, Palei AC, Granger JP. Immune mechanisms linking obesity and preeclampsia. Biomolecules. 2015;5:3142–76. https://doi.org/10.3390/biom5043142.
10. Gallos ID, Sivakumar K, Kilby MD, Coomarasamy A, Thangaratinam S, Vatish M. Preeclampsia is associated with, and preceded by, hypertriglyceridaemia: a meta-analysis. BJOG. 2013;120:1321–32. https://doi.org/10.1111/1471-0528.12375.
11. Zavalza-Gómez AB. Obesity and oxidative stress: a direct link to preeclampsia? Arch Gynecol Obstet. 2011;283:415–22. https://doi.org/10.1007/s00404-010-1753-1.
12. Gupta S, Aziz N, Sekhon L, Agarwal R, Mansour G, Li J, et al. Lipid peroxidation and antioxidant status in preeclampsia: a systematic review. Obstet Gynecol Surv. 2009;64:750–9. https://doi.org/10.1097/OGX.0b013e3181bea0ac.
13. Morisaki N, Nagata C, Jwa SC, Sago H, Saito S, Oken E, et al. Pre-pregnancy BMI-specific optimal gestational weight gain for women in Japan. J Epidemiol. 2017;27:492–8. https://doi.org/10.1016/j.je.2016.09.013.
14. Masuyama H, Segawa T, Sumida Y, Masumoto A, Inoue S, Akahori Y, et al. Different profiles of circulating angiogenic factors and adipocytokines between early- and late-onset preeclampsia. BJOG. 2010;117:314–20. https://doi.org/10.1111/j.1471-0528.2009.02453.x.
15. Ramsay JE, Jamieson N, Greer IA, Sattar N. Paradoxical elevation in adiponectin concentrations in women with preeclampsia. Hypertension. 2003;42:891–4.
16. Otun HA, Lash GE, Innes BA, Bulmer JN, Naruse K, Hannon T, et al. Effect of tumour necrosis factor-α in combination with interferon-γ on first trimester extravillous trophoblast invasion. J Reprod Immunol. 2011;88:1–11. https://doi.org/10.1016/j.jri.2010.10.003.
17. Haider S, Knöfler M. Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium. Placenta. 2009;30:111–23. https://doi.org/10.1016/j.placenta.2008.10.012.

18. Kupferminc MJ, Peaceman AM, Wigton TR, Rehnberg KA, Socol ML. Tumor necrosis factor-alpha is elevated in plasma and amniotic fluid of patients with severe preeclampsia. Am J Obstet Gynecol. 1994;170:1752–7.
19. Pijnenborg R, McLaughlin PJ, Vercruysse L, Hanssens M, Johnson PM, Keith JC, et al. Immunolocalization of tumour necrosis factor-alpha (TNF-alpha) in the placental bed of normotensive and hypertensive human pregnancies. Placenta. 1998;19:231–9.
20. Opsjøn SL, Novick D, Wathen NC, Cope AP, Wallach D, Aderka D. Soluble tumor necrosis factor receptors and soluble interleukin-6 receptor in fetal and maternal sera, coelomic and amniotic fluids in normal and pre-eclamptic pregnancies. J Reprod Immunol. 1995;29:119–34.
21. Kaneko K, Sugitani M, Goto M, Murashima A. Tocilizumab and pregnancy: four cases of pregnancy in young women with rheumatoid arthritis refractory to anti-TNF biologics with exposure to tocilizumab. Mod Rheumatol. 2016;26:672–5. https://doi.org/10.3109/14397595.2016.1140256.
22. LaMarca B, Speed J, Fournier L, Babcock SA, Berry H, Cockrell K, et al. Hypertension in response to chronic reductions in uterine perfusion in pregnant rats: effect of tumor necrosis factor-alpha blockade. Hypertension. 2008;52:1161–7. https://doi.org/10.1161/HYPERTENSIONAHA.108.120881.
23. Weedon-Fekjær MS, Sheng Y, Sugulle M, Johnsen GM, Herse F, Redman CW, et al. Placental miR-1301 is dysregulated in early-onset preeclampsia and inversely correlated with maternal circulating leptin. Placenta. 2014;35:709–17. https://doi.org/10.1016/j.placenta.2014.07.002.
24. Molvarec A, Szarka A, Walentin S, Beko G, Karádi I, Prohászka Z, et al. Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia. Reprod Biol Endocrinol. 2011;9:124. https://doi.org/10.1186/1477-7827-9-124.
25. Chrelias G, Makris GM, Papanota AM, Spathis A, Salamalekis G, Sergentanis TN, et al. Serum inhibin and leptin: risk factors for pre-eclampsia? Clin Chim Acta. 2016;463:84–7. https://doi.org/10.1016/j.cca.2016.10.013.
26. Naruse K, Yamasaki M, Umekage H, Sado T, Sakamoto Y, Morikawa H. Peripheral blood concentrations of adiponectin, an adipocyte-specific plasma protein, in normal pregnancy and preeclampsia. J Reprod Immunol. 2005;65:65–75.
27. Khosrowbeygi A, Ahmadvand H. Positive correlation between serum levels of adiponectin and homocysteine in pre-eclampsia. J Obstet Gynaecol Res. 2013;39:641–6. https://doi.org/10.1111/j.1447-0756.2012.02015.x.
28. Masuyama H, Nobumoto E, Inoue S, Hiramatsu Y. Potential interaction of brain natriuretic peptide with hyperadiponectinemia in preeclampsia. Am J Physiol Endocrinol Metab. 2012;302:E687–93. https://doi.org/10.1152/ajpendo.00548.2011.
29. Naruse K, Yamasaki Y, Tsunemi T, Onogi A, Noguchi T, Sado T, et al. Increase of high molecular weight adiponectin in hypertensive pregnancy was correlated with brain-type natriuretic peptide stimulation on adipocyte. Pregnancy Hypertens. 2011;1:200–5. https://doi.org/10.1016/j.preghy.2011.07.004.
30. D'Anna R, Baviera G, Corrado F, Giordano D, De Vivo A, Nicocia G, et al. Adiponectin and insulin resistance in early- and late-onset pre-eclampsia. BJOG. 2006;113:1264–9.
31. Mori T, Shinohara K, Wakatsuki A, Watanabe K, Fujimaki A. Adipocytokines and endothelial function in preeclamptic women. Hypertens Res. 2010;33:250–4. https://doi.org/10.1038/hr.2009.222.
32. Utzschneider KM, Carr DB, Tong J, Wallace TM, Hull RL, Zraika S, et al. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. Diabetologia. 2005;48:2330–3.
33. Lappas M, Yee K, Permezel M, Rice GE. Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. J Endocrinol. 2005;186:457–65.

34. Chen D, Dong M, Fang Q, He J, Wang Z, Yang X. Alterations of serum resistin in normal pregnancy and preeclampsia. Clin Sci. 2005;108:81–4.
35. Yura S, Sagawa N, Itoh H, Kakui K, Nuamah MA, Korita D, et al. Resistin is expressed in the human placenta. J Clin Endocrinol Metab. 2003;88:1394–7.
36. Dechend R, Müller DN, Wallukat G, Homuth V, Krause M, Dudenhausen J, et al. AT1 receptor agonistic antibodies, hypertension, and preeclampsia. Semin Nephrol. 2004;24:571–9.
37. Zhao L, Bracken MB, Dewan AT, Chen S. Association between the SERPINE1 (PAI-1) 4G/5G insertion/deletion promoter polymorphism (rs1799889) and pre-eclampsia: a systematic review and meta-analysis. Mol Hum Reprod. 2013;19:136–43. https://doi.org/10.1093/molehr/gas056.
38. Ito A, Suganami T, Miyamoto Y, Yoshimasa Y, Takeya M, Kamei Y, et al. Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy. J Biol Chem. 2007;282:25445–52.
39. Madan JC, Davis JM, Craig WY, Collins M, Allan W, Quinn R, et al. Maternal obesity and markers of inflammation in pregnancy. Cytokine. 2009;47:61–4. https://doi.org/10.1016/j.cyto.2009.05.004.
40. Naruse K, Noguchi T, Sado T, Tsunemi T, Shigetomi H, Kanayama S, et al. Chemokine and free fatty acid levels in insulin-resistant state of successful pregnancy: a preliminary observation. Mediat Inflamm. 2012;2012:432575. https://doi.org/10.1155/2012/432575.
41. Fasshauer M, Waldeyer T, Seeger J, Schrey S, Ebert T, Kratzsch J, et al. Serum levels of the adipokine visfatin are increased in pre-eclampsia. Clin Endocrinol (Oxf). 2008;69:69–73.
42. Demir BC, Atalay MA, Ozerkan K, Doster Y, Ocakoglu G, Kucukkomurcu S. Maternal adiponectin and visfatin concentrations in normal and complicated pregnancies. Clin Exp Obstet Gynecol. 2013;40:261–7.
43. Montagnana M, Lippi G, Albiero A, Salvagno GL, Franchi M, Guidi GC. Serum pro-inflammatory cytokines in physiological and pre-eclamptic pregnancies. Gynecol Endocrinol. 2008;24:113–6. https://doi.org/10.1080/09513590801895575.
44. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. Am J Reprod Immunol. 1997;37:240–9.
45. De Falco M, De Luca L, Onori N, Cavallotti I, Artigiano F, Esposito V, et al. Apelin expression in normal human tissues. In Vivo. 2002;16:333–6.
46. Simsek Y, Celik O, Yilmaz E, Karaer A, Dogan C, Aydin S, et al. Serum levels of apelin, salusin-alpha and salusin-beta in normal pregnancy and preeclampsia. J Matern Fetal Neonatal Med. 2012;25:1705–8. https://doi.org/10.3109/14767058.2011.660221.
47. Cobellis L, De Falco M, Mastrogiacomo A, Giraldi D, Dattilo D, Scaffa C, et al. Modulation of apelin and APJ receptor in normal and preeclampsia complicated placentas. Histol Histopathol. 2007;22:1–8. 10.14670/HH-22.1.
48. Inuzuka H, Nishizawa H, Inagaki A, Suzuki M, Ota S, Miyamura H, et al. Decreased expression of apelin in placentas from severe pre-eclampsia patients. Hypertens Pregnancy. 2013;32:410–21. https://doi.org/10.3109/10641955.2013.813535.
49. Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J Lipid Res. 1988;29:1451–60.
50. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res. 1999;424:83–95.
51. Murdolo G, Piroddi M, Luchetti F, Tortoioli C, Canonico B, Zerbinati C, et al. Oxidative stress and lipid peroxidation by-products at the crossroad between adipose organ dysregulation and obesity-linked insulin resistance. Biochimie. 2013;95:585–94. https://doi.org/10.1016/j.biochi.2012.12.014.
52. Nakakita B, Mogami H, Kondoh E, Tsukamoto T, Yanagita M, Konishi I. Case of soluble fms-like tyrosine kinase 1 apheresis in severe pre-eclampsia developed at 15 weeks' gestation. J Obstet Gynaecol Res. 2015;41:1661–3. https://doi.org/10.1111/jog.12760.
53. Thadhani R, Hagmann H, Schaarschmidt W, Roth B, Cingoez T, Karumanchi SA, et al. Removal of soluble Fms-like tyrosine Kinase-1 by dextran sulfate apheresis in preeclampsia. J Am Soc Nephrol. 2016;27:903–13. https://doi.org/10.1681/ASN.2015020157.

54. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011;108:1451–5. https://doi.org/10.1073/pnas.1011293108.
55. Schaeffler I, Gross P, Buettner R, Bollheimer C, Buechler C, Neumeier M, et al. Fatty acid-induced induction of Toll-like receptor-4/nuclear factor-kappaB pathway in adipocytes links nutritional signalling with innate immunity. Immunology. 2009;126:233–45. https://doi.org/10.1111/j.1365-2567.2008.02892.x.
56. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care. 2008;31:1858–63. https://doi.org/10.2337/dc08-0039.
57. Chen X, Scholl TO. Association of elevated free fatty acids during late pregnancy with preterm delivery. Obstet Gynecol. 2008;112:297–303. https://doi.org/10.1097/AOG.0b013e3181802150.
58. Villa PM, Laivuori H, Kajantie E, Kaaja R. Free fatty acid profiles in preeclampsia. Prostaglandins Leukot Essent Fatty Acids. 2009;81:17–21. https://doi.org/10.1016/j.plefa.2009.05.002.
59. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell. 2008;134:933–44. https://doi.org/10.1016/j.cell.2008.07.048.
60. Frigolet ME, Gutiérrez-Aguilar R. The role of the novel lipokine palmitoleic acid in health and disease. Adv Nutr. 2017;8:173S–81S. https://doi.org/10.3945/an.115.011130.
61. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005;436:356–62.
62. Vaisbuch E, Romero R, Mazaki-Tovi S, Erez O, Kim SK, Chaiworapongsa T, et al. Retinol binding protein 4—a novel association with early-onset preeclampsia. J Perinat Med. 2010;38:129–39. https://doi.org/10.1515/JPM.2009.140.
63. Masuyama H, Inoue S, Hiramatsu Y. Retinol-binding protein 4 and insulin resistance in pre-eclampsia. Endocr J. 2011;58:47–53.
64. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem. 2006;52:405–13.
65. Fasshauer M, Seeger J, Waldeyer T, Schrey S, Ebert T, Kratzsch J, et al. Serum levels of the adipokine adipocyte fatty acid-binding protein are increased in preeclampsia. Am J Hypertens. 2008;21:582–6. https://doi.org/10.1038/ajh.2008.23.

Autophagy in Preeclampsia

8

Akitoshi Nakashima, Aiko Aoki, Tae Kusabiraki, Tomoko Shima, Osamu Yoshino, Shi-Bin Cheng, Surendra Sharma, and Shigeru Saito

Abstract

Autophagy is an evolutionarily conserved process in eukaryotes by which cytoplasmic cargo sequestered inside double-membrane vesicles is delivered to the lysosome for degradation. Recently, there is increasing evidence that modulating autophagy accumulates during pregnancy. In early pregnancy, trophoblasts and the fetus experience hypoxic and low-nutrient conditions; nevertheless, extravillous trophoblasts (EVTs) invade the uterine myometrium up to one third of its depth and migrate along the lumina of spiral arterioles, replacing the maternal endothelial lining. An enhancement of autophagy induced by physiological hypoxia occurs during the invasion and vascular remodeling in EVTs. However, soluble endoglin, which is increased in sera in preeclamptic cases, suppresses EVT invasion or vascular remodeling by inhibiting autophagy in vivo. In addition, a substance selectively degraded by autophagy, p62/SQSTM1, accumulates in EVT cells in preeclamptic placental biopsy samples showing impaired autophagy in vivo. On the other hand, there are some reports about autophagy activation in preeclamptic placentas. Though changes in autophagy may affect the fates of mothers and babies, controversy remains for the evaluation of autophagy status in preeclampsia. In this chapter, we will introduce the role of autophagy in embryogenesis, implantation, and maintaining pregnancy and discuss the autophagy status in preeclampsia.

A. Nakashima, M.D., Ph.D. • A. Aoki, M.D., Ph.D. • T. Kusabiraki, M.D. • T. Shima, M.D., Ph.D. • O. Yoshino, M.D., Ph.D. • S. Saito, M.D., Ph.D. (✉)
Faculty of Medicine, Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan
e-mail: s30saito@med.u-toyama.ac.jp

S.-B. Cheng, M.D., Ph.D. • S. Sharma, M.D., Ph.D.
Department of Pediatrics, Women and Infants Hospital, Brown University, Providence, RI, USA

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_8

Keywords
Autophagy • Extravillous trophoblasts • Hypoxia • p62 • Soluble endoglin

8.1 Introduction

The placenta mediates the exchange of nutrients and oxygen between the mother and fetus under hypoxic and low-nutrient conditions during the early gestation period [1, 2]. A hypoxia, which generally impairs cell growth, is an especially preferable condition for early placentation to augment trophoblast invasion [3–5], indicating that trophoblasts possess evolutionary mechanisms allowing them to adjust to the stress. Meanwhile, disruption of the regulating mechanisms may contribute to placental dysfunction, which results in miscarriage, preeclampsia, or fetal growth restriction (FGR). Now, autophagy is well known as the adjusting cellular mechanism for maintaining homeostasis, and a failure of autophagy is also known to be associated with some human diseases [6]. Though autophagy is stringently orchestrated to maintain homeostasis, it is a question for autophagy researchers how dysregulated autophagy is involved in human diseases. In our field, preeclampsia and FGR are life-threatening diseases for the baby as well as a mother. Severe preeclampsia is often complicated by FGR. Ten million women develop preeclampsia each year, and 76,000 mothers among them die each year all over the world [7]. There is growing evidence for the role of autophagy in the pathophysiology of preeclampsia. This chapter introduces our findings and some related papers and points out some problems for autophagy-related assays of the placenta.

8.2 The Role of Autophagy in Reproduction

Macroautophagy (herein referred to as autophagy) was originally discovered as a nonselective bulk degradation process for long-lived proteins or cytoplasmic components during starvation. Autophagy is involved in cellular energy metabolism but also in quality control of cellular proteins (by removing aggregated proteins, damaged organelles, lipid droplets, and intruding viruses or pathogens from the cells) (Fig. 8.1) [8]. This machinery can be deployed in some cellular processes: phagocytosis, exocytosis, secretion, antigen presentation, and regulation of inflammatory signaling [9]. Though the lysosome, which contains hydrolytic enzymes that can break down virtually all kinds of biomolecules, serves as the final step of the autophagic pathway by fusing to the autophagosome, the lysosome itself is also involved in various cell processes, including secretion, plasma membrane repair, cell signaling, and energy metabolism. Consequently, the autophagy pathway mediates human diseases such as neurodegenerative diseases, tumor progression or suppression, infectious diseases, metabolic diseases, and inflammatory diseases [10–14].

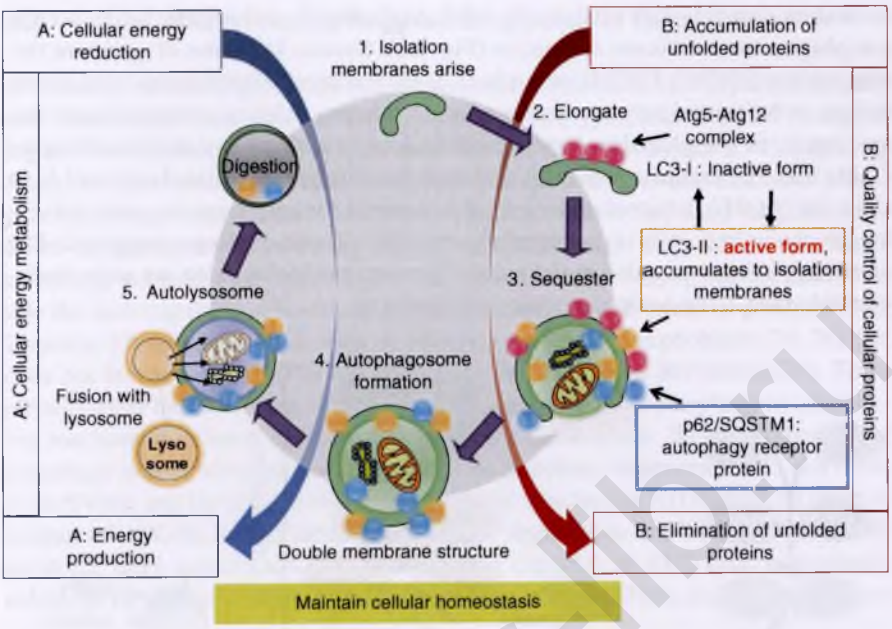


Fig. 8.1 Autophagy machinery and functions. Autophagy stimulation stress, such as hypoxia or starvation, induces the isolation membrane in cytoplasm. The isolation membrane elongates and closes, known as the "autophagosome," and fuses with lysosomes, known as the "autolysosome," resulting in degradation of the cytoplasmic components by lysosomal hydrolases. This machinery is involved in cellular energy metabolism or quality control of cellular proteins

Mice lacking beclin 1 (BECN1), the mammalian orthologue of yeast Atg6, in luteal cells of the ovaries showed normal ovulation, fertilization, implantation, and ovary size [15]. Mice lacking Atg5 or Atg7 are born normally but die within the first day after birth without suckling [16, 17], suggesting less contribution of autophagy for reproduction. Autophagy has, however, more relation to the anteroposterior implantation window. By using an experimentally delayed implantation model mouse, which was created by ovariectomy on day 4 of pregnancy with daily progesterone injection from day 5 (day 1, vaginal plug), autophagy makes dormant blastocysts survive longer in the presence than the absence of 17β-estradiol [18]. After fertilization, autophagy is highly induced in the fertilized oocytes within 4 h after fertilization [19], and then autophagy is transiently suppressed from the late one-cell to middle two-cell stages and reactivated after the late two-cell stage, suggesting that reactivation of autophagy is involved in the degradation of maternal proteins in oocytes [20–22]. This is because autophagy-deficient embryos, Atg5-null oocytes fertilized with Atg5-null sperm, showed reduction of protein synthesis and did not develop to the eight-cell stage [19].

The PI3K complex containing BECN1 and Ambra1 (activating molecule in beclin1-regulated autophagy) and the ULK1-FIP200 (focal adhesion kinase family interacting protein of 200 kD) complex initiate a process of the autophagosome

nucleation step [11, 23], while Atg3, Atg5, Atg7, Atg12, and Atg16L1 enhance the autophagosome membrane elongation (Fig. 8.2). Genetic knockout of upstream factors, such as BECN1, FIP200, or Ambra1, is embryonically lethal because of severe defects in the central nervous system or heart, whereas deletion of downstream factors results in a slightly low birth weight in fetuses without any defects of organs (Table 8.1). For instance, pups with defects in microtubule-associated protein 1 light chain 3B (LC3B), a homologue of Apg8p essential for autophagy in yeast, survive longer than their wild type counterparts [24]. These findings suggested that autophagy-related proteins at the autophagosome nucleation step are more indispensable than at the autophagosome initiation step.

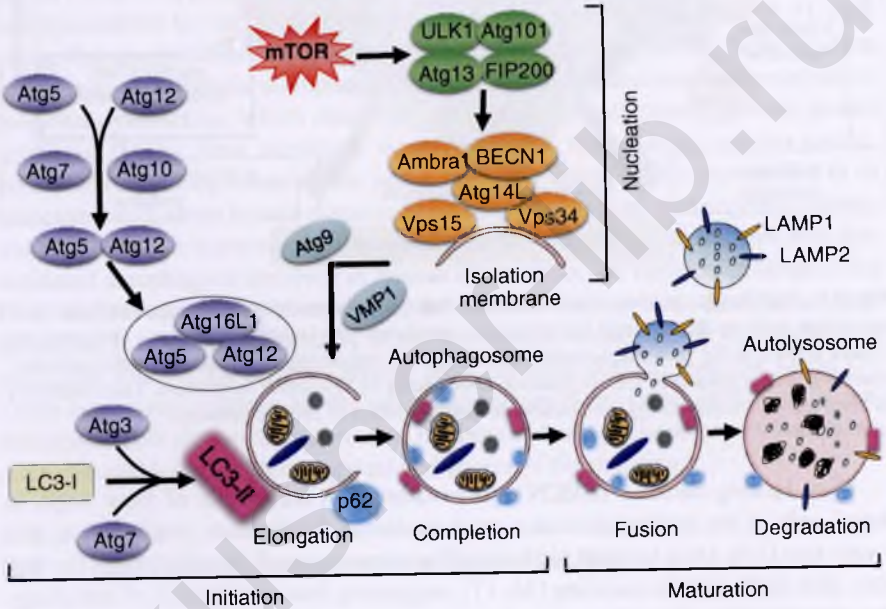


Fig. 8.2 Autophagy cascade. There are three steps in the autophagy pathway. The first step is enucleation. Autophagy induction signals stimulate the PI3K complex via the ULK1 complex. The second step is to elongate the isolation membrane and complete the autophagosome. The final step is the maturation step; the autophagosome fuses with lysosomes, forms the autolysosome, and digests the contents within itself

Table 8.1 The phenotypes of autophagy-related proteins in embryogenesis

Target gene	Phenotype
<Autophagosome nucleation step>	
BCLN1	Early embryonic death (E7.5 or earlier): Defects in proamniotic canal closure
FIP200	Embryonic death (E13.5–E16.5): Heart failure and liver degeneration
Ambra1	Embryonic death (E10–E14): Defects in neural tube development in midbrain/hindbrain, exenecphaly, spina bifida
<Autophagosome elongation step>	
Atg3, Atg5, Atg7, Atg16L1	Death within a day after birth because of suckling defect in neonates

8.3 The Role of Autophagy on Trophoblast Functions

In humans, villous trophoblasts and extravillous trophoblasts (EVTs) are differentiated from trophoblastic stem cells. EVTs have two cell types, interstitial EVTs migrating into the myometrium through the endometrium and endovascular EVTs migrating along the spiral arterioles. The EVTs invade into spiral arteries and then form a "trophoblastic plug" in the arteries to allow the growth of the embryo and the placenta in a low-oxygen environment during the early pregnant period (Fig. 8.3). Activation of autophagy is observed in interstitial EVTs, which invaded deeper, but not shallower, into the maternal decidua basalis at the implantation site, at 7 weeks of gestation [25]. Hypoxia, 2% oxygen tension, induces autophagy in primary trophoblasts [25, 26], but does not induce autophagy in Atg5 knockout mouse embryo fibroblasts [27]. Thus, physiological hypoxia can activate autophagy, suggesting that autophagy is a regulating mechanism in harsh conditions during early placentation. To clarify the role of autophagy in EVT invasion under hypoxia, two autophagy-suppressed EVT cell lines, HTR/SVneo and HchEpC1b cells [28], by stably transfecting ATG4B^{C74A}, an inactive mutant of ATG4B, which inhibits autophagic degradation and lipidation of LC3B paralogs, were constructed [29]. The invasive capacity of EVT cells is generally enhanced by hypoxia, which activates autophagy in trophoblasts, and the suppression

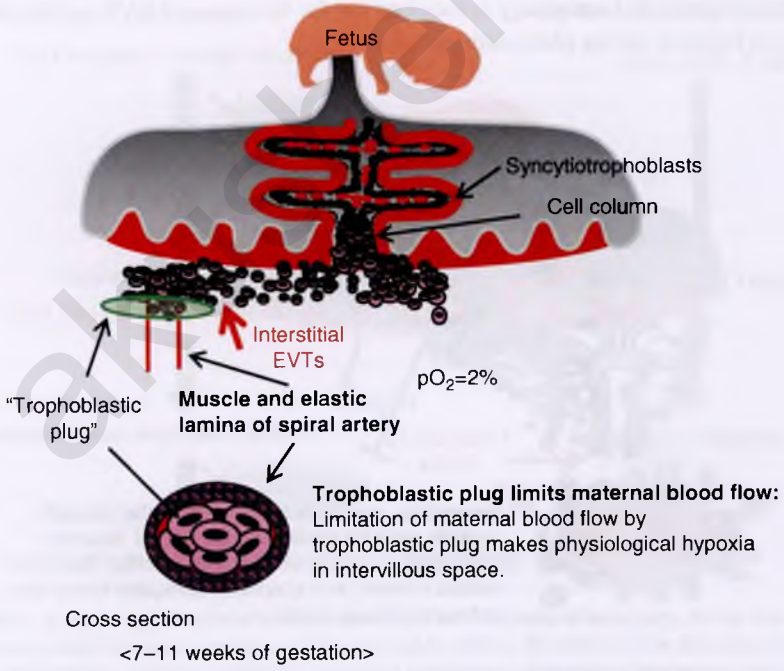


Fig. 8.3 The process of placentation in the early stage of gestation during 7–11 weeks of gestation. EVT cells invade the maternal side, and reduce maternal blood flow to the placenta by a trophoblastic plug, resulting in physiological hypoxia in the intervillous space. Even under hypoxia, interstitial EVT cells continue to invade the maternal side

of autophagy attributed to the inhibition of invasion in the autophagy-suppressed EVT cell lines, compared with that of the autophagy-normal EVT cell lines, under 2% oxygen tension [25]. In addition, strong expression of hypoxia-inducible factor (HIF)-1α by cobalt chloride, an inhibitor of HIF prolyl hydroxylase, which induced autophagy activation, decreased cellular ATP levels in the autophagy-suppressed cells greater than in autophagy-normal cells, showing that autophagy supports EVT invasion as a cellular energy-producing machinery [30]. Another report demonstrated that excessive induction of autophagy by glucose oxidase reduced trophoblast invasion without an increase in apoptosis [31].

After 12 weeks of gestation, in which oxygen tension is increased to moderate hypoxia (approximately 8%) by removing the trophoblastic plug from the uterine spiral arteries accompanied by endovascular EVT invasion, EVTs gradually disrupt elastic fibers in the spiral arteries and replace the fibers (Fig. 8.4). This vascular remodeling of the arteries produces proper perfusion to placenta for sustaining fetal growth. A tube formation assay using EVT cells and human umbilical vascular endothelial cells (HUVECs) has been used for evaluating the ability of vascular remodeling by EVTs [32]. In this assay using less than 8% oxygen tension, mimicking physiological oxygen tension after 12 weeks of gestation, the remodeling ability in the autophagy-suppressed cells was lower than that in the autophagy-normal EVT cells. In addition, hypoxia did not affect the cellular proliferation between the autophagy-normal and suppressed EVT cells under hypoxia. Taken together, these findings suggest that autophagy is required for the functions of EVTs under physiological hypoxia during placentation.

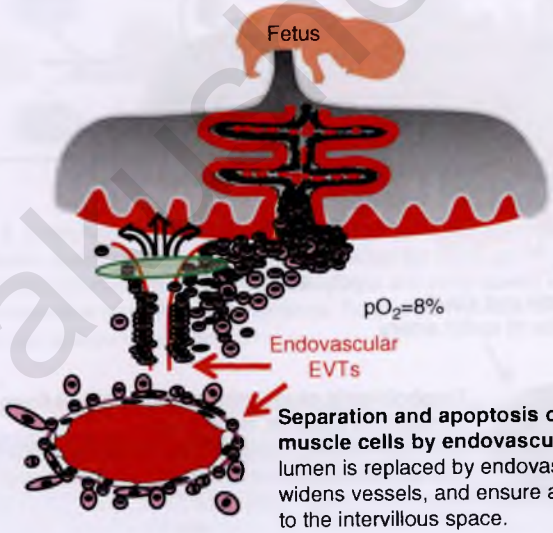


Fig. 8.4 The process of placentation in the early stage of gestation during 12–16 weeks of gestation. Endovascular EVT cells increase the blood flow into the intervillous space by dilating vessels and replacing vascular lumen with EVT cells in the spiral arteries

Although suppression of autophagy by ATG4B^{C74A} did not affect the expression levels of HIF1α under hypoxia in trophoblast cells [25], HIF1α seems to be controlled partially by chaperone-mediated autophagy (CMA) [33]. HIF1α is basically degraded by the oxygen-dependent proteasome under normoxia; on the other hand, CMA-mediated HIF1α degradation via LAMP2A, a lysosomal transporter protein, is in response to nutrient deprivation, but not low oxygen tension in the rat liver [33]. CMA might be important for regulating HIF1α levels in EVTs because hypoxic and starved circumstances simulate the endometrium during the early pregnant period, during which EVTs invade.

8.4 The Role of Autophagy in Preeclampsia or FGR: Pros and Cons

BECN1 initiates autophagosome formation in mammals; the expression of BECN1 in the placenta was reported to be higher in FGR without preeclampsia [34] and not significantly different in preeclampsia [35]. As mentioned before, the impairment of the invasion and vascular remodeling under hypoxia, which were observed in autophagy-deficient EVT cell lines, causes poor placentation, the first stage in the etiology of preeclampsia [36, 37] (Fig. 8.5). Sera from preeclamptic patients induce

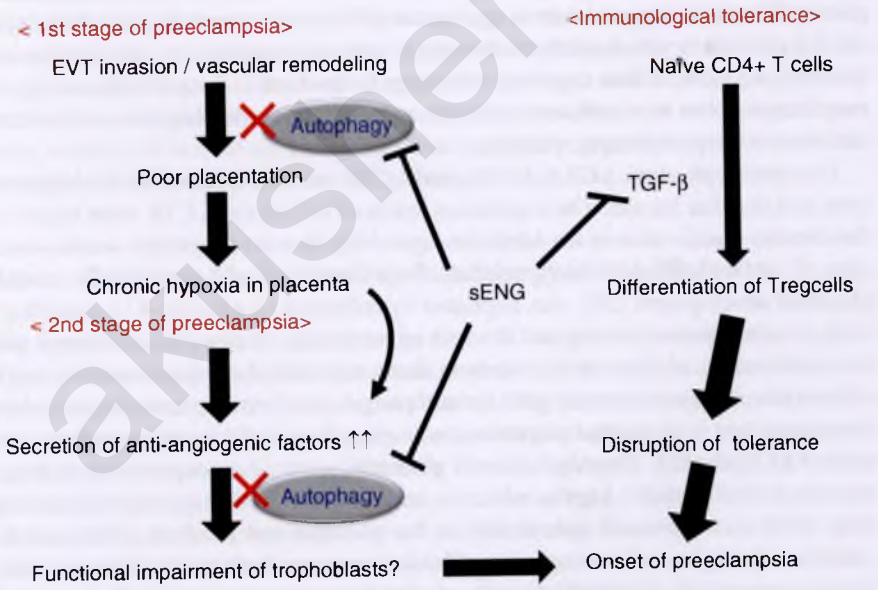


Fig. 8.5 A two-stage disorder of preeclampsia from the viewpoint of autophagy. At the first stage, autophagy inhibition in trophoblasts can be induced by sENG. The failure of the first stage induces poor placentation, resulting in chronic hypoxia in the placenta. Hypoxia augments anti-angiogenic factor secretion from villi, especially soluble endoglin, and chronic hypoxia might cooperate with autophagy inhibition, resulting in the impairment of trophoblasts functions. Soluble endoglin also might increase inflammation in the preeclamptic placenta by decreasing regulatory T cells. This vicious cycle of autophagy inhibition might disrupt homeostasis in preeclamptic placentas

hypertension, proteinuria, and FGR in pregnant IL-10$^{-/-}$ mice, indicating that various factors, including soluble endoglin (sENG) and soluble Flt-1, contribute to the occurrence of preeclampsia in the mice [32]. Among the factors in the sera of preeclamptic women, sENG suppressed invasion and vascular remodeling in EVT cell lines by inhibiting autophagy. TGF-β treatment abolished the effects on EVT invasion by sENG [25]. In human placental bed biopsy samples, which contained cytokeratin 7-positive EVT cells, p62, a substrate of autophagy, was highly expressed in EVT cells, suggesting that autophagy inhibition was present in preeclamptic placentas. In addition, the sera from normotensive women induced autophagy with gestational age in peripheral blood mononuclear cells, while the sera from women with preeclampsia did not [38]. In women with oocyte donation pregnancies, an independent risk factor of preeclampsia or gestational hypertension [39–43] in which a fetus is a complete allograft to mothers, p62 positive rates in EVT cells were significantly higher in women with oocyte donation than for normal pregnancies [44]. On the other hand, there are some reports that demonstrated autophagy activation in preeclamptic placentas. Autophagic vacuoles were observed by electron micrographs in the syncytiotrophoblast layer of human placentas [45, 46], and autophagic vacuoles were seen more in FGR [45]. In preeclampsia, an increase in LC3-II and decrease in p62 were reported in the placentas of women with hypertensive disorders in pregnancy, compared with normotensive pregnancies [47]. The combination of ceramide overload-induced autophagy and oxidative stress-reduced hydrolase activity impaired placental function in preeclampsia accompanied by a reduction in *N*-acylsphingosine amidohydrolase 1, which catalyzes the degradation of ceramide into sphingosine and free fatty acid [48]. Taken together, autophagy is involved in the pathophysiology of preeclampsia, but it is still controversial as to whether autophagy is activated or inhibited in the preeclamptic placenta.

In a mouse placenta, LC3A, LC3B, and LC3C are all expressed in the labyrinth zone and decidua basalis. The expression levels of LC3A and LC3B were higher in the decidua basalis than in the labyrinth layer [49]. In a rat pregnancy model, invasion of rat trophoblasts, which required down-regulation of galectin-4 for normal placental development [50], was regulated by reduction of galectin-4 by autophagy [51]. A recent paper investigated the role of autophagy in placenta and found that the birth weight of fetus delivered from dams with labyrinth layer-specific Atg7-deleted placentas, an essential gene for autophagosome formation, was significantly lower than that with normal placentas, indicating that inhibition of autophagy was related to FGR [52]. The Atg7-deleted placentas were also associated with mitochondrial dysfunction. Atg9b, which is required for autophagosome formation (Fig. 8.2), was expressed specifically in the placenta and pituitary gland; on the other hand, Atg9a, a homolog of Atg9b, was expressed ubiquitously in multiple human organs [53]. To clarify the role of autophagy in preeclampsia, Kojima et al. mated Atg9a knockout mice with heterozygous p57^{Kip2} mice, which develop hypertension and proteinuria in dams [54]. In pups with heterozygous or homozygous deletion of Atg9a, the incidence of fetal death was increased compared with wild type counterparts [55]. In addition, the body weights of Atg9a knockout pups were significantly lower than those in of Atg9a heterozygous knockout or wild type pups.

Taken together, these findings suggest that autophagy is involved in normal placentation and fetal development in rodent pregnancy.

8.5 Protein Aggregation in Preeclampsia

So far, protein aggregation, which is caused by autophagy suppression, has been found in several neurodegenerative diseases [6, 56]. A correlation between protein aggregation and the pathogenesis of preeclampsia has recently been proposed [57]. The accumulation of aggregated proteins, such as transthyretin, a transporter of thyroxine and retinol, or amyloid precursor protein (APP), which is also seen in neurodegenerative diseases, was also observed in placentas with preeclampsia [58, 59]. Furthermore, urinary congophilia, which indicates the existence of aggregated amyloid proteins in urine, can be diagnosed using the Congo red (CR) dot test, in which urine is mixed with CR [58, 60]. The positive rate of this test was significantly higher in women with severe preeclampsia than in the healthy pregnant controls, indicating that aggregated proteins are not only in the placentas but also in the blood stream of women with preeclampsia. In the field of neurodegenerative diseases, in which the role of autophagy has been most investigated, there is a notion that cytotoxic aggregated proteins cannot be attenuated in neural cells, which are non-proliferating. In other words, aggregated proteins can be halved with one cell division in a proliferating cell. This notion suggests that degradation of aggregated proteins by autophagy is more important for maintaining homeostasis in non-proliferating cells. In placental development, proliferation of villous cytotrophoblasts is decreased after the first trimester, and then the proliferation rate becomes even lower in the third trimester. Thus, accumulation of aggregated proteins, which is driven by autophagy suppression, may be greater in placentas after the second trimester because they are less proliferative than those in the first trimester.

8.6 Cautions for Estimating Autophagy in the Placenta

The symptoms of preeclampsia emerge from 20 weeks of gestation in pregnant women. However, preeclamptic placentas, rather than normal placentas in the second trimester, are hard to obtain for evaluation. This is a fundamental problem for placental researchers. The importance of estimating autophagy is to see the autophagy flux, the autophagic activity (Fig. 8.6). For evaluating the autophagy flux, there is no single "gold standard" method to monitor the activity [61, 62]. On the western blotting, the activated autophagy flux can be evaluated by the increase in the LC3-II/actin ratio in response to lysosomal inhibitors, such as bafilomycin A1 or chloroquine, compared with that without inhibitors. This indicates that the dynamics of the autophagy flux are assessable by blockade of the flux by inhibitors. Some papers, however, use transcriptional up-regulation or protein expression of LC3 as an indicator of autophagic activity, but increases of LC3 mRNA or protein do not necessarily indicate the activation of autophagy [61, 62]. In a similar way to the western blotting, numbers of LC3 dots, which are parallel to increases of LC3-II, are often

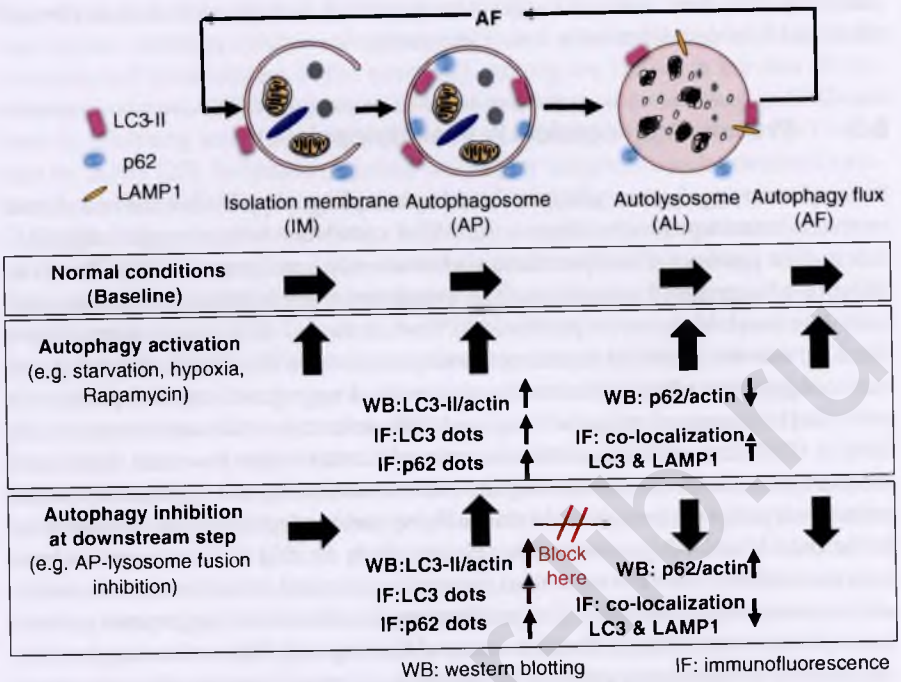


Fig. 8.6 Different autophagic structures for regulation of autophagy. The depicted pictures are the isolation membrane (IM), autophagosome (AP), autolysosome (AL), and autophagy flux (AF). Autophagy flux indicates the speed of the autophagy cycle and autophagic activity. Under normal conditions, basal autophagy constitutively flows. When autophagy is activated with starvation, hypoxia, or rapamycin, there is an increase in all types of autophagic structures. When autophagy is suppressed, for example, by lysosomal inhibitors, at any step after closure of the autophagosome, the number of autophagosomes but not autolysosomes is increased

used to evaluate the autophagy flux in immunofluorescence. This method, however, should be used with caution to evaluate the autophagy status in placentas, because the increase of LC3 dots, which indicates an increase in the number of autophagosomes, but not autolysosomes, is observed when any downstream step of autophagosome formation is blocked; for instance, LC3 dots are increased when the fusion of autophagosomes and lysosomes is impaired (Fig. 8.6). Thus, an increase in LC3 dots could show autophagy activation or suppression in placentas. To precisely evaluate the autophagy status, all the autophagy pathways from the nucleation step to autolysosome formation should be investigated (Figs. 8.2 and 8.6). Then, what should we use for evaluating autophagy in placentas? We recommend the p62 protein, a substrate of autophagy, as an inhibitory indicator of autophagy in the placenta, because autophagy-suppressed EVT cells constitutively expressed higher p62 than autophagy-normal cells in two different trophoblast cell lines [25] (Fig. 8.6). As for the step of autolysosome formation, co-localization of LC3 dots and lysosomal-associated membrane protein 1 (LAMP1) is useful for confirming the autolysosome formation in the placenta (Fig. 8.6). In this scenario, marked accumulation of p62 has been reported in liver-specific autophagy-deficient mice, and

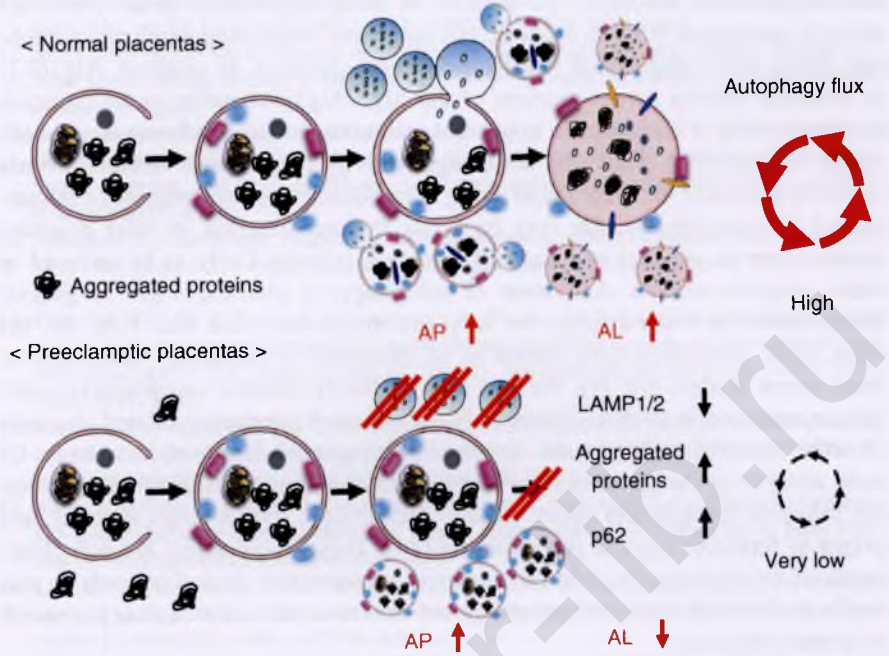


Fig. 8.7 The hypothesis of autophagy inhibition in preeclamptic placentas. Depicted are the relative amounts of AP and AL. When stress activates autophagy in normal placentas, all types of autophagic structures are increased and high autophagy flux can be maintained. On the other hand, some stress may induce the accumulation of aggregated proteins in preeclamptic placentas because preeclamptic placentas are in the inhibitory status of autophagy with impairment of lysosomes

the accumulation was also seen in human hepatocellular carcinomas, suggesting a correlation with the pathophysiology of tumorigenesis [63]. The accumulated p62, which was located in cytoplasmic inclusion bodies, was also involved in cellular injury [64]. In addition, Rubicon, a BCLN1-interacting negative regulator of autophagosome-lysosome fusion, might be a useful marker for evaluation of autophagy in placentas, because Rubicon inhibited the autophagy flux accompanied by p62 accumulation in a mouse model, and higher expression of Rubicon was observed in nonalcoholic fatty liver disease [65]. Finally, although it is based on our preliminary data that the number of lysosomes was decreased in the preeclamptic placentas, a decrease in autophagy flux by the impairment of lysosomes may have induced the deposits of aggregated proteins in the preeclamptic placentas (Fig. 8.7).

8.7 Future Directions for Autophagy Research on Preeclampsia

Autophagy has been known to mediate cancer development, cardiovascular diseases, immune-related diseases, neurodegeneration, and aging pathophysiology [66]. Insufficiency of autophagy occurs with aging, resulting in an increase in

neurodegenerative diseases, and ablation of autophagy-related genes correlates with the incidences of some diseases, but not others. Regarding aging and autophagy, aging is an independent risk factor for preeclampsia. In addition, Atg16L1, an essential protein for recruitment of the Atg5-Atg12 complex to the isolation membrane (Fig. 8.2), is known to prevent endotoxin-induced inflammasome activation in mice [67]. Thus, the autophagic activity to eliminate inflammasomes could be gradually decreased with aging. Considering the involvement of inflammation in preeclampsia, age may be a risk for preeclampsia in older pregnant women than in younger because inflammation is more likely to be induced in older pregnant women. Activation of autophagy by pharmacologic or genetic manipulation prolonged longevity in yeast, nematodes, and flies [68]. On the other hand, premature labor seems to be mediated by autophagy inhibition, at least mouse models [69, 70]. We now have to clarify whether autophagy is favorable or unfavorable for preeclampsia, FGR, and other pregnancy-related diseases, in order to develop therapeutic options involving modulation of autophagy. Of note, analysis of the placenta must take into account the age, time of onset, severity, infection, coagulation status, genetic background, immunological status, and origin of fertilized eggs in preeclampsia [44]. These could affect the autophagy status of the placenta. In addition, technical advances are needed not only to precisely evaluate the status of autophagy but also to accelerate autophagy research in human placentas.

References

1. James JL, Stone PR, Chamley LW. The effects of oxygen concentration and gestational age on extravillous trophoblast outgrowth in a human first trimester villous explant model. Hum Reprod. 2006;21(10):2699–705. https://doi.org/10.1093/humrep/del212. del212 [pii].
2. Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG, Roberts CT. Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. Hum Reprod Update. 2010;16(4):415–31. https://doi.org/10.1093/humupd/dmp046.
3. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature. 1996;379(6560):88–91. https://doi.org/10.1038/379088a0.
4. Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest. 2005;115(10):2679–88. https://doi.org/10.1172/JCI26390.
5. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature. 2006;441(7092):437–43. https://doi.org/10.1038/nature04871. nature04871 [pii].
6. Nixon RA. The role of autophagy in neurodegenerative disease. Nat Med. 2013;19(8):983–97. https://doi.org/10.1038/nm.3232.
7. Vata PK, Chauhan NM, Nallathambi A, Hussein F. Assessment of prevalence of preeclampsia from Dilla region of Ethiopia. BMC Res Notes. 2015;8:816. https://doi.org/10.1186/s13104-015-1821-5.
8. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell. 2011;147(4):728–41. https://doi.org/10.1016/j.cell.2011.10.026. S0092-8674(11)01276-1 [pii].
9. Boya P, Reggiori F, Codogno P. Emerging regulation and functions of autophagy. Nat Cell Biol. 2013;15(7):713–20. https://doi.org/10.1038/ncb2788. ncb2788 [pii].
10. Beau I, Mehrpour M, Codogno P. Autophagosomes and human diseases. Int J Biochem Cell Biol. 2011;43(4):460–4. https://doi.org/10.1016/j.biocel.2011.01.006. S1357-2725(11)00008-2 [pii].

11. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008;132(1):27–42. https://doi.org/10.1016/j.cell.2007.12.018. S0092-8674(07)01685-6 [pii].
12. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069–75. https://doi.org/10.1038/nature06639. nature06639 [pii].
13. Sarkar S. Chemical screening platforms for autophagy drug discovery to identify therapeutic candidates for Huntington's disease and other neurodegenerative disorders. Drug Discov Today Technol. 2013;10(1):e137–44. https://doi.org/10.1016/j.ddtec.2012.09.010. S1740-6749(12)00070-4 [pii].
14. Schneider JL, Cuervo AM. Autophagy and human disease: emerging themes. Curr Opin Genet Dev. 2014;26:16–23. https://doi.org/10.1016/j.gde.2014.04.003. S0959-437X(14)00035-5 [pii].
15. Gawriluk TR, Ko C, Hong X, Christenson LK, Rucker EB 3rd. Beclin-1 deficiency in the murine ovary results in the reduction of progesterone production to promote preterm labor. Proc Natl Acad Sci U S A. 2014;111(40):E4194–203. https://doi.org/10.1073/pnas.1409323111. 1409323111 [pii].
16. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol. 2005;169(3):425–34. https://doi.org/10.1083/jcb.200412022. jcb.200412022 [pii].
17. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, et al. The role of autophagy during the early neonatal starvation period. Nature. 2004;432(7020):1032–6. https://doi.org/10.1038/nature03029. nature03029 [pii].
18. Lee JE, Oh HA, Song H, Jun JH, Roh CR, Xie H, et al. Autophagy regulates embryonic survival during delayed implantation. Endocrinology. 2011;152(5):2067–75. https://doi.org/10.1210/en.2010-1456. en.2010-1456 [pii].
19. Tsukamoto S, Kuma A, Murakami M, Kishi C, Yamamoto A, Mizushima N. Autophagy is essential for preimplantation development of mouse embryos. Science. 2008;321(5885):117–20. https://doi.org/10.1126/science.1154822. 321/5885/117 [pii].
20. Aoki F, Worrad DM, Schultz RM. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. Dev Biol. 1997;181(2):296–307. https://doi.org/10.1006/dbio.1996.8466. S0012-1606(96)98466-3 [pii].
21. Piko L, Clegg KB. Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. Dev Biol. 1982;89(2):362–78. 0012-1606(82)90325-6 [pii].
22. Schultz RM. Regulation of zygotic gene activation in the mouse. Bioessays. 1993;15(8):531–8. https://doi.org/10.1002/bies.950150806.
23. Longatti A, Tooze SA. Vesicular trafficking and autophagosome formation. Cell Death Differ. 2009;16(7):956–65. https://doi.org/10.1038/cdd.2009.39. cdd200939 [pii].
24. Cann GM, Guignabert C, Ying L, Deshpande N, Bekker JM, Wang L, et al. Developmental expression of LC3alpha and beta: absence of fibronectin or autophagy phenotype in LC3beta knockout mice. Dev Dyn. 2008;237(1):187–95. https://doi.org/10.1002/dvdy.21392.
25. Nakashima A, Yamanaka-Tatematsu M, Fujita N, Koizumi K, Shima T, Yoshida T, et al. Impaired autophagy by soluble endoglin, under physiological hypoxia in early pregnant period, is involved in poor placentation in preeclampsia. Autophagy. 2013;9(3):303–16. https://doi.org/10.4161/auto.22927.
26. Chen B, Longtine MS, Nelson DM. Hypoxia induces autophagy in primary human trophoblasts. Endocrinology. 2012;153(10):4946–54. https://doi.org/10.1210/en.2012-1472.
27. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem. 2008;283(16):10892–903. https://doi.org/10.1074/jbc.M800102200. M800102200 [pii].
28. Omi H, Okamoto A, Nikaido T, Urashima M, Kawaguchi R, Umehara N, et al. Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection of E6/E7/hTERT and its transcriptional profile during hypoxia and reoxygenation. Int J Mol Med. 2009;23(2):229–36.
29. Fujita N, Noda T, Yoshimori T. Atg4B(C74A) hampers autophagosome closure: a useful protein for inhibiting autophagy. Autophagy. 2009;5(1):88–9. 7183 [pii]

30. Yamanaka-Tatematsu M, Nakashima A, Fujita N, Shima T, Yoshimori T, Saito S. Autophagy induced by HIF1alpha overexpression supports trophoblast invasion by supplying cellular energy. PLoS One. 2013;8(10):e76605. https://doi.org/10.1371/journal.pone.0076605.
31. Gao L, Qi HB, Kamana KC, Zhang XM, Zhang H, Baker PN. Excessive autophagy induces the failure of trophoblast invasion and vasculature: possible relevance to the pathogenesis of preeclampsia. J Hypertens. 2015;33(1):106–17. https://doi.org/10.1097/HJH.0000000000000366.
32. Kalkunte S, Boij R, Norris W, Friedman J, Lai Z, Kurtis J, et al. Sera from preeclampsia patients elicit symptoms of human disease in mice and provide a basis for an in vitro predictive assay. Am J Pathol. 2010;177(5):2387–98. https://doi.org/10.2353/ajpath.2010.100475. S0002-9440(10)60291-X [pii].
33. Ferreira JV, Fofo H, Bejarano E, Bento CF, Ramalho JS, Girao H, et al. STUB1/CHIP is required for HIF1A degradation by chaperone-mediated autophagy. Autophagy. 2013;9(9):1349–66. https://doi.org/10.4161/auto.25190. 25190 [pii].
34. Hung TH, Chen SF, Lo LM, Li MJ, Yeh YL, Hsieh TT. Increased autophagy in placentas of intrauterine growth-restricted pregnancies. PLoS One. 2012;7(7):e40957. https://doi.org/10.1371/journal.pone.0040957. PONE-D-12-09139 [pii].
35. Oh SY, Choi SJ, Kim KH, Cho EY, Kim JH, Roh CR. Autophagy-related proteins, LC3 and Beclin-1, in placentas from pregnancies complicated by preeclampsia. Reprod Sci. 2008;15(9):912–20. https://doi.org/10.1177/1933719108319159. 15/9/912 [pii].
36. Saito S, Nakashima A. A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling. J Reprod Immunol. 2014;101–102:80–8. https://doi.org/10.1016/j.jri.2013.06.002.
37. Bainbridge SA, Roberts JM, von Versen-Hoynck F, Koch J, Edmunds L, Hubel CA. Uric acid attenuates trophoblast invasion and integration into endothelial cell monolayers. Am J Physiol Cell Physiol. 2009;297(2):C440–50. https://doi.org/10.1152/ajpcell.00593.2008. 00593.2008 [pii].
38. Kanninen TT, Jayaram A, Jaffe Lifshitz S, Witkin SS. Altered autophagy induction by sera from pregnant women with pre-eclampsia: a case-control study. BJOG. 2014;121(8):958–64. https://doi.org/10.1111/1471-0528.12755.
39. Wolff KM, McMahon MJ, Kuller JA, Walmer DK, Meyer WR. Advanced maternal age and perinatal outcome: oocyte recipiency versus natural conception. Obstet Gynecol. 1997;89(4):519–23. https://doi.org/10.1016/S0029-7844(97)00051-3.
40. Wiggins DA, Main E. Outcomes of pregnancies achieved by donor egg in vitro fertilization—a comparison with standard in vitro fertilization pregnancies. Am J Obstet Gynecol. 2005;192(6):2002–6; discussion 6–8. https://doi.org/10.1016/j.ajog.2005.02.059.
41. Salha O, Sharma V, Dada T, Nugent D, Rutherford AJ, Tomlinson AJ, et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. Hum Reprod. 1999;14(9):2268–73.
42. Keegan DA, Krey LC, Chang HC, Noyes N. Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes. Fertil Steril. 2007;87(4):776–81. https://doi.org/10.1016/j.fertnstert.2006.08.105.
43. Henne MB, Zhang M, Paroski S, Kelshikar B, Westphal LM. Comparison of obstetric outcomes in recipients of donor oocytes vs. women of advanced maternal age with autologous oocytes. J Reprod Med. 2007;52(7):585–90.
44. Nakabayashi Y, Nakashima A, Yoshino O, Shima T, Shiozaki A, Adachi T, et al. Impairment of the accumulation of decidual T cells, NK cells, and monocytes, and the poor vascular remodeling of spiral arteries, were observed in oocyte donation cases, regardless of the presence or absence of preeclampsia. J Reprod Immunol. 2016;114:65–74. https://doi.org/10.1016/j.jri.2015.07.005.
45. Curtis S, Jones CJ, Garrod A, Hulme CH, Heazell AE. Identification of autophagic vacuoles and regulators of autophagy in villous trophoblast from normal term pregnancies and in fetal growth restriction. J Matern Fetal Neonatal Med. 2013;26(4):339–46. https://doi.org/10.3109/14767058.2012.733764.
46. Hung TH, Hsieh TT, Chen SF, Li MJ, Yeh YL. Autophagy in the human placenta throughout gestation. PLoS One. 2013;8(12):e83475. https://doi.org/10.1371/journal.pone.0083475. PONE-D-13-28637 [pii].

47. Akaishi R, Yamada T, Nakabayashi K, Nishihara H, Furuta I, Kojima T, et al. Autophagy in the placenta of women with hypertensive disorders in pregnancy. Placenta. 2014;35(12):974–80. https://doi.org/10.1016/j.placenta.2014.10.009. S0143-4004(14)00821-2 [pii].
48. Melland-Smith M, Ermini L, Chauvin S, Craig-Barnes H, Tagliaferro A, Todros T, et al. Disruption of sphingolipid metabolism augments ceramide-induced autophagy in preeclampsia. Autophagy. 2015;11(4):653–69. https://doi.org/10.1080/15548627.2015.1034414.
49. Hiyama M, Kusakabe KT, Takeshita A, Sugi S, Kuniyoshi N, Imai H, et al. Nutrient starvation affects expression of LC3 family at the feto-maternal interface during murine placentation. J Vet Med Sci. 2015;77(3):305–11. https://doi.org/10.1292/jvms.14-0490.
50. Arikawa T, Simamura E, Shimada H, Nishi N, Tatsuno T, Ishigaki Y, et al. Expression pattern of Galectin 4 in rat placentation. Placenta. 2012;33(10):885–7. https://doi.org/10.1016/j.placenta.2012.07.013.
51. Arikawa T, Liao S, Shimada H, Inoue T, Sakata-Haga H, Nakamura T, et al. Galectin-4 expression is down-regulated in response to autophagy during differentiation of rat trophoblast cells. Sci Rep. 2016;6:32248. https://doi.org/10.1038/srep32248.
52. Muralimanoharan S, Gao X, Weintraub S, Myatt L, Maloyan A. Sexual dimorphism in activation of placental autophagy in obese women with evidence for fetal programming from a placenta-specific mouse model. Autophagy. 2016;12(5):752–69. https://doi.org/10.1080/15548627.2016.1156822.
53. Yamada T, Carson AR, Caniggia I, Umebayashi K, Yoshimori T, Nakabayashi K, et al. Endothelial nitric-oxide synthase antisense (NOS3AS) gene encodes an autophagy-related protein (APG9-like2) highly expressed in trophoblast. J Biol Chem. 2005;280(18):18283–18290. M413957200 [pii]. https://doi.org/10.1074/jbc.M413957200.
54. Kanayama N, Takahashi K, Matsuura T, Sugimura M, Kobayashi T, Moniwa N, et al. Deficiency in p57Kip2 expression induces preeclampsia-like symptoms in mice. Mol Hum Reprod. 2002;8(12):1129–35.
55. Kojima T, Yamada T, Akaishi R, Furuta I, Saitoh T, Nakabayashi K, et al. Role of the Atg9a gene in intrauterine growth and survival of fetal mice. Reprod Biol. 2015;15(3):131–8. https://doi.org/10.1016/j.repbio.2015.05.001. S1642-431X(15)00043-1 [pii].
56. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci. 2003;4(1):49–60. https://doi.org/10.1038/nrn1007.
57. Cheng SB, Nakashima A, Sharma S. Understanding pre-eclampsia using Alzheimer's etiology: an intriguing viewpoint. Am J Reprod Immunol. 2016;75(3):372–81. https://doi.org/10.1111/aji.12446.
58. Buhimschi IA, Nayeri UA, Zhao G, Shook LL, Pensalfini A, Funai EF, et al. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. Sci Transl Med. 2014;6(245):245ra92. https://doi.org/10.1126/scitranslmed.3008808. 6/245/245ra92 [pii].
59. Kalkunte SS, Neubeck S, Norris WE, Cheng SB, Kostadinov S, Vu Hoang D, et al. Transthyretin is dysregulated in preeclampsia, and its native form prevents the onset of disease in a preclinical mouse model. Am J Pathol. 2013;183(5):1425–36. https://doi.org/10.1016/j.ajpath.2013.07.022. S0002-9440(13)00543-9 [pii].
60. McCarthy FP, Adetoba A, Gill C, Bramham K, Bertolaccini M, Burton GJ, et al. Urinary congophilia in women with hypertensive disorders of pregnancy and preexisting proteinuria or hypertension. Am J Obstet Gynecol. 2016;215(4):464.e1–7. https://doi.org/10.1016/j.ajog.2016.04.041.
61. Klionsky DJ, Cuervo AM, Seglen PO. Methods for monitoring autophagy from yeast to human. Autophagy. 2007;3(3):181–206.
62. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. Cell. 2010;140(3):313–26. https://doi.org/10.1016/j.cell.2010.01.028. S0092-8674(10)00063-2 [pii].
63. Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, et al. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. J Cell Biol. 2011;193(2):275–84. https://doi.org/10.1083/jcb.201102031.
64. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell. 2007;131(6):1149–63. https://doi.org/10.1016/j.cell.2007.10.035.

65. Tanaka S, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, et al. Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. Hepatology. 2016;64(6):1994–2014. https://doi.org/10.1002/hep.28820.
66. Kroemer G. Autophagy: a druggable process that is deregulated in aging and human disease. J Clin Invest. 2015;125(1):1–4. https://doi.org/10.1172/JCI78652.
67. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2008;456(7219):264–8. https://doi.org/10.1038/nature07383. nature07383 [pii].
68. Madeo F, Zimmermann A, Maiuri MC, Kroemer G. Essential role for autophagy in life span extension. J Clin Invest. 2015;125(1):85–93. https://doi.org/10.1172/JCI73946.
69. Agrawal V, Jaiswal MK, Mallers T, Katara GK, Gilman-Sachs A, Beaman KD, et al. Altered autophagic flux enhances inflammatory responses during inflammation-induced preterm labor. Sci Rep. 2015;5:9410. https://doi.org/10.1038/srep09410. srep09410 [pii].
70. Cao B, Macones C, Mysorekar IU. ATG16L1 governs placental infection risk and preterm birth in mice and women. JCI Insight. 2016;1(21):e86654. https://doi.org/10.1172/jci.insight.86654.

Animal Models in Preeclampsia

9

Keiichi Kumasawa

Abstract

Preeclampsia is a pregnancy-specific disorder characterized by hypertension and excessive proteinuria after 20 weeks' gestation. It is an important cause of maternal and fetal morbidity and mortality worldwide. The disease is almost exclusive to humans and termination of the pregnancy continues to be the only effective and fundamental treatment. The disorder is considered to be multifactorial, although most cases of preeclampsia are characterized by abnormal maternal uterine vascular remodeling by fetally derived placental trophoblast cells. In spite of many previous researches, mechanism of preeclampsia is not clearly and sufficiently elucidated. Recently "two-stage theory" is widely adopted as a pathology of preeclampsia. The first stage involves abnormal placentation characterized by poor trophoblast invasion, incomplete vascular remodeling of spiral arteries, and placental hypoxia. The second stage is manifested as the maternal syndrome of hypertension and proteinuria with systemic endothelial dysfunction. Each step is associated with various factors, and numerous animal models have been used to study those various aspects of preeclampsia. For first stage, many models were reported from the points of immune responses, abnormal trophoblast invasion, placental oxygen dysregulation, and inappropriate maternal vascular damage. For second stage, antiangiogenesis factors, such as soluble fms-like tyrosine kinase 1 (sFLT1) and soluble endoglin (sENG), were adopted for exploit of model mice. However, preeclampsia is almost exclusive to humans mainly because of morphology of placenta; the same pathology in human preeclampsia is difficult to mimic in model

K. Kumasawa, M.D., Ph.D.
Department of Obstetrics and Gynecology, Osaka University, Suita, Osaka, Japan
e-mail: kumasawa@gyne.osaka-u.ac.jp; k1kumasawa@gmail.com

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_9

animals. Therefore, various types of animal models are required. Investigations into the pathophysiology and treatment of preeclampsia will need to continue, albeit at a frustratingly slow pace. There remains a pressing need for novel approaches for pathology and therapy; new models will be needed for this complex and devastating disorder.

Keywords
Model animal • Immune response • Trophoblast invasion • Anti-angiogenesis

9.1 Introduction

Preeclampsia is diagnosed by hypertension and proteinuria after 20 weeks' gestation, which causes critical maternal and fetal morbidity and mortality. Especially in developing countries, preeclampsia is still a major cause of maternal death. Though preeclampsia is a common disease affecting about 5% of all human pregnancies, there are only very rare reports of it in other species [1, 2]. In addition, thus far, mechanism of preeclampsia is not to be seen, and fundamental therapy is to terminate pregnancy, leading to increase of preterm birth.

A variety of animal models have been developed in an attempt to elucidate causative factors, study the pathogenesis of the disease, and develop management and therapeutic options. Although many animal models share features with the hypertensive disorders of pregnancy, most of them do not include the overall spectrum of symptoms seen in the human disease, mainly because of the difference of morphology of placenta.

9.2 Pathology

Pathology of preeclampsia is not sufficiently elucidated, in spite of many previous researches. Recently, a "two-stage theory" has been proposed to describe the pathogenesis of preeclampsia and is widely accepted. Poor placentation is happened before 11 weeks of gestation, and endothelial dysfunction is happened after a 12 weeks of gestation. The first stage involves abnormal placentation characterized by poor trophoblast invasion, incomplete vascular remodeling of spiral arteries, and placental hypoxia. The second stage is manifested as the maternal syndrome of hypertension and proteinuria with systemic endothelial dysfunction. Immune responses are closely related with both steps. In addition, autophagy might be involved in each step of the pathophysiology of preeclampsia [3].

The change between the two stages is thought to be due to the release of factors from the abnormally developed placenta into the maternal circulation. Recent reports revealed an imbalance of angiogenic and anti-angiogenic factors, as well as an immune imbalance in the pathophysiology of preeclampsia. However, the cause and trigger of these mechanisms are still unknown.

9.3 Animal Models

An ideal animal model of preeclampsia would exhibit all the symptoms seen in preeclamptic women, including hypertension, proteinuria, intrauterine growth restriction (IUGR), endothelial dysfunction, and an imbalance of angiogenic/anti-angiogenic factors, all of which arise secondary to poor trophoblast invasion and resolve after delivery of the placenta [4]. Differences in placentation among mammals make the search for an ideal model difficult (Table 9.1).

9.3.1 What Kind of Animals?

Placentas in primates are most similar to those in humans. However, even among primates, spontaneous preeclampsia has only been reported in baboon twins and in the patas monkey, and not all of the current criteria for preeclampsia were assessed in these animals [5, 6]. Among laboratory models, the guinea pig and other caviomorph rodents have a placental morphology and placental invasion that is most similar to those found in humans, including the presence of populations of cells that resemble cytotrophoblast cells and extravillous trophoblast cells [7]. As we state in this chapter, many kinds of preeclampsia model animals were reported, although each of them has obvious limitations. For example, mice have shallow trophoblast invasion, a 3-week gestation, and three trophoblast layers versus a single layer of syncytial trophoblasts characteristic of human pregnancy and placentation [8, 9]. Therefore, although the mouse model is particularly useful for genetic manipulation, it is less useful for studying trophoblast invasion and vascular remodeling, which are critical steps in the pathogenesis of preeclampsia. These limitations also apply to many of the pharmacologically induced models of preeclampsia.

The vast majority of work with animal models of preeclampsia has been performed in the rat and mouse. These rodents share a hemochorial placental type with humans, and their placentas display both interstitial and endovascular trophoblast invasion as well as remodeling of maternal arteries, albeit to a lesser extent than in humans. Rat placentas exhibit more invasion than mouse placentas. Although they do not progress to eclampsia, there are numerous rodent models that display some or all of the key features of preeclampsia. These models have been extremely useful in elucidating the pathophysiology and potential causes of the disease.

9.3.2 Immune Response

There are several lines of evidence supporting a role for maternal immune response in the development of preeclampsia. First, several immune-associated risk factors increase the probability of developing preeclampsia, including preexisting autoimmune disease [10, 11]. Second, primiparity, a change of partner and a short initial coitus-to-conception interval are all risk factors for preeclampsia, suggesting that the response to paternal antigens plays a critical role [11, 12]. This hypothesis is supported by the ability of

Table 9.1 Animal models in preeclampsia

Category	Description	Species	Phenotype[a]	Comment	Ref
Natural	Natural	Baboon, Patas monkey	EBP	Similar placental morphology with human	[5, 6]
Immune response	Low-dose endotoxion	Rat	EBP. UP, low platelet counts	Low-dose endotoxin administration	[18]
	Th1/Th2 imbalance model	Mice	Fetal resorption EBP. UP	IL-4, IL-12 administration	[20]
	Cytokine administration/ reduction models	Rat, mouse	EBP. UP	TNFα, IL-6, IL-10 administration	[15, 16, 21–26]
	Autoantibodies to angiotensin II type I receptors (AT1-AAs)	Mouse	EBP. UP	Administration of angiotensin receptor antibodies	[29, 30]
Trophoblast invasion	Doxycycline-administrated model	Rat	Reduced trophoblast invasion	No elevation of preeclampsia	[31]
	STOX1	Mouse	EBP. UP	Overexpression of STOX1	[32]
Oxygen	Reduced uterine perfusion pressure (RUPP) model	Rat, mouse	EBP. UP	Surgical occlusion of blood supply	[33–47]
	Hypoxia-inducible factor 1α (HIF-1α)-related models	Mouse	EBP. UP	Genetic manipulation of HIF1 levels	[48–50]
Anti-angiogenesis	sFLT1 overexpression	Rat, mouse	EBP. UP, fetal growth restriction	Overexpression of sFLT1	[51, 54]
	sENG overexpression	Rat	EBP. UP	Overexpression of sENG	[55]
Preexisting/ spontaneous	Renin angiotensin-related models	Mouse	EBP. UP	Overexpression of angiotensinogen and rennin	[56–58]
	BPH/5 mouse	Mouse	EBP. UP	Natural	[59–61]
Others	Insulin-induced models of preeclampsia	Rat	EBP. UP	Insulin pellets administration	[62, 63]
	Stress induced model animals	Rat	EBP. UP	Sonic stimulation, stimulation of celiac ganglions, cold stimulation	[64, 65]
	L-NAME-treated rat	Rat	EBP. UP	Administration of L-NAME	[66]
	Placental overexpression of adenosine	Mouse	EBP. UP	Placental overexpression of adenosine	[67]

[a]*EBP* elevated blood pressure, *UP* proteinuria

seminal plasma to suppress the female immune response to paternal antigens [13, 14]. Finally, serum concentrations of inflammatory cytokines, such as TNFα and IL-6, are significantly increased; however, placental production of the anti-inflammatory cytokine IL-10 is decreased, in women with preeclampsia [15–17].

Various kinds of animal models have been developed to elucidate the role of the immune response, particularly inflammation, in the pathogenesis of preeclampsia.

9.3.2.1 Administration of Low-Dose Endotoxin

Administration of low-dose endotoxin to pregnant rats results in elevated systolic blood pressure, proteinuria, and continuous peripheral blood low platelet counts, but endotoxin-treated nonpregnant rat didn't exhibit the preeclamptic phenotype. In addition, the model revealed the evidence of glomerular fibrinogen deposition in the kidneys, but changes of serum anti-angiogenic factors have not been reported in this model [18] but showed no change in endotoxin-treated nonpregnant rats. Peripheral blood platelet counts decreased significantly in pregnant rats after endotoxin infusion.

9.3.2.2 IL-4 and IL-12 Administration: Th1/Th2 Imbalance Model

It has been proposed that immune responses in mammalian normal pregnancy are not T helper 1 (Th1)-like, but T helper 2 (Th2)-like, and upregulation of Th1 responses and downregulation of Th2 responses occur in preeclampsia [19]. Transfer of either IL-4 and/or IL-12 stimulated splenocytes from BALB/C virgin female mice into BALB/C pregnant mice mated with either C57BL/6 or BALB/C male mice resulted in fetal resorption and glomerular nephritis associated with hypertension and proteinuria [20].

9.3.2.3 Cytokine Administration/Reduction Models (TNFα, IL-6, IL-10)

Two inflammatory cytokines that are elevated in the serum of women with preeclampsia, TNFα [15, 21] and IL-6 [16], have been adopted to create animal models that demonstrate the role of inflammation in preeclampsia. TNFα-infused pregnant rats and baboons exhibit hypertension, proteinuria, and elevated serum concentrations of sFLT1 [22, 23]. Elevated blood pressure of the model has been found to be associated with a reduction of renal neuronal nitric oxide synthase.

IL-6 administration causes similar increases in blood pressure and proteinuria in pregnant rats, although serum sFLT1 levels in these animals were not assessed [24]. The preeclamptic phenotype isn't observed in nonpregnant rat.

Exposure of an IL-10-knockout mouse to a hypoxic environment (9.5% O_2) during pregnancy resulted in preeclampsia symptoms, hypertension, proteinuria, and lower level of serum sENG, whereas only fetal growth restriction occurred in wild-type mice exposed to hypoxia [25]. In addition, inhibition of IL-10 by monoclonal antibody during early gestation leads to hypertension in pregnant baboons [26].

9.3.2.4 Autoantibodies to Angiotensin II Type I Receptors (AT1-AAs)

AT1-AAs have been detected in some women with preeclampsia, and these might be associated with disease risk [27, 28].

In both rats and mice, infusion of AT1-AAs during pregnancy induces symptoms of preeclampsia, including hypertension, proteinuria, and vascular remodeling defects in placenta [29, 30]. In addition, this treatment also increases HIF1α expression, indicating a potential pathophysiological link between placental hypoxia and immune factors [30]. In addition, the models reveal elevated levels of serum sFLT1, sENG, and TNFα.

9.3.3 Trophoblast Invasion

Poor trophoblast invasion of maternal spiral arteries is a key feature of preeclampsia set in early pregnancy. An animal model that mimics the process from reduced trophoblast invasion to preeclamptic symptoms has not been reported.

9.3.3.1 Doxycycline-Administrated Model

Doxycycline-administrated pregnant rats are a potential candidate for this aspect of preeclampsia. In this model, doxycycline inhibits matrix metalloproteinase that leads to reduced trophoblast invasion and following reduced placental perfusion [31]. Doxycycline also resulted in intrauterine growth retardation. In their model rats, whether doxycycline treatment alone causes preeclampsia symptoms was not assessed.

9.3.3.2 Storkhead Box 1 (STOX1)

In 2005, STOX1 was discovered as a transcription factor responsible for the preeclampsia in Dutch families. STOX1 belongs to the enlarged Forkhead Box gene family. STOX1 is able to modulate trophoblast proliferation and migration. STOX1 is maternally expressed in column extravillous trophoblasts in the placenta. Doridot L and colleagues generated mice overexpressing human *STOX1*. Wild-type (WT) female mice crossed with transgenic males overexpressing *STOX1* reveal hypertension, proteinuria, an increased plasma level of soluble anti-angiogenic factors, as well as kidney and placenta histological alterations [32].

9.3.4 Oxygen Dysregulation

Several model animals have been used to elucidate the role of oxygen deprivation and hypoxia-reperfusion injury in the pathophysiology of preeclampsia.

9.3.4.1 Reduced Uterine Perfusion Pressure (RUPP) Model

Clinical and experimental evidence continues to implicate reduced placental perfusion, leading to hypertension and vascular dysfunction, preeclampsia. The relationship between reduced uteroplacental perfusion and preeclampsia has been demonstrated in a variety of animals. They are called "reduced uterine perfusion pressure (RUPP)" model.

The first RUPP model described in 1940 by Ogden and colleagues demonstrated pregnancy-associated hypertension in anesthetized dogs by partial occlusion of the

infrarenal abdominal aorta [33]. Their report was followed by later studies in pregnant dogs using bilateral ligation of the utero-ovarian arteries and constriction below the renal arteries which resulted in hypertension, proteinuria, and glomerular endotheliosis [34, 35]. Variations of these techniques, generally involving partial or complete occlusion of the abdominal aorta and/or occlusion of one or both uterine arteries, have been used with varying degrees of success in many animals including rabbits [36], dogs [37, 38], rhesus monkeys [39], baboons [40], and sheep [41]. In general, these models revealed decrease of the blood flow to the uterus by 40–80%. One of the typical work on baboons demonstrated that uteroplacental ischemia occurring secondary to unilateral uterine artery ligation resulted in hypertension, proteinuria, and renal histological changes including endotheliosis and deposition of fibrin and fibrinoid deposits [42]. All these changes were associated with a reduced platelet count and an increase in circulating sFLT1. However, in the study, placental histology was not performed., Although primate models are promising in the study of pathophysiology of preeclampsia, they are expensive and contain legal and ethical issues. Therefore other animal models are frequently adopted.

Particularly, RUPP rat is most used model, in which the ovarian arteries that supply the uterus are surgically narrowed to reduce uteroplacental blood flow [37]. Thus far RUPP rat model has been used in more than 90 publications.

The RUPP model exhibits proteinuria, hypertension, increased levels of serum sFLT-1 and the IL-6, reduced levels of placental growth factor (PlGF), and fetal growth restriction [43–46]. RUPP rat model is frequently used as an excellent potential model to test treatments that address any of the symptoms of preeclampsia. However, placentas in the RUPP model do not exhibit reduced trophoblast invasion, a key aspect of preeclampsia. Moreover, a fundamental weak point of this model is that surgical occlusion of arteries is not associated with the pathology of human preeclampsia [47].

9.3.4.2 Hypoxia-Inducible Factor 1α (HIF-1α)-Related Models

HIF1α is a transcription factor that plays a crucial role in mediating cellular and systemic responses to hypoxia. Then various genes are expressed by HIF1α, under low oxygen conditions. HIF1α-overexpressing transgenic mice revealed elevated blood pressure and proteinuria and IUGR [48]. Moreover knockdown of the HIF1α inhibitor CITED2, which is widely expressed in embryo and in placenta, leads to smaller placenta, IUGR, and IUFD [49]. Catechol-O-methyltransferase (COMT) enzyme produces 2-methoxyestradiol (2-ME). 2-ME is a natural metabolite of estradiol that is generated by the placenta, and inhibits HIF1α, and is reported to be elevated during the third trimester of normal human pregnancy. Knockout of COMT leads mice to suppressed placental HIF-1α expression, hypertension, proteinuria, and elevated serum level of sFLT1 [50]. In addition, injection of 2-ME suppresses HIF-1α in the mice model and reverses preeclamptic symptoms [50]. HIF-1α-associated models show incomplete remodeling of maternal spiral arteries, fetal and placental growth restriction, hypertension, and proteinuria. Models that induce a hypoxic response in the placenta exhibit multiple aspects of preeclampsia and could be useful to test potential treatments for the disorder.

9.3.5 Anti-angiogenesis

The pathophysiology of preeclampsia remains largely unknown. As stated above as "two-stage theory," it has been hypothesized that placental ischemia is a critical event in early period of pregnancy, leading to placental production of soluble factors that cause maternal endothelial dysfunction, resulting in the clinical findings of hypertension, proteinuria, IUGR, and edema. In 2005, Maynard and colleagues reported placental sFLT1, an antagonist of VEGF and placental growth factor (PlGF), is upregulated in preeclampsia and falls after delivery rapidly [51]. In the placenta, sFLT1 is expressed mainly in the trophoblast cells [52]. The trophoblast layer is located between the umbilical capillaries as the fetal side and the maternal blood vessels.

In 2003, Koga K et al. also reported that the concentrations of sFLT1 in serum from women with preeclampsia (median 7791 pg/mL) were >6-fold higher than those from control (1132 pg/mL, $p < 0.0001$). The serum levels of sFLT1 decreased markedly after delivery in both groups [53].

Since the dawn of discovery of sFLT1 as a key factor of preeclampsia, numerous researches were reported in relation to sFLT1 using human serum samples. In addition, several model animals were reported using sFLT1.

In 2006, Venkatesha et al. reported soluble sENG, placenta-derived soluble TGF-beta co-receptor, is elevated in the serum of preeclamptic women, and it correlates with disease severity and falls after delivery.

9.3.5.1 sFLT1 Model Animals

Overexpression of anti-angiogenic factors, such as sFlt1 and sENG, in rodents represents one of the important classes of animal models of preeclampsia.

Overexpression of circulating sFLT1 in pregnant rats [51] or in the placenta of pregnant mice [54] contains two famous models which caused hypertension, proteinuria, and renal damage characteristic of preeclampsia.

In 2003, Maynard and colleagues administered sFLT1 systemically to pregnant rats using adenoviral vector and then induces hypertension, proteinuria, and glomerular endotheliosis in dams. These observations suggest that excess circulating sFLT1 contributes to the pathogenesis of preeclampsia. Although hypertension is also observed in sFLT1-injected nonpregnant rats, and the effect of sFLT1 is not dependent on pregnancy, discovery by Maynard, Karumanchi SA et al. was innovative and a historic event [51].

In 2011, Kumasawa and colleagues established an experimental model to test the role of sFLT1 in preeclampsia using a lentiviral vector-mediated placenta-specific expression system [54]. The model mice showed hypertension and proteinuria during pregnancy, and the symptoms regressed after parturition. Using the mice, Kumasawa et al. reported low-dose pravastatin induced the VEGF-like angiogenic factor placental growth factor (PlGF) and ameliorated the symptoms [54].

9.3.5.2 sENG Model

Overexpression of sENG in pregnant rats also increases blood pressure and proteinuria, although not to the same extent as overexpression of sFLT1, and the symptoms are most severe when both sFLT-1 and sENG are simultaneously overexpressed

[55]. Thus, these models might be most relevant for studying the downstream pathophysiology and treatment of preeclampsia, rather than its initial, pregnancy-specific cause. In addition, as shown in previous report, sFLT1 can be a strong target for new therapy despite of initial triggers in early pregnancy [54].

9.3.6 Preexisting Hypertension Model/Spontaneous Model

Preexisting hypertension, chronic hypertension, is a risk factor of superimposed preeclampsia. It is difficult to distinguish between a patient with preeclampsia and a pregnant patient who had preexisting hypertension if physicians don't know her previous data of blood pressure. It is important to study models that assess the role of underlying hypertension in the evolution of preeclampsia.

Many studies have explored the link between chronic hypertension and preeclampsia through alterations in the renin-angiotensin system, a major endocrine regulator of blood pressure. The model described above involving injection of autoantibodies to angiotensin II type I receptors (AT1-AAs) exhibits preeclampsia symptoms because the antibodies activate the AT1 receptor, which mediates most of the blood pressure increasing activities of angiotensin II [29]. This has been shown by co-injection of an AT1 receptor antagonist (losartan) and anti-AT1-receptor autoantibodies; the antagonist blocks the autoantibodies to trigger preeclampsia in pregnant mice [29].

9.3.6.1 Renin Angiotensin-Related Models of Preeclampsia

Human angiotensinogen-overexpressing female mice or rats mated with human renin-overexpressing males develop transient elevated blood pressure late in pregnancy associated with proteinuria, IUGR [56–58]. These changes were thought to occur secondary to elevated secretion of placental renin. Mouse offspring with both transgenes also exhibit chronic hypertension [57].

9.3.6.2 The BPH/5 Mouse

The BPH/5 mouse strain, which is mildly hypertensive, has been discovered and used to study the relation between preexisting hypertension and preeclampsia. These mice develop preeclampsia-like symptoms, including late gestational hypertension, proteinuria, endothelial dysfunction, poor placental development, and abnormal maternal uterine arteries [59, 60]. In addition, the elevated blood pressure decreased to nonpregnancy levels 2 days after delivery. This model has been used to test potential therapeutic agents for preeclampsia. Administration of the angiogenic factor VEGF121 prevents the development of preeclampsia-like symptoms in BPH/5 mice [61]. The result indicates the potential treatment for patients with chronic hypertension.

9.3.7 Others

9.3.7.1 Insulin-Induced Models of Preeclampsia

Insulin resistance and hyperinsulinemia are associated with essential hypertension. And pregnancy is accompanied with insulin resistance and hyperinsulinemia

following placental secretion of diabetogenic hormones including growth hormone, cortisol, and placental lactogen. Chronic hyperinsulinemia and increased insulin resistance was tried to induce a preeclamptic state in animals. In pregnant rats with chronic hyperinsulinemia, induced by sustained release insulin pellets administration, blood pressure increased in late gestational days, but no change of urinary protein excretion was documented [62]. The model with chronic exogenous hyperinsulinemia is associated with reduced urinary excretion of nitric oxide metabolites. Total NO production was decreased, and treatment with L-arginine normalized blood pressure and increased NO production and renal eNOS protein levels. Administration of L-arginine also improved fetal weight despite no changes in the levels of serum insulin and glucose [63]. As diabetes itself is associated with increased oxidative stress and increased reactive oxygen species (ROS) generation, which results in decreased NO production and increased peroxynitrite generation, conclusions regarding the role of insulin in preeclampsia remain to be seen (Fig. 9.1).

9.3.7.2 Stress-Induced Model Animals

Many risk factors for preeclampsia are associated with the effects of stress, whereas low-stress situations appear to be protective against preeclampsia. To investigate the association between stress and the development of preeclampsia, rats were exposed to a sonic stimulus in addition to the stress associated with overcrowd between days 7 and 14 of pregnancy. Chronic stress in pregnant rats resulted in decreased maternal weight gain, elevated blood pressure, increased vasomotility and proteinuria, lower endothelium-derived relaxing factor release, and lower fetal weight. A greater number of fetuses had higher adrenal weight, higher blood pressure, and lower vascular relaxation [64]. An animal model of HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome was developed by means of stimulation of the celiac ganglion in rats. An increase in blood pressure, serum aspartate

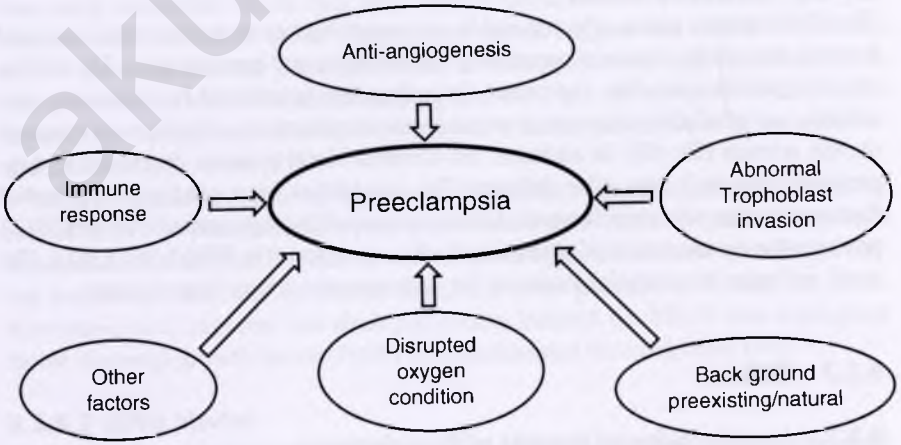


Fig. 9.1 Various aspect of preeclampsia

transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), norepinephrine, and epinephrine was found in the endotoxin-treated to the celiac ganglion pregnant rats compared with control rats treated with the saline solution. A significant decrease in platelet count was found in endotoxin-treated pregnant rats compared with the control rats [65]. In another rat model, chronic local cold stimulation of the soles of the paws at 0 degree induced preeclampsia-like symptoms including proteinuria, a decrease in trophoblast invasion, raised catecholamine secretion, and fibrinoid deposits of the labyrinth [65]. This model suggests that the cause of preeclampsia is involved in chronic stimulation of the sympathetic nerve system.

9.3.7.3 N^{ω}-Nitro-L-Arginine Methyl Ester Hydrochloride (L-NAME)-Treated Rat

L-NAME has been used extensively as a paradigmatic inhibitor of NO synthase. The L-NAME-treated rat revealed early and late onset preeclamptic symptoms: an elevation in systolic blood pressure (SBP) throughout pregnancy. L-NAME groups showed a decrease in SBP after delivery of the pups [66].

9.3.7.4 Adenosine-Related Placental Dysfunction Model

Adenosine is one of the key signaling molecules that orchestrate the cellular response to hypoxia, energy depletion, and tissue damage. Iriyama T. et al. exploited adenosine-related animal model of preeclampsia: genetically engineered pregnant mice with elevated adenosine exclusively in placentas. They also revealed that elevated placental adenosine was enough to induce preeclamptic features, including hypertension, proteinuria, small fetuses, and impaired placental vasculature [67].

9.4 Challenges and Future Directions

Despite the publication of over 30,000 articles on the etiology, prediction, diagnosis and treatment of preeclampsia, pathophysiology of the disease remains to be seen. Thus far we can't decide whether preeclampsia is composed if one or many diseases.

For example, aging is one of the critical risk factors of preeclampsia. Is it possible to make model animals which mimics advanced maternal age with preeclampsia?

Can we accurately predict those women who will develop to the disease by using a single set of parameters? In addition, if diagnosed early enough, can the disorder be prevented or ameliorated?

Moreover what will be an effective prevention drug? Many models have been developed to address these questions, but many other models will be needed before we have the necessary tools to sufficiently understand and overcome preeclampsia. Thus far, we have mimicked the vascular pathology of preeclampsia successfully; it is most likely that targeting vascular abnormalities (e.g., statins) will shed light on the therapy of preeclampsia. This field requires further research by further researchers.

References

1. Stout C, Lemmon WB. Glomerular capillary endothelial swelling in a pregnant chimpanzee. Am J Obstet Gynecol. 1969;105:212–5.
2. Van Wagenen G. Vital statistics from a breeding colony. Reproduction and pregnancy outcome in Macaca mulatta. J Med Primatol. 1972;1:2–28.
3. Nakashima A, Yamanaka-Tatematsu M, Fujita N, Koizumi K, Shima T, Yoshida T, Nikaido T, Okamoto A, Yoshimori T, Saito S. Impaired autophagy by soluble endoglin, under physiological hypoxia in early pregnant period, is involved in poor placentation in preeclampsia. Autophagy. 2013;9:303–16.
4. McCarthy FP, Drewlo S, Kingdom J, Johns EJ, Walsh SK, Kenny LC. Peroxisome proliferator-activated receptor-gamma as a potential therapeutic target in the treatment of preeclampsia. Hypertension. 2011;58:280–6.
5. Gille JH, Moore DG, Sedgwick CJ. Placental infarction: a sign of pre-eclampsia in a patas monkey (Erythrocebus patas). Lab Anim Sci. 1977;27:119–21.
6. Palmer AE, London WT, Sly DL, Rice JM. Spontaneous preeclamptic toxemia of pregnancy in the patas monkey (Erythrocebus patas). Lab Anim Sci. 1979;29:102–6.
7. Mess A, Zaki N, Kadyrov M, Korr H, Kaufmann P. Caviomorph placentation as a model for trophoblast invasion. Placenta. 2007;28:1234–8.
8. Malassine A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update. 2003;9:531–9.
9. Georgiades P, Ferguson-Smith AC, Burton GJ. Comparative developmental anatomy of the murine and human definitive placentae. Placenta. 2002;23:3–19.
10. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ. 2005;330:565.
11. Trogstad L, Magnus P, Stoltenberg C. Pre-eclampsia: risk factors and causal models. Best Pract Res Clin Obstet Gynaecol. 2011;25:329–42.
12. Basso O, Christensen K, Olsen J. Higher risk of pre-eclampsia after change of partner. An effect of longer interpregnancy intervals? Epidemiology. 2001;12:624–9.
13. Maitra U, Davis S, Reilly CM, Li L. Differential regulation of Foxp3 and IL-17 expression in CD4 T helper cells by IRAK-1. J Immunol. 2009;182(9):5763.
14. Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlstrom AC, Care AS. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol Reprod. 2009;80:1036–45.
15. Kupferminc MJ, Peaceman AM, Wigton TR, Rehnberg KA, Socol ML. Tumor necrosis factor-alpha is elevated in plasma and amniotic fluid of patients with severe preeclampsia. Am J Obstet Gynecol. 1994;170:1752–7; discussion 1757–9.
16. Vince GS, Starkey PM, Austgulen R, Kwiatkowski D, Redman CW. Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. Br J Obstet Gynaecol. 1995;102:20–5.
17. Makris A, Xu B, Yu B, Thornton C, Hennessy A. Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. Placenta. 2006;27:445–51.
18. Faas MM, Schuiling GA, Baller JF, Visscher CA, Bakker WW. A new animal model for human preeclampsia: ultra-low-dose endotoxin infusion in pregnant rats. Am J Obstet Gynecol. 1994;171:158–64.
19. Saito S, Umekage H, Sakamoto Y, Sakai M, Tanebe K, Sasaki Y, Morikawa H. Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with pre-eclampsia. Am J Reprod Immunol. 1999;41:297–306.
20. Hayakawa S, Fujikawa T, Fukuoka H, Chisima F, Karasaki-Suzuki M, Ohkoshi E, Ohi H, Kiyoshi Fujii T, Tochigi M, Satoh K, Shimizu T, Nishinarita S, Nemoto N, Sakurai I. Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. J Reprod Immunol. 2000;47:121–38.

21. Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. J Clin Endocrinol Metab. 2001;86:2505–12.
22. LaMarca BB, Bennett WA, Alexander BT, Cockrell K, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of tumor necrosis factor-alpha. Hypertension. 2005;46:1022–5.
23. Sunderland NS, Thomson SE, Heffernan SJ, Lim S, Thompson J, Ogle R, McKenzie P, Kirwan PJ, Makris A, Hennessy A. Tumor necrosis factor alpha induces a model of preeclampsia in pregnant baboons (Papio hamadryas). Cytokine. 2011;56:192–9.
24. Orshal JM, Khalil RA. Interleukin-6 impairs endothelium-dependent NO-cGMP-mediated relaxation and enhances contraction in systemic vessels of pregnant rats. Am J Physiol Regul Integr Comp Physiol. 2004;286:R1013–23.
25. Lai Z, Kalkunte S, Sharma S. A critical role of interleukin-10 in modulating hypoxia-induced preeclampsia-like disease in mice. Hypertension. 2011;57:505–14.
26. Orange S, Rasko JE, Thompson JF, Vaughan J, Olive E, Pedler M, Horvath JS, Hennessy A. Interleukin-10 regulates arterial pressure in early primate pregnancy. Cytokine. 2005;29:176–85.
27. Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol. 2010;63:534–43.
28. Abou-Nassar K, Carrier M, Ramsay T, Rodger MA. The association between antiphospholipid antibodies and placenta mediated complications: a systematic review and meta-analysis. Thromb Res. 2011;128:77–85.
29. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, Hicks MJ, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nat Med. 2008;14:855–62.
30. Wenzel K, Rajakumar A, Haase H, Geusens N, Hubner N, Schulz H, Brewer J, Roberts L, Hubel CA, Herse F, Hering L, Qadri F, Lindschau C, Wallukat G, Pijnenborg R, Heidecke H, Riemekasten G, Luft FC, Muller DN, Lamarca B, Dechend R. Angiotensin II type 1 receptor antibodies and increased angiotensin II sensitivity in pregnant rats. Hypertension. 2011;58:77–84.
31. Verlohren S, Geusens N, Morton J, Verhaegen I, Hering L, Herse F, Dudenhausen JW, Muller DN, Luft FC, Cartwright JE, Davidge ST, Pijnenborg R, Dechend R. Inhibition of trophoblast-induced spiral artery remodeling reduces placental perfusion in rat pregnancy. Hypertension. 2010;56:304–10.
32. Doridot L, Passet B, Mehats C, Rigourd V, Barbaux S, Ducat A, Mondon F, Vilotte M, Castille J, Breuiller-Fouche M, Daniel N, le Provost F, Bauchet AL, Baudrie V, Hertig A, Buffat C, Simeoni U, Germain G, Vilotte JL, Vaiman D. Preeclampsia-like symptoms induced in mice by fetoplacental expression of STOX1 are reversed by aspirin treatment. Hypertension. 2013;61:662–8.
33. Ogden E, Hildebrand G, Page EW. Rise in blood pressure during ischaemia of the gravid uterus. Prac Soc Exper Biol Med. 1940;43:49e51.
34. Hodari AA. Chronic uterine ischemia and reversible experimental "toxemia of pregnancy". Am J Obstet Gynecol. 1967;97:597–607.
35. Abitbol MM, Pirani CL, Ober WB, Driscoll SG, Cohen MW. Production of experimental toxemia in the pregnant dog. Obstet Gynecol. 1976;48:537–48.
36. Losonczy G, Brown G, Venuto RC. Increased peripheral resistance during reduced uterine perfusion pressure hypertension in pregnant rabbits. Am J Med Sci. 1992;303:233–40.
37. Abitbol MM. A simplified technique to produce toxemia in the pregnant dog. Am J Obstet Gynecol. 1981;139:526–34.
38. Woods LL, Brooks VL. Role of the renin-angiotensin system in hypertension during reduced uteroplacental perfusion pressure. Am J Phys. 1989;257:R204–9.
39. Combs CA, Katz MA, Kitzmiller JL, Brescia RJ. Experimental preeclampsia produced by chronic constriction of the lower aorta: validation with longitudinal blood pressure measurements in conscious rhesus monkeys. Am J Obstet Gynecol. 1993;169:215–23.

40. Cavanagh D, Rao PS, Tsai CC, O'Connor TC. Experimental toxemia in the pregnant primate. Am J Obstet Gynecol. 1977;128:75–85.
41. Clark KE, Durnwald M, Austin JE. A model for studying chronic reduction in uterine blood flow in pregnant sheep. Am J Phys. 1982;242:H297–301.
42. Makris A, Thornton C, Thompson J, Thomson S, Martin R, Ogle R, Waugh R. McKenzie P, Kirwan P, Hennessy A. Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. Kidney Int. 2007;71:977–84.
43. Gadonski G, LaMarca BB, Sullivan E, Bennett W, Chandler D, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of interleukin 6. Hypertension. 2006;48:711–6.
44. Granger JP, LaMarca BB, Cockrell K, Sedeek M, Balzi C, Chandler D, Bennett W. Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. Methods Mol Med. 2006;122:383–92.
45. Balta O, Boztosun A. Deveci K, Gulturk S, Ekici F, Kaya A, Cetin A, Cetin M. Reduced uterine perfusion pressure model is not successful to mimic severe preeclampsia. Placenta. 2011;32:675–80.
46. George EM, Cockrell K, Aranay M, Csongradi E, Stec DE, Granger JP. Induction of heme oxygenase 1 attenuates placental ischemia-induced hypertension. Hypertension. 2011;57:941–8.
47. McCarthy FP, Kingdom JC, Kenny LC, Walsh SK. Animal models of preeclampsia; uses and limitations. Placenta. 2011;32:413–9.
48. Tal R, Shaish A, Barshack I, Polak-Charcon S, Afek A, Volkov A, Feldman B, Avivi C, Harats D. Effects of hypoxia-inducible factor-1alpha overexpression in pregnant mice: possible implications for preeclampsia and intrauterine growth restriction. Am J Pathol. 2010;177:2950–62.
49. Withington SL, Scott AN, Saunders DN, Lopes Floro K, Preis JI, Michalicek J, Maclean K, Sparrow DB, Barbera JP, Dunwoodie SL. Loss of Cited2 affects trophoblast formation and vascularization of the mouse placenta. Dev Biol. 2006;294:67–82.
50. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, Parry S, Augustin HG, Gattone VH, Folkman J, Strauss JF, Kalluri R. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453:1117–21.
51. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111:649–58.
52. Fan X, Rai A, Kambham N, Sung JF, Singh N, Petitt M, Dhal S, Agrawal R, Sutton RE, Druzin ML, Gambhir SS, Ambati BK, Cross JC, Nayak NR. Endometrial VEGF induces placental sFLT1 and leads to pregnancy complications. J Clin Invest. 2014;124:4941–52.
53. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, Takeda S, Yano T, Tsutsumi O, Taketani Y. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. J Clin Endocrinol Metab. 2003;88:2348–51.
54. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011;108:1451–5.
55. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12:642–9.
56. Takimoto E, Ishida J, Sugiyama F, Horiguchi H, Murakami K, Fukamizu A. Hypertension induced in pregnant mice by placental renin and maternal angiotensinogen. Science. 1996;274:995–8.
57. Falcao S, Stoyanova E, Cloutier G, Maurice RL, Gutkowska J, Lavoie JL. Mice overexpressing both human angiotensinogen and human renin as a model of superimposed preeclampsia on chronic hypertension. Hypertension. 2009;54:1401–7.
58. Brosnihan KB, Hering L, Dechend R, Chappell MC, Herse F. Increased angiotensin II in the mesometrial triangle of a transgenic rat model of preeclampsia. Hypertension. 2010;55:562–6.

59. Davisson RL, Hoffmann DS, Butz GM, Aldape G, Schlager G, Merrill DC, Sethi S, Weiss RM, Bates JN. Discovery of a spontaneous genetic mouse model of preeclampsia. Hypertension. 2002;39:337–42.
60. Dokras A, Hoffmann DS, Eastvold JS, Kienzle MF, Gruman LM, Kirby PA, Weiss RM, Davisson RL. Severe feto-placental abnormalities precede the onset of hypertension and proteinuria in a mouse model of preeclampsia. Biol Reprod. 2006;75:899–907.
61. Woods AK, Hoffmann DS, Weydert CJ, Butler SD, Zhou Y, Sharma RV, Davisson RL. Adenoviral delivery of VEGF121 early in pregnancy prevents spontaneous development of preeclampsia in BPH/5 mice. Hypertension. 2011;57:94–102.
62. Podjarny E, Bernheim J, Katz B, Green J, Mekler J, Bursztyn M. Chronic exogenous hyperinsulinemia in pregnancy: a rat model of pregnancy-induced hypertension. J Am Soc Nephrol. 1998;9:9–13.
63. Bursztyn M, Podjarny E, Dahan R, Raz I, Bernheim J. Insulin-induced hypertension, L-arginine, and endothelial nitric oxide synthase in pregnant rats. Hypertens Pregnancy. 2003;22:267–74.
64. Takiuti NH, Kahhale S, Zugaib M. Stress in pregnancy: a new Wistar rat model for human preeclampsia. Am J Obstet Gynecol. 2002;186:544–50.
65. Kanayama N, She L, Maehara K, Kajiwara Y, Terao T. Induction of HELLP syndrome-like biochemical parameters by stimulation of the celiac ganglion in rats. J Hypertens. 1996;14:453–9.
66. Baijnath S, Murugesan S, Mackraj I, Gathiram P, Moodley J. The effects of sildenafil citrate on urinary podocin and nephrin mRNA expression in an L-NAME model of pre-eclampsia. Mol Cell Biochem. 2017;427:59–67.
67. Iriyama T, Wang W, Parchim NF, Song A, Blackwell SC, Sibai BM, Kellems RE, Xia Y. Hypoxia-independent upregulation of placental hypoxia inducible factor-1alpha gene expression contributes to the pathogenesis of preeclampsia. Hypertension. 2015;65:1307–15.

The Differences Between Early- and Late-Onset Pre-eclampsia

10

Anne Cathrine Staff and Christopher W.G. Redman

Abstract

Early- and late-onset pre-eclampsia (PE) exhibit important clinical differences both regarding associations to foetal growth restriction as well as short- and long-term health consequences for the mother and offspring. There is no definition consensus, but dichotomizing into preterm or term delivery (i.e. before or from gestational week (GW) 37) or into a very premature delivery or not (i.e. before or from gestational week 34) is common. The early-onset type is linked to poor placentation and foetal growth restriction, whereas maternal factors were suggested to cause the late-onset disease, without a placental impact. As an alternative model, we have suggested that both forms represent placental dysfunction (stage 1) prior to development of its clinical signs (stage 2), but both the causes of the placental malperfusion and its timing differ. In early-onset pre-eclampsia, the placental dysfunction is 'extrinsic' to the placenta, with incomplete spiral artery remodelling (an early pregnancy event). The cause of late-onset pre-eclampsia is 'intrinsic' to the growing and ageing placenta, restricting intervillous perfusion. Both pathways lead to secondary syncytiotrophoblast stress and release of pro-inflammatory factors into the maternal circulation. Maternal factors may increase the risk on many levels for the two stages of pre-eclampsia and contribute to the risk for both early- and late-onset forms. Our revised two-stage model of pre-eclampsia is in line with the clinical and biomarker heterogeneity of early- and late-onset disease. In conclusion, we present evidence that placental malperfusion and dysfunction cause both early- and onset pre-eclampsia, albeit for different reasons and with a different timing.

A.C. Staff, M.D., Ph.D. (✉)
Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway

Faculty of Medicine, University of Oslo, Oslo, Norway
e-mail: uxnnaf@ous-hf.no; a.c.staff@medisin.uio.no

C.W.G. Redman, M.D., Ph.D.
University of Oxford, Oxford, UK

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_10

Keywords
Decidua • Pre-eclampsia • Placenta • Hypertension • Malperfusion • Pathophysiology • Pregnancy • Spiral artery • Two-stage

10.1 Introduction: What Is Pre-eclampsia?

Hypertensive disorders of pregnancy represent a clinical challenge worldwide. In particular, pre-eclampsia (PE), defined as new-onset hypertension (≥140/90 mmHg) and proteinuria diagnosed after gestational week 20 or new-onset pre-eclampsia-associated signs in the absence of proteinuria [1, 2], is a leading cause for maternal and perinatal mortality and morbidity worldwide [3, 4]. However, the exact mortality and morbidity figures remain unknown [5]. It is generally stated that pre-eclampsia can only occur after 20 weeks, but a well-documented case of eclampsia has been reported at 16 weeks [6]. Pre-eclampsia requires the presence of a placenta (or a recently delivered placenta, as in postpartum pre-eclampsia, reviewed in [7]), but the relative contributions of maternal predisposing factors versus placental factors to its pathophysiology are not well delineated [7].

Pre-eclampsia presents in heterogeneous forms, with varying degrees and severity of maternal and foetal affection. Acute maternal complications of pre-eclampsia include eclampsia, stroke, placental abruption, disseminated intravascular coagulation, HELLP (hemolysis, elevated liver enzymes, and low platelets), liver haemorrhage or rupture, pulmonary oedema, adult respiratory distress syndrome, acute renal failure and death [8]. Globally, pre-eclampsia complications account for more than 50,000 maternal deaths annually [8, 9]. In developing countries, where lack of access to appropriate maternal care is a major problem, maternal death rates are as high as 15% as compared to 0–1.8% in industrialized countries [8]. Perinatal complications of pre-eclampsia include stillbirth, iatrogenic preterm delivery, foetal growth restriction (FGR), neonatal complications and later sequelae [10]. Pre-eclampsia and eclampsia are reported to affect 1–8% of all pregnancies (as reviewed in [11] and [12]). Many low- and middle-income countries (LMIC) lack however population-based medical birth statistics and may also lack definition as well as reliable documentation of hypertensive pregnancy subtypes [13].

Pre-eclampsia is often subclassified by clinical severity determined by maternal and/or foetal outcome characteristics, the latter including the presence or absence of foetal growth restriction (FGR), as it has higher perinatal morbidity and mortality than pre-eclampsia without FGR [14]. Also, a clinical subclassification into early- and late-onset of pre-eclampsia is widely used when dichotomizing severity of disease. Many experts favour classifying pre-eclampsia with the higher blood pressures (e.g. >160 mmHg systolic and >110 mmHg diastolic) as severe [15]. This classification is however not supported by many clinicians, as blood pressures are iatrogenically controlled in pregnancy, and not necessarily reflecting the severity of the underlying (placental) problem. Also, the clinical situation of any form of pre-eclampsia may be seen as severe [2], as pre-eclampsia is by nature unstable and the

clinical situation for both mother and foetus may deteriorate rapidly, hence justifying intense surveillance and rapid clinical action into the postpartum period.

10.2 Definitions of Early- and Late-Onset Pre-eclampsia

There is today a weak consensus on the definition of early- versus late-onset pre-eclampsia. The ISSHP (International Society for the Study of Hypertension in Pregnancy) published in 2013 the results of a survey on pre-eclampsia severity definitions among 22 expert members of the International Committee of ISSHP [15]. The majority of these experts (73%) considered 'early-onset' pre-eclampsia as occurring before 34 weeks, and 73% agreed to name pre-eclampsia occurring before 37 weeks as 'preterm pre-eclampsia'. A challenge with this definition is however that exact dating of first pre-eclampsia 'occurrence' is usually not documented in population-based medical birth registries nor in most local settings. Antenatal pregnancy follow-up, including routine blood pressure and urine screening, differs largely between countries and populations, affecting the time of detection of the first onset of pre-eclampsia signs ('occurrence'). Also, the onset of pre-eclampsia presents in highly variable ways, as reviewed by us previously [7], challenging a uniform definition of pre-eclampsia 'onset' and 'occurrence'. The classical sequence is identification of pregnancy-induced hypertension (PIH) first and proteinuria second, but the converse sequence occurs in a minority of cases [16]. Therefore, the proxy of defining early-onset and premature pre-eclampsia as related to the gestational age at delivery has been used in many publications, as delivery is a definite date and reliably documented in most populations. However, reliable gestational age at delivery may be lacking in low-resource settings, owing to the absence of ultrasound-based dating of the pregnancy or even lack of a reliable due date determined from the last menstrual period. A delivery date as the time for identifying a pre-eclampsia onset subtype would be very problematic if there is a big difference in delay between 'onset' and delivery across countries. Most countries with adequate antenatal and obstetric care follow international guidelines that recommend delivery of pre-eclampsia after gestational week (GW) 37, in order to avoid severe maternal or foetal crises. The international guidelines recommend conservative management of clinically reassuring pre-eclamptic pregnancies until GW 34, whereas there are no clear conclusions for the management of non-severe pre-eclampsia in GW 34–37 [17]. We anticipate that in very-early-onset pre-eclampsia, the mean follow-up time before iatrogenic delivery may vary more than for late-onset pre-eclampsia, especially between hospitals in low- and high-resource settings, as prolongation of pregnancy in order to delay delivery and reduce prematurity risks requires hospitalization, which is costly. We however argue that a gestational age threshold defined on the time of delivery, not the time of onset, may be the most reliable method when comparing population data. Most current literature dichotomizes pre-eclampsia into pre-eclamptic deliveries before or from term (with a threshold for early-onset disease at GW 37) or into very preterm (with a threshold for early-onset disease at GW 34). These terms correspond with the 'preterm

pre-eclampsia' and 'early-onset pre-eclampsia' definitions by ISSHP but use gestational age at delivery instead of the less objectively documented 'occurrence' definition by ISSHP [15]. Ideally, both times should be recorded, if available. The gestational age thresholds used are pragmatic. They are based on clinical terminology (prematurity defined as before GW 37), or the threshold below which maternal corticosteroids for foetal lung maturation are indicated (GW 34) when delivery is imminent, but not on clearly defined pathophysiological or risk factors.

10.3 Clinical Features of Early- and Late-Onset Pre-eclampsia

Whatever definition for early- and late-onset pre-eclampsia of the above options is used, late onset and early onset of the disease differ in both short- and long-term clinical outcomes for the mother and her offspring.

10.3.1 Differences in Short-Term Outcomes and Risk of Recurrence

The main clinical difference between the early- and late-onset pre-eclampsia is that the latter is usually less severe [18]. This is consistent with its shorter duration and with its effective management in countries with adequate antenatal care and induced delivery of all cases after GW 37. Early-onset pre-eclampsia is heavily burdened by the neonatal aspects of the syndrome, which includes not only prematurity but an increased risk for foetal growth restriction and small for gestational age babies [19, 20]. However, term pre-eclampsia is not without dangers for the mother or offspring, even if neonates are not growth restricted and may even be large for dates [19]. Eclampsia and HELLP are examples of life-threatening crises for both the pregnant woman and her foetus that are common in late gestation and post-term manifestations of pre-eclampsia, with high rates when calculated relative to the diminishing denominator of ongoing pregnancies [21]. Studies show that 20% of HELLP syndrome [22] and 55% of eclampsia cases [16] present at term (from GW 37). In other words, term pre-eclampsia is not benign just because the foetus is less threatened by FGR.

Table 10.1 summarizes important clinical features that are more commonly found in early-onset as compared to late-onset pre-eclampsia, including the higher recurrence risk of the former.

10.3.2 Differences in Long-Term Health Outcomes

In general, early-onset pre-eclampsia carries a higher risk of future disease for the mother than late-onset pre-eclampsia, including a higher risk of cardiovascular disease (CVD) [38] (Table 10.2). The association between pre-eclampsia and

Table 10.1 Clinical features of early-onset pre-eclampsia

More common in early-onset pre-eclampsia	Reference(s)
Higher rate of foetal growth restriction	[19, 20, 23, 24]
Higher perinatal mortality and morbidity	[18, 25]
More often placental pathology	[26–29]
Higher maternal mortality and morbidity	[18, 30–32]
More often abnormal uterine and umbilical artery Doppler findings	[18, 24]
Lower nulliparity rate	[33]
Better diagnosed and predicted by angiogenic biomarkers	[34, 35]
Higher recurrence rate	[11, 33, 36, 37]

Table 10.2 Higher rates of remote postpartum medical complications for the woman after premature pre-eclampsia

	Reference(s)
Premature death due to CVD	[31, 41, 42]
CVD, including hypertension	[31]
Remote metabolic syndrome	[44]
Remote renal disease	[45, 46]
Remote retinal disease	[47]

subsequent cardiovascular mortality and morbidity strengthens with more severe pre-eclampsia, including early onset, recurrent disease and neonatal morbidity [30, 31, 39–42]. The risk for coronary heart disease, stroke and other cardiovascular events is highest among women who develop both maternal signs of pre-eclampsia (hypertension and proteinuria) and manifest abnormal placentation function, such as foetal growth restriction, especially with preterm delivery [43].

The surviving offspring of pre-eclamptic pregnancies also risks more remote health problems following early-onset as compared to late-onset disease, such as CVD [48–50]. However, the prematurity itself may explain many of the observed associations, because the prematurely delivered child may suffer from impaired development and growth of its organs. The risk of later hypertension in a child is as expected increased with any form of preterm delivery, with or without pre-eclampsia, but the underlying endothelial dysfunction seems to differ [51]. However, even late-onset pre-eclampsia confers increased risks of later arterial disease in the child, as reviewed by Davis et al. [48].

10.4 Epidemiology of Early- and Late-Onset Pre-eclampsia Rates and Risk Factors

Reliable population-based registry data from countries with a high maternal mortality from pre-eclampsia, which are low- and middle-income countries (LMIC), are lacking. A recent global survey by WHO included more than 270 thousand deliveries but did not report rates of early- and late-onset pre-eclampsia subtypes [12]. Many industrialized countries report similar proportions of early-onset versus late-onset pre-eclampsia, namely, 1/3 versus 2/3, when dichotomizing into preterm and

term pre-eclampsia (before or from GW 37+ 0 days). This is reported from the Medical Birth Registry of Norway [11], a high-income setting, as well as in a small case-control study from Thailand [52], the latter a LMIC setting. However, some data suggest relatively higher rates of the clinically challenging early-onset pre-eclampsia form in some LMIC settings [12]. A large region-based report from the Reunion Island reported almost 2/3 of pre-eclamptic pregnancies delivered before GW 37 (61%) and as many as 31% of pre-eclampsia cases delivered before GW 34 [53], representing a substantial problem with prematurity of the neonates and consequently more long-term health risks.

The general risk factors for pre-eclampsia are well-documented and reviewed in Chaps. 1 and 2 of this book. They include nulliparity, older maternal age, multiples, renal disease, chronic inflammatory diseases such as diabetes mellitus, obesity, chronic hypertension and some form of autoimmune diseases, as well as previous pre-eclampsia, a family history of pre-eclampsia and cardiovascular disease, new sexual partner or more than 10 years since last pregnancy with current partner, as well as use of barrier contraception prior to pregnancy. Publications comparing global pre-eclampsia rates have in general not focused on epidemiological differences in risk factors for early- versus late-onset pre-eclampsia. A recent relevant study [54] of a large cohort of singleton deliveries in Washington State, USA, concluded that African-American descent, chronic hypertension and congenital anomalies were more strongly associated with very-early-onset pre-eclampsia (delivery before GW 34), while younger maternal age, nulliparity and diabetes mellitus were more associated with late-onset disease.

The total rates for pre-eclampsia seem to have declined in many industrialized countries over the last decade [11, 55], despite increasing rates of obesity, an important risk factor for pre-eclampsia. Increasing international trends for induced delivery in women post-term, in order to avoid foetal demise, may be an explanation for the decrease observed in Europe and Australia [11, 55]. A Norwegian population-based study showed however that there was a small decrease in both early- and late-onset pre-eclampsia (defined as delivery before or after GW 37) from 1999 to 2008, after adjusting for maternal age and parity [11]. In addition to more aggressive post-term induction policies of many countries, low-dose aspirin has also been introduced as prophylaxis for pre-eclampsia in women at increased risk, possibly also contributing to a small decreasing trend of pre-eclampsia in high-income settings.

10.5 Previous Pathophysiological Understanding of Early- and Late-Onset Pre-eclampsia

Evidence pointing to the placenta as important in pre-eclampsia was presented more than hundred years ago, in a report by Schmorl in 1893, reporting trophoblasts in the lungs of women dying of pre-eclampsia (reviewed in [56]). Further major insight was gained by Robertson et al. (1967), who discovered that pre-eclampsia is associated with poor placentation, with shallow remodelling of maternal uteroplacental

spiral arteries during the first half of pregnancy [57]. Poor placentation is however more than shallow endovascular trophoblast invasion and is reviewed elsewhere in this book (Chap. 3).

It has been suggested that early- and late-onset pre-eclampsia are different diseases, due to differing risk factors and clinical presentation [20, 23, 54], but it is also argued that more data are needed to determine whether they really represent two distinct disorders or a spectrum of disease severity across a range of gestational ages [18]. Our current view is that both forms represent subtypes of the pre-eclampsia syndrome, where the causes and timing of placental dysfunction, the triggering event for the maternal vascular response, differ [58].

Before the 1990s, the secondary maternal manifestations of pre-eclampsia (with new-onset hypertension and proteinuria) were mistakenly regarded as primary. A 1990 publication of Redman [59] argued that the disordered tissue must be the placental (foetal) trophoblast, as a foetus is not needed (the same syndrome occurs with a complete hydatidiform mole), neither is the uterus (women with abdominal pregnancy may develop pre-eclampsia). Also, pre-eclampsia is cured after removal of the placenta, except in some rare cases of postpartum disease (reviewed in [7]). Thus, Redman argued that 'in its origins pre-eclampsia is a trophoblastic disease, not a disorder of the hemodynamic, renal, or even endothelial system, although processes involving these systems may be essential to its evolution' [59]. The concept of pre-eclampsia representing an endothelial cell dysfunction disorder was introduced by Roberts and coworkers in 1989 [60].

In general, placental histopathology is more common in early-onset than late-onset pre-eclampsia [26–29]. This includes more shallow endovascular trophoblast invasion with incomplete physiological remodelling of the maternal uteroplacental spiral arteries, as well as more placental parenchymal pathology. However, no uteroplacental artery or placental villous lesion has been identified to be uniquely associated with the early- or late-onset form of the disease. The findings of placentas in severe early-onset FGR also overlap with severe early-onset pre-eclampsia placentas [27].

The classical two-stage placental model of pre-eclampsia, which was introduced by Redman in 1991 [56], is often cited. The first stage was suggested to result from poor placentation, with shallow remodelling of uteroplacental spiral arteries during the first half of pregnancy. The ensuing uteroplacental malperfusion, placental oxidative and endoplasmic reticulum stress, then causes placental hypersecretion of pro-inflammatory and antiangiogenic factors into the maternal circulation, as reviewed by us previously [7, 58]. Maternal endothelium is the primary target for these inflammatory factors. The ensuing systemic endothelium dysfunction and excessive vascular inflammatory response can explain all the maternal features of the second stage of pre-eclampsia. The original two-stage model assumes that some degree of poor placentation occurs in all forms of pre-eclampsia, but this is incompatible with the fact that in most late-onset pre-eclampsia cases, delivering at or post term, the neonates are not growth restricted [19]. The original two-stage model is therefore applicable mainly to early-onset pre-eclampsia.

The understanding by most researchers until now has therefore been that early-onset pre-eclampsia is ascribed to poor placentation, whereas the late-onset form has been ascribed to predominantly maternal factors, with a normal placenta and a normal placentation. Many of the late-onset forms also fit with the concept named 'maternal' pre-eclampsia, proposed as a distinct pre-eclampsia subgroup by Ness and Roberts in 1996 [61]. Ness and Roberts suggested two types of pre-eclampsia, where 'placental pre-eclampsia' was described as having a 'placental circulation problem'. The suggested 'maternal pre-eclampsia' type has no evidence of poor placentation and, therefore, according to the authors, a normal placenta circulation. Consequently, late-onset or term pre-eclampsia has been believed to reflect an abnormal maternal reaction to placental-shed factors from a normally functioning placenta. A preset higher level of vascular inflammation has been believed to mediate this abnormal response [62], such as is predicted occurring in maternal obesity, diabetes mellitus and chronic hypertension, all risk factors for pre-eclampsia.

The major discovery by Karumanchi's team of how angiogenic and antiangiogenic factors of placental origin contribute to the pre-eclampsia syndrome [63, 64] helped to link stages 1 and 2 concepts of the original two-stage model of pre-eclampsia. Their roles are reviewed elsewhere in this book (Chap. 11). Not all pre-eclamptic pregnancies are however characterized by dysregulated circulating angiogenic factors, such as high maternal circulating levels of sFlt1 (soluble VEGF receptor-1) and low levels of PlGF (placenta growth factor). In general, early-onset pre-eclampsia is better diagnosed (and predicted) by a dysregulated circulating anti-angiogenic biomarker pattern (e.g. low PlGF and high sFlt1) than late-onset pre-eclampsia [65], as reviewed by us [34] and as documented indirectly for a large merged cohort of hypertensive pregnancy disorders [35].

10.6 Two Placental Pathways to Pre-eclampsia

Neither the two-stage model nor the 'placental and maternal' pre-eclampsia model can completely explain all the clinical aspects of the disease, neither the FGR heterogeneity nor why angiogenic factor biomarkers better identify and predict early-onset than late-onset disease. We argue that both pre-eclampsia and FGR represent non-specific syndromic entities, where there are many overlapping pathophysiological steps in their causation. Placentally derived biomarkers do not seem to distinguish well between these syndromes. For example, they partially identify preterm FGR, similarly to preterm pre-eclampsia, with respect to diagnosis [66–69] or prediction [70, 71].

We have recently proposed an alternative model, where both early- and late-onset pre-eclampsia develop in two stages. The first stage includes malperfusion and placental dysfunction, which leads to similar placental stress signalling to the mother that causes her clinical signs (stage 2). The key feature is that the cause and timing for the placental dysfunction and malperfusion differ between the early- and late-onset pre-eclampsia forms [7, 34]. Our concept is depicted in the figure.

The first placental pathway to stage 1 of the disease is the one previously described for early-onset pre-eclampsia (Fig. 10.1, Pathway A). Here, the placental dysfunction (stage 1) is extrinsically caused, as the incomplete remodelling of the maternal spiral arteries is underlying the abnormal uteroplacental perfusion, leading to the secondary syncytiotrophoblast (STB) stress and the release of pro-inflammatory factors into the maternal circulation. This early perturbation of placental growth and function is associated with FGR. The second placental pathway (Fig. 10.1, Pathway B), of late-onset pre-eclampsia, has an 'intrinsic' cause of placental dysfunction (stage 1 disease). This arises because the maturing placenta outgrows the capacity of the uterus and supporting maternal functions. The placenta becomes relatively under-perfused, with restricted intervillous perfusion, also causing syncytiotrophoblast (STB) stress, but at a later gestational age than the first pathway [7] in a previously normal placenta. This particularly happens with placental overgrowth associated with maternal obesity and large placentas. Late-onset pre-eclampsia is not usually associated with FGR and in line with our concept of a late STB stress. Hence, we suggest that both placental pathways ('extrinsic' and 'intrinsic') cause the same type of placental dysfunction (stage 1) and STB stress but in different contexts. We have in our revised model extended the original two-stage model, where stage 1 (placental dysfunction) was presented as an early event in pregnancy, by suggesting that stage 1 may also occur, for another reason, at or beyond term or even in relatively large placentas, as suggested for the 'intrinsic' placental pathway.

We have previously summarized the evidence for increased syncytiotrophoblast damage and stress in pre-eclampsia that we hypothesize is the common feature of stage 1 in both early- and late-onset pre-eclampsia [7]. In general, these findings have not been extensively investigated in early- versus late-onset pre-eclampsia. One of the problems is to identify satisfactory controls at term. Placentas that are exposed to uterine contractions and labour differ from those electively delivered without labour. Endoplasmic reticulum (ER) stress, a part of the general unfolded protein response, has been demonstrated in early-onset pre-eclampsia, but not in late-onset pre-eclampsia [72], as well as in FGR [73], and its relation to subtypes of placental dysfunction needs further investigation. Many post-term placentas exhibit similar morphological findings as early-onset pre-eclampsia [74], which is in our opinion indicative of STB stress by the congested and ageing placenta. In summary, we suggest that there are two causes of uteroplacental malperfusion underlying pre-eclampsia, as illustrated in the two pathways to stage 1 in Fig. 10.1.

A consequence of our revised model of two placental causes of pre-eclampsia is that the foetal growth restriction associated with early- and not late-onset pre-eclampsia is better explained than in any previous models. As the first pathway to stage 1 disease of pre-eclampsia is established early in pregnancy (Fig. 10.1, Pathway A), during the remodelling of uteroplacental spiral arteries in the first half of pregnancy, the placental dysfunction (stage 1) is reached early and is more likely to affect foetal growth, compatible with FGR being prevalent in early-onset pre-eclampsia. In late-onset disease, stage 1 is reached much later in pregnancy (Fig. 10.1, Pathway B); therefore, the offspring is rarely growth restricted.

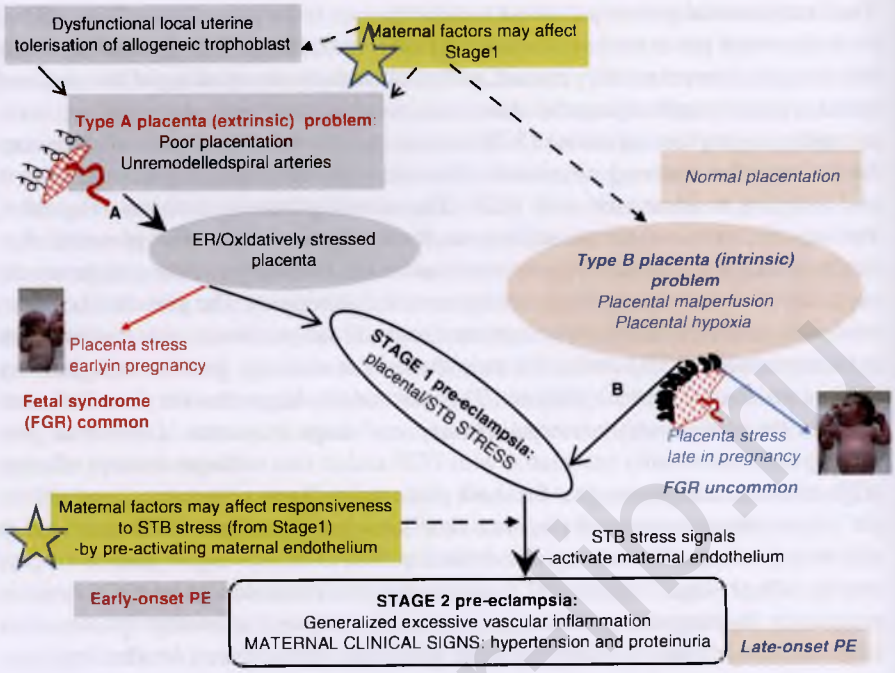


Fig. 10.1 Revised two-stage pre-eclampsia (PE) model: all PE is dependent on placenta syncytiotrophoblast (STB) stress, and maternal risk factors may impact on several levels and both stages. Pathway A (associated with early-onset PE) illustrates the 'extrinsic' cause, and pathway B (associated with late-onset PE) illustrates the 'intrinsic' cause for placental malperfusion and dysfunction (stage 1), leading to the clinically recognized maternal syndrome of pre-eclampsia (stage 2). Pathway B is what is seen in postmature placentas, where the size of the term placenta may restrict intervillous perfusion. These two pathways (A and B) to placental stress are further detailed previously by us [7]

Our revised model also explains the patterns of circulating anti- and pro-angiogenic biomarkers in pregnancy [34]. In general, the angiogenic biomarkers work best for early-onset pre-eclampsia, and not so well for late-onset pre-eclampsia. This is because, in terms of cellular mechanisms, these circulating factors are not biomarkers of pre-eclampsia, but of syncytiotrophoblast (STB) cellular stress. Hence, late-onset pre-eclampsia cannot be predicted early in pregnancy because there is no STB stress at that time and therefore no change in the stress biomarkers. Similarly, and as illustrated in Fig. 10.1, early-onset pre-eclampsia (and FGR) is predictable by dysregulated angiogenic circulating markers early in pregnancy because they reflect STB stress as an early consequence of poor placentation. At term, the circulating angiogenic biomarkers demonstrate that all pregnant women are beginning to develop a degree of STB stress. Hence, the background of 'normality' at this stage more closely resembles that of pre-eclampsia, which makes a pre-eclampsia diagnosis based on circulating biomarkers more difficult in late gestational ages. What seems to be normal is, in fact, not normal.

The revised model indicates that late-onset pre-eclampsia also depends on the placenta, not only on maternal factors and the maternal vasculature. If a dysfunctional maternal vasculature and metabolism were the sole cause of pre-eclampsia, it would develop independently of pregnancy, both in susceptible women and men, which is not the case. Our conclusion therefore is that there is no pre-eclampsia that does not involve the placenta (stage 1). In contrast to the original 'maternal pre-eclampsia' model, we propose that placental malperfusion underlies late-onset pre-eclampsia, as it does in early-onset disease, but the causation is different. It results from a villous constraint mechanism at the end of any normal pregnancy, more prematurely reached in cases of large placentas, such as in twins and moles [12, 15].

The original two-stage model of pre-eclampsia could not explain the FGR heterogeneity of early- and late-onset disease nor the impact of maternal risk. With respect to maternal risk factors, the new model incorporates the possibility that they act on multiple levels, in particular impeding placentation [7]. In this way they can potentially affect both stages of pre-eclampsia, as illustrated by the stars in Fig. 10.1. Maternal factors (such as chronic vascular disease, which includes women with obesity, autoimmune diseases, insulin resistance and chronic arterial disease) may not only impede placentation affecting placental growth and function (converging on stage 1 pathology of placental dysfunction) but also amplify maternal vascular sensitivity to placenta-shed factors (converging on stage 2, generating the maternal clinical signs). We predict that pre-pregnancy chronic vascular inflammation might affect placentation through several pathways (Fig. 10.1, Pathway A), including altering local decidual inflammatory interactions. Maternal factors may cause uteroplacental spiral artery inflammation and contribute to reduced endovascular trophoblast invasion into these arteries but not into the decidual interstitium, as is observed in early-onset pre-eclampsia [75]. On the other hand, maternal factors, such as obesity, may contribute to late uteroplacental malperfusion even with normal placentation, through increasing the risk of the 'intrinsic' placenta pathology in large and congested placentas (Fig. 10.1, Pathway B). Thirdly, we suggest that maternal risk factors may affect maternal vasculature responsiveness to placenta-shed inflammatory factors, explaining why a slight problem in stage 1 could result in clinical disease (stage 2). We also suggest that increasing inflammatory factors shed from an ageing placenta towards term could be sufficient to provoke clinically recognized stage 2 of pre-eclampsia in some susceptible women, such as in women with pre-pregnancy chronic vascular inflammation.

10.7 The New Two-Stage Model in Relation to Early- and Late-Onset Pre-eclampsia

We argue above that our revised two-stage model of pre-eclampsia (Fig. 10.1), with many potential combinations of maternal and placental risk factors and pathways, explains the heterogeneity of pre-eclampsia syndrome, including why FGR is more common in early- than late-onset disease.

Our revised two-stage model fits with other aspects of pre-eclampsia epidemiology, such as increasing risk of post-term HELLP and eclampsia [21], as all post-mature placentas have the potential to reach stage 1, owing to increasing STB stress in an ageing and enlarging placenta. We suggest that 'the pregnancy is a race', where delivery 'wins the prize of a normal pregnancy', while other post-term pregnancies lose the race and develop pre-eclampsia. An increasing trend over the last decade in developed countries of inducing post-term pregnancies to reduce the risk of postpartum foetal deaths might indeed have contributed to the lack of increasing rates of pre-eclampsia we have seen in several countries [11], despite a global trend of increasing obesity rates, the latter expectedly increasing pre-eclampsia rates according to epidemiological risk factors for pre-eclampsia and our revised model.

The new two-stage model could explain some rare forms of postpartum pre-eclampsia and eclampsia or even eclampsia diagnosed prior to the agreed 20 GW threshold for diagnosing pre-eclampsia. As seen from Fig. 10.1, any extreme forms of pre-pregnancy vascular inflammation, such as in systemic lupus, could result in very rapid developments of stage 1 and stage 2, justifying the rare forms of eclampsia seen very early in pregnancy in some of these patients. As for the rare, but well-documented, postpartum cases of eclampsia and pre-eclampsia (revised in [56]), they have all in common that placental tissue was recently present in the mother. Although placenta-derived circulating biomarkers such as sFlt1 fall rapidly after delivery of the placenta, we postulate that some inflammatory mediators from placenta remain postpartum. As parturition in itself may lead to unspecific vascular inflammation, we postulate that an added burden of vascular inflammation postpartum of any 'non-placental' cause, such as in endometritis and following delivery surgery, may lead to maternal clinical disease (stage 2) despite the absence of major placenta tissue in some susceptible women. However, the evidence is not yet available, and these are hypotheses that need to be tested.

Conclusion

We present evidence that placental malperfusion is the cause for both early- and onset pre-eclampsia, albeit for different reasons and with a different timing. Poor placentation and inadequate uteroplacental spiral artery remodelling leading to placental malperfusion with secondary placental oxidative and ER stress is the main cause for the early-onset type ('extrinsic placental pathway'). The late-onset type has little or no evidence of poor placentation but seems to be secondary to an intraplacental cause of placental malperfusion ('intrinsic placental pathway'), due to the growing placenta reaching its size limit, resulting in secondary malperfusion and a hypoxic placenta. Both pathways converge on STB and placenta stress, stimulating release of multiple inflammatory factors into the maternal circulation that disrupt endothelial function, of which hypertension and proteinuria are two signs. We argue that the placenta is the cause in all cases of pre-eclampsia and that maternal predisposing factors may affect both pathways leading to the first stage (placental dysfunction), as well as accelerating the steps towards the second stage of the disease (clinical presentation

with new-onset hypertension and proteinuria). Our revised two-stage model of pre-eclampsia is consistent with the clinical presentations of early- and late-onset pre-eclampsia, in addition to being compatible with what maternal placenta-associated circulating biomarkers can diagnose and predict regarding early- and late-onset disease.

References

1. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122–31.
2. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97–104.
3. Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel JP, et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121(Suppl 1):14–24.
4. Vogel JP, Souza JP, Mori R, Morisaki N, Lumbiganon P, Laopaiboon M, et al. Maternal complications and perinatal mortality: findings of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121(Suppl 1):76–88.
5. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013;170(1):1–7.
6. Lindheimer MD, Spargo BH, Katz AI. Eclampsia during the 16th gestational week. JAMA. 1974;230(7):1006–8.
7. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia—two placental causes of preeclampsia? Placenta. 2014;35(Suppl):S20–S5.
8. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol. 2012;36(1):56–9.
9. Duley L. Pre-eclampsia and the hypertensive disorders of pregnancy. Br Med Bull. 2003;67:161–76.
10. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA, Giannone PJ. Maternal preeclampsia and neonatal outcomes. J Pregnancy. 2011;2011:214365.
11. Klungsoyr K, Morken NH, Irgens L, Vollset SE, Skjaerven R. Secular trends in the epidemiology of pre-eclampsia throughout 40 years in Norway: prevalence, risk factors and perinatal survival. Paediatr Perinat Epidemiol. 2012;26(3):190–8.
12. Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP. Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis. PLoS One. 2014;9(3):e91198.
13. Firoz T, Sanghvi H, Merialdi M, von Dadelszen P. Pre-eclampsia in low and middle income countries. Best Pract Res Clin Obstet Gynaecol. 2011;25(4):537–48.
14. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy. 2003;22(2):143–8.
15. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013;3(1):44–7.
16. Douglas KA, Redman CW. Eclampsia in the United Kingdom. BMJ. 1994;309(6966):1395–400.
17. Broekhuijsen K, van Baaren GJ, van Pampus MG, Ganzevoort W, Sikkema JM, Woiski MD, et al. Immediate delivery versus expectant monitoring for hypertensive disorders of pregnancy between 34 and 37 weeks of gestation (HYPITAT-II): an open-label, randomised controlled trial. Lancet. 2015;385(9986):2492–501.

18. Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. Obstet Gynecol Surv. 2011;66(8):497–506.
19. Xiong X, Demianczuk NN, Saunders LD, Wang FL, Fraser WD. Impact of preeclampsia and gestational hypertension on birth weight by gestational age. Am J Epidemiol. 2002;155(3):203–9.
20. Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease? BJOG. 2004;111(4):298–302.
21. Caughey AB, Stotland NE, Escobar GJ. What is the best measure of maternal complications of term pregnancy: ongoing pregnancies or pregnancies delivered? Am J Obstet Gynecol. 2003;189(4):1047–52.
22. Sibai BM, Ramadan MK, Usta I, Salama M, Mercer BM, Friedman SA. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome). Am J Obstet Gynecol. 1993;169(4):1000–6.
23. Moore MP, Redman CW. Case-control study of severe pre-eclampsia of early onset. Br Med J (Clin Res Ed). 1983;287(6392):580–3.
24. Verlohren S, Melchiorre K, Khalil A, Thilaganathan B. Uterine artery Doppler, birthweight and timing of preeclampsia onset: providing insights into the dual etiology of late-onset preeclampsia. Ultrasound Obstet Gynecol. 2014;44:293–8.
25. Murphy DJ, Stirrat GM. Mortality and morbidity associated with early-onset preeclampsia. Hypertens Pregnancy. 2010;19:221–31.
26. Sebire NJ, Goldin RD, Regan L. Term preeclampsia is associated with minimal histopathological placental features regardless of clinical severity. J Obstet Gynaecol. 2005;25(2):117–8.
27. Mifsud W, Sebire NJ. Placental pathology in early-onset and late-onset fetal growth restriction. Fetal Diagn Ther. 2014;36(2):117–28.
28. Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. Am J Obstet Gynecol. 2003;189(4):1173–7.
29. Ogge G, Chaiworapongsa T, Romero R, Hussein Y, Kusanovic JP, Yeo L, et al. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. J Perinat Med. 2011;39(6):641–52.
30. Funai EF, Friedlander Y, Paltiel O, Tiram E, Xue X, Deutsch L, et al. Long-term mortality after preeclampsia. Epidemiology. 2005;16(2):206–15.
31. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. Hypertension. 2010;56(1):166–71.
32. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. Obstet Gynecol. 2001;97(4):533–8.
33. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension. 2009;53(5):812–8.
34. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. Am J Obstet Gynecol. 2015;213(4 Suppl):S9–4.
35. Burke O, Benton S, Szafranski P, von Dadelszen P, Buhimschi SC, Cetin I, et al. Extending the scope of pooled analyses of individual patient biomarker data from heterogeneous laboratory platforms and cohorts using merging algorithms. Pregnancy Hypertens. 2016;6(1):53–9.
36. Hnat MD, Sibai BM, Caritis S, Hauth J, Lindheimer MD, MacPherson C, et al. Perinatal outcome in women with recurrent preeclampsia compared with women who develop preeclampsia as nulliparas. Am J Obstet Gynecol. 2002;186(3):422–6.
37. Ebbing C, Rasmussen S, Skjaerven R, Irgens LM. Risk factors for recurrence of hypertensive disorders of pregnancy, a population based cohort study. Acta Obstet Gynecol Scand. 2017;96(2):243–50.
38. Staff AC, Redman CW, Williams D, Leeson P, Moe K, Thilaganathan B, et al. Pregnancy and long-term maternal cardiovascular health: progress through harmonization of research cohorts and biobanks. Hypertension. 2016;67(2):251–60.
39. Wikstrom AK, Haglund B, Olovsson M, Lindeberg SN. The risk of maternal ischaemic heart disease after gestational hypertensive disease. BJOG. 2005;112(11):1486–91.

40. Ness RB, Hubel CA. Risk for coronary artery disease and morbid preeclampsia: a commentary. Ann Epidemiol. 2005;15(9):726–33.
41. Skjaerven R, Wilcox AJ, Klungsoyr K, Irgens LM, Vikse BE, Vatten LJ, et al. Cardiovascular mortality after pre-eclampsia in one child mothers: prospective, population based cohort study. BMJ. 2012;345:e7677.
42. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ. 2001;323(7323):1213–7.
43. Newstead J, von Dadelszen DP, Magee LA. Preeclampsia and future cardiovascular risk. Expert Rev Cardiovasc Ther. 2007;5(2):283–94.
44. Stekkinger E, Zandstra M, Peeters LL, Spaanderman ME. Early-onset preeclampsia and the prevalence of postpartum metabolic syndrome. Obstet Gynecol. 2009;114(5):1076–84.
45. Ihle BU, Long P, Oats J. Early onset pre-eclampsia: recognition of underlying renal disease. Br Med J (Clin Res Ed). 1987;294(6564):79–81.
46. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. Preeclampsia and the risk of end-stage renal disease. N Engl J Med. 2008;359(8):800–9.
47. Auger N, Fraser WD, Paradis G, Healy-Profitos J, Hsieh A, Rheaume MA. Preeclampsia and long-term risk of maternal retinal disorders. Obstet Gynecol. 2017;129(1):42–9.
48. Davis EF, Newton L, Lewandowski AJ, Lazdam M, Kelly BA, Kyriakou T, et al. Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. Clin Sci (Lond). 2012;123(2):53–72.
49. Davis EF, Lazdam M, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y, et al. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. Pediatrics. 2012;129(6):e1552–e61.
50. Fugelseth D, Ramstad HB, Kvehaugen AS, Nestaas E, Stoylen A, Staff AC. Myocardial function in offspring 5-8years after pregnancy complicated by preeclampsia. Early Hum Dev. 2011;87(8):531–5.
51. Lazdam M, de la Horra A, Pitcher A, Mannie Z, Diesch J, Trevitt C, et al. Elevated blood pressure in offspring born premature to hypertensive pregnancy: is endothelial dysfunction the underlying vascular mechanism? Hypertension. 2010;56(1):159–65.
52. Aksornphusitaphong A, Phupong V. Risk factors of early and late onset pre-eclampsia. J Obstet Gynaecol Res. 2013;39(3):627–31.
53. Iacobelli S, Bonsante F, Robillard PY. Pre-eclampsia and preterm birth in Reunion Island: a 13 years cohort-based study. Comparison with international data. J Matern Fetal Neonatal Med. 2016;29(18):3035–40.
54. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol. 2013;209(6):544.e1–e12.
55. Roberts CL, Ford JB, Algert CS, Antonsen S, Chalmers J, Cnattingius S, et al. Population-based trends in pregnancy hypertension and pre-eclampsia: an international comparative study. BMJ Open. 2011;1(1):e000101.
56. Redman CW. Current topic pre-eclampsia and the placenta. Placenta. 1991;12(4):301–8.
57. Robertson WB, Brosens I, Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J Pathol Bacteriol. 1967;93:581–92.
58. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. Hypertension. 2013;61(5):932–42.
59. Redman CW. Platelets and the beginnings of preeclampsia. N Engl J Med. 1990;323(7):478–80.
60. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol. 1989;161(5):1200–4.
61. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. Am J Obstet Gynecol. 1996;175(5):1365–70.
62. Roberts JM, Redman CWG. Preeclampsia—more than pregnancy-induced hypertension. Lancet. 1993;341(8858):1447–51.
63. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350(7):672–83.

64. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111(5):649–58.
65. Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. Am J Obstet Gynecol. 2010;202(1):40–7.
66. Benton SJ, McCowan LM, Heazell AE, Grynspan D, Hutcheon JA, Senger C, et al. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction. Placenta. 2016;42:1–8.
67. Herraiz I, Droge LA, Gomez-Montes E, Henrich W, Galindo A, Verlohren S. Characterization of the soluble fms-like tyrosine kinase-1 to placental growth factor ratio in pregnancies complicated by fetal growth restriction. Obstet Gynecol. 2014;124(2 Pt 1):265–73.
68. Molvarec A, Gullai N, Stenczer B, Fugedi G, Nagy B, Rigo J Jr. Comparison of placental growth factor and fetal flow Doppler ultrasonography to identify fetal adverse outcomes in women with hypertensive disorders of pregnancy: an observational study. BMC Pregnancy Childbirth. 2013;13:161.
69. Triunfo S, Lobmaier S, Parra-Saavedra M, Crovetto F, Peguero A, Nadal A, et al. Angiogenic factors at diagnosis of late-onset small-for-gestational age and histological placental underperfusion. Placenta. 2014;35(6):398–403.
70. Ghosh SK, Raheja S, Tuli A, Raghunandan C, Agarwal S. Can maternal serum placental growth factor estimation in early second trimester predict the occurrence of early onset pre-eclampsia and/or early onset intrauterine growth restriction? A prospective cohort study. J Obstet Gynaecol Res. 2013;39(5):881–90.
71. Schoofs K, Grittner U, Engels T, Pape J, Denk B, Henrich W, et al. The importance of repeated measurements of the sFlt-1/PlGF ratio for the prediction of preeclampsia and intrauterine growth restriction. J Perinat Med. 2014;42(1):61–8.
72. Yung HW, Atkinson D, Campion-Smith T, Olovsson M, Charnock-Jones DS, Burton GJ. Differential activation of placental unfolded protein response pathways implies heterogeneity in causation of early- and late-onset pre-eclampsia. J Pathol. 2014;234(2):262–76.
73. Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS, et al. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. Am J Pathol. 2008;173(2):451–62.
74. Jones CJ, Fox H. Ultrastructure of the placenta in prolonged pregnancy. J Pathol. 1978;126(3):173–9.
75. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in pre-eclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension. 2013;62(6):1046–54.

Part V

Prediction of Preeclampsia

sFlt-1/PLGF

11

Holger Stepan and Janine Hoffmann

Abstract

Due to novel scientific knowledge about molecular pathomechanisms, there is a new understanding of preeclampsia as a placental disease. Angiogenic factors were shown to influence placentation, and in the last decade, intensive research emerged particularly the antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) and the pro-angiogenic placental growth factor (PlGF) to be most relevant in this process. Technical efforts and the development of commercially available automated methods firstly enabled maternal serum measurements of these factors and the introduction into daily clinical use. In various clinical studies, the additional value of sFlt-1 and PlGF for diagnosis and even prediction of preeclampsia have been confirmed. This major advance firstly allows a better diagnosis and distinction of preeclampsia, a disease with an extremely heterogeneous clinical appearance, from various different gestation-associated symptoms or diseases. A more precise diagnosis and the feasibility of prediction opened new horizons in clinical management of preeclampsia. This article gives an overview about the latest scientific knowledge about the angiogenic factors sFlt-1 and PlGF and provides actual recommendations for its clinical use for suspected preeclampsia.

Keywords

Preeclampsia • Placental disease • Pregnancy • sFlt-1 • PlGF

H. Stepan, M.D. (✉) • J. Hoffmann, M.D.
Department of Obstetrics, University of Leipzig, Leipzig, Germany
e-mail: holger.stepan@medizin.uni-leipzig.de; janine.hoffmann@medizin.uni-leipzig.de

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_11

11.1 Introduction

Since molecular pathways and pathophysiology are better understood, today, angiogenic factors play an increasingly important role in diagnosis, prognosis, and even early prediction of preeclampsia. The current standard diagnostic approach by measurement of only blood pressure and proteinuria must be considered outdated because it is strongly limited for diagnosis and even insufficient for prognosis and prediction. Particularly preexisting hypertension and/or proteinuria hampers the diagnostic power of the old standard diagnostic tools. Because, in turn, the right diagnosis is decisively required for determination of the correct subsequent management, preeclampsia has been subject to various innovative studies. The current knowledge about the role of angiogenic factors in the pathology and pathophysiology of preeclampsia now opens new horizons. Intensive investigations of the last years showed the diagnostic and predictive value of angiogenic factors and amended current standard diagnostic, yet. Therefore, preeclampsia became less erratic and unpredictable than it had been before. With this newly developed understanding, preeclampsia has to be considered as ischemic placental disease on the basis of endothelial disorders [1]. Special research focused on the antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) which is the soluble vascular endothelial growth factor receptor-1 (VEGFR-1) and the pro-angiogenic placental growth factor (PlGF). As will be demonstrated in the following, the angiogenic imbalance plays an important role in the development of preeclampsia. With the recent introduction of automated methods, the biomarker sFlt-1 and PlGF can be reliably assessed in clinical practice. These factors are even more interesting because they do not only reflect the presence but also the severity of the disease. The additional value of the biomarkers sFlt-1 and PLGF and its ratio sFlt-1/PlGF tremendously influenced clinical routine management of patients with suspected preeclampsia by extending current diagnostic standards and improving diagnostic reliability.

11.2 Anti-angiogenesis Is an Important Mechanism in the Pathogenesis of Preeclampsia

Preeclampsia as a placental disease does not exist without the placenta, correlates with placental pathology, and ends with the removal of the placenta [2, 3]. Various factors are involved in the placentation process and therefore also in the development of preeclampsia. By its multifactorial pathogenesis, multiple tissues and organ systems are affected. The end-stage manifestation of preeclampsia involves the whole maternal body, foremost the kidneys, the liver, and the brain. The fetal manifestation foremost comprises intrauterine growth restriction and often causes preterm birth with all its complications (Fig. 11.1).

At the molecular level, an imbalance of pro-angiogenic and antiangiogenic factors such as vascular endothelial growth factor (VEGF), soluble endoglin (sEng), sFlt-1, and PlGF is considered to be substantially involved in the pathogenesis of placental dysfunction. The observation that cancer patients, who were treated with

VEGF blockers, developed preeclamptic symptoms and typical morphologic changes of endothelial cells [4, 5] initially indicated that VEGF might be involved in a signaling chain, meaningful for the pathogenesis of preeclampsia. As one of the components of the vasodilator system, VEGF is today known to be necessary for the normal placentation process. Also PlGF and other factors such as angiopoietin 1 and angiopoietin 2 [6, 7] are involved in the vascular remodeling process, each with its specific but overlapping activity spectra.

The maternal protein sFlt-1 is a splice variant of the VEGF receptor. By disturbance of the placental adaption process, it is substantially involved into the pathogenesis of preeclampsia [2, 5, 8, 9]. Lacking from the transmembrane and the cytoplasmatic domain, sFlt-1 binds as an antagonist to the receptor-binding domains of VEGF and PLGF with high affinity. This causes a reduction of free VEGF and PLGF serum concentrations so that interaction with the surface endothelial factors is inhibited and the signaling chain in the endothelial cells disrupted (Figs. 11.1 and 11.2). Consequently, spiral artery transformation is disturbed and additionally, typical changes of endothelial morphology are also triggered in other tissues. Endothelial cells lose their flat appearance and therewith their function [1, 2]. These effects, most evidently in the glomerular endothelium, lead to the classic histological findings with cell swelling,

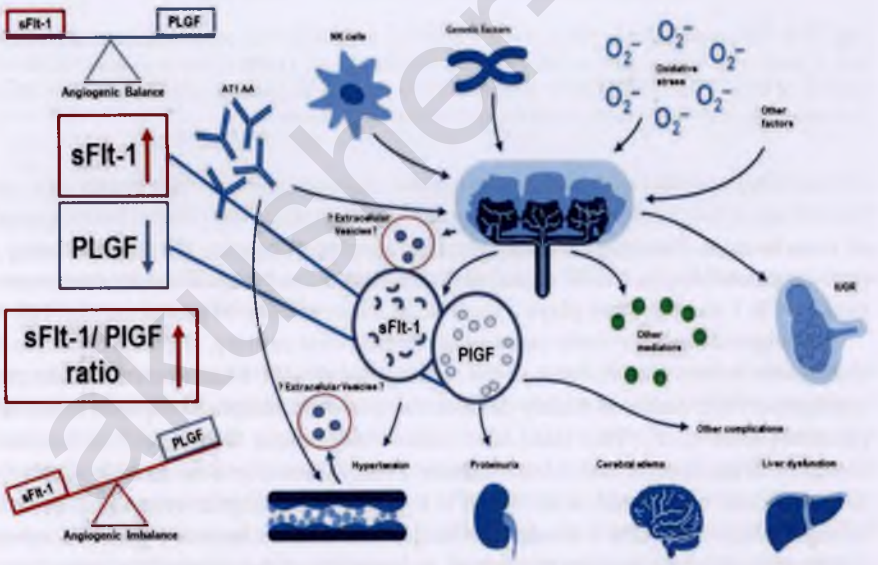


Fig. 11.1 In normal pregnancies, sFlt-1 and PlGF are in a physiological angiogenic balance. Various factors and mediators influence the trophoblast invasion and placentation and in case of preeclampsia cause excessive production and liberation of sFlt-1 by different cascades. By binding of the pro-angiogenic PlGF, high sFlt-1 levels result in an unphysiological increase of the sFlt-1/PlGF ratio (angiogenic imbalance). Measurement of sFlt-1/PlGF ratio helps to identify women with preeclampsia and those who are likely to develop preeclampsia. AT1 AA = angiotensin-converting enzyme autoantibodies. NK cells = natural killer cells (adapted from Wang A et al. [103])

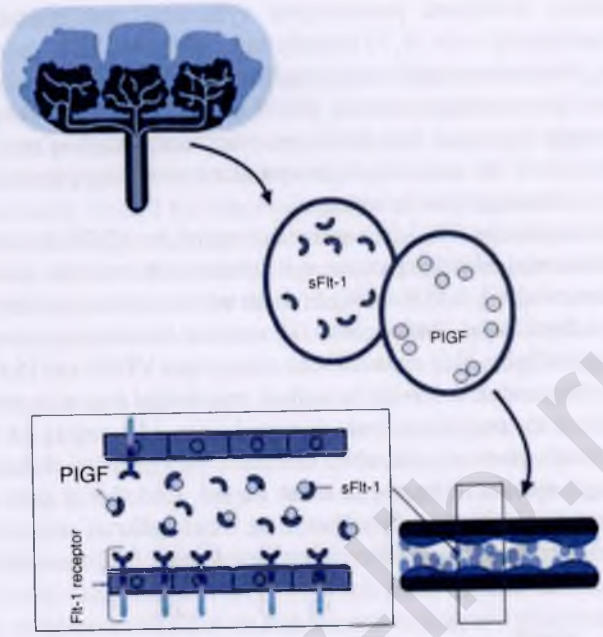


Fig. 11.2 An excessive production of sFlt-1 is known in patients with preeclampsia. As an antagonist, it binds with high affinity to free PlGF in maternal serum. Thereby, pro-angiogenic effects by binding of PlGF to membranous Flt-1 receptors (= a vascular endothelial growth factor) are inhibited and are thought to be responsible for endothelial dysfunction

the so-called endotheliosis, and microvascular obstruction [2, 8]. The angiogenic factors influence the function of various tissues which explains the clinical heterogeneity of preeclampsia. Because the cardiovascular adaptation process during pregnancy is partly regulated by the VEGF signal pathway, it also can be disturbed by overexpression of sFlt-1 and therefore plays a key role in the syndrome of preeclampsia [10].

Investigated since the early years of the twenty-first century, altered expression of angiogenic factors with increase of the antiangiogenic sFlt-1 and decrease of the pro-angiogenic PlGF has been widely demonstrated in preeclamptic compared to healthy patients (Table 11.1). This state, also called "angiogenic imbalance," is foremost observed in the second and third trimester. Firstly shown in a rat model, where the animals were transfected with the sFlt-1 gene, preeclamptic symptoms became stronger with rising sFlt-1 levels [2]. Actually, a relation between maternal serum concentrations of the angiogenic factors and severity of the disease has been shown in a lot of human studies. Severity is negatively correlated with the pro-angiogenic factor PlGF and positively with the antiangiogenic factor sFlt-1 [11–17].

Important pioneering clinical research gave hope that angiogenic factors could also be used for prediction of preeclampsia. Increased maternal sFlt-1 serum concentrations and decreased PlGF serum concentrations were found about 5 weeks prior to the onset of the disease [11, 18]. A dynamic of maternal sFlt-1 serum concentrations during pregnancy can not only be observed in preeclamptic patients. In

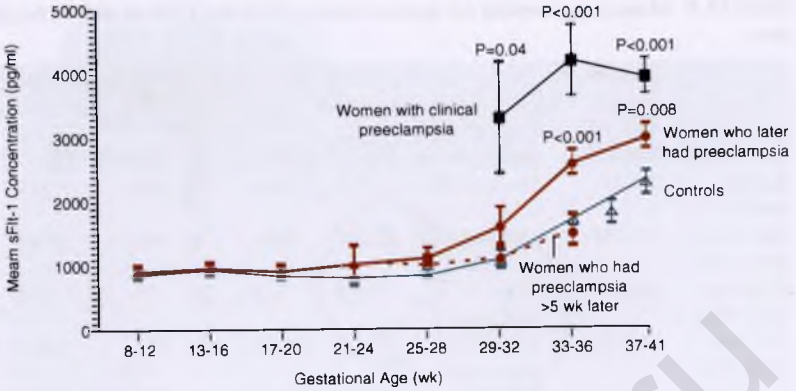
Table 11.1 Measurement results for maternal serum sFlt-1 and PlGF as well as the sFlt-1/PlGF ratio

References	Number of patients with PE (controls)	Time of measurement	Median sFlt-1 (pg/mL)		Median PlGF (pg/mL)		Median sFlt-1/PlGF ratio	
			PE	Controls	PE	Controls	PE	Controls
Levine et al. [11]	23 (23)	Onset of PE	4382	1643	137	669	32.0	2.5
Verlohren et al. [12]	71 (268)	Onset of PE	12,981	2641	76	342	354.5	19.4
Thadhani et al. [87]	40 (80)	First trimester	1048	973	23	63	45.6	15.4
De Vivo et al. [14]	52 (52)	Second trimester	20,330	7169	200	961	106.7	12.7
		Third trimester	44,870	12,560	91	852	411.7	19.2
Kusanovic et al. [98]	62 (1560)	12th gw	1426	1726	24	34	62.5	51.1
		22nd gw	1637	1612	214	330	5.9	4.8
Lim et al. [99]	40 (100)	14th–21st gw	4945	2788	100	175	75.5	19.4
Kim et al. [100]	46 (100)	16th–18th gw	3861	2353	86	146	39.8	15.8
Sunderji et al. [16]	39 (388)	20th–36th gw	91,514	2416	12	447	7626.2	5.4
Stepan et al. [101]	12 (38)	21st–22nd gw	1927	452	119	184	16.2	2.5
Tsatsaris et al. [17]	19 (31)	30th–38th gw	2690	120	67	586	39.9	0.2
Ohkuchi et al. [15]	34 (144)	32nd gw	10,471	3019[d]	53	549	195.0	5.5
Shibata et al. [13]	26 (27)	34th–35th gw	5221	1857	86	228	60.7	8.1

Various studies at various time points of gestational age show a significant increase of sFlt-1 and sFlt-1/PlGF ratio and a significant decrease of PlGF in patients with manifest or later preeclampsia (adapted from Lapaire et al. [102])

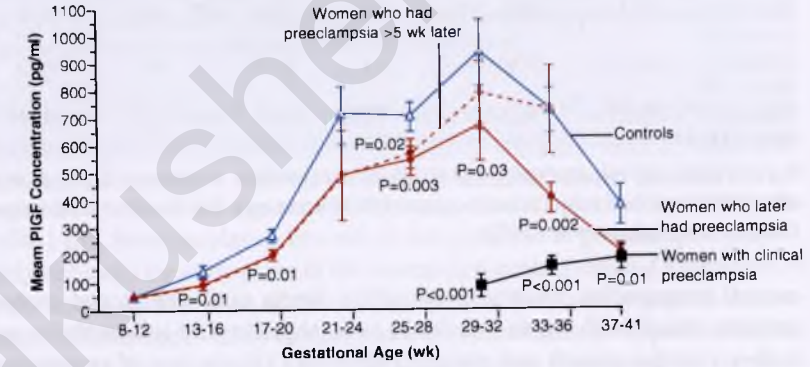
normal pregnancies, there are low sFlt-1 levels until the second trimester. An increase after 33–36 weeks was found to be physiological and might be associated with a vascular growth and placental ripening [11]. In case of preeclampsia, this physiological increase is extremely exceeded and occurs much earlier than in uneventful pregnancies (Fig. 11.3). Since sFlt-1 binds to free PLGF, their free serum concentrations are associated with each other. So, also the decrease of free PlGF serum concentrations is physiological in healthy pregnancies at term but significantly stronger in patients with preeclampsia [19, 20] (Fig. 11.4).

Inhomogeneity of preeclampsia challenges the handling of the disease. Different subtypes or even different entities are discussed, such as early onset vs. late onset, severe vs. mild forms, preeclampsia in high-risk vs. low-risk patients, or preeclampsia with intrauterine growth restriction (IUGR) vs. without IUGR. Furthermore the involved organs, the liver, kidney, cardiovascular system, brain, and/or placenta, often differ between patients with preeclampsia [21]. Interestingly, the increase of



No. of specimans								
Controls	20	44	56	9	72	21	70	21
Before preeclampsia	21	43	56	6	75	23	57	19
>5 wk before preeclampsia	21	43	56	6	71	19	8	—
During preeclampsia	—	—	—	—	—	2	14	26

Fig. 11.3 A physiological increase of sFlt-1 can be observed in healthy pregnancies. In patients with preeclampsia and those who will later develop a preeclampsia, this increase is exceeded. In the second and third trimester, mean differences from normal are the higher the earlier gestational age and the more severe or acute the disease (Levin et al. [11] reused with permission)



No. of specimans								
Controls	20	44	56	9	72	21	70	21
Before preeclampsia	21	43	56	6	75	23	57	19
>5 wk before preeclampsia	21	43	56	6	71	19	8	—
During preeclampsia	—	—	—	—	—	2	14	26

Fig. 11.4 The pro-angiogenic factor PlGF physiologically decreases in the third trimester of healthy pregnancies. In patients with preeclampsia, significantly lower PlGF levels can be observed and cause impaired placental and endothelial function. As for sFlt-1 in the second and third trimester, mean differences of PlGF serum levels in patients with preeclampsia differ strongly from normal with earlier gestational age and severity of the disease (Levin et al. [11] reused with permission)

sFlt-1 correlates with the severity of clinical disease, and sFlt-1 levels rapidly normalize after the delivery of the placenta [11, 22]. Angiogenic profiles are even known to depend on the type of hypertensive disorder and are also altered in patients with chronic diseases [23–29].

Trigger factors for the development of preeclampsia are expected to be multifactorial. Increased sFlt-1 levels in patients with preeclampsia but also in patients with chronic diseases support the hypothesis that hypoxia is one of the key factors triggering excessive sFlt-1 liberation [30, 31]. Whether the excessive sFlt-1 released by the placenta is the primary event or a secondary effect has not been finally clarified, yet. However, a lot of factors and different mediators affecting the highly complex pathomechanism of preeclampsia are identified. New, current research has focused on extracellular vesicles which are supposed to trigger the pathogenesis of preeclampsia and are linked to the angiogenic factors. Extracellular vesicles are involved in the corpuscular level of the cell-to-cell communication. Depending on their tissue origin (e.g., endothelial, vascular smooth muscle, blood cells), they differ in concentration and content. Cell-to-cell communication can be realized by exocytosis and endocytosis. Specific contents might induce inflammation and procoagulatory processes which in turn contribute to the development or progression of preeclampsia. There are some recent studies that showed altered extracellular vesicles even in nonpregnant women with risk factors for preeclampsia (e.g., age, parity, obesity, diabetes, or autoimmune diseases or placental abnormalities) [32–37]. Placental risk factors, primarily impaired perfusion and hypoxia, are supposed to cause the release of extracellular vesicles. This underlines the hypothesis that extracellular vesicles are involved in the pathogenesis of preeclampsia, if not causatively then probably at least significantly [38, 39].

Also genetic or immune factors are supposed to influence the cytotrophoblast invasion and placentation, probably by induction of excessive production of antiangiogenic factors [40, 41] (Fig. 11.1). So, natural killer (NK) cells are thought to be an immune modulator that substantially disturbs the development of immunotolerance, the induction of angiogenic factors, and the vascular remodeling required for normal placentation [40]. Also the renin-angiotensin-aldosterone axis is known to be involved. Vascular response to vasoconstrictive agents, such as angiotensin II, is increased in patients with preeclampsia. Angiotensin II as one of various AT1 receptor autoantibodies (AT1 AA) has been shown to induce an increased release of the antiangiogenic factors sFlt-1 and sEng [42, 43].

If heterogeneity of the disease might be better explained by other, hitherto still unknown variables is one of the still open questions. Pathogenesis and molecular pathomechanisms of preeclampsia are not yet completely investigated, but nevertheless, the current state of research reached a milestone. The main achievements of science are the detection and investigation of the meaning of angiogenic factors, primarily sFlt-1 and PlGF, for the development of preeclampsia. With implication of sFlt-1 and PlGF into clinical practice, diagnosis, prognosis, and prediction are today more reliable than ever before.

11.3 Diagnosis of Preeclampsia with the Use of Angiogenetic Factors

Classically, the diagnosis of preeclampsia is based on the new onset of elevated blood pressure (≥140/90 mmHg) and proteinuria (≥300 mg/24 h or a protein-creatinine ratio in spontaneous urine) after the 20^0 gestational weeks. Clinical signs like edemas, renal or hematological dysfunction, as well as hepatic, pulmonary, or neurological symptoms, and IUGR are common but occur with a large variety and not in each patient. Severe preeclampsia is diagnosed if at least one of the following clinical signs complicates pregnancy: blood pressure ≥160/110 mm Hg, renal dysfunction (creatinine ≥79.6 μmol/L (0.9 mg/dL) or oliguria <500 mL/24 h), hepatic dysfunction (elevated liver enzymes, persistent upper abdominal pain), lung edemas, hematologic diseases (thrombocyte count <100 Gpt/L, hemolysis), neurologic symptoms (severe headache, visual defects), or fetal growth restriction (estimated fetal weight <5th percentile) [44, 45]. Because of the high heterogeneity and the fact that clinical signs are not pathognomonic, diagnostic is challenging in clinical practice. Particularly pregnancy-associated symptoms and other comorbidities with chronic or acute diseases like autoimmune diseases, gastrointestinal disorders, migraine, or epilepsy often hamper diagnostics. In patients with preexisting proteinuria or kidney diseases, the classical clinical signs hypertension and proteinuria are diagnostically not useful. Furthermore, a precise diagnosis or classification with regard to severity is not possible by the classical standard of care. Women with preeclamptic signs or symptoms are generally rather overclassified by its use.

Now, the angiogenic factors sFlt-1 and PlGF and particularly the sFlt-1/PlGF ratio offer additional help. Since sFlt-1 levels were shown to be directly proportional to the severity of proteinuria, and inversely correlated to platelet count and gestational age-adjusted neonatal birth weight, they found their way into clinical use [46, 47]. With this parameter, preeclampsia can be reliably detected with high sensitivity and specificity. Patients with preeclampsia have significantly higher sFlt-1 levels and lower PlGF levels and therefore significantly raised sFlt-1/PlGF ratios. The sFlt-1/PlGF ratio has been shown to be the most reliable diagnostic parameter [12, 26] (Fig. 11.5). With a high sensitivity and specificity of up to 84% and 95%, respectively, the sFlt-1/PlGF ratio is also helpful for differentiation between patients with preeclampsia and gestational and chronic hypertension. Compared to normal pregnancies, sFlt-1 levels and sFlt-1/PlGF ratios are slightly increased in patients with chronic or gestational hypertension and higher in the later (≥34^0 gestational weeks) than in the earlier gestational phase (<34^0 gestational weeks). In comparison, patients with preeclampsia have a significantly stronger increase of sFlt-1 levels and sFlt-1/PlGF ratios which is highest in early onset (<34^0 gestational weeks) and severe forms and in case of coincident SGA [2, 11, 25, 30, 46, 48–55] (Fig. 11.6). Compared to healthy controls, maternal serum sFlt-1 concentrations were 43 times higher in early-onset and threefold higher in late-onset disease (≥34^0 gestational weeks). PLGF serum concentrations were 21 times vs. fivefold lower, respectively [52].

In a cross-sectional study of Tripathi et al., sFlt-1 measurements demonstrated 89% sensitivity and 90% specificity in early preeclampsia as compared to 55% sensitivity and 58% specificity in late preeclampsia [30]. Since sFlt-1 is considered to be produced by the placenta [56], this observation supports the hypothesis that

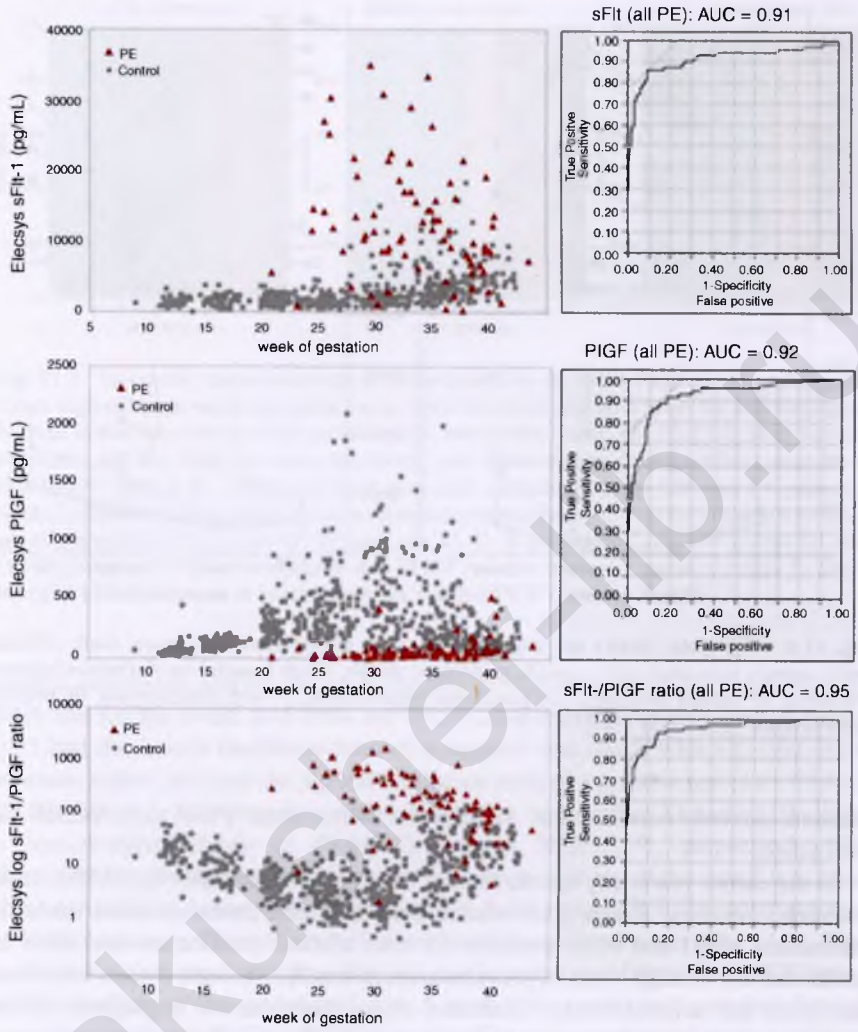


Fig. 11.5 The sFlt-1/PlGF ratio can reliably be used for discrimination between patients with normal pregnancies and those with preeclampsia. Highest sensitivities and specificities (AUC 0.95) were shown for the sFlt-1/PlGF ratio compared to measurements of only sFlt-1 (AUC 0.91) and/or PlGF (AUC 0.92) (Verlohren et al. [12] reused with permission). *PE* preeclampsia, *AUC* area under the curve

early-onset preeclampsia is more placenta derived than late-onset preeclampsia. Late forms are considered to be predominantly a maternal disease or a disturbed maternal response to still unknown factors. Different angiogenic states in early and late pregnancy but also in mild and severe forms indicate that the classic definition of preeclampsia has to be revised. Probably, preeclampsia should rather be classified by different entities. Assuming that preeclamptic symptoms can be related to a severe placental disease but also can occur because of other, not primarily or less

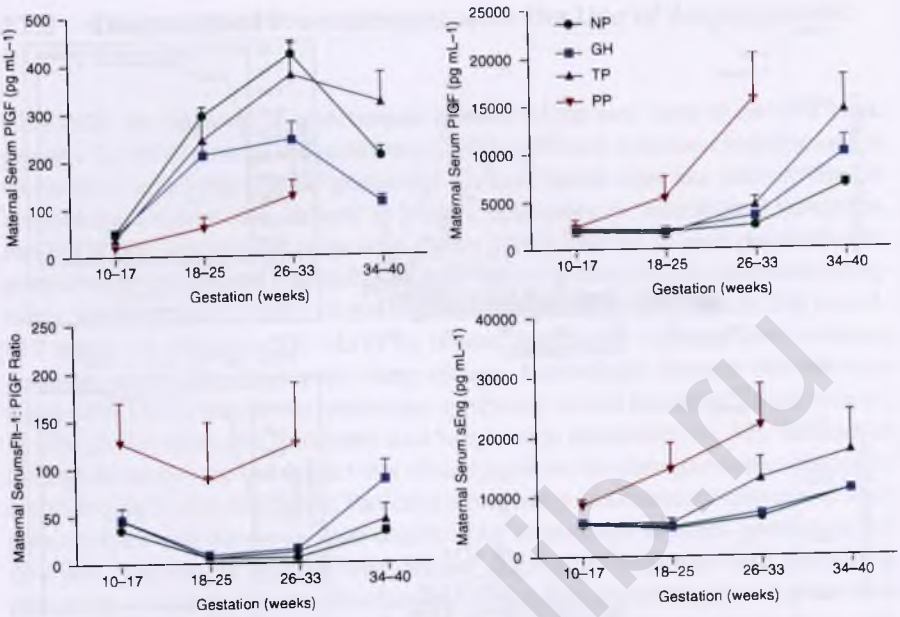


Fig. 11.6 Angiogenic factors are useful for diagnosis of hypertensive disease. Each different entity appears associated with a specific angiogenic profile, which is useful for distinction between the forms. *NP* normal pregnancy, *GH* gestational hypertension, *TP* term preeclampsia, *PP* preterm preeclampsia (Noori et al. [55])

placenta-derived reasons, the biomarkers sFlt-1 and PlGF are useful for distinction.

In the earlier years, angiogenic factors were only measureable by ELISA in the research laboratory. Recently introduced, fully automated measurements made the biomarkers sFlt-1 and PlGF available for wide clinical practice use and allow its additional use in diagnostics of preeclampsia. Since 2010 several studies have been published that demonstrated a faster and easier assessment of angiogenic factors with these commercially available methods (e.g., Roche Elecsys® immunoassay (Mannheim, Germany)) [12, 15, 16, 57–59]. Beneath the benefit in diagnostics, this even allows clinical researches in large patient groups. For the first time, cutoff values could have been evaluated.

At first, Verlohren et al. evaluated a gestation-wide cutoff of 85 for the sFlt-1/PlGF ratio. With a high sensitivity of 89% and specificity of 97%, sFlt-1/PlGF ratio today is considered as a useful additional diagnostic parameter for preeclampsia [12] (Fig. 11.7, left image).

Because clinical manifestation and consequences of preeclampsia differ highly and dependently on gestational age, early-onset and late-onset preeclampsia are also clinically differentiated. Therefore, various authors have pointed out that one cutoff throughout the whole pregnancy might be not sufficient. In a large multicenter case-control study, new gestational phase-specific cutoff values were defined for the diagnosis of preeclampsia [60]. In early pregnancy (20^0–33^6 gestational

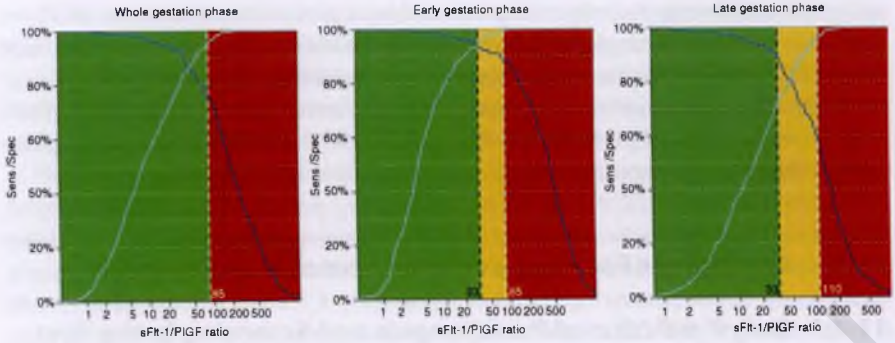


Fig. 11.7 This model demonstrates the different cutoffs for the sFlt-1/PlGF ratio for ruling in (red zones, high-risk) and ruling out (green zones, low-risk) preeclampsia. A lower but intermediate risk is given in the intermediate zone (yellow zones, intermediate risk). The light blue lines represent sensitivity and the dark blue lines specificity. Left Gerhard plot: gestation-wide cutoff for the sFlt-1/PlGF ratio is 85. Middle Gerhard plot: early gestational phase (20^0 and 33^6 gestational weeks) cutoff of the sFlt-1/PlGF ratio for ruling out preeclampsia is 33 and for ruling in 85. Patients are at intermediate risk if sFlt-1/PlGF ratio was 33–85. Right Gerhard plot: late gestational phase ($\geq 34^0$ gestational weeks) cutoff of the sFlt-1/PlGF ratio for ruling out preeclampsia is 33 and for ruling in 110. Patients are at intermediate risk if sFlt-1/PlGF ratio was 33–110

weeks), sensitivity and specificity of the sFlt-1/PlGF ratio cutoff ≤ 33 were 95% and 94% and for the cutoff ≥ 85 88% and 99.5%, respectively. An sFlt-1/PlGF ratio of ≤ 33 had the lowest likelihood ratio of a negative test (0.05; 95% CI, 0.02–0.13), whereas values ≥ 85 had the highest likelihood ratio of a positive test (176; 95% CI, 24.88–1245). In later pregnancy, after 34^0 weeks, the cutoffs ≤ 33 and ≥ 110 yielded a sensitivity/specificity of 89.6%/73.1% and 58.2/95.5%, respectively [60]. Summarizing the findings of this study, the high sensitivity of the cutoff was found to be meaningful during early pregnancy, but the high specificity is more important in later pregnancy. The authors built a model with equivocal zones (Fig. 11.7). Thus, a low risk is indicated, and preeclampsia can be ruled out if the sFlt-1/PlGF ratio is ≤ 33 regardless of the gestational age. A high risk is indicated and preeclampsia must be considered if sFlt-1/PlGF ratio is ≥ 85 in early gestational phase ($<34^0$ gestational weeks) and if sFlt-1/PlGF ratio is ≥ 110 in late gestational phase ($\geq 34^0$ gestational weeks).

Patients in the zone between these lower and upper cutoffs, the so-called intermediate zone (sFlt-1/PlGF ratio 33–85 or 33–110, respectively), were also shown to be at risk for adverse outcomes, which was however mostly less severe than in early-onset preeclampsia [61, 62]. Moreover, this patient group had a higher comorbidity with chronic or acute diseases or had a twin pregnancy.

Altered angiogenic factors have also been shown in uneventful twin pregnancies [63]. Compared to healthy singleton pregnancies, sFlt-1/PlGF ratio was shown about twofold increased in healthy twin pregnancies [63–65]. Compared to singleton pregnancies, twin pregnancies are at a twofold higher risk to develop hypertensive disorders. Furthermore, twin pregnancies with hypertensive disorders, particularly with preeclampsia, are known to have more severe adverse outcomes with early preterm

and preterm delivery, placental abruption, and necessity of treatment in an intensive care unit compared to singleton preeclamptic pregnancies. The higher risk might be caused by relative ischemia and/or increased placental mass [65, 66].

Study data are poor in this field, but increased attention is crucial in twin pregnancies. From the knowledge today, the sFlt-1/PlGF ratio is also useful in twin pregnancies, but there is evidence that different cutoff values might be necessary [63].

11.4 Angiogenic Factors and Prediction of Preeclampsia

11.4.1 Early Prediction of Preeclampsia and Screening in the First Trimester

The importance of early detection of patients with increased risk for preeclampsia is growing, particularly since the preventive benefit of low-dose aspirin has recently clearly been confirmed by the ASPRE study [67–69]. The knowledge about an increased risk furthermore allows selection of patients profiting from more intensive care and feto-maternal monitoring during pregnancy which additionally helps to reduce severe adverse outcomes. Today many studies provide good evidence that angiogenic factors are useful for prediction of preeclampsia, already in the first trimester.

Traditionally, the a priori risk for preeclampsia is assessed from maternal characteristics and medical history. Despite available individualized screening tests, the National Institute for Health and Care Excellence (NICE) guidelines in the UK and also the American College of Obstetricians and Gynecologists (ACOG) guidelines in the USA still recommend the medical characteristics and history as the best and most useful screening approach for early selection and treatment of patients at risk for preeclampsia [70–73]. In the NICE guidelines, each patient with one high-risk factor or two moderate-risk factors is considered to have a high risk for preeclampsia [70]. Also in Germany, traditional a priori risk assessment is common. An individualized early preeclampsia screening test using PAPP-A and PlGF is neither generally recommended by the guidelines and has not yet been widespread [74].

As in the USA, for patients with high a priori risk for preeclampsia, treatment with low-dose aspirin is recommended [71–74]. By the NICE guidelines, only women with a history of preeclampsia in two or more previous pregnancies or women with a previous early-onset preeclampsia requiring preterm birth (<34^0 gestational weeks) obtain preventive low-dose aspirin.

In clinical routine, the a priori risk is evaluated by questionnaires, taking the individual parameter maternal age, racial origin, method of conception, cigarette smoking, history of chronic diseases, family and patient history of preeclampsia, parity, and maternal weight and height into account. Although known to be one of the most important risk factors for preeclampsia, poor detection rates (DR) have clearly been demonstrated if only the a priori risk was used as selection parameter. DR (false-positive rate (FPR) = 5%) of maximum 41/36.7/29.4% and DR (FPR = 10%) of maximum 53/47.5/40.3% have been assessed for early and intermediate/late preeclampsia, respectively [75–80].

With the combination of the a priori risk and the physiologic maternal parameters mean arterial pressure (MAP) and uterine artery pulsatility index (UtA-PI), significant increases of the DR for preeclampsia can already be achieved. With a 5% FPR, DR for early and intermediate/late preeclampsia were maximum 82/54.6/37% and with a 10% FPR 94/71.5/56.6%, respectively [76, 79–81].

Screening models that additionally integrate biochemical molecules (e.g., pregnancy-associated plasma protein-A (PAPP-A), PlGF, placental protein-13, inhibin-A, activin-A, sEng, pentraxin-3, and P-selectin), which are involved into the placentation process, provide the best prediction. These combination algorithms were shown to be superior to each known single screening parameter, which appears logically in view of the multifactorial causes of preeclampsia [75–83]. However, there is some heterogeneity in studies on which parameters should be integrated in the screening test at 11–13 gestational weeks. The best DR were observed if all maternal factors, all biophysical factors, and all available biochemical markers were incorporated [75, 80, 81, 83]. Despite very high DR (5% FPR) of 91.0/79.4/60.9% and DR (10% FPR) 95.2/88.3/71.1% for early/intermediate/late preeclampsia, such a model would be hard to realize in clinical practice and so is not useful. In a prospective multicenter study with 58,884 singleton pregnancies at 11^0–13^6 gestational weeks, the Fetal Medicine Foundation (FMF) firstly provided a clinically useful algorithm that included maternal factors, MAP and UtA-PI as physiological and PAPP-A and PlGF as biochemical factors [76]. In a recent large prospective study with 8775 singleton pregnancies, superior performance of the FMF-screening algorithm compared to screening by the NICE and ACOG guideline criteria was confirmed by O'Gorman et al. They furthermore found nearly equal results for the algorithm with and without PAPP-A [80, 81] (Fig. 11.8). So, for the combination of maternal factors, UtA-PI,

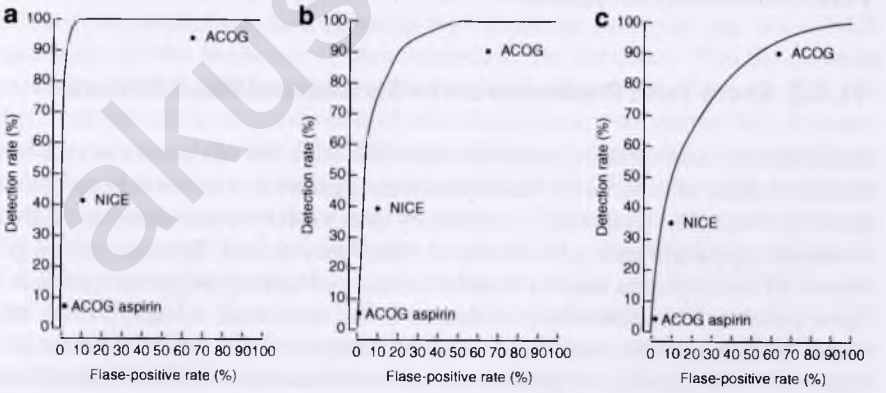


Fig. 11.8 The receiver operating characteristics demonstrate superiority of the used Fetal Medicine Foundation algorithm at (**a**) <32 gestational weeks, (**b**) <37 gestational weeks, and (**c**) ≥37 gestational weeks compared to screening by National Institute for Health and Care Excellence (NICE) and American College of Obstetricians and Gynecologists (ACOG) guidelines for early detection of preeclampsia. FMF algorithm combines maternal factors, MAP, UtA-PI, and PlGF. NICE and ACOG screening was performed using only maternal factors. Even the ACOG criteria leading to treatment with aspirin due to high risk (ACOG aspirin group) provided low test performance (O'Gorman et al. [81])

MAP, and PlGF, DR (5% FPR) of 94/66/32% and DR (10% FPR) of 100/75/43% for early/intermediate/late preeclampsia were demonstrated. Similarly, the combination of maternal factors, UtA-PI, MAP, PLGF, and PAPP-A, provided a DR (5% FPR) of 94/66/31% and DR (10% FPR) of 100/80/43%. Both were superior to the combination of maternal factors, UtA-PI, MAP, and PAPP-A (5% FPR, 88/61/29%; 10% FPR, 94/69/42%). Hence, today an algorithm with the use of maternal factors UtA-PI, MAP, and PLGF can be considered suitable for early preeclampsia screening in the first trimester and is feasible in daily clinical routine [80].

There is no evidence that sFlt-1 levels are useful for the first trimester screening. Although some studies have shown increased sFlt-1 levels in the first trimester in high-risk patients [84, 85], this was not confirmed by the majority of the studies [24, 86, 87].

A very recent study has demonstrated that screening for preeclampsia might be possible noninvasively in the first trimester using trophoblast retrieval and isolation from the cervix (TRIC), a method which is broadly known from the PAP smear assessment [88]. The authors hypothesized that molecular signatures from extravillous trophoblast cells (EVT) early in gestation reflect their disrupted function in at least a portion of pregnancies that later develop preeclampsia and also IUGR. They could demonstrate that expression of the proteins PAPPA, FLT1, ENG, AFP, PGF, and LGALS14, known to be associated with preeclampsia and/or IUGR, was altered in EVT cells at 6–20 gestational weeks in patients with later perinatal diseases after 20 weeks.

According to current knowledge, integration of more individualized screening methods into clinical practice must be considered reasonable. They reflect the patient's real risk more reliably and hence can help to carefully handle valuable resources by an individually risk-adapted pregnancy management. This is more important since reliable early tests are available and angiogenic factors, particularly PLGF, can routinely be assessed.

11.4.2 Short-Term Prediction in the Second and Third Trimester

Prediction of short-term outcome and therefore selection of patients at risk from healthy women are crucial for the clinical management. In clinical routine, women often present with symptoms or laboratory parameters of preeclampsia, such as headache, epigastric pain, proteinuria, or thrombocytopenia. In earlier times, prediction of preeclampsia was not feasible because of lacking prognostic markers in these patients. Since intensively evaluated in the last years, the sFlt-1/PlGF ratio offers reliable short-term prediction of preeclampsia-related adverse outcomes. It allows not only detection of patients at risk for adverse outcome due to preeclampsia in the next following days but also reassurance of patient and obstetrician and therefore helps to use resources efficiently.

In a prospective multicenter study of women with suspected preeclampsia prior to 35^0 gestational weeks, Chappell et al. demonstrated that low PlGF levels (<5th percentile) can predict occurrence of preeclampsia within 14 days [89]. Despite the low specificity of 55% but with a representative corresponding area under the

receiver operating curve (AUC) of 87%, a high sensitivity of 96%, and a high negative predictive value of 98%, low PlGF levels were concluded to be innovatively useful for management in women with suspected preeclampsia. This test performance exceeded all commonly used tests (range AUC 58–76%) until that time. Later, Rana et al. showed a better reliability for the sFlt-1/PlGF ratio ≥85 in prediction of preeclampsia-related adverse outcomes within the following 2 weeks if preeclampsia was clinically suspected in the third trimester [90]. They also showed an inverse correlation of sFlt-1/PlGF ratio and the time to delivery, which was stronger in earlier gestational phase (<34^0 gestational weeks) than later in pregnancy. If the sFlt-1/PlGF ratio was ≥85, 86% of patients (<34^0 gestational weeks) delivered within the next 2 weeks. With the PROGNOSIS study, a recent large prospective, multicenter, observational study, Zeisler et al. confirmed the predictive value of the sFlt-1/PlGF ratio in patients with any clinical or chemical symptoms of preeclampsia in the second and third trimester [91]. They evaluated its use for ruling in and ruling out preeclampsia/eclampsia and hemolysis, elevated liver enzymes, and low platelet count (HELLP)—syndrome within the following weeks after the first visit. Measuring angiogenic factors at the first time of presentation and regularly during the further pregnancy course with a fully automatic Elecsys® immunoassay, the authors could detect the sFlt-1/PlGF ratio of 38 as a useful cutoff point. With a negative predictive value of 99.3%, they primarily found sFlt-1/PlGF ratio ≤38 preeclampsia to be foremost reliable to rule out preeclampsia within the following week. With this high negative predictive value, precise short-term prognosis for 1 week is possible. Beneath ruling out, the cutoff value of 38 could furthermore be used to rule in preeclampsia/eclampsia/HELLP syndrome within 4 weeks in women with suspected preeclampsia. Although the positive predictive value of 36.7% for an sFlt-1/PlGF ratio above 38 may seem low, this test provides a tremendous improvement in prediction of preeclampsia. It has to be pointed out that, although with moderate precision, the sFlt-1/PlGF ratio today allows prediction of preeclampsia at the first place. With the classical parameters blood pressure and proteinuria, the predictive value is only 20% and therefore not useful for prediction of adverse pregnancy outcomes [92]. In a secondary analyses of the PROGNOSIS study, Zeisler et al. showed also a significant reduction of the time to delivery in women with sFlt-1/PlGF ratio >38, regardless of whether preeclampsia developed or not and independent of gestational age [93]. They calculated a 2.6-fold higher likelihood of preterm delivery and even a 2.9-fold greater likelihood of delivery on the day of the test if sFlt-1/PlGF ratio was >38. They also showed a 37% shorter time to delivery, with a median of 17 (interquartile range 10–26) remaining days. In contrast, women with an inconspicuous sFlt-1/PlGF ratio ≤38 had a time to delivery of 51 (interquartile range 30–75) remaining days. Correlation with sFlt-1/PlGF ratio and time to delivery was pronounced in patients who introduced themselves with symptoms in early gestational phase (24^0–33^6 gestational weeks) and in patients who later developed preeclampsia. Interestingly, there was also an association between increased sFlt-1/PlGF ratio >38 and iatrogenic preterm delivery which was not shown for patients with spontaneous preterm delivery [93].

Summarizing actual findings, an sFlt-1/PlGF ratio ≤38 is useful to reliably rule out preeclampsia within the following week. Contrarily, an sFlt-1/PlGF ratio >38 indicates a shortened time to delivery, an increased risk of developing preeclampsia within 4 weeks, and also a high risk for preterm birth because of other reasons than preeclampsia. This new knowledge is tremendously valuable for clinical routine and extremely helpful for determining further management in patients with any signs of preeclampsia in the second and third trimester. Whereas patients with an sFlt-1/PlGF ratio ≤38 can be reassured, increased attention has to be kept on patients with an sFlt-1/PlGF ratio >38 in order to prevent associated maternal and fetal adverse outcomes.

11.5 The sFlt-1/PlGF Ratio and Clinical Decision-Making

A flood of valuable studies provided new insights into the pathogenetic mechanisms of preeclampsia and knowledge about the high clinical value of the angiogenic factors sFlt-1 and PlGF or the sFlt-1/PlGF ratio, respectively. New opportunities of automated measurements offer its routine use and therefore change clinical management of preeclampsia gradually. Recently introduced into the NICE guidelines and also into the German guidelines, the sFlt-1/PlGF ratio has already been established as an aid in diagnosis in clinical practice. Allowing a more detailed and better diagnosis, the sFlt-1/PlGF ratio helps in determining further clinical management. Not as a single parameter but in combination with clinical symptoms and the classical diagnostic parameters blood pressure and proteinuria, the sFlt-1/PlGF ratio is useful to distinguish patients at short-term risk for adverse outcome due to preeclampsia in the second and third trimester. Beneath the gold standard tools, the special form of preeclampsia, the HELLP syndrome, needs to be additionally diagnosed or excluded, respectively, by the specific hematological and liver parameters.

In clinical routine, determination of the best further pregnancy management can only be assessed with the right diagnosis which in turn is not trivial. There are overlapping physiological changes accelerating with pregnancy progress or underlying chronic diseases that challenge diagnostics. As shown in a large clinical study, signs of preeclampsia develop in about 10% of all pregnancies but were associated to manifest preeclampsia in only 5–20% of all these women [94–96]. This reflects a tendency to overtreatment in clinical practice which likewise causes high costs and waste of valuable resources. On the other hand, unknown preeclampsia might result in severe adverse outcomes and correct diagnosis therefore must not be missed. Hence, obstetric procedures should foremost gain detection of patients with a manifest disease and those at high risk who need a more intensive care or hospitalization. On the other hand, protection from overtreatment should be warranted, too. As the sFlt-1/PlGF ratio allows a better discrimination, it can be recommended for clinical use. On the basis of current knowledge, our working group published a consensus paper stating actual recommended management of women with signs and symptoms of preeclampsia and asymptomatic high-risk patients [97]:

Firstly, if the sFlt-1/PlGF ratio is <38, preeclampsia can reliably be ruled out for at least 1 week, irrespective of gestational age. These patients currently have no preeclampsia and a low risk to develop preeclampsia, at least in the following week. Further ambulant management should depend on the course of symptoms, with controls in case of persistence or progression. Thereby, sFlt-1/PlGF has its great value in reassuring the clinician and the patient.

Secondly, if the sFlt-1/PlGF ratio is 38–85 in the early pregnancy phase (<34^0 gestational weeks) or 38–110 in later pregnancy phase (≥34^0 gestational weeks), increased attention is crucial because of an increased risk for adverse pregnancy outcome. Current preeclampsia or placenta-related disorders can be ruled out, but especially women in the early gestational phase are at risk for preterm birth and severe adverse outcomes. Therefore, monitoring should be enhanced with controls in 1–2 weeks in early gestational age (<34^0 gestational weeks). In later gestational age (≥34^0 gestational weeks) birth induction should be discussed.

Thirdly, patients with an sFlt-1/PlGF ratio >85 (<34^0 gestational weeks) or >110 (≥34^0 gestational weeks) are highly likely to have a manifest preeclampsia and to develop associated adverse outcome. Therefore, hospitalization and close clinical controls with repeated measurement, depending on the clinical situation every 2–4 days, are useful to determine the trend and the next treatment. If sFlt-1/PlGF ratio is severely elevated (>655 at <34^0 weeks and >201 at ≥34^0week), there is a risk for the need of delivery within the following 48 h. Therefore, a prompt application of antenatal corticoids is indicated to accelerate fetal lung maturation and to improve fetal outcome. Intravenous treatment with magnesium for neuroprotection has to be considered.

Clinical decision-making with the additional use of the sFlt-1/PlGF ratio was even investigated in the first prospective, multicenter study [95]. In total, there were 16.9% of cases in which decision of hospitalization was changed after knowledge of the ratio. In 13/118 women (11%), physicians revised their initial decision of hospitalization and went on with ambulant controls. In 5.9% of cases, physicians revised their decision toward hospitalization although ambulant care was firstly decided by classical management. Follow-up confirmed the right decision after knowledge of the sFlt-1/PlGF ratio so that the sFlt-1/PlGF ratio can be concluded to be helpful in finding the correct diagnosis and so to make reliable, clinical decisions [95].

Although sFlt-1/PlGF ratio is useful for diagnosis and prediction in the second and third trimester, the only option of definite therapy is delivery. So, even if prediction is better and therefore further management can be optimized, maternal and fetal complications cannot be avoided by its use. With regard to morbidity and mortality, there is still a need for more investigation comparing clinical managements with and without the sFlt-1/PlGF ratio. Also sufficient data about the economic benefit of sFlt-1/PlGF ratio usage in clinical routine are missing. Particularly, because low-dose aspirin provides an effective preventive treatment, further investigation of clinical and economic benefits of a first trimester screening for preeclampsia should be promoted and routine assessment discussed more intensely.

Although there are still a lot of open questions about the use of sFlt-1/PlGF ratio in clinical settings, scientific efforts led to a better understanding of preeclampsia. New horizons were opened with the implication of angiogenic factors, explicitly with the sFlt-1/PlGF ratio into clinical routine. As an aid in diagnosis, together with clinical signs, the sFlt-1/PlGF ratio significantly improved diagnostic and also prediction of preeclampsia. Identification of the heterogeneous clinical syndrome of preeclampsia and especially distinction of real preeclampsia from other diseases with preeclamptic symptoms is now more reliable and provides more diagnostic confidence in patients and obstetricians.

References

1. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol. 1989;161:1200–4.
2. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111:649–58. https://doi.org/10.1172/JCI17189.
3. Ossada V, Jank A, Stepan H. The impact of uterine curettage postpartum on maternal sFlt-1 concentration. J Perinat Med. 2016;44:351–4. https://doi.org/10.1515/jpm-2015-0104.
4. Yang JC, et al. A randomized double-blind placebo controlled trial of bevacizumab (anti-VEGF antibody) demonstrating a prolongation in time to progression in patients with metastatic renal cancer. Proc Am Soc Clin Oncol. 2002;21:5a.
5. Patel TV, Morgan JA, Demetri GD, George S, Maki RG, Quigley M, Humphreys BD. A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib. J Natl Cancer Inst. 2008;100(4):282. https://doi.org/10.1093/jnci/djm311.
6. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69:1–7. https://doi.org/10.1095/biolreprod.102.014977.
7. Zygmunt M, Herr F, Münstedt K, Lang U, Liang OD. Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2003;110(Suppl 1):S10–8.
8. Stillman IE, Karumanchi SA. The glomerular injury of preeclampsia. J Am Soc Nephrol. 2007;18:2281–4. https://doi.org/10.1681/ASN.2007020255.
9. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111:707–16. https://doi.org/10.1172/JCI17423.
10. Lu F, Longo M, Tamayo E, Maner W, Al-Hendy A, Anderson GD, et al. The effect of overexpression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol. 2007;196:396.e1–396.e7; discussion 396.e7. https://doi.org/10.1016/j.ajog.2006.12.024.
11. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, KF Y, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350:672–83. https://doi.org/10.1056/NEJMoa031884.
12. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, et al. An automated method for the determination of the sFlt-1/PlGF ratio in the assessment of preeclampsia. Am J Obstet Gynecol. 2010;202:161. https://doi.org/10.1016/j.ajog.2009.09.016.
13. Shibata E, Rajakumar A, Powers RW, Larkin RW, Gilmour C, Bodnar LM, et al. Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. J Clin Endocrinol Metab. 2005;90:4895–903. https://doi.org/10.1210/jc.2004-1955.

14. de VA, Baviera G, Giordano D, Todarello G, Corrado F, D'anna R. Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia. Acta Obstet Gynecol Scand. 2008;87:837–42. https://doi.org/10.1080/00016340802253759.
15. Ohkuchi A, Hirashima C, Suzuki H, Takahashi K, Yoshida M, Matsubara S, Suzuki M. Evaluation of a new and automated electrochemiluminescence immunoassay for plasma sFlt-1 and PlGF levels in women with preeclampsia. Hypertens Res. 2010;33:422–7. https://doi.org/10.1038/hr.2010.15.
16. Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, Hodges-Savola C. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm pre-eclampsia: a prospective clinical study. Am J Obstet Gynecol. 2010;202:40.e1–7. https://doi.org/10.1016/j.ajog.2009.07.025.
17. Tsatsaris V, Goffin F, Munaut C, Brichant J-F, Pignon M-R, Noel A, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab. 2003;88:5555–63. https://doi.org/10.1210/jc.2003-030528.
18. Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J Matern Fetal Neonatal Med. 2005;17:3–18. https://doi.org/10.1080/14767050400028816.
19. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol. 2003;188:177–82.
20. Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ. Preeclampsia is associated with reduced serum levels of placenta growth factor. Am J Obstet Gynecol. 1998;179:1539–44.
21. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol. 2013;209:544.e1–544.e12. https://doi.org/10.1016/j.ajog.2013.08.019.
22. Levine RJ, Karumanchi SA. Circulating angiogenic factors in preeclampsia. Clin Obstet Gynecol. 2005;48:372–86.
23. Cohen AL, Wenger JB, James-Todd T, Lamparello BM, Halprin E, Serdy S, et al. The association of circulating angiogenic factors and HbA1c with the risk of preeclampsia in women with preexisting diabetes. Hypertens Pregnancy. 2014;33:81–92. https://doi.org/10.3109/10641955.2013.837175.
24. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med. 2008;21:9–23. https://doi.org/10.1080/14767050701830480.
25. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. Am J Obstet Gynecol. 2012;206:58. https://doi.org/10.1016/j.ajog.2011.07.037.
26. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, et al. L13. The routine measurement of the sFlt1/PlGF ratio allows differential diagnosis of hypertensive pregnancy disorders and has prognostic potential in preeclamptic patients. Pregnancy Hypertens. 2011;1:245–6. https://doi.org/10.1016/j.preghy.2011.08.014.
27. Kim MY, Buyon JP, Guerra MM, Rana S, Zhang D, Laskin CA, et al. Angiogenic factor imbalance early in pregnancy predicts adverse outcomes in patients with lupus and antiphospholipid antibodies: results of the PROMISSE study. Am J Obstet Gynecol. 2016;214:108. https://doi.org/10.1016/j.ajog.2015.09.066.
28. Spradley FT. Metabolic abnormalities and obesity's impact on the risk for developing pre-eclampsia. Am J Physiol Regul Integr Comp Physiol. 2017;312:R5–R12. https://doi.org/10.1152/ajpregu.00440.2016.
29. Vellanki K. Pregnancy in chronic kidney disease. Adv Chronic Kidney Dis. 2013;20:223–8. https://doi.org/10.1053/j.ackd.2013.02.001.

30. Tripathi R, Rath G, Jain A, Salhan S. Soluble and membranous vascular endothelial growth factor receptor-1 in pregnancies complicated by pre-eclampsia. Ann Anat. 2008;190:477–89. https://doi.org/10.1016/j.aanat.2008.08.002.
31. Munaut C, Lorquet S, Pequeux C, Blacher S, Berndt S, Frankenne F, Foidart J-M. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. Hum Reprod. 2008;23:1407–15. https://doi.org/10.1093/humrep/den114.
32. Stepanian A, Bourguignat L, Hennou S, Coupaye M, Hajage D, Salomon L, et al. Microparticle increase in severe obesity: not related to metabolic syndrome and unchanged after massive weight loss. Obesity (Silver Spring). 2013;21:2236–43. https://doi.org/10.1002/oby.20365.
33. Zhang X, McGeoch SC, Johnstone AM, Holtrop G, Sneddon AA, MacRury SM, et al. Platelet-derived microparticle count and surface molecule expression differ between subjects with and without type 2 diabetes, independently of obesity status. J Thromb Thrombolysis. 2014;37:455–63. https://doi.org/10.1007/s11239-013-1000-2.
34. Sabatier F, Darmon P, Hugel B, Combes V, Sanmarco M, Velut J-G, et al. Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. Diabetes. 2002;51:2840–5.
35. Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M, et al. Effects of severe hypertension on endothelial and platelet microparticles. Hypertension. 2003;41:211–7.
36. Lacroix R, Plawinski L, Robert S, Doeuvre L, Sabatier F, Martinez de Lizarrondo S, et al. Leukocyte- and endothelial-derived microparticles: a circulating source for fibrinolysis. Haematologica. 2012;97:1864–72. https://doi.org/10.3324/haematol.2012.066167.
37. Dignat-George F, Camoin-Jau L, Sabatier F, Arnoux D, Anfosso F, Bardin N, et al. Endothelial microparticles: a potential contribution to the thrombotic complications of the antiphospholipid syndrome. Thromb Haemost. 2004;91:667–73. https://doi.org/10.1160/TH03-07-0487.
38. Tannetta DS, Dragovic RA, Gardiner C, Redman CW, Sargent IL. Characterisation of syncytiotrophoblast vesicles in normal pregnancy and pre-eclampsia: expression of Flt-1 and endoglin. PLoS One. 2013;8:e56754. https://doi.org/10.1371/journal.pone.0056754.
39. Ali A, Ganai J, Muthukrishnan S, Kohli S. Evaluation of autonomic dysfunction in obese and non-obese hypertensive subjects. J Clin Diagn Res. 2016;10:YC01–3. https://doi.org/10.7860/JCDR/2016/18780.7923.
40. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med. 2006;12:1065–74. https://doi.org/10.1038/nm1452.
41. Wilson ML, Goodwin TM, Pan VL, Ingles SA. Molecular epidemiology of preeclampsia. Obstet Gynecol Surv. 2003;58:39–66. https://doi.org/10.1097/01.OGX.0000042241.12428.C9.
42. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jüpner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest. 1999;103:945–52. https://doi.org/10.1172/JCI4106.
43. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, et al. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nat Med. 2008;14:855–62. https://doi.org/10.1038/nm.1856.
44. ACOG Committee on Obstetric Practice. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet. 2002;77:67–75.
45. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013;3:44–7. https://doi.org/10.1016/j.preghy.2012.11.001.
46. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee Kim Y, Gonçalves LF, et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am J Obstet Gynecol. 2004;190:1541–1547; discussion 1547–50. https://doi.org/10.1016/j.ajog.2004.03.043.

47. Veas CJ, Aguilera VC, Muñoz IJ, Gallardo VI, Miguel PL, González MA, et al. Fetal endothelium dysfunction is associated with circulating maternal levels of sE-selectin, sVCAM1, and sFlt-1 during pre-eclampsia. J Matern Fetal Neonatal Med. 2011;24:1371–7. https://doi.org/10.3109/14767058.2011.556204.
48. Herse F, Verlohren S, Wenzel K, Pape J, Muller DN, Modrow S, et al. Prevalence of agonistic autoantibodies against the angiotensin II type 1 receptor and soluble fms-like tyrosine kinase 1 in a gestational age-matched case study. Hypertension. 2009;53:393–8. https://doi.org/10.1161/HYPERTENSIONAHA.108.124115.
49. Kim YN, Lee DS, Jeong DH, Sung MS, Kim KT. The relationship of the level of circulating antiangiogenic factors to the clinical manifestations of preeclampsia. Prenat Diagn. 2009;29:464–70. https://doi.org/10.1002/pd.2203.
50. Masuyama H, Segawa T, Sumida Y, Masumoto A, Inoue S, Akahori Y, Hiramatsu Y. Different profiles of circulating angiogenic factors and adipocytokines between early- and late-onset pre-eclampsia. BJOG. 2010;117:314–20. https://doi.org/10.1111/j.1471-0528.2009.02453.x.
51. Ohkuchi A, Hirashima C, Matsubara S, Suzuki H, Takahashi K, Arai F, et al. Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. Hypertens Res. 2007;30:151–9. https://doi.org/10.1291/hypres.30.151.
52. Wikström A-K, Larsson A, Eriksson UJ, Nash P, Nordén-Lindeberg S, Olovsson M. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. Obstet Gynecol. 2007;109:1368–74. https://doi.org/10.1097/01.AOG.0000264552.85436.a1.
53. Levine RJ, Lam C, Qian C, KF Y, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355:992–1005. https://doi.org/10.1056/NEJMoa055352.
54. Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim K-H. Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy. Am J Obstet Gynecol. 2007;197:28.e1–6. https://doi.org/10.1016/j.ajog.2007.04.010.
55. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation. 2010;122:478–87. https://doi.org/10.1161/CIRCULATIONAHA.109.895458.
56. Vuorela P, Helske S, Hornig C, Alitalo K, Weich H, Halmesmäki E. Amniotic fluid—soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet Gynecol. 2000;95:353–7.
57. Benton SJ, Hu Y, Xie F, Kupfer K, Lee S-W, Magee LA, von Dadelszen P. Angiogenic factors as diagnostic tests for preeclampsia: a performance comparison between two commercial immunoassays. Am J Obstet Gynecol. 2011;205:469.e1–8. https://doi.org/10.1016/j.ajog.2011.06.058.
58. Schiettecatte J, Russcher H, Anckaert E, Mees M, Leeser B, Tirelli AS, et al. Multicenter evaluation of the first automated Elecsys sFlt-1 and PlGF assays in normal pregnancies and preeclampsia. Clin Biochem. 2010;43:768–70. https://doi.org/10.1016/j.clinbiochem.2010.02.010.
59. Wothe D, Gaziano E, Sunderji S, Romero R, Kusanovic JP, Rogers L, et al. Measurement of sVEGF R1 and PlGF in serum: comparing prototype assays from Beckman Coulter, Inc. to R&D Systems microplate assays. Hypertens Pregnancy. 2011;30:18–27. https://doi.org/10.3109/10641950903322881.
60. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, et al. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. Hypertension. 2014;63:346–52. https://doi.org/10.1161/HYPERTENSIONAHA.113.01787.
61. Rana S, Schnettler WT, Powe C, Wenger J, Salahuddin S, Cerdeira AS, et al. Clinical characterization and outcomes of preeclampsia with normal angiogenic profile. Hypertens Pregnancy. 2013;32:189–201. https://doi.org/10.3109/10641955.2013.784788.

62. Hoffmann J, Ossada V, Weber M, Stepan H. An intermediate sFlt-1/PlGF ratio indicates an increased risk for adverse pregnancy outcome. Pregnancy Hypertens. 2017;10:165–70. https://doi.org/10.1016/j.preghy.2017.08.003.
63. Dröge L, Herraìz I, Zeisler H, Schlembach D, Stepan H, Küssel L, et al. Maternal serum sFlt-1/PlGF ratio in twin pregnancies with and without pre-eclampsia in comparison with singleton pregnancies. Ultrasound Obstet Gynecol. 2015;45:286–93. https://doi.org/10.1002/uog.14760.
64. Bdolah Y, Lam C, Rajakumar A, Shivalingappa V, Mutter W, Sachs BP, et al. Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia? Am J Obstet Gynecol. 2008;198:428. https://doi.org/10.1016/j.ajog.2007.10.783.
65. Rana S, Hacker MR, Modest AM, Salahuddin S, Lim K-H, Verlohren S, et al. Circulating angiogenic factors and risk of adverse maternal and perinatal outcomes in twin pregnancies with suspected preeclampsia. Hypertension. 2012;60:451–8. https://doi.org/10.1161/HYPERTENSIONAHA.112.195065.
66. Sibai BM, Hauth J, Caritis S, Lindheimer MD, MacPherson C, Klebanoff M, et al. Hypertensive disorders in twin versus singleton gestations. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Am J Obstet Gynecol. 2000;182:938–42.
67. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de PMC, et al. Aspirin versus Placebo in pregnancies at high risk for preterm preeclampsia. N Engl J Med. 2017;377:613–22. https://doi.org/10.1056/NEJMoa1704559.
68. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, et al. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. Obstet Gynecol. 2010;116:402–14. https://doi.org/10.1097/AOG.0b013e3181e9322a.
69. Roberge S, Villa P, Nicolaides K, Giguère Y, Vainio M, Bakthi A, et al. Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. Fetal Diagn Ther. 2012;31:141–6. https://doi.org/10.1159/000336662.
70. National Collaborating Centre for Women's and Children's Health (UK). Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy. NICE Clinical Guidelines. 2010.
71. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122:1122–31. https://doi.org/10.1097/01.AOG.0000437382.03963.88.
72. Committee Opinion No. 638: First-Trimester Risk Assessment for Early-Onset Preeclampsia. Obstet Gynecol. 2015;126:e25–7. https://doi.org/10.1097/AOG.0000000000001049.
73. Committee Opinion Summary No. 638: First-Trimester Risk Assessment for Early-Onset Preeclampsia. Obstet Gynecol. 2015;126:689. https://doi.org/10.1097/01.AOG.0000471175.95314.42
74. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe. S1-Leitlinie: Diagnostik und Therapie hypertensiver Schwangerschaftserkrankungen. 12/2013.
75. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn. 2011;31:66–74. https://doi.org/10.1002/pd.2660.
76. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther. 2013;33:8–15. https://doi.org/10.1159/000341264.
77. Rolnik DL, Wright D, Poon LC, Syngelaki A, O'Gorman N, de Paco Matallana C, et al. ASPRE trial: performance of screening for preterm pre-eclampsia. Ultrasound Obstet Gynecol. 2017. https://doi.org/10.1002/uog.18816.
78. Poon LCY, Kametas NA, Chelemen T, Leal A, Nicolaides KH. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. J Hum Hypertens. 2010;24:104–10. https://doi.org/10.1038/jhh.2009.45.
79. Poon LCY, Akolekar R, Lachmann R, Beta J, Nicolaides KH. Hypertensive disorders in pregnancy: screening by biophysical and biochemical markers at 11-13 weeks. Ultrasound Obstet Gynecol. 2010;35:662–70. https://doi.org/10.1002/uog.7628.

80. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, et al. Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol. 2017;49:751–5. https://doi.org/10.1002/uog.17399.
81. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, et al. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. Ultrasound Obstet Gynecol. 2017;49:756–60. https://doi.org/10.1002/uog.17455.
82. Poon LC, Wright D, Rolnik DL, Syngelaki A, Delgado JL, Tsokaki T, et al. ASPRE trial: effect of aspirin in prevention of preterm preeclampsia in subgroups of women according to their characteristics and medical and obstetrical history. Am J Obstet Gynecol. 2017. https://doi.org/10.1016/j.ajog.2017.07.038.
83. Poon LCY, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension. 2009;53:812–8. https://doi.org/10.1161/HYPERTENSIONAHA.108.127977.
84. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. Am J Obstet Gynecol. 2008;199:266.e1–6. https://doi.org/10.1016/j.ajog.2008.06.069.
85. Smith GCS, Crossley JA, Aitken DA, Jenkins N, Lyall F, Cameron AD, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. Obstet Gynecol. 2007;109:1316–24. https://doi.org/10.1097/01.AOG.0000265804.09161.0d.
86. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, Thadhani R. Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. Hypertension. 2007;50:137–42. https://doi.org/10.1161/HYPERTENSIONAHA.107.087700.
87. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. J Clin Endocrinol Metab. 2004;89:770–5. https://doi.org/10.1210/jc.2003-031244.
88. Bolnick JM, Kohan-Ghadr H-R, Fritz R, Bolnick AD, Kilburn BA, Diamond MP, et al. Altered biomarkers in trophoblast cells obtained noninvasively prior to clinical manifestation of perinatal disease. Sci Rep. 2016;6:32382. https://doi.org/10.1038/srep32382.
89. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, et al. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. Circulation. 2013;128:2121–31. https://doi.org/10.1161/CIRCULATIONAHA.113.003215.
90. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125:911–9. https://doi.org/10.1161/CIRCULATIONAHA.111.054361.
91. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med. 2016;374:13–22. https://doi.org/10.1056/NEJMoa1414838.
92. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gynecol. 2001;97:261–7.
93. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Soluble fms-ike tyrosine kinase-1-to-Placental growth factor ratio and time to delivery in women with suspected preeclampsia. Obstet Gynecol. 2016;128:261–9. https://doi.org/10.1097/AOG.0000000000001525.
94. Milne F, Redman C, Walker J, Baker P, Black R, Blincowe J, et al. Assessing the onset of pre-eclampsia in the hospital day unit: summary of the pre-eclampsia guideline (PRECOG II). BMJ. 2009;339:b3129. https://doi.org/10.1136/bmj.b3129.
95. Klein E, Schlembach D, Ramoni A, Langer E, Bahlmann F, Grill S, et al. Influence of the sFlt-1/PlGF ratio on clinical decision-making in women with suspected preeclampsia. PLoS One. 2016;11:e0156013. https://doi.org/10.1371/journal.pone.0156013.

96. Chaiworapongsa T, Chaemsaithong P, Korzeniewski SJ, Yeo L, Romero R. Pre-eclampsia part 2: prediction, prevention and management. Nat Rev Nephrol. 2014;10:531-40. https://doi.org/10.1038/nrneph.2014.103.
97. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, et al. Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. Ultrasound Obstet Gynecol. 2015;45:241–6. https://doi.org/10.1002/uog.14799.
98. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. J Matern Fetal Neonatal Med. 2009;22:1021–38. https://doi.org/10.3109/14767050902994754.
99. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. Obstet Gynecol. 2008;111:1403–9. https://doi.org/10.1097/AOG.0b013e3181719b7a.
100. Kim S-Y, Ryu H-M, Yang J-H, Kim M-Y, Han J-Y, Kim J-O, et al. Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia. J Korean Med Sci. 2007;22:873–7. https://doi.org/10.3346/jkms.2007.22.5.873.
101. Stepan H, Unversucht A, Wessel N, Faber R. Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion. Hypertension. 2007;49:818–24. https://doi.org/10.1161/01.HYP.0000258404.21552.a3.
102. Lapaire O, Shennan A, Stepan H. The preeclampsia biomarkers soluble fms-like tyrosine kinase-1 and placental growth factor: current knowledge, clinical implications and future application. Eur J Obstet Gynecol Reprod Biol. 2010;151:122–9. https://doi.org/10.1016/j.ejogrb.2010.04.009.
103. Wang A, Rana S, Karumanchi SA. Preeclampsia: the role of angiogenic factors in its pathogenesis. Physiology (Bethesda). 2009;24:147–58. https://doi.org/10.1152/physiol.00043.2008.

Ambulatory Blood Pressure Measurement and Home Blood Pressure Measurement

12

Hirohito Metoki

Abstract

Preeclampsia is a combination of high blood pressure and protein urea. Therefore, blood pressure measurements are important because their accuracy is associated with correct diagnosis of preeclampsia. Blood pressure is generally used for diagnosis and treatment and is measured in a medical environment. However, in nonpregnant adults, ambulatory and home blood pressure measurements have recently been recommended for monitoring blood pressure, home blood pressure should be a priority when office and home blood pressure values differ. The beneficial evidence of measuring blood pressure outside the office setting in pregnant women has been recently increasing. Out-of-office blood pressure may improve the accuracy of classifying hypertension during pregnancy. Classification may aid in identifying novel prognostic factors and improving treatment.

Keywords

Blood pressure • Pregnancy • Ambulatory measurement • Home measurement

12.1 Introduction

Blood pressure measurement during pregnancy is important because hypertensive disorders of pregnancy (HDP) are defined by blood pressure level [1]. Blood pressure can be monitored in several ways. Classification of blood pressure measurements and their characteristics are shown in Table 12.1.

H. Metoki
Division of Public Health, Hygiene and Epidemiology, Tohoku Medical and Pharmaceutical University Faculty of Medicine, Komatsushima 4-4-1, Aoba-ku, Sendai, 981-8558, Japan
e-mail: hmetoki@tohoku-mpu.ac.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_12

Table 12.1 Characteristics of each type of blood pressure measurement during pregnancy

	Clinic blood pressure	Ambulatory blood pressure	Home blood pressure
Frequency of measurement	Low	High	High
Measurement standardization	Possible	Unnecessary	Possible
Reproducibility	Unfavorable	Favorable	Most favorable
White-coat phenomenon	Present	Absent	Absent
Drug efficacy assessment	Possible	Appropriate	Optimal
Evaluation of the duration of drug efficacy	Impossible	Possible	Most favorable
Evaluation of short-term variability (variations at 15- to 30-min intervals)	Impossible	Possible	Impossible
Evaluation of diurnal changes (evaluation of nocturnal blood pressure)	Impossible	Possible	Possible
Evaluation of changes between 2 days	Impossible	Impossible	Possible
Evaluation of long-term changes (seasonal variation and during pregnancy)	Possible	Impossible	Favorable

Blood pressure measured in a medical environment is called office blood pressure, clinic blood pressure, or conventional blood pressure. Although office blood pressure measured in a medical environment is often used for diagnosis and treatment, its reproducibility is limited because standardized measurement is not performed in many cases.

For nonpregnant adults, ambulatory and home blood pressure measurements have recently been recommended for monitoring blood pressure outside the office setting to diagnose hypertension. Monitoring blood pressure outside the office setting in a nonmedical environment has recently been recommended to diagnose and treat hypertension [2]. Diagnosing hypertension during pregnancy is slightly difficult because hypertensive disorder of pregnancy has several subclassifications (preeclampsia, gestational hypertension, eclampsia, and superimposed preeclampsia) and large hemodynamic changes occur during pregnancy (Fig. 12.1). Although there is a little evidence of monitoring blood pressure outside the office setting, they are widely adopted recently. In Canada, 83% of obstetricians attempt to exclude white-coat hypertension, and 78 and 12% of them encourage blood pressure monitoring at home and while ambulatory in nonmedical settings, respectively [3].

Over the last 30 years, out-of-office blood pressure measurement has been increasingly used because of its prognostic significance in nonpregnant adults. The comparison of office blood pressure and out-of-office blood pressure in relation to hypertension based on office blood pressure does not make sense because of the close colinearity between office blood pressure and definition of hypertension based on office blood pressure. Cardiovascular prognosis is generally used for comparison among measurements (Fig. 12.2). This is the same for pregnant women. When new parameters are compared with office blood pressure, other indexes, such as the prognosis of newborns, should be included in a gold standard as Hermida et al. discussed [4]. Instead of preeclampsia diagnosis based on office blood pressure, maternal and fetal prognoses should be used to compare which blood pressure has a better prognostic significance for preeclampsia diagnosis (Fig. 12.2). The beneficial evidence of measuring ambulatory and home blood pressures in pregnant women has been recently increasing.

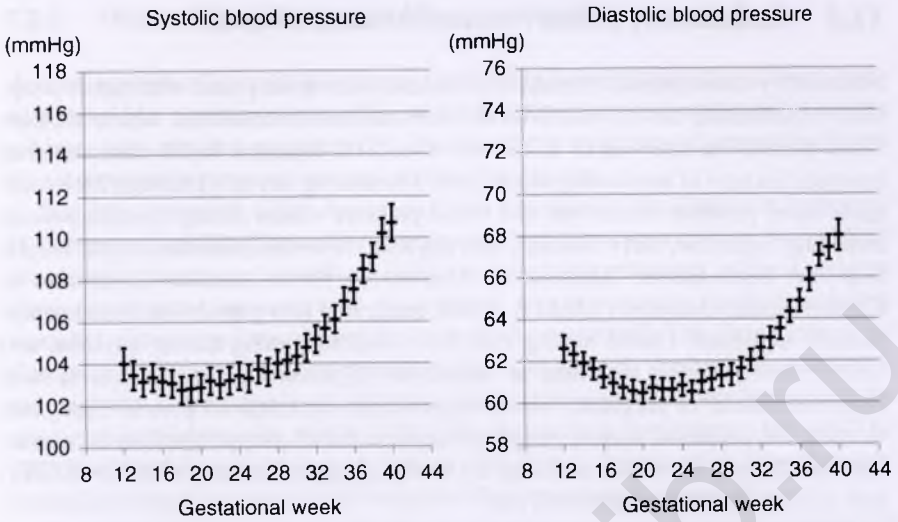


Fig. 12.1 Blood pressure values during normal pregnancy (n = 425) (Modified from Metoki H, et al. *Clin Exp Hypertens*. 2012)

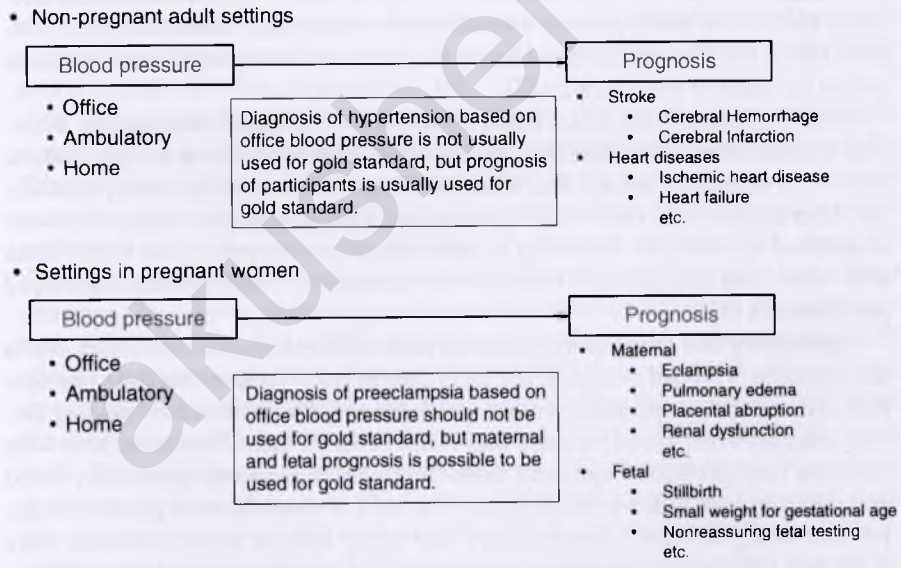


Fig. 12.2 Comparison of office blood pressure measurements in nonpregnant and pregnant adult women. When office blood and out-of-office blood pressures are compared with the definition of hypertension based on office blood pressure, the comparison does not make sense because of the close colinearity between office blood pressure and definition of hypertension based on office blood pressure. Cardiovascular prognosis is generally used instead of diagnosis of hypertension. Instead of preeclampsia diagnosis based on office blood pressure, maternal and fetal prognoses should be used to compare which blood pressure has a better prognostic significance for preeclampsia diagnosis

12.2 Ambulatory Blood Pressure Measurements

Ambulatory blood pressure measurements have been widely used with the development of automatic devices based on the cuff-oscillometric method, which measure blood pressure at intervals of 15–30 min over 24 h. Japanese health insurance has covered this type of monitoring since 2008. Monitoring not only includes 24-h averaged blood pressure values but also blood pressure values during specific periods including nighttime, early morning, and daytime. In nonpregnant adults, the results from the International Database of Ambulatory blood pressure in relation to Cardiovascular Outcome (IDACO), which integrated four population-based cohort studies, suggested 130/80 mmHg over 24 h, 140/85 mmHg during daytime, and 120/70 mmHg during nighttime as criteria for hypertension [5]. No criteria have been established for pregnant women. Based on the data derived from 276 pregnant women, the proposed normal ranges of daytime blood pressure before 16 weeks, between 26 and 30 weeks, and after 30 weeks of gestation are <130/77, <133/81, and <135/86 mmHg, respectively [6].

Diurnal blood pressure variation can be calculated from blood pressure values during specific periods. Diurnal systolic and diastolic blood pressure variations are 12–14 and 18–19%, respectively [6]. Diurnal variations of blood pressure and heart rate become attenuated during normal late pregnancy. This is because 80–90% of individuals with extreme hypertension or severe preeclampsia have attenuated nocturnal declines in blood pressure (non-dippers) or increased nocturnal blood pressure (risers) [6]. The nocturnal decline in blood pressure is reported to be attenuated before the onset of preeclampsia [7].

When ambulatory and office blood pressures are compared, masked and white-coat hypertension can be classified. Based on the ambulatory blood pressure values, 32% of hypertensive women had white-coat hypertension during early pregnancy [8]. After exclusion of white-coat hypertension, 22% of pregnant women who were considered hypertensive according to ambulatory blood pressure and 8% of those with white-coat hypertension based on the ambulatory blood pressure developed preeclampsia [8].

Ambulatory and office blood pressures have different clinical definitions. Ayala and Hermida [9] found no significant difference in blood pressure between primiparous and multiparous pregnant women after adjusting for possible confounding factors, whereas office blood pressure may differ between them. Newborns who were small for their gestational age were more closely associated with ambulatory blood pressure than with office blood pressure. The risks of small for their gestational age per 10 mmHg elevation in ambulatory and office systolic blood pressures were 1.74- and 1.40-fold higher, respectively, and office systolic blood pressure did not reach statistical significance [10]. A recent study [11] followed 87 high-risk women with normal office blood pressure and revealed that nocturnal and masked hypertension had 4.72- and 7.81-fold higher risk of preeclampsia/eclampsia, respectively, than normotension.

12.3 Home Blood Pressure Measurements

According to the Japanese guidelines for treating hypertension, home blood pressure should be a priority when office and home blood pressure values differ among subjects who are not pregnant [2]. The beneficial evidence of measuring home blood pressure in pregnant women has been increasing.

In a joint statement of the American Heart Association, the American Society of Hypertension, and the Preventive Cardiovascular Nurses Association, home blood pressure monitoring is theoretically ideal for determining changes in blood pressure during pregnancy because it is the optimal way to provide multiple readings recorded at the same time of day over prolonged periods [12]. The Guidelines of the European Society of Hypertension state that home blood pressure measurement, although not commonly practiced, has a considerable potential for improving the management of pregnant women [13]. The recent task force report from ACOG (2013) recommends that pregnant women with chronic hypertension or with poorly controlled blood pressure should measure blood pressure at home [14].

In general, blood pressure slightly decreases in normal pregnant women and reaches a nadir at ~20 weeks of gestation. Systolic and diastolic blood pressures increase by 10 and 7 mmHg, respectively, throughout the 40 weeks of gestation [15].

The same study found that both environmental temperature and gestational age affect home blood pressure values. Notably, home systolic/diastolic blood pressures during pregnancy changed by 12.8/12.5 and 3.1/3.0 mmHg in women who delivered in January and July, respectively [16].

In most international guidelines, 135/85 mmHg of home blood pressure is now widely used as a criterion for the diagnosis of hypertension for nonpregnant adults. The reference value was initially proposed based on the results of a cross-sectional study because no prognosis-based study is available. From cross-sectional analysis performed in the Ohasama study, the mean + 1 standard deviation (SD), mean + 2 SD, and the 95th percentile values obtained as reference upper limits for home blood pressure from subjects identified as normotensive based on screening blood pressure were 125/77, 137/86, and 134/83 mmHg, respectively [17]. A cross-sectional study of 4021 multiethnic pregnant women was conducted at an outpatient clinic of a university hospital in Switzerland. The study reported that the values for automated blood pressure in 495% of pregnant women were 128/74, 128/78, and 131/78 mmHg at 12, 20, and 36 weeks of gestation, respectively [18]. Denolle et al. [19] analyzed data from six French hospitals. They proposed that the upper normal limits of mean home blood pressure + 2 SD were 118/73, 117/73, and 121/80 mmHg and 116/70, 113/70, and 118/76 mmHg in 495% of pregnant women during the first, second, and third trimesters, respectively. The mean home blood pressure values (± SD) among 101 normotensive Japanese women were 101.8 ± 7.9/59.8 ± 5.8 and 110.1 ± 9.7/66.8 ± 7.7 mmHg at 20 and 40 weeks of gestation, respectively [20]. The home blood pressure values were almost identical between these two studies.

A recent cross-sectional study based on longitudinal observation clearly showed a correlation between office and home blood pressures among pregnant women. Based on the standard major axis method, home blood pressure values equivalent to an office blood pressure of 140/90 mmHg were 120.8/83.5 mmHg, 126.0/85.2 mmHg, and 136/89.3 mmHg in the first, second, and third trimesters, respectively [21]. The results suggest that diagnostic thresholds based on home blood pressure in pregnant women at or near term are comparable with those for nonpregnant women. Although the consensus how the pregnant women with relatively high home blood pressure like 130/84 mmHg during early pregnancy should be treated is not obtained yet, women have high risk of HDP. When home and office blood pressure values are compared, office blood pressure is significantly higher in primiparous women compared with multiparous women, whereas home blood pressure does not significantly differ between the groups [22].

Similar to ambulatory blood pressure measurement, white-coat hypertension can also be defined by home blood pressure measurement. The white-coat effect defined by home blood pressure is more evident in primiparous rather than in multiparous women [23]. One study found that 76% of 57 hypertensive pregnant women who tele-transmitted their blood pressure values measured at home had white-coat hypertension [24].

The prognostic significance of home blood pressure has recently been reported. Inoue et al. [25] measured urinary salt excretion and home blood pressure for 7 consecutive days before the 20th and after the 30th gestational week. They found that higher home blood pressure before the 20th gestational week and older age were significantly associated with the development of hypertensive disorders in pregnancy after adjustment for a family history of hypertension and serum uric acid in the first trimester. The adjusted odds ratio was 1.15 for home systolic blood pressure and 1.14 for home diastolic blood pressure [25]. A recent study has shown that birth weight was more significantly associated with home blood pressure than with office blood pressure. Although higher quintiles of home mean and diastolic blood pressure showed higher risk for a 500 g lower infant birth weight, the association was weak in office blood pressure (Fig. 12.3). An elevation of 1-SD in home and office diastolic blood pressures was associated with a 1.28- and 1.06-fold higher risk for a 500 g lower birth weight, respectively (Fig. 12.4) [26].

Long-term prognosis is also an important issue. Home and office blood pressures measured 7 years after delivery were associated with maternal gestational hypertension defined by office blood pressure [27]. In such situations, other indexes instead of office blood pressure during pregnancy may reflect the ideal association between gestational hypertension and long-term prognosis. A feasibility trial was conducted using a recently developed mobile application that transmits blood pressure measured at home [28, 29].

12.4 Summary

Prognostic factors may differ according to the pathophysiology among hypertensive pregnant women. Out-of-office blood pressure may improve the accuracy of classifying hypertension during pregnancy. Fine classification may help to identify

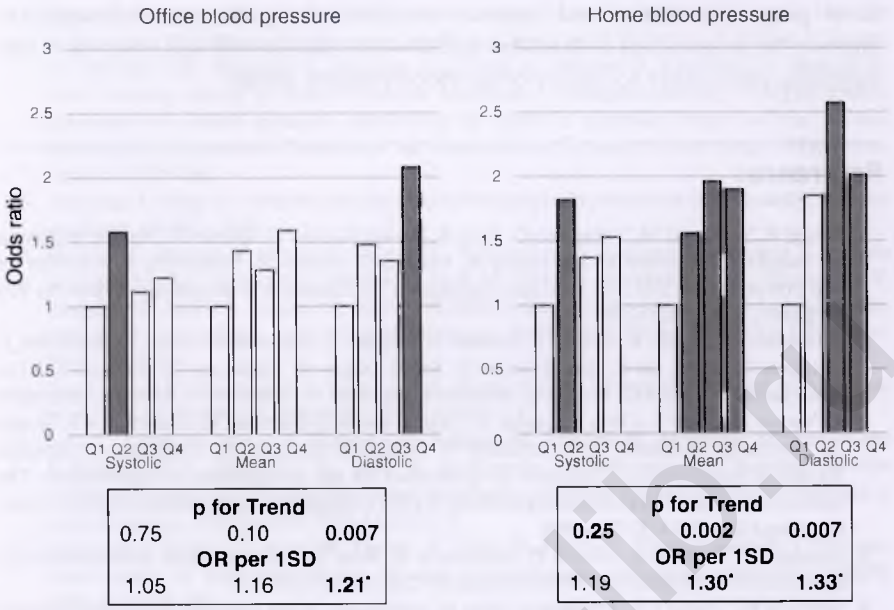


Fig. 12.3 Association between office and home blood pressures before the 20th gestational week and infant birth weight. Horizontal line shows quartiles of systolic, mean, and diastolic blood pressure values before the 20th gestational week of pregnancy measured at office and home. Vertical line shows proportional odds ratios for having 500 g lower infant birth weight. All models were adjusted by maternal age, pre-pregnancy body mass index, GWG, parity, history of PIH, family history of hypertension, smoking status, alcohol intake, GDM, delivery week, infant's sex, season of conception, and gestational week at BP measurement (Figure from Iwama N, et al. *Hypertens Res.* 2016)

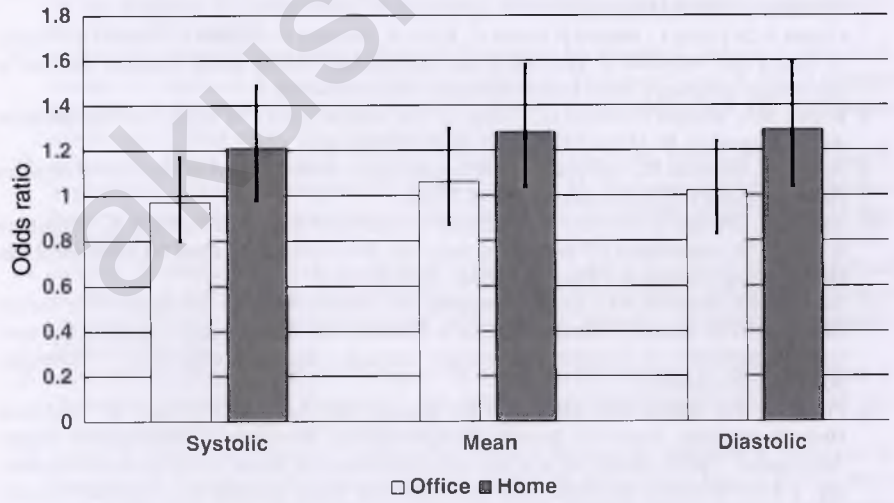


Fig. 12.4 Comparison of effects between office and home blood pressures on infant birth weight. Vertical line shows proportional odds ratios for having 500 g lower infant birth weight when office and home blood pressures were included into the model as continuous variables simultaneously per 1-significant difference in the variables. All models were adjusted by maternal age, pre-pregnancy BMI, GWG, parity, history of PIH, family history of hypertension, smoking status, alcohol intake, GDM, delivery week, infant's sex, season of conception, and gestational week at BP measurement (Figure from Iwama N, et al. *Hypertens Res.* 2016)

novel prognostic factors and improve treatment. Early treatment strategies to improve the prognosis of both mother and newborn should be based on an early fine diagnosis, particularly for out-of-office blood pressure values.

References

1. Takagi K, Yamasaki M, Nakamoto O, Saito S, Suzuki H, Seki H, Takeda S, Ohno Y, Sugimura M, Suzuki Y, Watanabe K, Matsubara K, Makino S, Metoki H, Yamamoto T. A review of best practice guide 2015 for care and treatment of hypertension in pregnancy. Hypertens Res Pregnancy. 2015;3:65–103.
2. Shimamoto K, Ando K, Fujita T, Hasebe N, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ishimitsu T, Ito M, Ito S, Itoh H, Iwao H, Kai H, Kario K, Kashihara N, Kawano Y, Kim-Mitsuyama S, Kimura G, Kohara K, Komuro I, Kumagai H, Matsuura H, Miura K, Morishita R, Naruse M, Node K, Ohya Y, Rakugi H, Saito I, Saitoh S, Shimada K, Shimosawa T, Suzuki H, Tamura K, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Umemura S. Japanese Society of Hypertension Committee for guidelines for the management of hypertension. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2014). Hypertens Res. 2014;37:253–390.
3. Dehaeck U, Thurston J, Gibson P, Stephanson K, Ross S. Blood pressure measurement for hypertension in pregnancy. J Obstet Gynaecol Can. 2010;32:328–34.
4. Hermida RC, Ayala DE. Prognostic value of ambulatory blood pressure monitoring in pregnancy. J Hypertens. 2010;28:1110–1.
5. Brown MA, Robinson A, Bowyer L, Buddle ML, Martin A, Hargood JL, Cario GM. Ambulatory blood pressure monitoring in pregnancy: what is normal? Am J Obstet Gynecol. 1998;178:836–42.
6. Kikuya M, Hansen TW, Thijs L, Björklund-Bodegård K, Kuznetsova T, Ohkubo T, Richart T, Torp-Pedersen C, Lind L, Ibsen H, Imai Y, Staessen JA. International database on ambulatory blood pressure monitoring in relation to cardiovascular outcomes investigators. Diagnostic thresholds for ambulatory blood pressure monitoring based on 10-year cardiovascular risk. Circulation. 2007;115:2145–52.
7. Cugini P, Di Palma L, Battisti P, Leone G, Pachí A, Paesano R, Masella C, Stirati G, Pierucci A, Rocca AR, Morabito S. Describing and interpreting 24-hour blood pressure patterns in physiologic pregnancy. Am J Obstet Gynecol. 1992;166:54–60.
8. Brown MA, Mangos G, Davis G, Homer C. The natural history of white coat hypertension during pregnancy. Br J Obstet Gynaecol. 2005;112:601–6.
9. Ayala DE, Hermida RC. Influence of parity and age on ambulatory monitored blood pressure during pregnancy. Hypertension. 2001;38:753–8.
10. Eguchi K, Ohmaru T, Ohkuchi A, Hirashima C, Takahashi K, Suzuki H, Kario K, Matsubara S, Suzuki M. Ambulatory BP monitoring and clinic BP in predicting small for-gestational-age infants during pregnancy. J Hum Hypertens. 2016;30:62–7.
11. Salazar MR, Espeche WG, Leiva Sisnieguez BC, Balbín E, Leiva Sisnieguez CE, Stavile RN, March CE, Grassi F, Santillan C, Cor S, Carbajal HA. Significance of masked and nocturnal hypertension in normotensive women coursing a high-risk pregnancy. J Hypertens. 2016;34(11):2248–52.
12. Pickering TG, Miller NH, Ogedegbe G, Krakoff LR, Artinian NT, Goff D. American Heart Association, American Society of Hypertension, Preventive Cardiovascular Nurses Association. Call to action on use and reimbursement for home blood pressure monitoring: a joint scientific statement from the American Heart Association, American Society of Hypertension, and Preventive Cardiovascular Nurses Association. Hypertension. 2008;52:10–29.

13. Parati G, Stergiou GS, Asmar R, Bilo G, de Leeuw P, Imai Y, Kario K, Lurbe E, Manolis A, Mengden T, O'Brien E, Ohkubo T, Padfield P, Palatini P, Pickering T, Redon J, Revera M, Ruilope LM, Shennan A, Staessen JA, Tisler A, Waeber B, Zanchetti A, Mancia G, ESH Working Group on Blood Pressure Monitoring. European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. J Hypertens. 2008;26:1505–26.
14. American College of Obstetricians and Gynaecologists. Hypertension in pregnancy. Practice guideline. ACOG. 2013.
15. Metoki H, Ohkubo T, Obara T, Akutsu K, Yamamoto M, Ishikuro M, Sakurai K, Iwama N, Katagiri M, Sugawara J, Hirose T, Sato M, Kikuya M, Yagihashi K, Matsubara Y, Yaegashi N, Mori S, Suzuki M, Imai Y, BOSHI Study Group. Daily serial hemodynamic data during pregnancy and seasonal variation: the BOSHI study. Clin Exp Hypertens. 2012;34:290–6.
16. Rey E, Pilon F, Boudreault J. Home blood pressure levels in pregnant women with chronic hypertension. Hypertens Pregnancy. 2007;26:403–14.
17. Imai Y, Satoh H, Nagai K, Sakuma M, Sakuma H, Minami N, Munakata M, Hashimoto J, Yamagishi T, Watanabe N, Yabe T, Nishiyama A, Nakatsuka H, Koyama H, Abe K. Characteristics of a community-based distribution of home blood pressure in Ohasama in northern Japan. J Hypertens. 1993;11:1441–9.
18. Ochsenbein-Kolble N, Roos M, Gasser T, Huch R, Huch A, Zimmermann R. Cross sectional study of automated blood pressure measurements throughout pregnancy. BJOG. 2004;111:319–25.
19. Denolle T, Daniel JC, Calvez C, Ottavioli JN, Esnault V, Herpin D. Home blood pressure during normal pregnancy. Am J Hypertens. 2005;18:1178–80.
20. Metoki H, Ohkubo T, Watanabe Y, Nishimura M, Sato Y, Kawaguchi M, Hara A, Hirose T, Obara T, Asayama K, Kikuya M, Yagihashi K, Matsubara Y, Okamura K, Mori S, Suzuki M, Imai Y, BOSHI Study Group. Seasonal trends of blood pressure during pregnancy in Japan: the babies and their parents' longitudinal observation in Suzuki Memorial Hospital in Intrauterine Period study. J Hypertens. 2008;26:2406–13.
21. Mikami Y, Takai Y, Era S, Ono Y, Saitoh M, Baba K, Suzuki H, Seki H. Provisional criteria for the diagnosis of hypertension in pregnancy using home blood pressure measurements. Hypertens Res. in press.
22. Ishikuro M, Obara T, Metoki H, Ohkubo T, Yamamoto M, Akutsu K, Sakurai K, Iwama N, Katagiri M, Yagihashi K, Yaegashi N, Mori S, Suzuki M, Kuriyama S, Imai Y. Blood pressure measured in the clinic and at home during pregnancy among nulliparous and multiparous women: the BOSHI study. Am J Hypertens. 2013;26:141–8.
23. Ishikuro M, Obara T, Metoki H, Ohkubo T, Iwama N, Katagiri M, Nishigori H, Narikawa Y, Yagihashi K, Kikuya M, Yaegashi N, Hoshi K, Suzuki M, Kuriyama S, Imai Y. Parity as a factor affecting the white-coat effect in pregnant women: the BOSHI study. Hypertens Res. 2015;38:770–5.
24. Denolle T, Weber JL, Calvez C, Getin Y, Daniel JC, Lurton O, Cheve MT, Marechaud M, Bessec P, Carbonne B, Razafintsalama T. Diagnosis of white coat hypertension in pregnant women with teletransmitted home blood pressure. Hypertens Pregnancy. 2008;27:305–13.
25. Inoue M, Tsuchihashi T, Hasuo Y, Ogawa M, Tominaga M, Arakawa K, Oishi E, Sakata S, Ohtsubo T, Matsumura K, Kitazono T. Salt intake, home blood pressure, and perinatal outcome in pregnant women. Circ J. 2016;80:2165–72.
26. Iwama N, Metoki H, Ohkubo T, Ishikuro M, Obara T, Kikuya M, Yagihashi K, Nishigori H, Sugiyama T, Sugawara J, Yaegashi N, Hoshi K, Suzuki M, Kuriyama S, Imai Y, BOSHI Study Group. Maternal clinic and home blood pressure measurements during pregnancy and infant birth weight: the BOSHI study. Hypertens Res. 2016;39:151–7.

27. Hosaka M, Asayama K, Staessen JA, Tatsuta N, Satoh M, Kikuya M, Ohkubo T, Satoh H, Imai Y, Nakai K. Relationship between maternal gestational hypertension and home blood pressure in 7-year-old children and their mothers: Tohoku Study of Child Development. Hypertens Res. 2015;38:776–82.
28. Marko KI, Krapf JM, Meltzer AC, Oh J, Ganju N, Martinez AG, Sheth SG, Gaba ND. Testing the feasibility of remote patient monitoring in prenatal care using a mobile App and connected Devices: a prospective observational trial. JMIR Res Protoc. 2016;5:e200.
29. Ganapathy R, Grewal A, Castleman JS. Remote monitoring of blood pressure to reduce the risk of preeclampsia related complications with an innovative use of mobile technology. Pregnancy Hypertens. 2016;6:263–5.

MicroRNA

13

Toshihiro Takizawa, Akihide Ohkuchi, Shigeki Matsubara, Toshiyuki Takeshita, and Shigeru Saito

Abstract

Aberrant expression of microRNAs (miRNAs) occurs in the preeclamptic placenta, where it causes dysregulation of functional molecules. The human placenta expresses a unique set of miRNAs (e.g., chromosome 19 miRNA cluster miRNAs). miRNAs, including placenta-specific miRNAs, are released from the placental villous trophoblast into the maternal circulation via exosomes. Because placenta-specific miRNAs are detectable in maternal blood, information about the placenta can be obtained during routine pregnancy screening via minimally invasive tests such as blood sampling, rather than highly invasive tests such as tissue biopsy. Radical treatment for preeclampsia (PE) is termination of the pregnancy, but in cases of early-onset PE, the pregnancy should be prolonged as long as possible to improve the infant's prognosis. Prediction of PE in the first trimester could make it possible to prevent PE and develop novel therapeutic strategies to treat PE. This chapter explores the predictive utility of plasma placenta-specific miRNAs.

Keywords

Placenta-specific microRNA • Exosome • Maternal plasma • Early-onset preeclampsia

T. Takizawa, M.D., Ph.D. (✉)
Department of Molecular Medicine and Anatomy, Nippon Medical School, Tokyo, Japan
e-mail: t-takizawa@nms.ac.jp

A. Ohkuchi, M.D., Ph.D. • S. Matsubara, M.D., Ph.D.
Department of Obstetrics and Gynecology, Jichi Medical University, Tochigi, Japan

T. Takeshita, M.D., Ph.D.
Department of Obstetrics and Gynecology, Nippon Medical School, Tokyo, Japan

S. Saito, M.D., Ph.D.
Department of Obstetrics and Gynecology, Faculty of Medicine, University of Toyama, Toyama, Japan

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_13

13.1 MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA molecules that are single-stranded in their functional form (approximately 22 nucleotides in length). miRNAs bind to partial complementary sequences within the 3′-untranslated region of coding RNAs (target mRNAs) to regulate gene expression [1]. This post-transcriptional regulation by miRNAs is involved in a variety of physiological cell functions and the molecular pathology of diseases [2]. To date, 2588 types of human mature miRNAs [1881 types of precursor miRNAs (pre-miRNAs)] have been registered in a database [University of Manchester: miRBase Release 21. http://www.mirbase.org/index.shtml (2014). Accessed 31 March 2017.].

Moreover, recent next-generation sequencing studies have revealed that miRNAs are more complex and more diverse than previously thought [3, 4]. Human miRNAs have been roughly classified into two main categories according to their modes of biogenesis, i.e., canonical and noncanonical miRNAs [5] (Fig. 13.1). Canonical

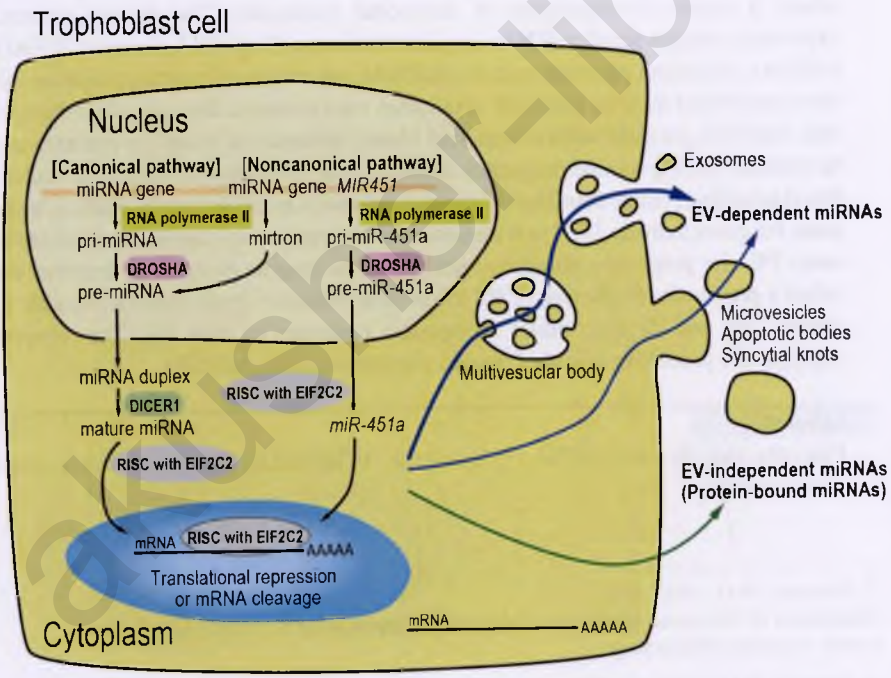


Fig. 13.1 The biogenesis of microRNAs (miRNAs) and miRNA secretory pathways. Canonical miRNAs are generated from the DROSHA-dependent and DICER1-dependent pathway. Noncanonical miRNAs are produced from DROSHA-independent pathways and DICER1-independent pathways. Mirtrons are miRNA precursors generated from a DROSHA-independent pathway. Endogenous short hairpin RNAs are produced from another DROSHA-independent pathway (not shown). *miR-451a* is generated from a DICER1-independent pathway. miRNAs are secreted via extracellular vesicle (EV)-dependent and EV-independent pathways. EV-dependent miRNAs are packaged inside membrane-bound vesicles; EVs include not only exosomes but also other EVs (i.e., microvesicles, apoptotic bodies, and syncytial knots). EV-independent miRNAs bind to proteins [e.g., EIF2C2 (also known as AGO2)]. RISC, RNA-induced silencing complex

miRNAs are generated from the DROSHA-dependent and DICER1-dependent pathway [5], as described below. miRNA genes are transcribed as long primary transcripts (pri-miRNAs) by RNA polymerase II in the nucleus. The pri-miRNAs are then cleaved into pre-miRNAs (approximately 70 nucleotides) by the RNase III DROSHA. The pre-miRNAs are exported to the cytoplasm and subsequently processed into miRNA duplexes (approximately 22 nucleotides) by the RNase III enzyme DICER1 that produces mature miRNAs. One strand of the mature miRNAs is loaded into the RNA-induced silencing complex (RISC), which contains EIF2C2 (also known as AGO2). The incorporated miRNA binds partial complementary sequences within the 3′ untranslated region of target mRNAs and mediates gene suppression by translational repression or mRNA cleavage.

Noncanonical miRNAs are produced from alternative biogenesis pathways, i.e., DROSHA-independent pathways and DICER1-independent pathways [5]. Mirtrons are miRNA precursors generated from a DROSHA-independent pathway; they are spliced and debranched short introns forming pre-miRNA hairpins. Approximately 500 mirtrons including confidents and candidates have been discovered [6]. Although mirtrons can produce functional miRNAs [7], their functional roles in the human placenta remain to be elucidated. Endogenous short hairpin RNAs (e.g., 7-methylguanosine capped pre-miR-320a) are miRNA precursors generated from another DROSHA-independent pathway [8]. *miR-451a* is generated from a DICER1-independent pathway [9]. After DROSHA cleavage of pri-miR-451a, the pre-miR-451a is directly incorporated into EIF2C2 without DICER1 cleavage. It has been shown that *miR-451a* regulates human erythropoiesis by targeting *RAB14* [10]. Deep sequencing has also revealed the isoforms of miRNAs (isomiRs) that differ by a few bases from their corresponding canonical mature sequences [11, 12]; isomiRs can function as regular miRNAs [13, 14].

In human trophoblast cells, the miRNA biogenesis pathway is functional and produces miRNAs. Donker et al. investigated the expression of miRNA-processing molecules (e.g., *DROSHA*, *XPO5*, *DICER1*, and *EIF2C2*) in villous trophoblast cells in vitro, i.e., primary trophoblast cells isolated from term placentas [15]. The miRNA-processing molecules are expressed in both primary cytotrophoblast (CTB) cells and differentiated multinucleated syncytia [i.e., syncytiotrophoblast (STB) cells]; interestingly, hypoxia has an insignificant influence on the expression of the key molecules in these cells [15]. In villous trophoblast cells in vivo, the expression of miRNA-processing molecules has also been studied. Forbes et al. reported that DICER1 is abundant in CTB cells [16, 17]. EIF2C2 is present in both STB and CTB cells [15, 18]. Placenta-specific miRNAs (e.g., *miR-517b-3p*) described below are produced in both STB and CTB cells [19, 20].

13.2 Placenta-Derived MicroRNAs

To data, approximately 800 different miRNAs species are known to be expressed in the human placenta [21]. However, the biological significance of most miRNAs in the human placenta is still largely unknown. Some miRNAs have been shown to regulate trophoblast development and function; these trophoblast-associated miRNAs and their validated targets are summarized in Table 13.1.

Table 13.1 miRNAs involved in the regulation of trophoblast cell functions

miRNA	Validated targets	Function	References
let-7a-5p	*MAPK1*, *MYC*	Proliferation	[70]
miR-18a-5p	*ESR1*	Invasion, apoptosis	[71]
miR-19b-3p	*CYP19A1*, *GCM1*	Differentiation	[72]
miR-20a-5p	*FOXA1*	Proliferation, migration, and invasion	[73]
miR-29b-3p	*ITGB1*, *MCL1*, *MMP2*, *VEGFA*	Apoptosis, invasion, and angiogenesis	[74]
miR-34a-5p	*MYC*	Invasion	[75]
miR-101-3p	*ERP44*	Apoptosis	[76]
miR-106a-5p	*CYP19A1*	Differentiation	[72]
miR-125b-1-3p	*S1PR1*	Invasion	[77]
miR-135b-5p	*CXCL12*	Invasion	[78]
miR-137	*ESRRA*	Proliferation and migration	[79]
miR-155-5p	*CYR61*	Angiogenesis	[49]
	CCND1	Proliferation and migration	[80]
miR-193b-3p	*TGFB2*	Migration and invasion	[21]
miR-195-5p	*ACVR2A*	Invasion	[81]
miR-204-5p	*MMP9*	Invasion	[82]
miR-205-5p	*MED1*	Differentiation	[83]
miR-210-3p	*CCNE1*, *EFNA3*, *HOXA9*	Proliferation and invasion	[51]
	HSD17B1	Steroidogenesis	[84]
	KCMF1	Invasion	[85]
	STAT6	Inflammatory	[86]
miR-376c-3p	*ALK5*, *ALK7*	Proliferation and invasion	[87]
miR-377-3p	*MAPK1*, *MYC*	Proliferation	[70]
miR-378a-5p	*NODAL*	Proliferation, survival, migration, and invasion	[88]
miR-515-5p	*CYP19A1*, *FZD5*, *GCM1*	Differentiation	[53]
miR-518c-3p	*HSA17B1*	Steroidogenesis	[51]
miR-519d-3p	*CXCL6*, *FOXL2*, *NR4A2*	Migration	[89]
	MMP2	Invasion	[90]
miR-520c-3p	*CD44*	Invasion	[43]
miR-675-5p	*NOMO1*	Proliferation	[91]

Human placental trophoblast cells express a unique set of miRNAs (i.e., placenta-specific miRNAs) in addition to the miRNAs that are ubiquitously expressed in tissues and organs, albeit with differing expression levels (e.g., *miR-21-5p*) [22, 23]. Most placenta-specific miRNAs are transcribed from three clusters on chromosomes 14 and 19: the chromosome 14 miRNA cluster (C14MC), the chromosome 19 miRNA cluster (C19MC), and the miR-371-3 cluster (in the vicinity of C19MC) [22, 23]. C14MC is formed of 54 types of miRNA genes, which produce 63 types of mature miRNAs; the C14MC miRNAs are imprinting genes that are expressed uniquely by maternally derived alleles [24]. The C14MC miRNAs are highly expressed in trophoblast cells during the first trimester, but this expression decreases toward the third trimester. C19MC is formed of 46 types of miRNA genes, which produce 58 types of mature miRNAs; the C19MC miRNAs are imprinting genes that are expressed uniquely by paternally derived alleles [25]. C19MC is only conserved among primate species, including humans [19, 26]. C19MC miRNA expression increases toward the third trimester, and C19MC miRNAs are strongly expressed in

trophoblast cells during the third trimester. The miR-371-3 cluster is formed of four types of miRNA genes (i.e., *mir-371a*, *mir-371b*, *mir-372*, and *mir-373*), and this cluster is primarily expressed in trophoblast cells and embryonic stem cells [22].

13.3 Placenta-Derived Exosomes

13.3.1 Placental miRNAs Are Released from the Villous Trophoblast into Maternal Circulation via Exosomes

Multivesicular bodies (MBVs) are intracellular organelles containing intraluminal vesicles and are formed by the inward budding of the outer endosomal membrane. When some MBVs fuse with the plasma membrane, intraluminal vesicles are released as exosomes outside cells by exocytosis. Intracellular miRNAs are released into the extracellular space via the exosome-dependent pathway as one of the miRNA secretory pathways [27, 28] and can consequently be detected in body fluids (blood, cerebrospinal fluid, urine, etc.) [29] (Fig. 13.1).

The surface of human chorionic villi covers two trophoblastic layers: the outer STB layer, which is in contact with maternal blood in the intervillous space, and the inner CTB layer, which is subjacent to the STB and supported by a basal lamina. Many MBVs are present in STB cells of the human placenta (Fig. 13.2). These MBVs are the main source of placenta-derived exosomes detected in maternal blood; STB-derived exosomes contain miRNAs including placenta-specific miRNAs [20, 30]. Thus, placenta-specific miRNAs within exosomes are specific

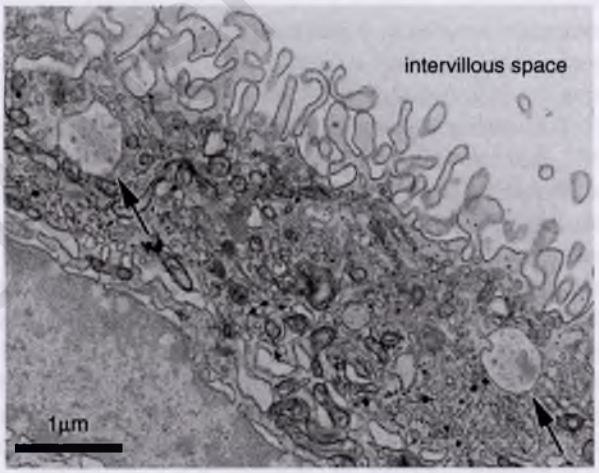


Fig. 13.2 Transmission electron micrograph of the syncytiotrophoblast (STB) in a terminal villus of the human full-term placenta. The STB covers the outermost surface of the villus and is in direct contact with maternal blood in the intervillous space. Multivesicular bodies are indicated with arrows. Well-developed microvilli and intracellular organelles (e.g., mitochondria, endoplasmic reticulum, and nucleus) are evident. Adapted from Takizawa et al. [69] with permission from Yodosha (Tokyo, Japan)

markers for placenta-derived exosomes together with placental alkaline phosphatase (PLAP) [31] and human endogenous retrovirus envelope proteins [syncytin-1 (ERVWE1) and syncytin-2 (HERV-FRD)] [32], which are found on the surface of exosome membranes. However, the extracellular vesicles (EVs) released from STB cells include not only exosomes but also other EVs. Therefore, caution is required when interpreting placenta-specific miRNAs that are present in maternal plasma. EVs are classified into exosomes, microvesicles, apoptotic bodies, and syncytial knots (syncytial nuclear aggregates) [33, 34]. Exosomes are the smallest EVs (30–100 nm) and are derived from MVBs, as mentioned above. Microvesicles are larger than exosomes (100 nm–2 μm) and are formed by direct budding from the plasma membrane.

Additionally, secreted miRNAs (e.g., plasma miRNAs) exist as EV-dependent and EV-independent miRNAs (Fig. 13.1). EV-dependent miRNAs are packaged inside membrane-bound vesicles. EV-independent miRNAs bind to EIF2C2 and high-density lipoproteins, and these miRNA-binding molecules protect miRNAs from RNase activity [35–37].

13.3.2 Placenta-Derived Exosomes in Maternal Blood during Normal Pregnancy

Plasma exosomes are a mixture of placenta-derived exosomes and maternal cell-derived exosomes during pregnancy. The concentration of plasma exosomes (measured by nanoparticle tracking analysis) increases toward the third trimester (approximately a 13-fold increase between the first and third trimesters) [38]. Among plasma exosomes isolated by differential ultracentrifugation, placenta-derived exosomes are detectable as early as the sixth week of pregnancy, and the concentration of placenta-derived exosomes (i.e., PLAP concentration in exosomes) increases during the first trimester (6–12 weeks) [39]. Moreover, the concentration of placenta-derived exosomes increases at least until the second trimester (22–24 weeks) [38]. From the point of view of placenta-derived EVs, including exosomes and microvesicles, but not from placenta-derived exosomes, placenta-derived EVs (accounting for ≤1% of plasma EVs [40]) are detectable from the second trimester and increase toward the third trimester [41].

In this chapter, we primarily explore the clinical implications of placenta-derived miRNAs and exosomes in patients with preeclampsia (PE); their physiological roles are beyond the scope of this discussion. Placenta-specific miRNAs are transferred into maternal cells mediated by exosomes, and their target genes are subsequently modulated in the recipient cells. For example, placenta-specific *miR-517a-3p*, a C19MC miRNA, is transferred from trophoblast cells to maternal natural killer cells and subsequently inhibits the target mRNA *PRKG1* [42]. Moreover, Takahashi et al. proposed a novel model of exosome-mediated cell–cell communication in which STB-derived exosomes, including C19MC miRNA *miR-520c-3p*, regulate cell invasion by targeting *CD44* mRNA in extravillous trophoblast cells [43]. More information can be obtained from other recent reviews [34, 44, 45].

13.4 Abnormal Expression of miRNAs in the Preeclamptic Placenta

Many reports have discussed abnormal expression of miRNAs in the placentas of women with PE [21, 46–53]. However, findings related to the types of miRNAs that are aberrantly expressed (i.e., PE-related miRNAs) are not always consistent between studies. This lack of consistency is likely attributable to a variety of factors, including differences in sample harvesting, preparation, and analytical methods, and different aspects of clinical study design including sample size and classifications based on clinical characteristics.

Although no consensus has been reached regarding the expression profile of PE-related miRNAs, increased expression of *miR-210-3p* in the placentas of women of women with PE is the sole common feature among many reports. *miR-210-3p* is a hypoxia-induced miRNA that is induced by HIF1A. *miR-210-3p* suppresses translation of a variety of genes (e.g., *E2F3* and *ISCU*), plays important roles in the hypoxic response (e.g., cell growth arrest and repression of mitochondrial metabolism), and may serve as a prognostic and therapeutic target (e.g., treatment of ischemic disorders) [54]. Because hypoxia is implicated as a key regulator of placental development, it is likely that increased expression of *miR-210-3p* in the placentas of women with PE is involved in the molecular pathogenesis of PE.

C19MC-derived miRNAs are also upregulated in the placentas of women with PE [21, 47, 51–53]. We conducted a comprehensive miRNA expression analysis to elucidate the changes in miRNA expression in the placentas of women with PE and found that aberrant expression of C19MC miRNA *miR-518c-3p*, as well as that of *miR-210-3p*, leads to dysregulation of *HSD17B1* in the placentas of women with PE [51]. HSD17B1 is a steroidogenic enzyme that catalyzes conversion of estrone to 17β-estradiol and is expressed predominantly in the human placenta [55]. Zhang et al. reported that the C19MC miRNA *miR-515-5p* suppresses target molecules (*CYP19A1*, *FZD5*, and *GCM1*) that play an important role in trophoblast differentiation (i.e., syncytium formation) [53]. They also reported that *miR-515-5p* expression and expression of its target molecules are inversely correlated in the placentas of women with PE. These findings suggest that aberrant expression of *miR-210-3p* and C19MC miRNAs occurs in the placentas of women with PE and causes dysregulation of molecules that function in the placenta.

Additionally, isomiRs (e.g., isomiRs of *miR-24-3p*) are differentially expressed between PE and normal samples with increased 3′ end addition of an adenosine [56]. Further functional studies will be needed to comprehensively clarify the role(s) of PE-related miRNAs in the onset and/or progression of PE.

13.5 Placenta-Derived Exosomes in Maternal Blood as Predictive Markers for PE

During pregnancy, placenta-derived exosomes and placenta-specific miRNAs are detectable in maternal blood, so information regarding the placenta can be obtained during routine pregnancy screening by minimally invasive tests such as blood

sampling rather than by highly invasive tests such as tissue biopsy (villus sampling and placental biopsy).

Pillay et al. reported differences in placenta-derived exosomes in plasma between patients with early-onset PE and late-onset PE (early onset, development of PE before 34 weeks of pregnancy; late onset, development of PE at or after 34 weeks of pregnancy) [57]. The concentration of placenta-derived exosomes increases significantly in women with early-onset PE (by approximately 3.6-fold) compared with that in women with normal pregnancies, whereas the concentration significantly decreases in women with late-onset PE compared with that in women with normal pregnancies and women with early-onset PE (both by approximately 0.4-fold). It appears that the release of exosomes from early-onset preeclamptic placentas into maternal plasma increases, whereas that from late-onset preeclamptic placentas decreases. These results may reflect differences in disease type (i.e., differences in the etiology and pathology of early- and late-onset PE).

Goswami et al. studied the release of placenta-derived EVs (including exosomes and microvesicles) into maternal plasma in pregnancies complicated by early- and late-onset PE using enzyme-linked immunosorbent assay with PLAP as an index [41]. The concentration of placenta-derived EVs in plasma significantly increases in patients with early-onset PE compared with that in women with normal pregnancies; however, they found no differences in the concentration of placenta-derived EVs between women with late-onset PE and women with normal pregnancies. Germain et al. reported that while the differences between early-onset and/or late-onset PE were not specified, the concentration of placenta-derived EVs (PLAP concentration in plasma EVs) in PE pregnancies is significantly elevated compared with that of normal pregnancies [58]. Dragovic et al. also analyzed the number of placenta-derived EVs from plasma samples of patients with late-onset PE by flow cytometry and nanoparticle tracking analysis and found no differences in the number of placenta-derived EVs between women with late-onset PE and women with normal pregnancies [40].

Taken together, these findings suggest that placenta-derived exosomes (and EVs) tend to increase significantly in the plasma of women with early-onset PE than those with a normal pregnancy. However, large-scale cohort studies of plasma exosomes during the first trimester are needed to determine whether this change in placenta-derived exosomes occurs before the onset of early-onset PE, i.e., whether it can be predicted.

13.6 Placenta-Specific miRNAs in Maternal Blood as Predictive Markers for PE

Miura et al. reported that 24 types of miRNA are "pregnancy-associated miRNAs" that are highly expressed in the human placenta and significantly decrease in maternal plasma after delivery [59]. These miRNAs include 16 types of C19MC miRNAs and 5 types of C14MC miRNAs, which are the abovementioned placenta-specific miRNAs. Miura et al. also showed significantly elevated ten types of C19MC miRNAs

(*miR-515-5p*, *miR-516a-5p*, *miR-516b-5p*, *miR-518b*, *miR-519d-3p*, *miR-520a-5p*, *miR-520h*, *miR-525-5p*, *miR-526b-5p*, and *miR-1323*) in the plasma of pregnant women with severe PE (20 patients) compared with the plasma of women with normal pregnancies (20 patients) [60]. Kotlabova et al. detected seven types of C19MC miRNAs (*miR-516-5p*, *miR-517-5p*, *miR-518b*, *miR-520a-5p*, *miR-520h*, *miR-525-5p*, and *miR-526a*) as "placenta-specific miRNAs" in the placenta and maternal plasma [61]. Five of the seven C19MC miRNAs (*miR-516-5p*, *miR-517-5p*, *miR-520a-5p*, m*iR-525-5p*, and *miR-526a*) increase significantly in the plasma of pregnant women with PE (63 patients) compared with the plasma of women with normal pregnancies (55 patients) [62]. Hromadnikova et al. conducted a preliminary plasma analysis of a small number of pregnant women with PE (five with late-onset PE and one with early-onset PE) in the first trimester of pregnancy before the onset of PE (12–16 weeks) [63]. The seven C19MC miRNAs reported by Kotlabova et al. [61] (see above) are markedly elevated beyond the cutoff values (mean plasma miRNA levels in pregnant women with normally progressing pregnancies +2 standard deviations), indicating that these C19MC miRNAs could serve as early predictive markers for PE (Fig. 13.3) [63]. Xu et al. examined plasma miRNAs from before (15–18 weeks) and after (35–39 weeks) the onset of severe PE in 20 women and reported decreased levels of *miR-18a* (*miR-18a-5p*?), *miR-19b1* (*miR-19b-3p*?), and *miR-92a1* (*miR-92a-3p*?) and an elevated level of *miR-210* (*miR-210-3p*?) not only after but also before the onset of PE, compared with the corresponding healthy controls (33 women with normal pregnancies) [52]. However, the significance of between-group differences was not specified, and primers for miRNAs were unclear, so their results are questionable. Wu et al. reported significantly elevated miRNAs (*miR-24-3p*, *miR-26a-5p*, *miR-103a-3p*, *miR-130b-3p*, *miR-181a-5p*, *miR-342-3p*, and *miR-574-5p*) in the plasma of pregnant women with severe PE but detected no C19MC miRNAs [64]. Interestingly, Hromadnikova et al. analyzed plasma from the first trimester of pregnancy (10–13 weeks) before the onset of gestational hypertension (GH), which is a different type of hypertensive pregnancy disorder than PE, in 18 pregnant women who developed GH and 28 women with normal pregnancies (control group) [65]. Four types of C19MC miRNAs (*miR-516-5p*, *miR-517-5p*, *miR-518b*, and *miR-520h*) are significantly elevated in the women who developed GH. In their clinical statistical analyses of plasma *miR-520h* alone or plasma *miR-520h* in combination with *miR-518b*, the respective predictive sensitivities, specificities, and positive likelihood ratios (95% confidence intervals) of GH are 73.30% and 63.30%, 92.90% and 92.90%, and 10.27 (2.6–40.4) and 8.87 (3.3–23.7). They concluded that plasma C19MC miRNAs in the first trimester of pregnancy are promising predictive markers for GH.

Williams et al. conducted a next-generation sequencing analysis of plasma and placental miRNAs (placental samples; maternal, fetal, and paternal plasma samples; and plasma samples from nonpregnant women) [66]. Plasma C19MC miRNAs are specifically derived from the placenta; moreover, plasma C19MC miRNA isomiRs specifically reflect the placenta from which it originated. According to calculations by Williams et al. [66], there are approximately 2×10^8 molecules of an individual type of C19MC miRNA per 1 mg of placental tissue and consequently 0.03 molecules of an individual type of C19MC miRNA per 1 mL of maternal plasma. Given

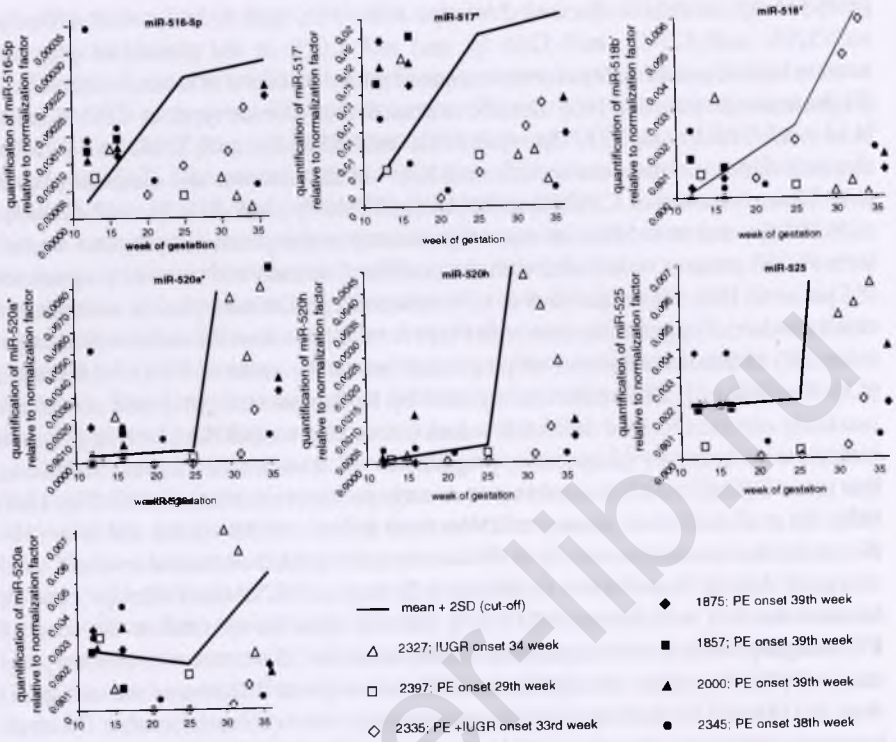


Fig. 13.3 C19MC miRNA expression levels in maternal plasma throughout gestation in seven patients who later developed preeclampsia (PE) and/or fetal growth restriction [formerly known as intrauterine growth retardation (IUGR)]. Seven types of C19MC miRNAs [*miR-516-5p*, *miR-517** (present ID *miR-517-5p*), *miR-518b*, *miR-520a** (present ID *miR-520a-5p*), *miR-520h*, *miR-525* (present ID *miR-525-5p*), and *miR-526a*] were employed as placenta-specific miRNAs. The cutoff (mean plasma miRNA level in pregnant women with normally progressing pregnancies +2 standard deviations) shows the maximum feasible specificity (percentage of those with normally progressing pregnancies identified as not having the condition) for each miRNA assay. Note the marked elevation of placenta-specific miRNA expression during the first trimester (12–16 weeks) in pregnant women who later developed PE and/or IUGR. Data were normalized to *miR-16-5p* and *let-7d-5p*. Adapted from Hromadnikova et al. [63] with permission from Elsevier

that ten copies per 1 mL of plasma are needed for current miRNA detection by real-time quantitative polymerase chain reaction under optimal conditions, C19MC miRNAs are detectable in maternal plasma even in the first trimester of pregnancy if ≥0.3 g of placental villous tissue is to grow within the uterus.

Reports of changes in the plasma miRNA profile in women with PE, as with those of miRNA profile changes in the preeclamptic placenta, have yet to provide consistent data for potential uses as biomarkers predictive of PE; however, it is likely that increases in plasma C19MC miRNAs and *miR-210-3p* are potentially predictive of PE in the first trimester of pregnancy [52, 63, 65].

13.7 Future Prospects

Plasma miRNAs are derived from a complex mixture of maternal cells and placental trophoblast cells during pregnancy. It has been difficult to specifically isolate placenta-derived exosomes from other types of EVs (i.e., placenta-derived microvesicles and maternally derived EVs) in maternal plasma. By obtaining information about other types of placenta-derived miRNAs in addition to placenta-specific miRNAs, it could be possible to obtain further insights into the miRNA-dysregulated molecular mechanism underlying the preeclamptic placenta. Therefore, standardization and development of methodologies to specifically isolate, purify, and quantify placenta-derived exosomes (and EVs) are eagerly awaited.

Radical treatment for PE is termination of the pregnancy, but in cases of early-onset PE, the pregnancy should be prolonged as long as possible to improve the infant's prognosis. Prediction of PE in the first trimester could make it possible to prevent PE and develop novel therapeutic strategies to treat PE. Thus, similar to other candidate PE predictive factors (e.g., soluble FLT1/PGF ratio [67, 68]), identifying qualitative and quantitative changes in plasma placenta-specific miRNAs and exosomes could be the key to predicting PE in the first trimester of pregnancy.

Acknowledgments This work was supported by Grants-in-Aids for Scientific Research and Private University Strategic Research Foundation Support Program (2013–2017) from the Ministry of Education, Culture, Sports, Science and Technology/Japan Society for the Promotion of Science, Japan.

References

1. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33. https://doi.org/10.1016/j.cell.2009.01.002.
2. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;12(12):861–74. https://doi.org/10.1038/nrg3074.
3. Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. Mol Cell. 2007;28(2):328–36. https://doi.org/10.1016/j.molcel.2007.09.028.
4. Castellano L, Stebbing J. Deep sequencing of small RNAs identifies canonical and non-canonical miRNA and endogenous siRNAs in mammalian somatic tissues. Nucleic Acids Res. 2013;41(5):3339–51. https://doi.org/10.1093/nar/gks1474.
5. Miyoshi K, Miyoshi T, Siomi H. Many ways to generate microRNA-like small RNAs: non-canonical pathways for microRNA production. Mol Gen Genomics. 2010;284(2):95–103. https://doi.org/10.1007/s00438-010-0556-1.
6. Ladewig E, Okamura K, Flynt AS, Westholm JO, Lai EC. Discovery of hundreds of mirtrons in mouse and human small RNA data. Genome Res. 2012;22(9):1634–45. https://doi.org/10.1101/gr.133553.111.
7. Schamberger A, Sarkadi B, Orban TI. Human mirtrons can express functional microRNAs simultaneously from both arms in a flanking exon-independent manner. RNA Biol. 2012;9(9):1177–85. https://doi.org/10.4161/rna.21359.
8. Xie M, Li M, Vilborg A, Lee N, Shu MD, Yartseva V, et al. Mammalian 5′-capped microRNA precursors that generate a single microRNA. Cell. 2013;155(7):1568–80. https://doi.org/10.1016/j.cell.2013.11.027.

9. Yang JS, Maurin T, Robine N, Rasmussen KD, Jeffrey KL, Chandwani R, et al. Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated microRNA biogenesis. Proc Natl Acad Sci U S A. 2010;107(34):15163–8. https://doi.org/10.1073/pnas.1006432107.
10. Kim M, Tan YS, Cheng WC, Kingsbury TJ, Heimfeld S, Civin CI. MIR144 and MIR451 regulate human erythropoiesis via RAB14. Br J Haematol. 2015;168(4):583–97. https://doi.org/10.1111/bjh.13164.
11. Morin RD, O'Connor MD, Griffith M, Kuchenbauer F, Delaney A, Prabhu AL, et al. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res. 2008;18(4):610–21. https://doi.org/10.1101/gr.7179508.
12. Vickers KC, Sethupathy P, Baran-Gale J, Remaley AT. Complexity of microRNA function and the role of isomiRs in lipid homeostasis. J Lipid Res. 2013;54(5):1182–91. https://doi.org/10.1194/jlr.R034801.
13. Xia J, Zhang W. A meta-analysis revealed insights into the sources, conservation and impact of microRNA 5′-isoforms in four model species. Nucleic Acids Res. 2014;42(3):1427–41. https://doi.org/10.1093/nar/gkt967.
14. Wyman SK, Knouf EC, Parkin RK, Fritz BR, Lin DW, Dennis LM, et al. Post-transcriptional generation of miRNA variants by multiple nucleotidyl transferases contributes to miRNA transcriptome complexity. Genome Res. 2011;21(9):1450–61. https://doi.org/10.1101/gr.118059.110.
15. Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. Mol Hum Reprod. 2007;13(4):273–9. https://doi.org/10.1093/molehr/gam006.
16. Forbes K, Farrokhnia F, Aplin JD, Westwood M. Dicer-dependent miRNAs provide an endogenous restraint on cytotrophoblast proliferation. Placenta. 2012;33(7):581–5. https://doi.org/10.1016/j.placenta.2012.03.006.
17. Forbes K. IFPA Gabor Than Award lecture: molecular control of placental growth: the emerging role of microRNAs. Placenta. 2013;34(Suppl):S27–33. https://doi.org/10.1016/j.placenta.2012.12.011.
18. Yang M, Chen Y, Chen L, Wang K, Pan T, Liu X, et al. miR-15b-AGO2 play a critical role in HTR8/SVneo invasion and in a model of angiogenesis defects related to inflammation. Placenta. 2016;41:62–73. https://doi.org/10.1016/j.placenta.2016.03.007.
19. Zhang R, Wang YQ, Su B. Molecular evolution of a primate-specific microRNA family. Mol Biol Evol. 2008;25(7):1493–502. https://doi.org/10.1093/molbev/msn094.
20. Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, et al. Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod. 2009;81(4):717–29. https://doi.org/10.1095/biolreprod.108.075481.
21. Zhou X, Li Q, Xu J, Zhang X, Zhang H, Xiang Y, et al. The aberrantly expressed miR-193b-3p contributes to preeclampsia through regulating transforming growth factor-beta signaling. Sci Rep. 2016;6:19910. https://doi.org/10.1038/srep19910.
22. Morales-Prieto DM, Ospina-Prieto S, Chaiwangyen W, Schoenleben M. Markert UR. Pregnancy-associated miRNA-clusters. J Reprod Immunol. 2013;97(1):51–61. https://doi.org/10.1016/j.jri.2012.11.001.
23. Morales-Prieto DM, Ospina-Prieto S, Schmidt A, Chaiwangyen W, Markert UR. Elsevier Trophoblast Research Award Lecture: origin, evolution and future of placenta miRNAs. Placenta. 2014;35(Suppl):S39–45. https://doi.org/10.1016/j.placenta.2013.11.017.
24. Seitz H, Royo H, Bortolin ML, Lin SP, Ferguson-Smith AC, Cavaille J. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. Genome Res. 2004;14(9):1741–8. https://doi.org/10.1101/gr.2743304.
25. Noguer-Dance M, Abu-Amero S, Al-Khtib M, Lefevre A, Coullin P, Moore GE, et al. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum Mol Genet. 2010;19(18):3566–82. https://doi.org/10.1093/hmg/ddq272.

26. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, et al. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet. 2005;37(7):766–70. https://doi.org/10.1038/ng1590.
27. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654–9. https://doi.org/10.1038/ncb1596.
28. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010;285(23):17442–52. https://doi.org/10.1074/jbc.M110.107821.
29. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010;56(11):1733–41. https://doi.org/10.1373/clinchem.2010.147405.
30. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, et al. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod. 2012;18(8):417–24. https://doi.org/10.1093/molehr/gas013.
31. Dragovic RA, Collett GP, Hole P, Ferguson DJ, Redman CW, Sargent IL, et al. Isolation of syncytiotrophoblast microvesicles and exosomes and their characterisation by multicolour flow cytometry and fluorescence Nanoparticle Tracking Analysis. Methods. 2015;87:64–74. https://doi.org/10.1016/j.ymeth.2015.03.028.
32. Vargas A, Zhou S, Ethier-Chiasson M, Flipo D, Lafond J, Gilbert C, et al. Syncytin proteins incorporated in placenta exosomes are important for cell uptake and show variation in abundance in serum exosomes from patients with preeclampsia. FASEB J. 2014;28(8):3703–19. https://doi.org/10.1096/fj.13-239053.
33. Mincheva-Nilsson L, Baranov V. The role of placental exosomes in reproduction. Am J Reprod Immunol. 2010;63(6):520–33. https://doi.org/10.1111/j.1600-0897.2010.00822.x.
34. Tannetta D, Masliukaite I, Vatish M, Redman C, Sargent I. Update of syncytiotrophoblast derived extracellular vesicles in normal pregnancy and preeclampsia. J Reprod Immunol. 2016. https://doi.org/10.1016/j.jri.2016.08.008.
35. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011;108(12):5003–8. https://doi.org/10.1073/pnas.1019055108.
36. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011;39(16):7223–33. https://doi.org/10.1093/nar/gkr254.
37. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011;13(4):423–33. https://doi.org/10.1038/ncb2210.
38. Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, et al. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS One. 2014;9(6):e98667. https://doi.org/10.1371/journal.pone.0098667.
39. Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, et al. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med. 2014;12:204. https://doi.org/10.1186/1479-5876-12-204.
40. Dragovic RA, Southcombe JH, Tannetta DS, Redman CW, Sargent IL. Multicolor flow cytometry and nanoparticle tracking analysis of extracellular vesicles in the plasma of normal pregnant and pre-eclamptic women. Biol Reprod. 2013;89(6):151. https://doi.org/10.1095/biolreprod.113.113266.
41. Goswami D, Tannetta DS, Magee LA, Fuchisawa A, Redman CW, Sargent IL, et al. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. Placenta. 2006;27(1):56–61. https://doi.org/10.1016/j.placenta.2004.11.007.

42. Kambe S, Yoshitake H, Yuge K, Ishida Y, Ali MM, Takizawa T, et al. Human exosomal placenta-associated miR-517a-3p modulates the expression of PRKG1 mRNA in Jurkat cells. Biol Reprod. 2014;91(5):129. https://doi.org/10.1095/biolreprod.114.121616.
43. Takahashi H, Ohkuchi A, Kuwata T, Usui R, Baba Y, Suzuki H, et al. Endogenous and exogenous miR-520c-3p modulates CD44-mediated extravillous trophoblast invasion. Placenta. 2017;50:25–31.
44. Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, et al. Placental exosomes in normal and complicated pregnancy. Am J Obstet Gynecol. 2015;213(4 Suppl):S173–81. https://doi.org/10.1016/j.ajog.2015.07.001.
45. Mouillet JF, Ouyang Y, Coyne CB, Sadovsky Y. MicroRNAs in placental health and disease. Am J Obstet Gynecol. 2015;213(4 Suppl):S163–72. https://doi.org/10.1016/j.ajog.2015.05.057.
46. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, et al. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol. 2007;196(3):261.e1–6. https://doi.org/10.1016/j.ajog.2007.01.008.
47. Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. Am J Obstet Gynecol. 2009;200(6):661.e1–7. https://doi.org/10.1016/j.ajog.2008.12.045.
48. Hu Y, Li P, Hao S, Liu L, Zhao J, Hou Y. Differential expression of microRNAs in the placentae of Chinese patients with severe pre-eclampsia. Clin Chem Lab Med. 2009;47(8):923–9. https://doi.org/10.1515/cclm.2009.228.
49. Zhang Y, Diao Z, Su L, Sun H, Li R, Cui H, et al. MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. Am J Obstet Gynecol. 2010;202(5):466.e1–7. https://doi.org/10.1016/j.ajog.2010.01.057.
50. Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K, Williams MA. Placental microRNA expression in pregnancies complicated by preeclampsia. Am J Obstet Gynecol. 2011;204(2):178.e12–21. https://doi.org/10.1016/j.ajog.2010.09.004.
51. Ishibashi O, Ohkuchi A, Ali MM, Kurashina R, Luo SS, Ishikawa T, et al. Hydroxysteroid (17-beta) dehydrogenase 1 is dysregulated by miR-210 and miR-518c that are aberrantly expressed in preeclamptic placentas: a novel marker for predicting preeclampsia. Hypertension. 2012;59(2):265–73. https://doi.org/10.1161/hypertensionaha.111.180232.
52. Xu P, Zhao Y, Liu M, Wang Y, Wang H, Li YX, et al. Variations of microRNAs in human placentas and plasma from preeclamptic pregnancy. Hypertension. 2014;63(6):1276–84. https://doi.org/10.1161/hypertensionaha.113.02647.
53. Zhang M, Muralimanoharan S, Wortman AC, Mendelson CR. Primate-specific miR-515 family members inhibit key genes in human trophoblast differentiation and are upregulated in preeclampsia. Proc Natl Acad Sci U S A. 2016. https://doi.org/10.1073/pnas.1607849113.
54. Chan YC, Banerjee J, Choi SY, Sen CK. miR-210: the master hypoxamir. Microcirculation. 2012;19(3):215–23. https://doi.org/10.1111/j.1549-8719.2011.00154.x.
55. Miettinen MM, Mustonen MV, Poutanen MH, Isomaa VV, Vihko RK. Human 17 beta-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression. Biochem J. 1996;314(Pt 3):839–45.
56. Guo L, Yang Q, Lu J, Li H, Ge Q, Gu W, et al. A comprehensive survey of miRNA repertoire and 3′ addition events in the placentas of patients with pre-eclampsia from high-throughput sequencing. PLoS One. 2011;6(6):e21072. https://doi.org/10.1371/journal.pone.0021072.
57. Pillay P, Maharaj N, Moodley J, Mackraj I. Placental exosomes and pre-eclampsia: maternal circulating levels in normal pregnancies and, early and late onset pre-eclamptic pregnancies. Placenta. 2016;46:18–25. https://doi.org/10.1016/j.placenta.2016.08.078.
58. Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. J Immunol. 2007;178(9):5949–56.
59. Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K, et al. Identification of pregnancy-associated microRNAs in maternal plasma. Clin Chem. 2010;56(11):1767–71. https://doi.org/10.1373/clinchem.2010.147660.

60. Miura K, Higashijima A, Murakami Y, Tsukamoto O, Hasegawa Y, Abe S, et al. Circulating chromosome 19 miRNA cluster microRNAs in pregnant women with severe pre-eclampsia. J Obstet Gynaecol Res. 2015;41(10):1526–32. https://doi.org/10.1111/jog.12749.
61. Kotlabova K, Doucha J, Hromadnikova I. Placental-specific microRNA in maternal circulation—identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol. 2011;89(2):185–91. https://doi.org/10.1016/j.jri.2011.02.006.
62. Hromadnikova I, Kotlabova K, Ondrackova M, Kestlerova A, Novotna V, Hympanova L, et al. Circulating C19MC microRNAs in preeclampsia, gestational hypertension, and fetal growth restriction. Mediat Inflamm. 2013;2013:186041. https://doi.org/10.1155/2013/186041.
63. Hromadnikova I, Kotlabova K, Doucha J, Dlouha K, Krofta L. Absolute and relative quantification of placenta-specific microRNAs in maternal circulation with placental insufficiency-related complications. J Mol Diagn. 2012;14(2):160–7. https://doi.org/10.1016/j.jmoldx.2011.11.003.
64. Wu L, Zhou H, Lin H, Qi J, Zhu C, Gao Z, et al. Circulating microRNAs are elevated in plasma from severe preeclamptic pregnancies. Reproduction. 2012;143(3):389–97. https://doi.org/10.1530/rep-11-0304.
65. Hromadnikova I, Kotlabova K, Hympanova L, Doucha J, Krofta L. First trimester screening of circulating C19MC microRNAs can predict subsequent onset of gestational hypertension. PLoS One. 2014;9(12):e113735. https://doi.org/10.1371/journal.pone.0113735.
66. Williams Z, Ben-Dov IZ, Elias R, Mihailovic A, Brown M, Rosenwaks Z, et al. Comprehensive profiling of circulating microRNA via small RNA sequencing of cDNA libraries reveals biomarker potential and limitations. Proc Natl Acad Sci U S A. 2013;110(11):4255–60. https://doi.org/10.1073/pnas.1214046110.
67. Liu Y, Zhao Y, Yu A, Zhao B, Gao Y, Niu H. Diagnostic accuracy of the soluble Fms-like tyrosine kinase-1/placental growth factor ratio for preeclampsia: a meta-analysis based on 20 studies. Arch Gynecol Obstet. 2015;292(3):507–18. https://doi.org/10.1007/s00404-015-3671-8.
68. Sovio U, Gaccioli F, Cook E, Hund M, Charnock-Jones DS, Smith GC. Prediction of preeclampsia using the soluble fms-like tyrosine kinase 1 to placental growth factor ratio: a prospective cohort study of unselected nulliparous women. Hypertension. 2017;69(4):731–8. https://doi.org/10.1161/hypertensionaha.116.08620.
69. Takizawa T, Ishibashi O, Matsubara S, Migita M, Takeshita T. Exosomes released from human placenta into maternal circulation: placenta-specific miRNAs (review). Exp Med (Yodosha, Tokyo, Japan [in Japanese]). 2011;29(3):392–8.
70. Farrokhnia F, Aplin JD, Westwood M, Forbes K. MicroRNA regulation of mitogenic signaling networks in the human placenta. J Biol Chem. 2014;289(44):30404–16. https://doi.org/10.1074/jbc.M114.587295.
71. Zhu X, Yang Y, Han T, Yin G, Gao P, Ni Y, et al. Suppression of microRNA-18a expression inhibits invasion and promotes apoptosis of human trophoblast cells by targeting the estrogen receptor alpha gene. Mol Med Rep. 2015;12(2):2701–6. https://doi.org/10.3892/mmr.2015.3724.
72. Kumar P, Luo Y, Tudela C, Alexander JM, Mendelson CR. The c-Myc-regulated microRNA-17~92 (miR-17~92) and miR-106a~363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. Mol Cell Biol. 2013;33(9):1782–96. https://doi.org/10.1128/mcb.01228-12.
73. Wang Y, Zhang Y, Wang H, Wang J, Pan Z, Luo S. Aberrantly up-regulated miR-20a in preeclampsic placenta compromised the proliferative and invasive behaviors of trophoblast cells by targeting forkhead box protein A1. Int J Biol Sci. 2014;10(9):973–82. https://doi.org/10.7150/ijbs.9088.
74. Li P, Guo W, Du L, Zhao J, Wang Y, Liu L, et al. microRNA-29b contributes to pre-eclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. Clin Sci (Lond). 2013;124(1):27–40. https://doi.org/10.1042/cs20120121.
75. Sun M, Chen H, Liu J, Tong C, Meng T. MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC. BMC Cell Biol. 2015;16:21. https://doi.org/10.1186/s12860-015-0068-2.
76. Zou Y, Jiang Z, Yu X, Zhang Y, Sun M, Wang W, et al. MiR-101 regulates apoptosis of trophoblast HTR-8/SVneo cells by targeting endoplasmic reticulum (ER) protein 44 during preeclampsia. J Hum Hypertens. 2014;28(10):610–6. https://doi.org/10.1038/jhh.2014.35.

77. Li Q, Pan Z, Wang X, Gao Z, Ren C, Yang W. miR-125b-1-3p inhibits trophoblast cell invasion by targeting sphingosine-1-phosphate receptor 1 in preeclampsia. Biochem Biophys Res Commun. 2014;453(1):57–63. https://doi.org/10.1016/j.bbrc.2014.09.059.
78. Tamaru S, Mizuno Y, Tochigi H, Kajihara T, Okazaki Y, Okagaki R, et al. MicroRNA-135b suppresses extravillous trophoblast-derived HTR-8/SVneo cell invasion by directly down regulating CXCL12 under low oxygen conditions. Biochem Biophys Res Commun. 2015;461(2):421–6. https://doi.org/10.1016/j.bbrc.2015.04.055.
79. TM L, Lu W, Zhao LJ. MicroRNA-137 affects proliferation and migration of placenta trophoblast cells in preeclampsia by targeting ERRalpha. Reprod Sci. 2016. https://doi.org/10.1177/1933719116650754.
80. Dai Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H, et al. MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. Placenta. 2012;33(10):824–9. https://doi.org/10.1016/j.placenta.2012.07.012.
81. Bai Y, Yang W, Yang HX, Liao Q, Ye G, Fu G, et al. Downregulated miR-195 detected in preeclamptic placenta affects trophoblast cell invasion via modulating ActRIIA expression. PLoS One. 2012;7(6):e38875. https://doi.org/10.1371/journal.pone.0038875.
82. Yu Y, Wang L, Liu T, Guan H. MicroRNA-204 suppresses trophoblast-like cell invasion by targeting matrix metalloproteinase-9. Biochem Biophys Res Commun. 2015;463(3):285–91. https://doi.org/10.1016/j.bbrc.2015.05.052.
83. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J. 2010;24(6):2030–9. https://doi.org/10.1096/fj.09-149724.
84. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, et al. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. J Cell Mol Med. 2012;16(2):249–59. https://doi.org/10.1111/j.1582-4934.2011.01291.x.
85. Luo R, Shao X, Xu P, Liu Y, Wang Y, Zhao Y, et al. MicroRNA-210 contributes to preeclampsia by downregulating potassium channel modulatory factor 1. Hypertension. 2014;64(4):839–45. https://doi.org/10.1161/hypertensionaha.114.03530.
86. Kopriva SE, Chiasson VL, Mitchell BM, Chatterjee P. TLR3-induced placental miR-210 down-regulates the STAT6/interleukin-4 pathway. PLoS One. 2013;8(7):e67760. https://doi.org/10.1371/journal.pone.0067760.
87. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, et al. MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension. 2013;61(4):864–72. https://doi.org/10.1161/hypertensionaha.111.203489.
88. Luo L, Ye G, Nadeem L, Fu G, Yang BB, Honarparvar E, et al. MicroRNA-378a-5p promotes trophoblast cell survival, migration and invasion by targeting Nodal. J Cell Sci. 2012;125(Pt 13):3124–32. https://doi.org/10.1242/jcs.096412.
89. Xie L, Mouillet JF, Chu T, Parks WT, Sadovsky E, Knofler M, et al. C19MC microRNAs regulate the migration of human trophoblasts. Endocrinology. 2014;155(12):4975–85. https://doi.org/10.1210/en.2014-1501.
90. Ding J, Huang F, Wu G, Han T, Xu F, Weng D, et al. MiR-519d-3p suppresses invasion and migration of trophoblast cells via targeting MMP-2. PLoS One. 2015;10(3):e0120321. https://doi.org/10.1371/journal.pone.0120321.
91. Gao WL, Liu M, Yang Y, Yang H, Liao Q, Bai Y, et al. The imprinted H19 gene regulates human placental trophoblast cell proliferation via encoding miR-675 that targets Nodal Modulator 1 (NOMO1). RNA Biol. 2012;9(7):1002–10. https://doi.org/10.4161/rna.20807.

Part VI

Therapy

Novel Therapies for Preeclampsia

14

Suzanne D. Burke and S. Ananth Karumanchi

Abstract

Preeclampsia is a complex, hypertensive gestational syndrome that lacks a clear pathogenesis, which has prevented development of effective therapies. Mechanistic evidence points to an augmented anti-angiogenic balance, with sFlt1 and other soluble factors contributing to maternal hypertension and proteinuria. This chapter will review novel research and therapeutic strategies, including recombinant proteins, small molecules, apheresis, and off-label use of medications.

Keywords

Preeclampsia • Pregnancy • Vascular endothelial growth factor (VEGF) • Anti-angiogenesis

14.1 Introduction

The pathogenesis of preeclampsia is incompletely understood, in which numerous genetic, immunological, and environmental factors interact. Placental insufficiency, resulting from failure of the trophoblastic remodeling of uterine spiral arterioles, is thought to be the primary insult that leads to the release of secreted factors that enter the mother's circulation, culminating in the clinical signs and symptoms of preeclampsia [1]. In this chapter, we will review the evidence for abnormal anti-angiogenic factors in the pathogenesis of preeclampsia and discuss novel therapeutic strategies based on these findings.

S.D. Burke, Ph.D. • S.A. Karumanchi, M.D. (✉)
Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA
e-mail: sananth@bidmc.harvard.edu

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_14

14.2 Soluble Anti-Aangiogenic Factors in the Pathogenesis of Maternal Syndrome

The most plausible explanations of genesis of at least two major phenotypes of preeclampsia, hypertension and proteinuria, are events that lead to an imbalance of pro- and anti-angiogenic factors in the circulation of pregnant women. Placenta-derived circulating anti-angiogenic factors as a potential mediator of the endothelial dysfunction and the maternal syndrome of preeclampsia have been an area of intense investigation. Of the various anti-angiogenic factors, soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin have been the subject of most studies (see Fig. 14.1) [2]. sFlt1 is a truncated form of the Flt1 (VEGFR1) receptor. It includes the extracellular ligand-binding domain, but not the transmembrane and intracellular domains; it is secreted (hence "soluble") and antagonizes both VEGF and PlGF (vascular endothelial and placental growth factors, respectively) in the circulation by binding and preventing their interaction with their endothelial receptors [3, 4]. Although sFlt1 is made in small amounts by other tissues (endothelial cells and monocytes), the placenta seems to be the major source of circulating sFlt1 during pregnancy as evidenced by the dramatic fall in circulating concentrations of sFlt1 after the delivery of the placenta [4].

In women with preeclampsia, circulating sFlt1 levels are increased, while free (unbound) PlGF and free VEGF levels are suppressed [4, 5]. The increase in sFlt1 precedes the onset of clinical disease by at least 5 weeks [6–8] and appears to be

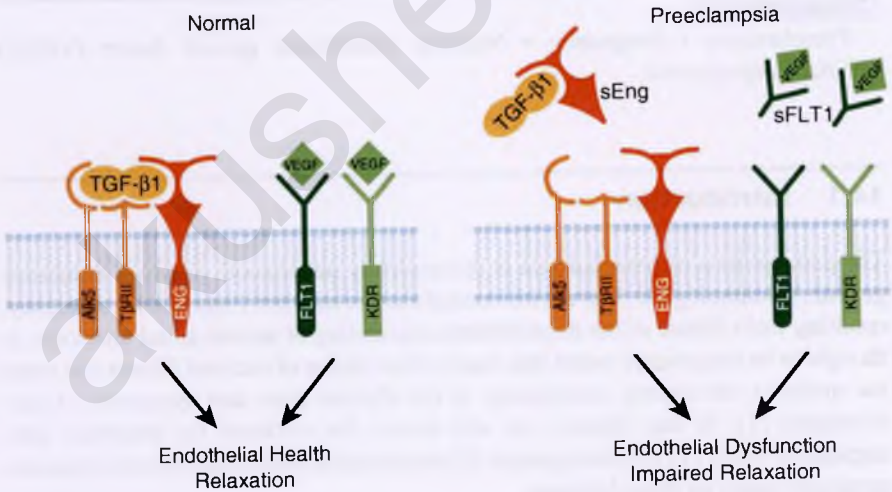


Fig. 14.1 sFlt1 and sEng cause endothelial dysfunction by antagonizing VEGF and TGF-β1 signaling. There is mounting evidence that VEGF and TGF-β1 are required to maintain endothelial health in several tissues, including the kidney and perhaps the placenta. During normal pregnancy, physiological levels of VEGF and TGF-β1 signaling in the vasculature maintain vascular homeostasis. In preeclampsia, excess placental secretion of sFlt1 and sEng (two endogenous circulating anti-angiogenic proteins) inhibits VEGF and TGF-β1 signaling (respectively) in the vasculature. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric oxide production, and release of procoagulant proteins. Figure reproduced with permission from Powe C et al. [1]

more pronounced in severe and early-onset preeclampsia [8, 9]. In several studies, decreased free PlGF levels were observed before 20 weeks gestation in women who later developed preeclampsia [8, 10, 11]. Although reduction in free PlGF may be predominantly explained by an increase in sFlt1, some data suggest the fall in PlGF may actually precede the rise in sFlt1, implicating other mechanisms such as decreased PlGF production [11]. In vitro studies confirm that excess placental sFlt1 production induces an anti-angiogenic state in the serum of preeclamptic women that can be rescued by exogenous VEGF and PlGF [4, 12]. Excess sFlt1 alone, when administered to pregnant rodents, induced albuminuria, hypertension, and renal pathological changes of glomerular endotheliosis by antagonizing circulating and local VEGF and PlGF and inducing endothelial dysfunction [4, 13, 14]. Interestingly, a significant percentage of cancer patients receiving VEGF signaling antagonists as adjuvant therapy develop hypertension and proteinuria [15–17]. Studies of genetically modified mice demonstrate that even a 50% reduction of renal VEGF production resulted in glomerular endotheliosis and proteinuria [18, 19]. These data suggest that excess circulating sFlt1, by neutralizing VEGF and PlGF, may play a causal role in the pathogenesis of the maternal syndrome in preeclampsia.

sFlt1 induces hypertension by impairing endothelial production of vasodilatory nitric oxide and promoting vasomotor sensitivity to vasopressors, including angiotensin II[20]. Other downstream targets of VEGF signaling such as vasodilatory prostacyclins and vasoconstrictors such as endothelin may also be involved [1]. The etiology of the proteinuria in preeclampsia is believed to be sFlt1-mediated loss of glomerular endothelial fenestrae that are normally maintained by podocyte-derived VEGF [19]. Whether secondary podocyte injury or podocyturia also contributes to proteinuria is still being debated [21]. The role of sFlt1 in preeclampsia is supported by clinical studies in a patient population considered high risk for the development of preeclampsia. The genes for sFlt1 and Flt1 are carried on chromosome 13, and the incidence of preeclampsia in mothers who carry fetuses with trisomy 13 is greatly increased, as compared with all other trisomies or with control pregnant patients [22, 23]. Women carrying trisomy 13 fetuses had higher placental sFlt1 expression and higher circulating sFlt1/PlGF levels compared to other trisomies or control pregnant women [24, 25]. In a recent genome-wide association study by McGinnis and Steinthorsdottir et al., after assessment of >7 million variants in >300,000 subjects of European descent, a single association signal was identified close to the *FLT1* gene in the fetal genome that was strongly associated with preeclampsia [26]. The finding was replicated in an independent European case-control cohort, and a total of three independent association signals were defined at this locus. As each risk variant contributed ~20% increased risk of preeclampsia, the combined effect of all three variants at the *FLT1* locus would be expected to increase disease risk threefold between offspring with the lowest and highest burden of risk alleles. These genetic studies strongly indicate that alterations in sFlt1 pathway may be a fundamental molecular defect in preeclampsia.

Endoglin (Eng) is an angiogenic receptor expressed on the surface of endothelial cells and placental syncytiotrophoblasts. Eng acts as a co-receptor for transforming growth factor-beta (TGF-beta), a potent pro-angiogenic molecule. Eng mRNA is upregulated in the preeclamptic placenta [27] (see Fig. 14.1). Similar to the secreted

sFlt1, the extracellular region of endoglin can be proteolytically cleaved, and soluble (s)Eng is released in excess quantities into the circulation of preeclamptic patients [28, 29]. In pregnant rats, sEng exacerbates the vascular damage mediated by sFlt1 resulting in severe preeclampsia-like illness, including the development of thrombocytopenia and fetal growth restriction, which are features of severe preeclampsia [27]. sEng in combination with sFlt1 also induces cerebral edema in mice [30]. Using trophoblast villous explant cultures at 5–8 weeks gestation, monoclonal antibodies to Eng as well as antisense Eng oligonucleotides stimulated trophoblast outgrowth and migration [31]. TGF-beta 1 and/or TGF-beta 3 inhibits trophoblast migration and invasion, and it appears that Eng mediates this effect. Therefore, it has been speculated that sEng produced by the placenta may be a compensatory mechanism to limit the effects of surface endoglin. In clinical studies, sEng was elevated not only during the disease but also before onset of symptoms [32, 33]. Elevations in sEng were particularly pronounced—therefore, potentially most useful for prediction—in women who developed preterm preeclampsia or preeclampsia with a small-for-gestational-age infant. Although the gestational pattern of sEng concentration tended to parallel the trajectory of the sFlt1/PlGF ratio, multivariate analysis indicated that each was significantly associated with preeclampsia. Indeed, a composite measure incorporating all three angiogenic molecules (sFlt1, sEng, and PlGF) was more strongly predictive of preeclampsia than the individual biomarkers [27]. Mechanistically, sEng has been shown to induce endothelial dysfunction by inhibiting TGF-beta-mediated endothelial nitric oxide signaling [27]. Consistent with these findings, transgenic mice overexpressing sEng develop hypertension [34]. In a more recent study, mice overproducing sEng were also found to develop podocyturia via a TGF-beta-independent pathway, which involves integrins [35]; however, the role of this non-TGF-beta pathway in preeclampsia-related proteinuria is still unclear. sEng has also been shown in culture studies to impair autophagy in extravillous trophoblasts [36]; it is therefore likely that sEng may also play a role in early placentation defects noted in preeclampsia.

Numerous alterations in signaling pathways in the placenta have been proposed to be upstream of the angiogenic imbalance, such as heme oxygenase, catechol-O-methyltransferase, and corin [37–40]. However, human studies describing the temporal relationship between angiogenic factors and the upstream pathways are still lacking.

14.3 Angiogenic Biomarkers

During the last 5 years, many diagnostic companies have developed highly sensitive, specific, and robust assays with rapid throughput to quantitate levels of total sFlt1 and free PlGF in plasma and serum [41–45]. Several groups have demonstrated that measurement of sFlt1 and PlGF can be used to differentiate preeclampsia from other diseases that mimic preeclampsia, such as chronic hypertension, gestational hypertension, kidney disease, and gestational thrombocytopenia [2]. Recently, Zeisler et al. demonstrated in a prospective multicenter trial that serum sFlt/PlGF can be used to rule out preeclampsia among patients with suspected disease with negative predictive value >99% [46]. Angiogenic markers have also been useful as surrogate markers in clinical trials [47, 48] (see Sect. 14.4).

14.4 Targeted Therapeutics

Multiple strategies for mitigating the clinical signs of preeclampsia have been tested. Based upon the recent advances presented above, a number of different strategies are being explored to allow carrying of the fetus to full term. These strategies target angiogenic factors using both in vitro and in vivo models to restore angiogenic balance. These include generation of recombinant proteins similar in function to VEGF, selective depletion of sFlt1 with antibodies and/or an extracorporeal device, as well as isolation of small molecules, such as siRNA or small molecule inhibitors of sFlt1 production or its effects (see Table 14.1 for summary).

1. *Recombinant growth factors*: In the recent years, a number of successful in vitro studies bolstered confidence in these targeted therapies. In 2010, Gilbert et al. showed that infusion of VEGF-121 lowered blood pressure and preserved renal function in rats with placental ischemia-induced hypertension. In the same year, Bergmann et al. showed that co-administration of adenoviruses expressing sFlt1 and VEGF in a rodent model of preeclampsia resulted in marked reduction of free sFlt1, allowing for renal recovery and normalized blood pressure [49]. These results are consistent with studies in pregnant rats where VEGF-121 was demonstrated to reverse preeclampsia-related signs and symptoms without adverse fetal effects [50]. VEGF-121 is the most freely diffusible isoform, which also lacks a heparin-binding domain; this makes VEGF-121 superior to VEGF-165 for therapeutic use. Interestingly, when pravastatin was administered to mice overexpressing placental specific sFlt1, the mice then showed increased placental growth factor expression and decreased sFlt1 levels in circulation, which allowed relief of preeclamptic symptoms [51]. More recently, recombinant PlGF was also shown to mitigate the signs and symptoms of preeclampsia in mouse and baboon models of preeclampsia [52, 53].
2. *Small molecules*: Small molecules such as sildenafil, a phosphodiesterase-5 inhibitor that enhances cGMP signaling, have been tested in various mouse and rat models of preeclampsia [20, 54, 55]. Because the uterine circulation is highly dependent on nitric oxide signaling, it is not surprising that compounds such as sildenafil have profound effects in reversing placental ischemia and improving fetal growth. In a recent clinical study of 50 patients, pregnancy duration was, on average, 4 days longer in sildenafil-treated women with severe preeclampsia, which was also accompanied by a significant reduction in maternal blood pressure [56]. However, there was no improvement in perinatal outcomes. Ongoing trials, such as STRIDOR, should shed additional light on the role of sildenafil in reversing placental ischemia and fetal growth restriction that accompanies preeclampsia [57]. Since hypoxia-inducible transcription factors (HIF) are a major driver of the abnormal anti-angiogenic factors, HIF inhibitors have been tested in preclinical models. Ouabain, a digoxin-like molecule, was recently shown to block sFlt1 production and ameliorate hypertension in rats with placental ischemia-induced hypertension [58]. Proton-pump inhibitors (PPI) were also shown to block sFlt1 production in cell culture studies and reverse hypertension in sFlt1-transgenic mice [59]. A double-blind placebo-controlled trial to evaluate the efficacy of esomeprazole (PPI) to treat early-onset preeclampsia (PIE) trial has been initiated [60].

Table 14.1 Novel therapeutics for preeclampsia

Therapy	Drug class/route of administration	Proposed mechanism	Reported benefits
Recombinant VEGF-121	Recombinant protein/s.c or i.v.	Restoration of angiogenic balance	• Decreased blood pressure • Improved renal function
Recombinant PlGF	Recombinant protein/s.c or i.v.	Restoration of angiogenic balance	• Decreased blood pressure • Improved renal function
Dextran sulfate cellulose column	Extracorporeal apheresis	Direct removal of positively charged sFlt1 and other molecules like LDL cholesterol	• Decreased blood pressure • Improved renal function • Increased time to delivery
Pravastatin	Statin/oral (i.p. for preclinical study)	Induction of PlGF and improved nitric oxide (NO) synthase	• Decreased blood pressure • Increase in fetal weight
Sildenafil	Phosphodiesterase type 5 inhibitors/ oral	Improves endothelial dysfunction by increasing NO bioavailability	• Decreased blood pressure • Decreased uterine artery RI, increased fetal growth
Ouabain	Cardiac glycoside/ oral and i.p (preclinical)	Inhibition of HIF1alfa	• Decreased blood pressure • No adverse effects in normal rodent gestation and offspring
Esomeprazole	Proton-pump inhibitor/oral (i.p. for preclinical study)	Reduction of HIF1alfa, upregulation of HO-1, restoration of angiogenic balance	• Decreased blood pressure
Aspirin	Oral medication	Anti-inflammatory and pro-angiogenic	• Prevents preterm preeclampsia
Relaxin	Recombinant peptide hormone/i.v. infusion or s.c. (preclinical)	Induces CV changes in pregnancy, reduced total peripheral resistance, increased cardiac output	• Decreased blood pressure • Decreased uterine artery RI
Choline	Essential nutrient/ oral	Downregulates sFlt1 production	• Decreased sFlt1 levels during pregnancy
Nicotinamide	Vitamin B3/oral	Inhibition of ADP ribosyl cyclase	• Decreased blood pressure • Improved renal function • Normalization of fetal growth

Abbreviations: *VEGF* vascular endothelial growth factor, *s.c.* subcutaneous, *i.p.* intraperitoneal, *PlGF* placental growth factor, *RI* resistance index, *NO* nitric oxide, *HO-1* heme oxygenase-1, *HIF1a* hypoxia-inducible factor 1-alpha, *i.v.* intravenous

3. *Targeted apheresis*: Thadhani et al. selectively removed positively charged sFlt1 molecules using a negatively charged dextran sulfate cellulose column [61]. These columns are frequently used in cases of familial hypercholesterolemia and certain autoimmune diseases. In a pilot study of eight pregnant women with preterm preeclampsia, dextran sulfate apheresis led to the

reduction in sFlt1 levels and improved proteinuria and hypertension, without any apparent adverse effects to either the mother or fetus. Extracorporeal removal of sFlt1 was performed up to four times in a single patient. She remained pregnant for 23 days. Two patients were treated twice; one remained pregnant for 15 days and the other for 19 days. In the untreated cohort, the average prolongation of pregnancy was 3.6 days. This seminal study demonstrates that selective removal of sFlt1 is well-tolerated and a safe method of prolonging gestation in severe preterm preeclamptic patients. More recently, using a plasma-specific dextran sulfate column, Thadhani et al. demonstrated that an 18% reduction in sFlt1 led to 44% reduction in protein/creatinine ratios that was associated with a prolongation of pregnancy >2 weeks compared with 3 days in untreated contemporaneous preeclampsia subjects [48]. If confirmed in a randomized trial, this approach could lead to targeted therapy for patients with preterm preeclampsia that present with an abnormal angiogenic profile. Adsorption columns using monoclonal antibodies that selectively deplete sFlt1 and sEng are currently being developed with better selectivity and clearance and without adverse effects.

4. *Aspirin therapy*: Strategies to prevent preeclampsia have been studied extensively over the last 20 years [62]. Unfortunately, several of these interventions, such as antioxidants or heparin, have not had much success [63, 64]. Aspirin therapy to prevent preeclampsia has been extensively studied. A comprehensive meta-analysis of antiplatelet agents to prevent preeclampsia, which included over 32,000 women of varying risk status from 31 trials, suggested antiplatelet agents have a modest benefit, with a relative risk of preeclampsia of 0.90 (95% CI 0.84–0.97) for aspirin-treated subjects [65]. More recent meta-analyses suggested aspirin therapy initiated $\leq$16 weeks of gestation was associated with a more significant reduction (~50% effect size) and a dose-response effect for the prevention of preeclampsia and fetal growth restriction with higher dosages of aspirin being associated with greater reduction of preeclampsia [66]. In a recent clinical trial, where only patients with low PlGF during the first trimester were enrolled, aspirin at a dose of 150 mg per day had a 62% reduction in preterm preeclampsia [47]. If these data can be confirmed, it would suggest that circulating angiogenic factors identify a select group of women with impending preeclampsia whose disease process may respond better to aspirin and who are inherently different than those with clinical risk factors.
5. *Other strategies*: Other investigators are exploring the safety and efficacy of relaxin (a vasodilator) in the treatment of preeclampsia [67]. Dietary choline, which acts by decreasing placental sFlt1 expression, has also been suggested as novel strategy to prevent preeclampsia [68]. Nicotinamide, a nontoxic vitamin, was shown to ameliorate preeclampsia in several animal models of the disease [69, 70] by blocking endothelin-1 signaling, downstream of sFlt1. A small prospective clinical study to evaluate nicotinamide has been initiated in North Carolina (personal communication, Dr. Kim Boggess). Results from these and other prospective therapeutic trials are eagerly awaited.

Conclusions

There are several reasons why little advancement has been made in development of targeted therapies for preeclampsia and its complications. Clinical trials involving pregnant women are (with necessity) subject to much greater regulation than those involving nonpregnant individuals [71]. Furthermore, mouse models of preeclampsia do not fully recapitulate the human condition [72]. Lack of a mechanistic understanding of the underlying events has also prevented development of novel therapies. Preeclampsia is a particularly challenging condition to target, as clinical signs develop well after initial placental insult. Since there is no way to predict poor placentation definitively, nor is there currently a method for correcting errors in placentation, therapy is aimed at treating the consequences of the abnormal placentation. During the last decade, a key role of circulating angiogenic factors in the pathogenesis of the maternal syndrome has emerged [2]. Development of novel angiogenic biomarkers should allow the development of innovative clinical trials targeting specific patient populations. The primary goal in management of a preeclamptic patient is prolongation of pregnancy to permit maximal growth of the fetus. This needs to be balanced with disease progression, with expedient delivery following signs of fetal distress, lack of growth, or worsening of maternal condition. In summary, there appears to be a shift in how clinicians and researchers are approaching preeclampsia—more focus is aimed at targeting vascular endothelial dysfunction due to angiogenic imbalance as a major contributor to the pathology observed in preeclampsia. There are several ongoing clinical studies for targeted therapies in preeclampsia. Whether these studies will improve perinatal outcomes remains to be seen.

References

1. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation. 2011;123(24):2856–69.
2. Karumanchi SA. Angiogenic factors in preeclampsia: from diagnosis to therapy. Hypertension. 2016;67(6):1072–9.
3. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A. 1993;90(22):10705–9.
4. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111(5):649–58.
5. Tsatsaris V, Goffin F, Munaut C, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab. 2003;88(11):5555–63.
6. Chaiworapongsa T, Romero R, Kim YM, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J Matern Fetal Neonatal Med. 2005;17(1):3–18.
7. Hertig A, Berkane N, Lefevre G, et al. Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia. Clin Chem. 2004;50(9):1702–3.

8. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350(7):672–83.
9. Chaiworapongsa T, Romero R, Espinoza J, et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am J Obstet Gynecol. 2004;190(6):1541–7; discussion 1547–1550.
10. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am J Obstet Gynecol. 2003;188(1):177–82.
11. Thadhani R, Mutter WP, Wolf M, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. J Clin Endocrinol Metab. 2004;89(2):770–5.
12. Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ Res. 2004;95(9):884–91.
13. Lu F, Longo M, Tamayo E, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol. 2007;196(4):396.e1–397; discussion 396.e7.
14. Szabat M, Johnson JD, Piret JM. Reciprocal modulation of adult beta cell maturity by activin A and follistatin. Diabetologia. 2010;53(8):1680–9.
15. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol. 2003;21(1):60–5.
16. Patel TV, Morgan JA, Demetri GD, et al. A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib. J Natl Cancer Inst. 2008;100(4):282–4.
17. Izzedine H, Escudier B, Lhomme C, et al. Kidney diseases associated with anti-vascular endothelial growth factor (VEGF): an 8-year observational study at a single center. Medicine. 2014;93(24):333–9.
18. Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med. 2008;358(11):1129–36.
19. Eremina V, Sood M, Haigh J, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111(5):707–16.
20. Burke SD, Zsengeller ZK, Khankin EV, et al. Soluble fms-like tyrosine kinase 1 promotes angiotensin II sensitivity in preeclampsia. J Clin Invest. 2016;126(7):2561–74.
21. Craici IM, Wagner SJ, Bailey KR, et al. Podocyturia predates proteinuria and clinical features of preeclampsia: longitudinal prospective study. Hypertension. 2013;61(6):1289–96.
22. Tuohy JF, James DK. Pre-eclampsia and trisomy 13. Br J Obstet Gynaecol. 1992;99(11):891–4.
23. Dotters-Katz SK, Humphrey WM, Senz KL, et al. Trisomy 13 and the risk of gestational hypertensive disorders: a population-based study. J Matern Fetal Neonatal Med. 2017;2:1–5.
24. Bdolah Y, Palomaki GE, Yaron Y, et al. Circulating angiogenic proteins in trisomy 13. Am J Obstet Gynecol. 2006;194(1):239–45.
25. Silasi M, Rana S, Powe C, et al. Placental expression of angiogenic factors in Trisomy 13. Am J Obstet Gynecol. 2011;204(6):546.e1–4.
26. McGinnis R, Steinthorsdottir V, Williams NO, et al. Variants in the fetal genome near FLT1 are associated with risk of preeclampsia. Nat Genet. 2017;49(8):1255–60.
27. Venkatesha S, Toporsian M, Lam C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12(6):642–9.
28. Kumar S, Pan CC, Bloodworth JC, et al. Antibody-directed coupling of endoglin and MMP-14 is a key mechanism for endoglin shedding and deregulation of TGF-beta signaling. Oncogene. 2014;33(30):3970–9.
29. Kaitu'u-Lino TJ, Tuohey L, Ye L, Palmer K, Skubisz M, Tong S. MT-MMPs in pre-eclamptic placenta: relationship to soluble endoglin production. Placenta. 2013;34(2):168–73.
30. Maharaj AS, Walshe TE, Saint-Geniez M, et al. VEGF and TGF-beta are required for the maintenance of the choroid plexus and ependyma. J Exp Med. 2008;205(2):491–501.

31. Caniggia I, Taylor CV, Ritchie JW, Lye SJ, Letarte M. Endoglin regulates trophoblast differentiation along the invasive pathway in human placental villous explants. Endocrinology. 1997;138(11):4977–88.
32. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355(10):992–1005.
33. Romero R, Nien JK, Espinoza J, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med. 2008;21(1):9–23.
34. Valbuena-Diez AC, Blanco FJ, Oujo B, et al. Oxysterol-induced soluble endoglin release and its involvement in hypertension. Circulation. 2012;126(22):2612–24.
35. Rossi E, Smadja DM, Boscolo E, et al. Endoglin regulates mural cell adhesion in the circulatory system. Cell Mol Life Sci. 2016;73(8):1715–39.
36. Nakashima A, Yamanaka-Tatematsu M, Fujita N, et al. Impaired autophagy by soluble endoglin, under physiological hypoxia in early pregnant period, is involved in poor placentation in preeclampsia. Autophagy. 2013;9(3):303–16.
37. Cudmore M, Ahmad S, Al-Ani B, et al. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation. 2007;115(13):1789–97.
38. Cui Y, Wang W, Dong N, et al. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. Nature. 2012;484(7393):246–50.
39. Ho L, Van Dijk M, Chye STJ, et al. ELABELA deficiency promotes preeclampsia and cardiovascular malformations in mice. Science. 2017;357(6352):707–13.
40. Kanasaki K, Palmsten K, Sugimoto H, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453(7198):1117–21.
41. Chappell LC, Duckworth S, Seed PT, et al. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. Circulation. 2013;128(19):2121–31.
42. Rana S, Powe CE, Salahuddin S, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125(7):911–9.
43. Sunderji S, Gaziano E, Wothe D, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. Am J Obstet Gynecol. 2010;202(1):40.e1–7.
44. Verdonk K, Visser W, Russcher H, Danser AH, Steegers EA, van den Meiracker AH. Differential diagnosis of preeclampsia: remember the soluble fms-like tyrosine kinase 1/placental growth factor ratio. Hypertension. 2012;60(4):884–90.
45. Verlohren S, Herraiz I, Lapaire O, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. Am J Obstet Gynecol. 2012;206(1):58.e1–8.
46. Zeisler H, Llurba E, Chantraine F, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med. 2016;374(1):13–22.
47. Rolnik DL, Wright D, Poon LC, et al. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. N Engl J Med. 2017;377:613–22.
48. Thadhani R, Hagmann H, Schaarschmidt W, et al. Removal of soluble fms-like tyrosine kinase-1 by Dextran sulfate apheresis in preeclampsia. J Am Soc Nephrol. 2016;27(3):903–13.
49. Bergmann A, Ahmad S, Cudmore M, et al. Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. J Cell Mol Med. 2010;14(6B):1857–67.
50. Li Z, Zhang Y, Ying Ma J, et al. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. Hypertension. 2007;50(4):686–92.
51. Kumasawa K, Ikawa M, Kidoya H, et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011;108(4):1451–5.
52. Makris A, Yeung KR, Lim SM, et al. Placental growth factor reduces blood pressure in a uteroplacental ischemia model of preeclampsia in nonhuman primates. Hypertension. 2016;67(6):1263–72.

53. Spradley FT, Tan AY, Joo WS, et al. Placental growth factor administration abolishes placental ischemia-induced hypertension. Hypertension. 2016;67(4):740–7.
54. Motta C, Grosso C, Zanuzzi C, et al. Effect of sildenafil on pre-eclampsia-like mouse model induced by L-name. Reprod Domest Anim. 2015;50(4):611–6.
55. Ramesar SV, Mackraj I, Gathiram P, Moodley J. Sildenafil citrate improves fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. Eur J Obstet Gynecol Reprod Biol. 2010;149(1):22–6.
56. Trapani A Jr, Goncalves LF, Trapani TF, Vieira S, Pires M, Pires MM. Perinatal and hemodynamic evaluation of sildenafil citrate for preeclampsia treatment: a randomized controlled trial. Obstet Gynecol. 2016;128(2):253–9.
57. Ganzevoort W, Alfirevic Z, von Dadelszen P, et al. STRIDER: Sildenafil Therapy In Dismal prognosis Early-onset intrauterine growth Restriction—a protocol for a systematic review with individual participant data and aggregate data meta-analysis and trial sequential analysis. Syst Rev. 2014;3:23.
58. Rana S, Rajakumar A, Geahchan C, et al. Ouabain inhibits placental sFlt1 production by repressing HSP27-dependent HIF-1alpha pathway. FASEB J. 2014;28(10):4324–34.
59. Onda K, Tong S, Beard S, et al. Proton pump inhibitors decrease soluble fms-like tyrosine kinase-1 and soluble endoglin secretion, decrease hypertension, and rescue endothelial dysfunction. Hypertension. 2017;69(3):457–68.
60. Cluver CA, Walker SP, Mol BW, et al. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): a study protocol. BMJ Open. 2015;5(10):e008211.
61. Thadhani R, Kisner T, Hagmann H, et al. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. Circulation. 2011;124(8):940–50.
62. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122–31.
63. Haddad B, Winer N, Chitrit Y, et al. Enoxaparin and aspirin compared with aspirin alone to prevent placenta-mediated pregnancy complications: a randomized controlled trial. Obstet Gynecol. 2016;128(5):1053–63.
64. Roberts JM, Myatt L, Spong CY, et al. Vitamins C and E to prevent complications of pregnancy-associated hypertension. N Engl J Med. 2010;362(14):1282–91.
65. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA, Group PC. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet. 2007;369(9575):1791–8.
66. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. Am J Obstet Gynecol. 2017;216(2):110–20.e116.
67. Unemori E, Sibai B, Teichman SL. Scientific rationale and design of a phase i safety study of relaxin in women with severe preeclampsia. Ann N Y Acad Sci. 2009;1160:381–4.
68. Jiang X, Bar HY, Yan J, et al. A higher maternal choline intake among third-trimester pregnant women lowers placental and circulating concentrations of the antiangiogenic factor fms-like tyrosine kinase-1 (sFLT1). FASEB J. 2013;27(3):1245–53.
69. Fushima T, Sekimoto A, Oe Y, et al. Nicotinamide ameliorates a preeclampsia-like condition in mice with reduced uterine perfusion pressure. Am J Physiol Renal Physiol. 2017;312(2):F366–72.
70. Li F, Fushima T, Oyanagi G, et al. Nicotinamide benefits both mothers and pups in two contrasting mouse models of preeclampsia. Proc Natl Acad Sci U S A. 2016;113(47):13450–5.
71. Fisk NM, Atun R. Market failure and the poverty of new drugs in maternal health. PLoS Med. 2008;5(1):e22.
72. Grigsby PL. Animal models to study placental development and function throughout normal and dysfunctional human pregnancy. Semin Reprod Med. 2016;34(1):11–6.

Pravastatin for Preeclampsia Prevention and Treatment

15

Guillermina Girardi

Abstract

Preeclampsia is a significant, multifactorial, multiorgan disease affecting 5–8% of all pregnancies and leading cause of maternal and fetal mortality. Despite improvements in the diagnosis, there is no effective method of prevention and treatment. While studies in women are of critical importance, investigation of pathological mechanisms in pregnant women is necessarily limited and the ability to establish cause and effect relationships, difficult. Mouse models were invaluable tools in the identification of pravastatin as a potential treatment to prevent pregnancy complications associated with placental dysfunction such as preeclampsia and intrauterine growth restriction.

Numerous epidemiological studies provided robust evidence demonstrating that pravastatin exposure during pregnancy does not affect fetal development. Several pilot studies suggest that pravastatin may be a good option to prevent and treat preeclampsia in women. A randomized clinical trial is necessary to confirm the effectiveness of pravastatin to treat preeclampsia.

Keywords

Preeclampsia • Obstetric antiphospholipid syndrome • Placental insufficiency • Pravastatin

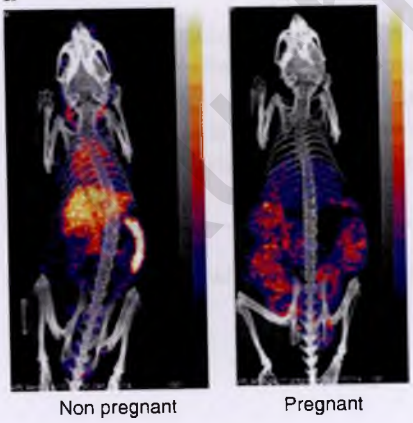
G. Girardi, Ph.D.
Division of Women Health, King's College, London, United Kingdom
e-mail: guillerminagirardi@gmail.com

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_15

15.1 Lessons from the Mouse

15.1.1 Pravastatin Prevents Adverse Pregnancy Outcomes in a Mouse Model of Antiphospholipid Syndrome (APS)

Placental insufficiency is one of the most severe APS-related complications for pregnant women. While studies in women are of critical importance, investigation of pathological mechanisms in pregnant women is necessarily limited and the ability to establish cause and effect relationships, difficult. Thus, mouse models are crucial for the identification of mediators and effectors in pregnancy complications associated with placental malperfusion, such as obstetric APS. Several in vitro studies demonstrated the binding of antiphospholipid (aPL) antibodies to trophoblasts [1, 2]. However, until recently, there was no direct evidence that aPL antibodies bind to the placenta in vivo. Using aPL antibodies labeled with radioactive indium (In^{-111}) and single photon emission computed tomography (SPECT/CT), the tissue distribution and binding of aPL antibodies in a pregnant mouse were visualized [3]. After injection of labeled aPL antibodies in the pregnant mouse, the antibodies were rapidly cleared from circulation when compared to the nonpregnant mouse, and the maximum accumulation of aPL antibodies was found in the fetal sacs (Fig. 15.1). Within the fetal sacs, a high count was found in the placenta [3]. Using noninvasive imaging, the in vivo real-time binding of aPL to the placenta was demonstrated (Fig. 15.1).



c

In^{111}- aPL (ng IgG/g tissue)	Pregnant	non pregnant
Blood	89±27*	261±110
Placenta	500±245	

Fig. 15.1 NanoSPECT/CT images of indium111-labeled aPL antibodies 1s. (**a**) Nonpregnant mouse injected with aPL antibody; (**b**) pregnant mouse injected with aPL antibody. In the pregnant mouse, aPL antibodies are rapidly cleared from circulation and are localized in the fetal sacs. (**c**) Within the fetal sacs, large amounts of the radioactive antibody are found in the placenta [3]. *Different from nonpregnant, $p < 0.05$

Pregnancy complications in APS have been erroneously attributed to placental thrombosis and infarcts. However, inflammation, in particular complement activation, plays a crucial role in trophoblast injury [4]. Interestingly, exposure to aPL antibodies and inflammatory mediators in uterus affects fetal neurodevelopment. Due to their large size, antibodies are not expected to pass the blood-brain barrier (BBB) under physiological conditions. Provocatively, during pregnancy, aPL antibodies cross the placenta and the BBB reaching the developing brain [3]. The BBB becomes more permeable during inflammation. Inflammatory mediators such as complement component C5a and TNF-α affect both endothelial and astroglial cells altering the BBB integrity. In addition, the immaturity of the fetal BBB, characterized by increased permeability, increases the susceptibility of the fetal brain to maternal autoantibodies and other proinflammatory and toxic insults.

Using in vivo SPECT/CT, it was demonstrated that aPL antibodies labeled with indium-111 are rapidly cleared from circulation in the pregnant mouse and large amounts of the radiolabeled antibody are entrapped in the placentas and fetal brains within the fetal sacs [3]. This was the first study to show in vivo in real time the passage of aPL antibodies to the fetus targeting the fetal brain [3]. Binding of aPL antibodies to the fetal brain was associated with complement deposition, measured using USPIO-labeled anti-C3 antibodies [5]. Interestingly aPL antibodies bind to the fetal brain and activate the complement cascade leading to abnormal fetal brain architecture and abnormal behavior in the offspring [5]. Increased anxiety was observed in the offspring of OAPS-mice, suggesting that in utero exposure to aPL antibodies causes abnormal cortical development and that C3 activation might be a footprint for adverse fetal outcomes. Complement activation is involved in the pathogenic effects of aPL antibodies on the placenta and the developing brain. A cross talk between coagulation and inflammation has been described in the pathogenesis of abnormal pregnancy outcomes in APS [6, 7]. Tissue factor (TF) was identified as a downstream mediator of complement activation [5]. TF, the major cellular activator of the coagulation cascade, is a key mediator in inflammation and trophoblasts injury in the mouse model of obstetric APS (OAPS-mice) [6, 7]. Using genetically modified mice with selective expression of TF in different tissues, an important role of complement-mediated TF expression in maternal neutrophils was demonstrated in OAPS-mice ([7], Fig. 15.2). aPL-induced TF expression modulates neutrophil activity toward a proinflammatory phenotype. In OAPS-mice, TF increases the respiratory burst and phagocytosis leading to subsequent trophoblast oxidative injury and adverse pregnancy outcomes. TF-mediated proinflammatory phenotype in OAPS involved the activation of protease-activated receptor 2 (PAR-2). Mice deficient in PAR-2 and treated with aPL antibodies exhibited reduced neutrophil activation and normal pregnancies, indicating that PAR-2 plays an important role in the pathogenesis of aPL antibody-induced fetal injury [7]. In addition, statins simvastatin and pravastatin downregulate TF and PAR-2 expression in neutrophils and thus prevent pregnancy loss [7]. In summary, TF signaling through PAR-2 mediates neutrophil activation and fetal death in OAPS. Statins prevent placental

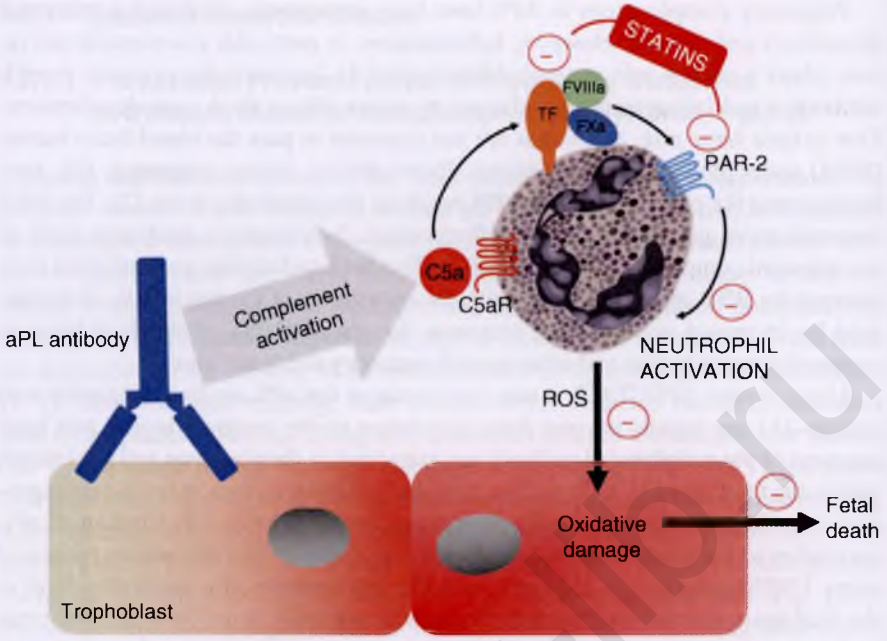


Fig. 15.2 Statins prevent neutrophil activation, trophoblast injury, and adverse pregnancy outcomes in a mouse model of obstetric APS—proposed mechanism. Maternal aPL antibodies bind to the placenta and activate the complement system. Interaction of complement split product C5a with its receptor C5aR triggers expression by neutrophils of tissue factor (TF), resulting in increased phagocytic capacity and generation of reactive oxygen species (ROS). Increased neutrophil activity leads to trophoblast injury and ultimately fetal death. TF-mediated neutrophil activation proceeds through engagement of protease-activated receptor 2 (PAR-2). Pravastatin prevents C5a-induced upregulation of TF and PAR-2 expression, thereby inhibiting the release of reactive oxygen species and trophoblast damage and protecting pregnancies

damage and rescue pregnancies suggesting that they may be an appropriate treatment for women with OAPS. Downregulation of TF and PAR-2 resulting in diminished neutrophil activity and survival of the fetuses is one of the many effects exerted by statins. Fig. 15.3 describes the pleiotropic effects of statins that may also contribute to the preventive effects of statins in pregnancy complications associated with placental insufficiency.

15.1.2 Pravastatin Prevents the Onset of Preeclampsia in Mouse Models

Placental insufficiency can result in serious pregnancy complications, including fetal growth restriction and preeclampsia (PE). The last decade has seen the emergence of abundant experimental evidence supporting a protective role for statins in

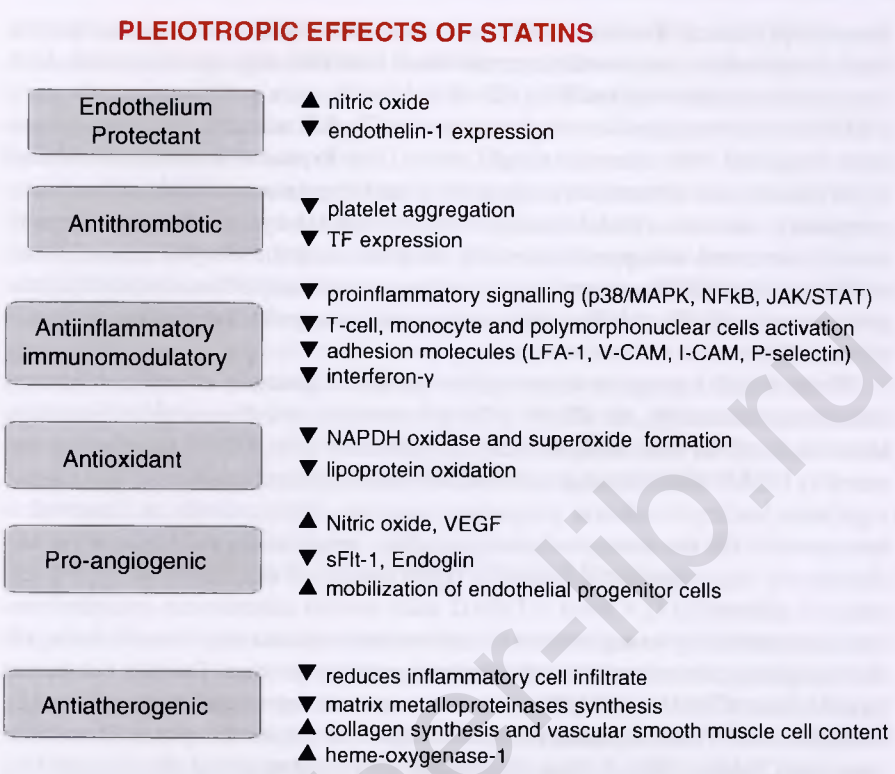


Fig. 15.3 Pleiotropic effects of statins. The beneficial effects of statins in preventing cardiovascular disease are not entirely due to cholesterol reduction. There is now compelling evidence that statins may diminish inflammation and oxidative stress, increase angiogenesis, inhibit the coagulation cascade, and protect the endothelium. These pleiotropic effects may explain the effects of pravastatin in preventing pregnancy complications associated with placental insufficiency

preventing preeclampsia in mice. Pravastatin, hydrosoluble statin with limited transport across the placenta and thus favorable pharmacokinetic profile in pregnancy [8–10], prevented the onset of PE in several animal models.

15.2 C1q KO and CBA/J × DBA/2 Mouse Models

Complement component C1q has an important role in trophoblast migration, spiral arteries remodeling, and normal placentation [11, 12]. Pregnant C1q-deficient (C1qKO) mice recapitulate the key features of human PE, hypertension, albuminuria, endotheliosis, decreased placental vascular endothelial growth factor (VEGF), and elevated levels of soluble VEGF receptor 1 (sVEGFR1 or sFlt-1), that correlate with increased fetal death [12]. In addition, decreased blood flow and increased oxidative stress are observed in placentas

from C1qKO mice. Treatment of C1qKOmice with pravastatin restored trophoblast invasiveness, improved placental blood flow and angiogenic balance, and, thus, prevented the onset of PE [12]. sFlt-1 levels were reduced, and placental VEGF levels were significantly increased in C1qKO mice treated with pravastatin compared with untreated C1qKO mice [12]. Pravastatin treatment reduced hypertension and albuminuria, signs of preeclampsia associated with adverse pregnancy outcomes. Renal damage and endothelial dysfunction were significantly attenuated with pravastatin [12]. Studies using the C1qKO mouse model of PE highlighted the causative role of impaired trophoblast invasion in the pathogenesis of PE and identified pravastatin as a good therapeutic option to prevent PE.

While C1qKO pregnant mice exhibit the wide spectrum of clinical features observed in human PE, the CBA/J × DBA/2 mouse model of recurrent miscarriage shows most of the clinical signs except hypertension [13]. CBA/J females impregnated by DBA/2 males develop endothelial dysfunction and angiogenic factors dysregulation leading to adverse pregnancy outcomes. Provocatively, as observed in humans with PE, the concept of primigravidity—epidemiological landmark of this disease—is also observed in CBA/J × DBA/2 mice, in which only the first pregnancy is affected [13]. CBA/J × DBA/2 mice exhibit albuminuria, endotheliosis, increased sensitivity to angiotensin II, and increased plasma leptin levels during the first pregnancy that correlates with bad pregnancy outcomes. Despite not having hypertension, CBA/J × DBA/2 females show severe endothelial dysfunction [13]. Antagonism of VEGF signaling by sFlt-1 seems to be involved in placental and fetal injury in CBA/J × DBA/2 mice. Pravastatin prevented maternal disease and fetal outcomes in the CBAxDBA model. Pravastatin restored angiogenic balance, ameliorated glomerular injury, diminished hypersensitivity to angiotensin II, and prevented intrauterine growth restriction leading to good pregnancy outcomes [13]. The CBA/J × DBA/2 model was another helpful tool, in the identification of pravastatin as a candidate therapy to prevent preeclampsia and its related maternal and fetal complications.

15.3 sFlt-1-Induced Mouse Models

Placenta-derived antiangiogenic factors, such as sFlt-1 and soluble endoglin (sENG), have been related to the progression of preeclampsia, and some studies suggest a causative effect [14, 15]. Systemic administration of adenoviral vectors expressing sFLT-1 to mice resulted in PE characterized by increased vascular reactivity to phenylephrine [16]. Interestingly, pravastatin improved the vascular reactivity in this murine model of PE and decreased sFlt-1 levels [16]. Using this model, a potential mechanism for the protective effects of pravastatin was postulated. In this study, the authors suggested that pravastatin's ability to prevent preeclampsia phenotype may be mediated through pleiotropic mechanisms involving a prosurvival/antiapoptotic mitogen-activated protein kinase (MAPK) pathway [17].

The beneficial effects of statins in PE were also observed in a mouse model of preeclampsia that highlights the importance of the placental origin of

antiangiogenic factors in the pathogenesis of placental insufficiency. Kumasawa et al. developed a model of PE that faithfully reproduces many of the human findings of late-onset PE by using a lentiviral vector-mediated placenta-specific expression system [18]. Transduction of the trophectoderm—which provides most of the main and functional components of the future placenta—of blastocyst-stage embryos with HIV-I-based self-inactivating lentiviral vectors expressing sFlt-1 resulted in the development of PE in mice. In this model, hypertension, proteinuria, and intrauterine growth restriction were observed. Pravastatin treatment of mice with sFlt-1-induced PE ameliorates symptoms by increasing VEGF-like angiogenic factor placental growth factor and diminishing sFlt-1. This study also suggests that pravastatin may be a good candidate drugs for preeclampsia treatment [18]. The protective effects of pravastatin in pregnancy were also observed in a rat model of placental ischemia-induced hypertension [19]. In the rat, pravastatin attenuated hypertension, oxidative stress, and angiogenic imbalance after placental ischemia induced by reduction of uteroplacental perfusion pressure (RUPP) [19].

15.4 From Mice to Women: Pilot Clinical Studies

Based on the pathophysiologic similarities between cardiovascular disease and preeclampsia and the promising results observed in animal models, there is an increasing interest in treating pregnant women with statins to prevent preeclampsia. Despite the abundant information provided by animal studies regarding the beneficial effects of statins in preventing pregnancy complications and its safety during pregnancy, translational studies and the organization of RCT were significantly delayed. This delay was due in part to some obstetricians misinterpreting and misusing the Food and Drug Administration (FDA) categories. Statins were classified as category X in 1979 not because they were teratogenic but because there was no indication for a pregnant woman to take statins and no data relating to the effects on a pregnant women and/or the fetus were available at that time (as opposed to the existence of evidence of harm) [20, 21]. Studying drugs in pregnancy for off-label indications such as pravastatin for preeclampsia prevention is still a challenge [22]. Importantly while atorvastatin and simvastatin were included in category X, pravastatin was never included in this classification. The FDA has received requests to improve the decades-old content and format of pregnancy prescription drug labeling since 1992. Epidemiological data collected to date demonstrate that statins are not major teratogens [23, 24]. Several reports on clinical and laboratory data regarding inadvertent exposure to statins in human pregnancy are available [25–27].

In 2015, based on the fact that the five-letter system left patients and physicians ill-informed and resulted in false assumptions about the actual meaning of the letters, the FDA replaced the former pregnancy risk letter categories on prescription and biological drug labeling with new and clearer information for both patients and healthcare providers [28]. Despite strong evidence of fetal safety now available and in particular recent studies of the transfer of pravastatin across the dually perfused placental supporting pravastatin's favorable pharmacokinetic profile in pregnancy [8], the ability to reshape and restate a medical consensus regarding the use of

statins in pregnancy has been difficult. That several RCT are currently active in the United States and Europe clearly shows a better understanding of clinicians and patients regarding the safe use of statins during pregnancy. Moreover, one of these trials is intended to determine the safety and pharmacokinetic data regarding the use of statins for preventing preeclampsia in high-risk pregnant women. Preliminary data from this study showed no identifiable safety risk associated with pravastatin use in pregnant women [29].

The first report to suggest the beneficial effects of pravastatin in preventing preeclampsia in women was published by our group. In this case study, a patient with antiphospholipid syndrome (APS) with a history of early preeclampsia leading to a stillbirth at week 26 developed preeclampsia in her second pregnancy, despite anticoagulant treatment [30]. Uterine artery Dopplers showed increased resistance and bilateral notching. To prevent intrauterine fetal death as in the previous pregnancy, the patient was supplemented with pravastatin. Addition of pravastatin (20 mg/daily) to standard of care therapy low molecular weight heparin plus low-dose aspirin (LMWH+LDA) normalized blood pressure and proteinuria and reversed abnormal uterine blood flow in this patient [30]. A live and healthy baby girl weighing 2830 g was delivered vaginally at 38 weeks, and no peripartum complications were observed. The patient stopped receiving statins prior to delivery in order to breastfeed, and, surprisingly, preeclampsia relapsed shortly after delivery. The patient was treated again with pravastatin and preeclamptic features disappeared (Fig. 15.4, [30]).

A pilot clinical study to examine the effects of pravastatin in women diagnosed with PE between 24 and 29 weeks was conducted by Brownfoot FC et al. [31]. In this study, four patients—significantly hypertensive (90–105/155–200 mmHg) and with proteinuria ranging from 840 to 2990 mg/24 h—were treated with daily pravastatin (40 mg) from the day of admission. All women presented growth restricted

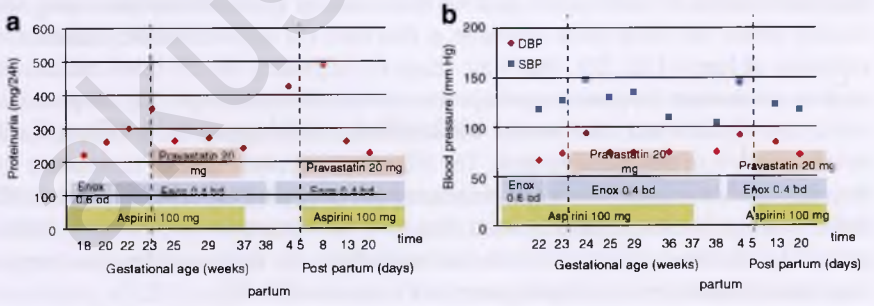


Fig. 15.4 Pravastatin improved blood pressure and proteinuria during pregnancy and postpartum in a patient with APS refractory to anticoagulation (enoxaparin and aspirin) [30]. (**a**) Urinary protein levels (mg/24 h) in the patient during pregnancy and postpartum. Proteinuria dropped significantly after pravastatin treatment was administered at 23 + 1 weeks and at day 5 in the postpartum. (**b**) Systolic (SBP) and diastolic (DBP) blood pressure variations during pregnancy and postpartum. A significant diminution in SBP and DBP was observed after pravastatin was added at 23 + 1weeks and 5 days postpartum. Ninety days after delivery, proteinuria and blood pressure remained within normal values

fetuses. The patients also received betamethasone and magnesium sulfate for lung maturation and neuroprotection. After pravastatin treatment, symptoms of PE resolved. Pravastatin stabilized blood pressure in three of the patients, and only one patient required antihypertensive medication nifedipine. Delivery in these women was triggered by fetal indications rather than worsening of maternal disease. Furthermore, serum sFlt-1, sENG, and endothelin-1 levels remained stable after pravastatin treatment. The authors concluded that these results supported the concept that pravastatin might be a candidate therapeutic strategy for preeclampsia.

While the studies mentioned above were intended to ameliorate preeclampsia after the onset, a study by Costantine et al. [29] investigated the effectiveness of pravastatin in preventing preeclampsia in high-risk patients. As an initial step in this multicenter, double-blind, placebo-controlled, randomized trial (clinicaltrials.gov NCT01717586) [29], a pilot study was performed to evaluate the utility of pravastatin in preventing preeclampsia in women with a history of severe preeclampsia in a prior pregnancy that required delivery before 34 weeks. Twenty subjects between 12 and 16 weeks of pregnancy were randomized to pravastatin (10 mg) ($n = 10$) or placebo ($n = 10$). Four women in the placebo group developed preeclampsia; three of them showed severe disease compared to the pravastatin group, suggesting a beneficial effect of pravastatin in preventing the onset of preeclampsia. A slight but not significant diminution in antiangiogenic factors sFlt-1 and sENG and an increase in PlGF were observed in the pravastatin group compared to placebo [29]. Birth weight was similar in both groups, and no congenital abnormalities or other adverse effects were observed in the two groups. No maternal, fetal, or neonatal death was observed [29]. As previously stated, this study provided important information regarding preliminary safety and pharmacokinetic data regarding the use of pravastatin in early pregnancy to prevent preeclampsia in high-risk women and justifies the use of pravastatin in a larger RCT that is currently taking place at Eunice Kennedy Shriver National Institute of Child Health and Human Development Obstetric-Fetal Pharmacology Research Units Network. A proof of principle, double-blind, randomized placebo-controlled, multicenter trial of pravastatin to ameliorate early-onset preeclampsia (sTAmP) was recently completed in the United Kingdom [32]. The aim of this trial was to establish whether a significant reduction of angiogenic markers by pravastatin will alleviate the severity of early-onset preeclampsia (PE) in women.

A recent study also demonstrated the beneficial effects of pravastatin in preventing preeclampsia in women with antiphospholipid syndrome (APS) [33]. Pregnancy complications in APS have been attributed to placental thrombosis and infarcts. Thus, treatment with low-dose aspirin (LDA) and heparinoids (low molecular weight heparin, LMWH) has become a conventional option for pregnant women with APS. However, treatment is ineffective in preventing adverse outcomes in a significant number of pregnancies [34, 35]. In particular, anticoagulant therapy showed to be ineffective in preventing PE and intrauterine growth restriction (IUGR) in women with APS increasing the need to explore other treatments to improve obstetrical outcome. In this study the effects of pravastatin on pregnancy outcome in women with APS that developed PE despite the use of anticoagulants was assessed. Twenty-one pregnant women with APS and an adverse obstetric history—which developed PE

APS	PE / IUGR	Doppler studies	LMWH + LDA	Pravastatin - times of administration (weeks)	Threshold achievement (BP <130/90 mmHg; Prot <300mg/dl, normal Dopplers) (days)	Pregnancy survival after diagnosis (weeks)	Time of delivery (weeks)	Neonatal outcome
YES N=10	YES severe	↑uterine arteries PI 4 bilateral Notching ↑rEDV	yes	NO		4.5 IQR[2-6] 3 stillbirths (25-26 w)	26.5 IQR[26-32]	BW: 900gr IQR[580-1100] All admitted at NICU 3 deaths 3 abnormal development
YES N=11	YES severe	↑uterine arteries PI 3 bilateral notching ↑rEDV	yes	YES 24 IQR [23-26]	14 IQR[10-15]	13 IQR[8-14]	36 IQR[35-38]	BW 2390gr IQR[2065-2770] 2 admitted at NICU (twins) All normal now

Fig. 15.5 Pravastatin improved maternal and fetal outcomes in OAPS refractory to antithrombotic therapy [33]. Improvement was observed at 14 days (IQR [10–15]). Pregnancy survival improved significantly with pravastatin treatment and deliveries occurred close to term diminishing the risks associated with prematurity. *LMWH* low molecular weight heparin, *LDA* low-dose aspirin, *PE* preeclampsia, *IUGR* intrauterine growth restriction, *PI* pulsatility index, *rEDV* reverse end diastolic volume (umbilical arteries), *BP* blood pressure, *Prot* proteinuria, *BW* birth weight, *NICU* neonatal intensive care unit

and/or severe IUGR despite being treated with LDA+LMWH since the beginning of pregnancy—participated in this study. A group of ten women with APS and severe PE and/or IUGR received only conventional LDA+LMWH treatment and served as control. Pravastatin (20 mg/day) was added to conventional treatment in 11 women when signs of preeclampsia and/or IUGR were observed. Uteroplacental blood hemodynamics were measured by Doppler. Progression of hypertension and proteinuria and fetal/neonatal outcomes were also evaluated. In the control group that received only antithrombotic therapy, deliveries occurred preterm, and only 6 of the 11 neonates survived. Neonates spent several months at the neonatology intensive care unit, and three show abnormal development ([33], Fig. 15.5).

In the group supplemented with pravastatin, placental blood flow increased, and hypertension and proteinuria stabilized as early as 10 days after treatment (median 14, IQR [10–15]) leading to live birth in all patients. In the group treated with pravastatin, pregnancies were significantly prolonged (13 weeks IQR [8–14]) compared to the group that only received LMWH+aspirin (4.5 weeks, IQR [2–6] ([33], Fig. 15.5). Delivery dates in the group supplemented with pravastatin were close to term allowing appropriate fetal development. No late sequelae were reported for the infants in the pravastatin group [33]. This study suggests that women with refractory obstetric APS may have improved pregnancy outcomes with pravastatin taken at the time of onset of PE or severe IUGR until the end of pregnancy.

Conclusion

The preclinical studies showed promising results on the beneficial effects of pravastatin in treating placental insufficiency and laid foundation for the human studies. The mouse studies and the pilot human studies emphasize the need of a

randomized clinical trial (RCT) to confirm these observations. Pravastatin was never included in category X by the FDA and due to its hydrophilic characteristics has a minimal transplacental transfer, diminishing fetal safety concerns. Regarding maternal safety concerns, large-scale, placebo-controlled, randomized clinical trials have established conclusive evidence that the long-term use of pravastatin in nonpregnant women is not associated with maternal risks. Long-term exposure does not compromise liver or muscle function when compared to placebo-treated nonpregnant women [36].

The new FDA classification that replaced the five-letter system together with the abundant evidence demonstrating that pravastatin is safe during pregnancy will facilitate the organization of RCT to investigate the effectiveness of pravastatin in preventing and treating preeclampsia. Other therapeutic strategies to prevent and/or treat preeclampsia have had limited success, and the only current available therapy is the delivery of the baby, associated with high risks due to prematurity. Treatment and prevention of PE with pravastatin is a promising option to prevent and treat preeclampsia but a RCT should be organized to confirm its effectiveness.

References

1. Di Simone N, Meroni PL, D'Asta M, et al. Pathogenic role of antibeta2glycoprotein I antibodies on human placenta: functional effects related to implantation and roles of heparin. Hum Reprod Update. 2015;21:97–118.
2. D'Ippolito S, Di Simone N, Di Nicuolo F, et al. Antiphospholipid antibodies: effects on trophoblast and endothelial cells. Am J Reprod Immunol. 2007;58:150–8.
3. Bertolaccini ML, Contento G, Lennen R, et al. Complement inhibition by hydroxychloroquine prevents placental and fetal brain abnormalities in antiphospholipid syndrome. J Autoimmun. 2016;75:30–8.
4. Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. Nat Med. 2004;10:1222–6.
5. Girardi G, Fraser J, Lennen R, Vontell R, Jansen M, Hutchison G. Imaging of activated complement using ultrasmall superparamagnetic iron oxide particles (USPIO) – conjugated vectors: an in vivo in utero non-invasive method to predict placental insufficiency and abnormal fetal brain development. Mol Psychiatry. 2015;20:1017–26.
6. Redecha P, Tilley R, Tencati M, Salmon JE, Kirchhofer D, Mackman N, Girardi G. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. Blood. 2007;110(7):2423–31.
7. Redecha P, Franzke CW, Ruf W, Mackman N, Girardi G. Neutrophil activation by the tissue factor/Factor VIIa/PAR2 axis mediates fetal death in a mouse model of antiphospholipid syndrome. J Clin Invest. 2008;118:3453–61.
8. Balan A, Szaingurten-Solodkin I, Swissa SS, Feinshtein V, Huleihel M, Holcberg G, Dukler D, Beharier O. The effects of pravastatin on the normal human placenta: lessons from ex-vivo models. PLoS One. 2017;12(2):e0172174.
9. Nanovskaya TN, Patrikeeva SL, Paul J, Costantine MM, Hankins GD, Ahmed MS. Transplacental transfer and distribution of pravastatin. Am J Obstet Gynecol. 2013;209(4):373.e1–5.
10. Zarek J, DeGorter MK, Lubetsky A, Kim RB, Laskin CA, Berger H, Koren G. The transfer of pravastatin in the dually perfused human placenta. Placenta. 2013;34(8):719–21.

11. Agostinis C, Bulla R, Tripodo C, Gismondi A, Stabile H, Bossi F, Guarnotta C, Garlanda C, De Seta F, Spessotto P, Santoni A, Ghebrehiwet B, Girardi G, Tedesco F. An alternative role of C1q in cell migration and tissue remodeling: contribution to trophoblast invasion and placental development. J Immunol. 2010;185(7):4420–9.
12. Singh J, Ahmed A, Girardi G. Role of complement component C1q in the onset of preeclampsia in mice. Hypertension. 2011;58(4):716–24.
13. Ahmed A, Singh J, Khan Y, Seshan SV, Girardi G. A new mouse model to explore therapies for preeclampsia. PLoS One. 2010;5(10):e13663.
14. Maynard SE, Karumanchi SA. Angiogenic factors and preeclampsia. Semin Nephrol. 2011;31(1):33–46.
15. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med. 2008;59:61–78.
16. Costantine MM, Tamayo E, Lu F, Bytautiene E, Longo M, Hankins GD, Saade GR. Using pravastatin to improve the vascular reactivity in a mouse model of soluble fms-like tyrosine kinase-1-induced preeclampsia. Obstet Gynecol. 2010;116(1):114–20.
17. Saad AF, Diken ZM, Kechichian TB, et al. Pravastatin effects on placental prosurvival molecular pathways in a mouse model of preeclampsia. Reprod Sci. 2016;23:1593–9.
18. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011;108(4):1451–5.
19. Bauer AJ, Banek CT, Needham K, Gillham H, Capoccia S, Regal JF, Gilbert JS. Pravastatin attenuates hypertension, oxidative stress, and angiogenic imbalance in rat model of placental ischemia-induced hypertension. Hypertension. 2013;61(5):1103–10.
20. Manson JM, Freyssinges C, Ducrocq MB, Stephenson WP. Postmarketing surveillance of lovastatin and simvastatin exposure during pregnancy. Reprod Toxicol. 1996;10:439–46.
21. Girardi G. Can statins prevent pregnancy complications? J Reprod Immunol. 2014;101–102:161–7.
22. Cleary KL, Roney K, Costantine MM. Challenges of studying drugs in pregnancy off-label indications: pravastatin for preeclampsia prevention. Semin Perinatol. 2014;38(8):523–7.
23. Zarek J, Koren G. The fetal safety of statins: a systematic review and meta-analysis. J Obstet Gynecol Can. 2014;36(6):506–9.
24. Karalis DG, Hill AN, Clifton S, Wild RA. The risks of statin use in pregnancy: a systematic review. J Clin Lipidol. 2016;10(5):1081–90.
25. Bateman BT, et al. Statins and congenital malformations: cohort study. BMJ. 2015;350:h1035.
26. Lecarpentier E, et al. Statins and pregnancy: between supposed risks and theoretical benefits. Drugs. 2012;72(6):773–88.
27. Winterfeld U, et al. Pregnancy outcome following maternal exposure to statins: a multicentre prospective study. BJOG. 2013;120(4):463–71.
28. www.drugs.com/pregnancy-categories.html.
29. Costantine MM, Clearly K, Hebert MF, Ahmed MS, Brown LM, Ren Z, Easterling TR, Haas DM, Haneline LS, Caritis SN, Venkataramanan R, West H, D'Alton M, Hankins G, Eunice Kennedy Shriver National Institute of Child Health and Human Development Obstetric-Fetal Pharmacology Research Units Network. Safety and pharmacokinetics of pravastatin used for the prevention of preeclampsia in high-risk pregnant women: a pilot randomized controlled trial. Am J Obstet Gynecol. 2016;214(6):720.e1–720.e17.
30. Lefkou E, Mamopoulos A, Fragakis N, Dagklis T, Vosnakis C, Nounopoulos E, Rousso D, Girardi G. Clinical improvement and successful pregnancy in a preeclamptic patient with antiphospholipid syndrome treated with pravastatin. Hypertension. 2014;63(5):e118–9.
31. Brownfoot FC, Tong S, Hannan NJ, Binder NK, Walker SP, Cannon P, Hastie R, Onda K, Kaitu'u-Lino TJ. Effects of pravastatin on human placenta, endothelium, and women with severe preeclampsia. Hypertension. 2015;66:687–97.
32. Ahmed A. New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications. Thromb Res. 2011;127(Suppl 3):S72–5.

33. Lefkou E, Mamopoulos A, Dagklis T, Vosnakis C, Rousso D, Girardi G. Pravastatin improves pregnancy outcomes in obstetric antiphospholipid syndrome refractory to antithrombotic therapy. J Clin Invest. 2016;126(8):2933–40.
34. Laskin CA, et al. Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA Trial. J Rheumatol. 2009;36(2):279–87.
35. Branch DW, Silver RM, Blackwell JL, Reading JC, Scott JR. Outcome of treated pregnancies in women with antiphospholipid syndrome: an update of the Utah experience. Obstet Gynecol. 1992;80(4):614–20.
36. Pfeffer MA, Keech A, Sacks FM, et al. Safety and tolerability of pravastatin in long term clinical trials: prospective pravastatin pooling (PPP) project. Circulation. 2002;105(20):2341–6.

Prevention and Treatment of Stroke and Eclampsia

16

Yasumasa Ohno

Abstract

Eclampsia and stroke during pregnancy are major causes of maternal and neonatal death in many countries. Herein, we describe the etiologies of and therapeutic strategies for eclampsia and stroke during pregnancy.

The incidence of eclampsia is 0.03–0.28% of deliveries, and its prognosis is improved. The incidence of pregnancy-associated stroke is 0.004–0.21%, and its maternal mortality rate ranges from 9 to 38%.

Hypertension is a risk factor for eclampsia and stroke. In pregnant patients whose blood pressure is greater than 180/120 mmHg, the use of $MgSO_4$ and antihypertensive agent is necessary. Clinicians should also pay attention to the presence of hypertension that first occurs during delivery. Repeated blood pressure measurements are necessary for the successful management of hypertension during labor.

In pregnant women with eclampsia or stroke, clinicians should give priority to emergent care and administer appropriate antihypertensive and anticonvulsive treatment. In cases of eclampsia or stroke that occurs before delivery, an emergent delivery should be considered. Discriminating between eclampsia and stroke based on neurological symptoms alone is difficult, and so computed tomography and/or magnetic resonance imaging-based brain scans should be obtained. When a stroke is detected, collaborative treatment with a neurosurgeon should be started as soon as possible. If a stroke is suspected at a primary medical facility or outside a medical facility, rapid maternal transport to an intensive medical facility is necessary. HELLP syndrome can markedly increase the degree of difficulty of neurosurgical operations. Indications for the surgical treatment of pregnancy-associated stroke that take account of the challenges encountered in the obstetrics setting should be developed.

Y. Ohno, M.D., Ph.D.
Ohno Ladies Clinic, Iwakura, Aichi, Japan
e-mail: yasumasa@ohno-clinic.jp

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_16

Keywords
Eclampsia • Stroke • Hypertension • Preeclampsia • Neuroimaging

16.1 Introduction

Eclampsia and stroke during pregnancy are major causes of maternal and neonatal death in many countries [1–3]. A WHO systematic analysis between 2003 and 2012 revealed that hypertensive disorder was the second most common cause of worldwide maternal death (14.0%) [4]. Stroke is also the second most common cause of maternal death in Japan (16.2%) [5]. Despite the ubiquity of these conditions and their public health impacts, neither their epidemiology nor therapeutic strategies for their treatment have been established. The lack of etiological information about eclampsia and stroke during pregnancy makes it difficult to propose appropriate management strategies for them. Close collaboration with neurosurgeons is necessary to prevent pregnancy-associated stroke; however, some medical facilities fail to establish good relationships with neurosurgeons, which can affect the prognosis of pregnant women that suffer strokes. In addition, some pregnant women develop hypertension after the onset of labor [6], but it is difficult to diagnose hypertension during early labor, and overlooked hypertension during labor can result in eclampsia and/or stroke. Here, we propose preventative and therapeutic strategies for eclampsia and stroke during pregnancy.

16.2 Eclampsia

16.2.1 Epidemiology and Pathophysiology

Eclampsia is defined as a seizure that occurs after 20 weeks' gestation in the absence of epilepsy and other basic disorders. Regarding the incidence of eclampsia, it has been reported to occur in 0.03–0.05% of deliveries in advanced countries [1, 2, 7], 0.04% of deliveries in Japan [8], and 0.28% of deliveries in developing countries [9]. A long-term Japanese survey of 518,024 deliveries revealed that eclampsia occurred antepartum, during labor, and postpartum in 19%, 37%, and 44% of cases, respectively, although it exhibited a good prognosis [8]. There are two different hypotheses regarding the pathogenesis of eclampsia. One involves cerebral ischemia caused by cerebral arterial vasospasm [10, 11], and the other involves cerebral hyperperfusion due to the breakdown of cerebral circulatory autoregulation [12–16]. The latter hypothesis is supported by recent evidence. Cerebral blood flow autoregulation operates normally within a mean arterial blood pressure range of 60–150 mmHg [17]. However, if the upper limit is greatly exceeded, hypertensive encephalopathy can occur. Schematic pathophysiology of eclampsia was shown in Fig. 16.1.

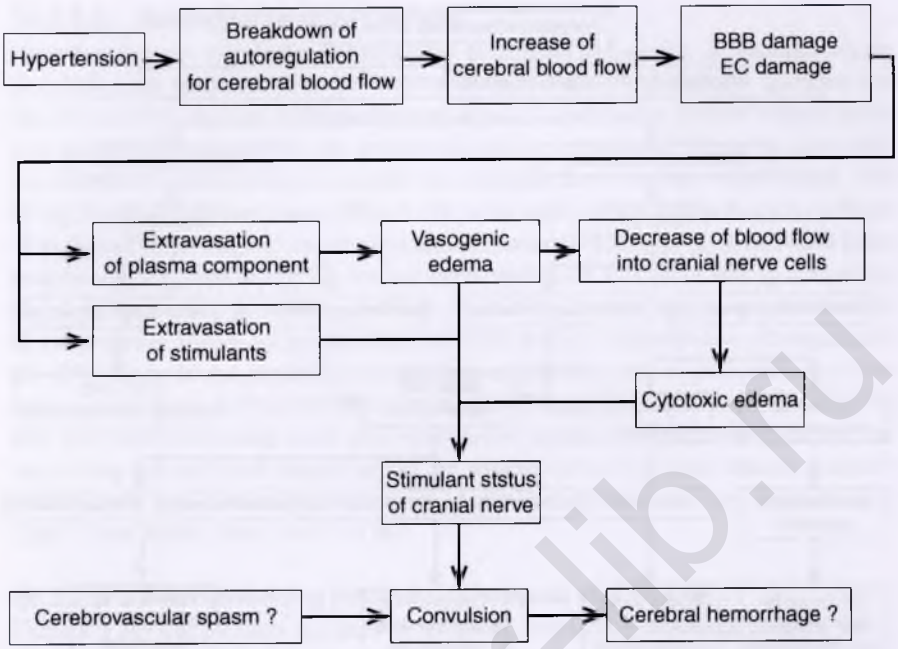


Fig. 16.1 Schematic pathophysiology of eclampsia. Acute hypertension induced loss of autoregulation with passive dilatation of cerebral arteries; hydrostatic pressure results in the extravasation of proteins and fluid into the interstitium. Convulsive activity in the brain is followed by a phase of hyperemia. These findings revealed that eclampsia is strongly related to reversible vasogenic edema. Vasogenic edema may increase the irritability of brain nerve cells, leading to convulsion. Correlation between convulsion and vasospasm is unclear, because the examination of vasospasm just at the point of convulsion is difficult. So, the possibility of vasospasm around the convulsion cannot be denied. Whether eclampsia leads to hypertensive cerebral hemorrhage or not is unclear. *BBB* blood-brain barrier, *EC* vascular endothelial cell

16.2.2 Treatment and Prevention

16.2.2.1 Initial Emergent Care

Schematic clinical management algorithm for eclampsia and suspected stroke case was demonstrated in Fig. 16.2. In pregnant women with eclampsia or stroke, clinicians should give priority to emergent care, including performing vital examinations, maintaining respiration, ensuring any required oxygen/intravenous drip infusions are provided, and monitoring the fetus' heart rate. Appropriate antihypertensive and anticonvulsive treatments are also necessary. In cases that occur before delivery, the termination of pregnancy by emergent delivery should be considered [18]. In some of eclamptic patient, fetal bradycardia continued even after the convulsion disappeared as shown in Fig. 16.3.

16.2.2.2 Anticonvulsive Therapy

The World Health Organization recommends the use of $MgSO_4$ based on the fact that $MgSO_4$ is more effective than diazepam and phenytoin at preventing maternal

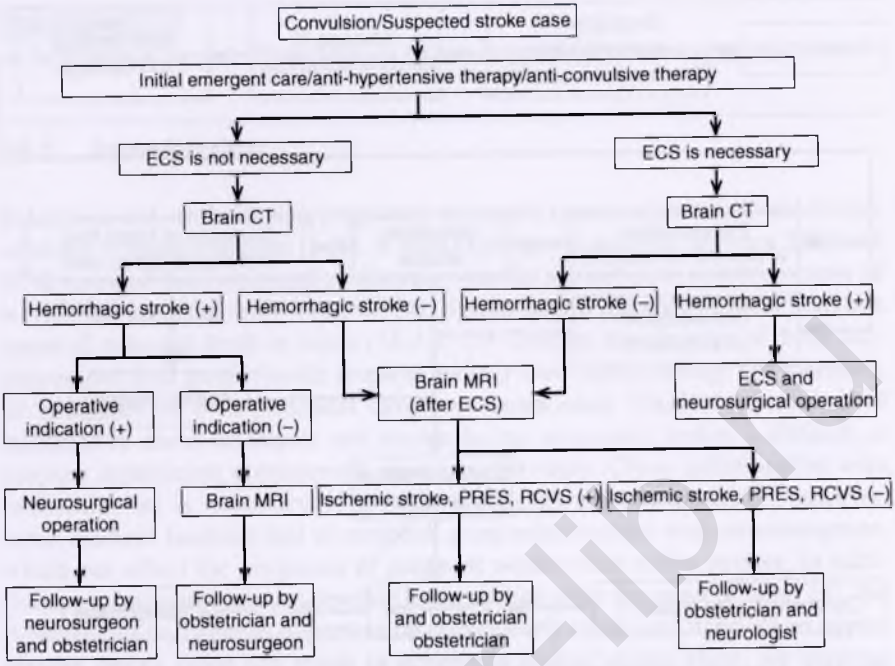


Fig. 16.2 Schematic clinical management algorithm for eclampsia and suspected stroke case. At the convulsion, we should give emergent care priority. In antenatal case, emergent delivery may be necessary. We should examine the presence of cerebral hemorrhage using CT as possible. If the patient accompanied with cerebral hemorrhage, collaborative management with neurosurgeon is necessary. For the patient without hemorrhage, we examine the presence of brain edema using MRI. *ECS* emergent cesarean section

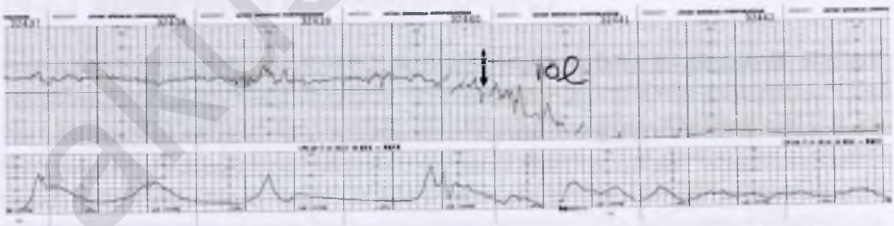


Fig. 16.3 Fetal heart rate pattern in eclamptic patient (Case 1). Fetal heart rate tracing shows fetal bradycardia following an intrapartum eclamptic convulsion. Fetal bradycardia continued even after the convulsion disappeared. Arrow, onset of convulsion

mortality and recurrent convulsions [19, 20]. If a patient's convulsions continue despite the administration of $MgSO_4$, anticonvulsants, such as diazepam, phenytoin, and phenobarbital, or intratracheal intubation should be performed [21].

16.2.2.3 Antihypertensive Therapy

Hypertension is a risk factor for recurrent eclampsia and stroke. In patients whose blood pressure is greater than 160/110 mmHg, the use of $MgSO_4$ (to decrease the risk of convulsions) and antihypertensive agent (to reduce the patient's blood pressure to 140–159 mmHg/90–109 mmHg) should be considered. While the preventative effects of antihypertensive agent on eclampsia have not been established, that of $MgSO_4$ has been confirmed [22]. On the other hand, while the preventative effects of antihypertensive agent (especially calcium channel blockers) on stroke have been established [23], that of $MgSO_4$ has not been confirmed. It is necessary to reduce the blood pressure of hypertensive mothers, especially in cases involving patients with blood pressure values of greater than 180/120 mmHg (hypertensive emergencies) [24–26]. Methyldopa, hydralazine, nifedipine, labetalol, and nicardipine can all be used as treatments for hypertension during labor. Among them, nicardipine is probably the most commonly used antihypertensive agent. If hypertension occurs, the supporting medical staff should report the patient's blood pressure data to a doctor immediately and discuss the necessity of medical intervention, as suggested by Case 3 (see below; Sect. 16.4.3) [18].

16.2.2.4 Discriminating Between Eclampsia and Stroke

There are several primary diseases of maternal convulsion as shown in Table 16.1. Discriminating between eclampsia, which has a good prognosis, and stroke, which has a poor prognosis, is very important in that. The main initial symptoms of stroke are impaired consciousness, hemiparesis, speech deficits, headache, and

Table 16.1 Primary diseases of maternal convulsion

Eclampsia [a]PRES [a]RCVS
Stroke
Hemorrhagic stroke (Cerebral hemorrhage, SAH, AVM, moyamoya disease) [a]PRES [a]RCVS
Ischemic stroke (Cerebral infarction, cerebral venous sinus thrombosis) [a]PRES [a]RCVS
Epilepsy
Cerebral angiopathy
Congenital brain disorder
Infectious encephalopathy
Brain tumor
Brain injury
Liver failure, renal failure
Metabolic disease, hypoglycemia
Hysteria
Drug induced
Antiphospholipid antibody syndrome
Autoimmune disease (SLE, TTP)

SAH subarachnoid hemorrhage, *AVM* arteriovenous malformation, *PRES* posterior reversible encephalopathy syndrome, *RCVS* reversible cerebrovascular spasm, *SLE* systemic lupus erythematosus, *TTP* thrombotic thrombocytopenic purpura
[a]PRES and RCVS were found in some of eclampsia and stroke

vomiting. Early convulsions are only seen in 6.6% of hemorrhagic stroke cases and 2.4% of ischemic stroke cases [27]. Cases of stroke in which convulsions are the initial symptom are relatively rare. However, when clinicians encounter pregnant women with convulsions, they should take the possibility of a stroke into account.

The American Stroke Association (ASA) proposed the following test: when facial or arm muscle weakness or a facial deficit is detected with or without convulsions, stroke should be strongly suspected (ACT FAST) [28, 29].

However, discriminating between eclampsia and stroke based on their neurological symptoms alone is difficult, and so neuroimaging technologies, including brain CT and MRI, should be used. In most intensive medical facilities, CT can be performed 24 h a day and is available for the diagnosis of hemorrhagic strokes. MRI, including T_2-weighted imaging (T_2WI), fluid-attenuated inversion recovery (FLAIR) imaging, diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) mapping, can provide detailed information about brain edema. The main pathophysiology of eclampsia is reversible vasogenic brain edema [12]. Eclamptic brain edema can localize in various regions, including the white matter of the occipital lobe, thalamus, putamen, and pons as well as combinations of these regions [12, 14, 30, 31]. A combination of DWI and ADC mapping should be used to discriminate between vasogenic and cytotoxic brain edema [12, 18]. PRES (posterior reversible encephalopathy syndrome) and RPLS (reversible posterior encephalopathy syndrome) are neurological syndromes involving reversible edema, mainly in the occipital lobe, and they exhibit neurological symptoms such as headache, convulsions, vomiting, visual disturbance, and neurological deficiencies. Thus, the neurological pathophysiology of eclampsia is equivalent to those of PRES/RPLS [12]. Some cases of eclampsia are complicated with reversible cerebral vasoconstriction syndrome (RCVS), which presents as repetitive severe headaches and multifocal cerebrovascular spasms and disappears within a few weeks [30]. It is unclear whether eclampsia can lead to cerebral hemorrhaging. Representative views of neuroimaging of eclampsia and stroke were shown in Figs. 16.4 and 16.5.

16.2.2.5 Collaboration with Neurosurgeons

When a stroke is detected, collaborative treatment with a neurosurgeon should be started as soon as possible. If a stroke is suspected at a primary medical facility or outside a medical facility, rapid maternal transport to an intensive medical facility is necessary. HELLP syndrome can markedly increase the degree of difficulty of neurosurgical operations. Obstetricians should highlight the importance and unique nature of HELLP syndrome to neurosurgeons, as demonstrated in Cases 2 and 3. Indications for the surgical treatment of strokes that take the challenges encountered in the obstetrics setting into account should be developed [18].

16.2.2.6 Hypertension During Labor (Labor-Onset Hypertension, LOH)

As shown in Case 1 (see below; Sect. 16.4.1), some pregnant women remain normotensive throughout pregnancy but then develop hypertension during labor (labor-onset hypertension, LOH). Patients who develop hypertension and are diagnosed

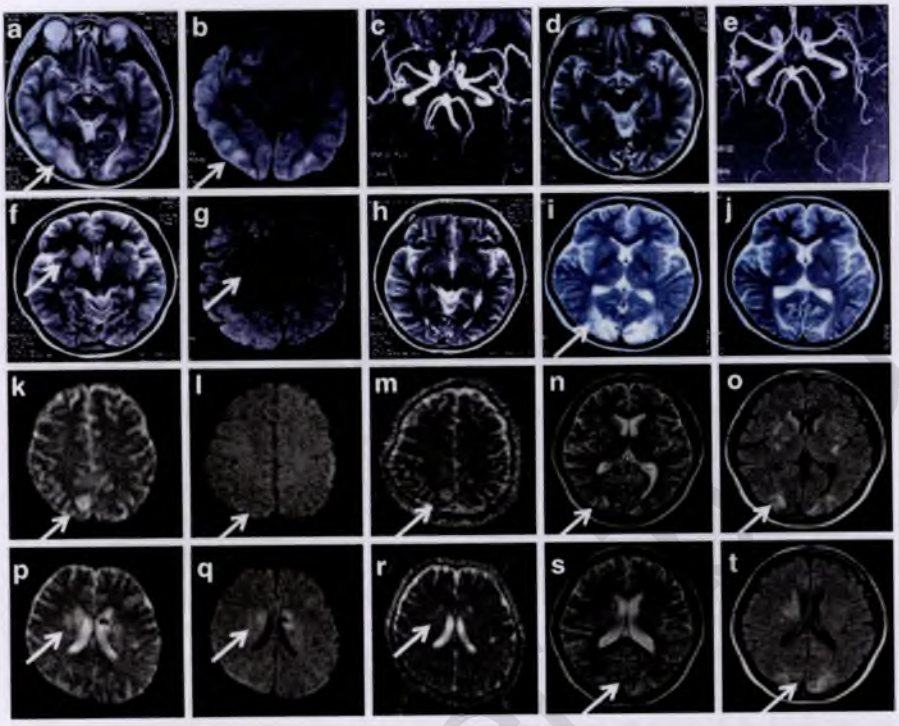


Fig. 16.4 MRI scans of eclampsia. (**a–e**) A 31-year-old Japanese primigravida woman who was complicated with convulsions and impaired consciousness at 4 h after delivery. Cytotoxic brain edema in the bilateral occipitoparietal lobes and cerebrovascular spastic changes were seen on T_2WI (**a**), DWI (**b**), and MRA (**c**), which were performed the day after the convulsions. The cytotoxic brain edema had disappeared by 31 days postpartum (**d**, **e**). (**f–h**) Case 1. Vasogenic brain edema was observed in the bilateral putamina and pontes on T_2WI (**f**) and DWI (**g**) scans obtained 60 min after the second episode of convulsions. It had disappeared by 36 days postpartum (**h**). (**i**, **j**) A 39-year-old Japanese primigravida woman who was complicated with preeclampsia and developed convulsions after a cesarean section at 32 weeks' gestation. Brain edema was seen in the bilateral occipital lobes on T_2WI performed just after the convulsions (**i**). It had disappeared at 8 days postpartum (**j**). (**k–m**) A 31-year-old Japanese primigravida woman who was complicated with preeclampsia and convulsions just before delivery. Vasogenic edema was noted in the right occipital lobe on T_2WI (**k**), DWI (**l**), and ADC (**m**) mapping scans performed at 2 h after delivery. Iso-low intensity DWI signals and high ADC are indicative of vasogenic brain edema. (**n**, **o**, **s**, **t**) A 32-year-old Japanese primigravida woman who was complicated with convulsions and placental abruption during labor. Multifocal brain edema was seen on T_2WI (**n**, **s**) and FLAIR (**o**, **t**) scans performed at 2 days after the patient underwent a cesarean section. As shown by these images, FLAIR was better than T_2WI at depicting the periventricular lesion. (**p–r**) A 30-year-old Japanese multigravida woman who was complicated with preeclampsia, convulsions, and HELLP syndrome at 5 h after delivery. Vasogenic edema was observed in the right occipital lobe on T_2WI (**p**), DWI (**q**), and ADC (**r**) mapping scans performed at 1-day postpartum. High-intensity DWI signals and high ADC are indicative of vasogenic brain edema (T_2 shine-through). Arrow, site of the brain edema

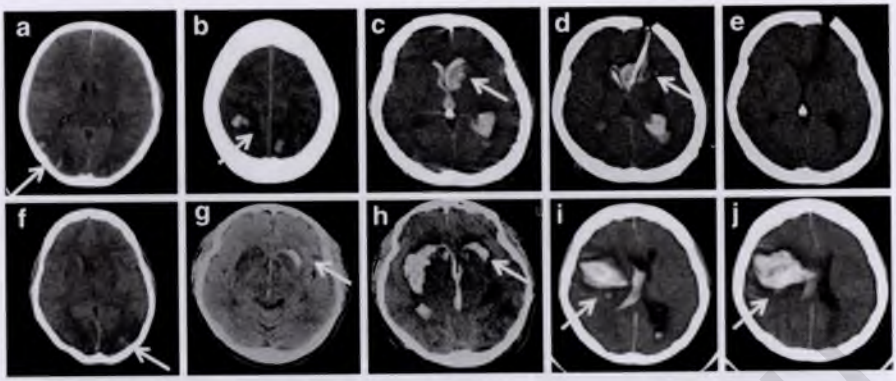


Fig. 16.5 CT scans of pregnancy-associated stroke. (**a**, **b**) Case 2. A CT scan obtained before the patient underwent a cesarean section detected multifocal cerebral hemorrhaging and massive brain edema. (**c–e**) Case 3. A CT scan obtained 60 min after delivery (**c**) detected a cerebral hemorrhage in the left caudate nucleus and ventricular release. Further CT scans, which were acquired just after the endoscopic hematoma evacuation procedure (**d**) and 17 days after the operation (**e**), demonstrated that the surgery had been successful. (**f**) A 34-year-old Japanese primigravida woman who was complicated with preeclampsia and convulsions at 29 weeks' gestation. A hemorrhagic infarction was seen in the bilateral occipital regions on the day after the patient underwent a cesarean section. (**g**, **h**) A 27-year-old Japanese primigravida woman who was complicated with placental abruption and continuously impaired consciousness after a cesarean section performed at 33 weeks' gestation and subsequently died. Severe cerebral hemorrhaging in the bilateral caudate nuclei, ventricular release, and massive brain edema were observed on a CT scan performed after the cesarean section. (**i**, **j**) A 37-year-old Japanese primigravida woman who was complicated with convulsions, impaired consciousness, and HELLP syndrome at 33 weeks' gestation and subsequently died. Severe cerebral hemorrhaging in the right cortex, ventricular release, and massive brain edema were seen on a CT scan obtained 2 days after the patient underwent a cesarean section. Arrow, site of the hemorrhage

with preeclampsia during the antenatal period are managed closely, and emergent delivery is considered if the patient's condition worsens. However, in patients with LOH, the onset of hypertension might be overlooked, and accurately assessing the risk of stroke is difficult. Such patients might be at risk of unexpected eclampsia or stroke during labor. Some previous studies have reported that LOH is a physiological change [31], whereas others have reported that LOH represents a late manifestation of preeclampsia that displays similar outcomes to preeclampsia [32].

We examined the cases of 1349 pregnant women who remained normotensive throughout pregnancy [6]. The patients were classified into four groups, the normotensive group, whose systolic blood pressure (SBP) remained below 140 mmHg throughout labor; the mild LOH group, whose maximum SBP during labor ranged from 140 to 159 mmHg; the severe LOH group, whose maximum SBP during labor ranged from 160 to 179 mmHg; and the emergent LOH group, whose maximum SBP during labor was greater than 180 mmHg. There were 1023 (76%), 241 (18%), 66 (5%), and 19 (1%) patients in the normotensive, mild LOH, severe LOH, and emergent LOH groups, respectively. Regarding the subjects' perinatal outcomes, SBP at delivery and the frequencies of antihypertensive agent use and interventional delivery differed significantly among the four groups. One of the patients in the emergent

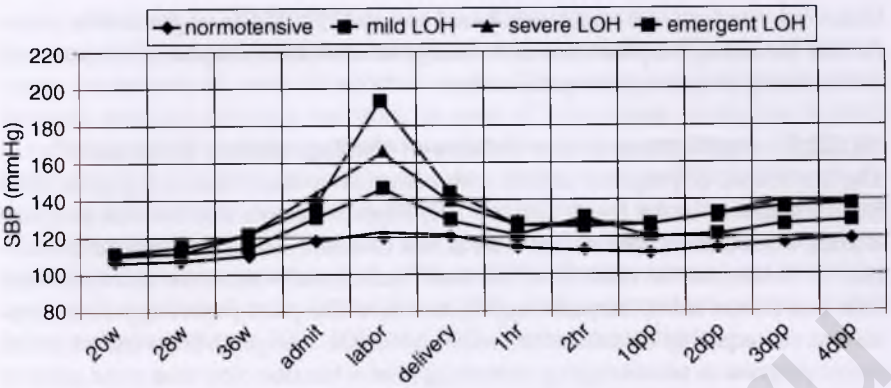


Fig. 16.6 Change of systemic blood pressure during labor and puerperium in patient without antenatal hypertensive disorder. There were 1023 (76%), 241 (18%), 66 (5%), and 19 (1%) patients in the normotensive, mild LOH, severe LOH, and emergent LOH groups, respectively. SBP at delivery and during puerperium differed significantly among the four groups. In all LOH groups, SBP after the delivery decreased remarkably and gradually elevated in the puerperium. *SBP* systolic blood pressure, *W* weeks' gestation, *admit* admission, *labor* during labor, *hr* hour after the delivery, *dpp* day postpartum, *LOH* labor-onset hypertension

LOH group developed eclampsia during labor. In all LOH groups, SBP after the delivery decreased remarkably. Blood pressure change around delivery in patient without antenatal hypertensive disorder [6] was shown in Fig. 16.6. Multivariate analysis extracted the following parameters as risk factors for severe/emergent LOH: being 35 years or older, a body mass index at delivery of ≥30, a SBP at 36 weeks' gestation of 130–139 mmHg, a SBP at admission of 130–139 mmHg, and a urinary protein score of 2+ during pregnancy. LOH is a risk factor for emergent hypertension and is associated with eclampsia and strokes during labor. We advocate that clinicians should pay attention to the presence of LOH. It might be possible to predict LOH using the extracted risk factors. In addition, it might be necessary to obtain repeated blood pressure measurements to ensure the successful management of LOH [6, 18]. The major difference between LOH and antenatal-onset preeclampsia/gestational hypertension is the presence of labor. Greater fetal demand in association with the reduced placental blood flow seen during labor might provide the stimulus for the renin-angiotensin reflex. So, from a clinical and pathophysiological point of view, we consider that it is necessary to treat these entities as two different conditions.

16.2.2.7 HBPM: A Tool for Predicting Eclampsia/Stroke

As shown in Case 2 (see below; Sect. 16.4.2), gestational proteinuria without hypertension can lead to preeclampsia or eclampsia within a few weeks. Multi-institutional Japanese research has revealed that 25% of cases of isolated gestational proteinuria progressed to preeclampsia [33]. On the other hand, home blood pressure measurement (HBPM) is increasingly being used in many countries and has been accepted well by hypertensive patients. The European Society of Hypertension has endorsed the use of HBPM in clinical practice as a useful adjunct to conventional office measurements [34]. SBP data obtained from HBPM showed a strong association with

stroke risk in a Japanese population-based survey [35]. HBPM might also be a useful tool for aiding the prediction and management of preeclampsia, eclampsia, and stroke during pregnancy and puerperium.

16.2.2.8 Verification of the Validity of the Expression "Eclampsia"

The first record of pregnant women with convulsions dates back to Egypt in 2200 BC. In addition, in the fourth century BC, Hippocrates pointed out that pregnant women who suffered convulsions were at risk of death. Bossler introduced the concept of "eclampsia" in 1739. Thus, the term "eclampsia" was coined at a time when little was known about the pathology of the brain. The pathophysiology of eclampsia was subsequently elucidated to involve reversible vasogenic brain edema due to recent progress in neuroimaging technology, and it became clear that some cases of eclampsia are complicated with hemorrhagic stroke. Confusion regarding convulsions, eclampsia, PRES, and stroke might hinder accurate understanding of the conditions that can cause convulsions in pregnant women. Therefore, we should discuss the validity of the expression "eclampsia" in the future.

16.3 Pregnancy-Associated Stroke

16.3.1 Epidemiology and Outcomes

The incidence of stroke in women of reproductive age is increased 1.5-fold by pregnancy [36]. Stroke during pregnancy is an extremely high-risk factor for both mother and child [37–39]. The incidence of pregnancy-associated stroke is 4.3–210 per 100,000 pregnancies [34, 40, 41]. Maternal mortality occurs in 9–38% of cases of pregnancy-associated stroke [40–44]. A WHO systematic analysis between 2003 and 2012 revealed that hemorrhage and hypertensive disorder were the cause of 27.1% and 14.0% of worldwide maternal death, respectively [4]. Stroke was the cause of 16.2% of maternal death in Japan [5]. Various population-based, single hospital-based, and nationwide studies have reported (Table 16.2) [8, 40, 43–47]. Western countries found that ischemic stroke is more frequent than hemorrhagic stroke. On the contrary, survey in Japan and Taiwan revealed that hemorrhagic stroke is more common than ischemic stroke (Table 16.2). This discrepancy might be related with

Table 16.2 Studies on pregnancy-associated stroke

Author	Year	Country	Study design	Stroke number	Incidence	Ischemic stroke (%)	Hemorrhagic stroke (%)
Sharshar	1995	France	Population	31	8.9	48	52
Kittner	1996	USA	Population	31	21.9	55	45
Jaigobin	2000	Canada	Single	34	67.1	62	38
Liang	2006	Taiwan	Single	26	38.9	35	65
Kuklina	2011	USA	Nationwide	4287	48.4	71	29
Ohno	2014	Japan	Prefecturewide	46	8.9	37	63
Yoshida	2017	Japan	Nationwide	151	10.2	25	74

Population population-based study, *Single* single hospital-based study, *Incidence* incidence per 100,000 deliveries

racial difference. A Japanese nationwide survey conducted in 2010–2011 [48] detected 134 cases of pregnancy-associated stroke, including 97 cases of hemorrhagic stroke and 37 cases of ischemic stroke. The survey revealed the following findings: maternal mortality was worse in cases of hemorrhagic stroke than in cases of ischemic stroke; underlying cerebrovascular diseases that were responsible for the hemorrhagic stroke were detected in 54 cases (56%), of which 87% had been undiagnosed before stroke onset (arteriovenous malformations were the most common underlying cerebrovascular disease); and hemorrhagic stroke occurred antepartum, during labor, and postpartum in 62%, 13%, and 25% of cases, respectively. According to a survey conducted in the United States [49], stroke occurred antepartum, during labor, and postpartum in 10%, 40%, and 50% of cases, respectively.

16.3.2 Treatment and Prevention

When a stroke is detected, collaborative treatment with a neurosurgeon should be started as soon as possible. If a stroke is suspected at a primary medical facility or outside a medical facility, rapid maternal transport to an intensive medical facility is necessary. Schematic clinical management algorithm for suspected stroke case was demonstrated in Fig. 16.2.

16.3.2.1 Cerebral Hemorrhaging

Cerebral hemorrhaging usually develops unilaterally, and it occurs more frequently in the putamen, thalamus, cerebellum, and pons (46–73%) than in the cortex (15–41%) [50, 51]. Neuroimaging of pregnancy-associated stroke was demonstrated in Fig. 16.5. A primary brain disorder, brain edema, increased intracranial pressure, and brain herniation due to cerebral hemorrhaging can all adversely affect maternal prognosis. Regarding symptoms, impaired consciousness, hemiparesis, aphasia, eyeball malpositioning, convulsions, and visual disturbance are seen. CT is useful for diagnosing cerebral hemorrhaging, as high-intensity images can be obtained in the acute phase. In pregnant patients that are complicated with convulsions, discriminating between eclampsia and stroke is difficult [52]. A previous study reported that 40% of eclampsia patients were complicated with cerebral hemorrhaging [53]. The surgical treatments for cerebral hemorrhaging include evacuation of the hematoma by craniotomy (Fig. 16.5c–e), a CT-based stereotactic method, and an endoscopic method. HELLP syndrome and diffuse intravascular coagulation (DIC) can markedly increase the degree of difficulty of neurosurgical operations. It is necessary to have a careful discussion with a neurosurgeon regarding whether surgical treatment or an emergent delivery should be given priority. A search for arteriovenous malformations (AVM) and moyamoya disease, which can cause cerebral hemorrhaging, is necessary in such cases.

16.3.2.2 Subarachnoid Hemorrhaging (SAH)

The frequency of SAH in pregnancy is 5.8 per 100,000 deliveries according to a nationwide survey carried out in the United States [54]. The main cause of SAH is the rupturing of intracranial aneurysms. The incidence of ruptured intracranial aneurysms in pregnancy is estimated to 3–10 per 100,000 pregnancies [42, 55, 56].

The frequency of aneurysmal SAH during pregnancy increases with advancing gestational age, and about 50% of ruptured aneurysms are found in the internal carotid artery [53]. Concerning the symptoms of SAH, severe headaches of sudden onset and/or impaired consciousness are frequently seen. Ruptured aneurysms in pregnant patients should be treated in the same manner as SAH in other patients. Surgical treatment should be carried out immediately to avoid rebleeding and ischemic complications due to vasospasm. In a previous study, the maternal mortality rate was 11% in the surgery group and 63% in the nonsurgery group [53]. In late pregnancy, i.e., when the fetus is mature, an emergent cesarean section should be performed followed by an aneurysm exclusion procedure. In early pregnancy, a surgical intervention to treat the aneurysm should be carried out while maintaining the pregnancy. The surgical treatments for ruptured aneurysms include microsurgical clipping and coiling embolization. In patients who present with high intracranial pressure caused by a massive intracerebral hematoma or acute hydrocephalus, emergency hematoma evacuation or ventricular drainage is required [57].

16.3.2.3 Cerebral Infarction

Ischemic stroke includes both arterial and venous infarctions. The incidence of ischemic stroke is 4–11 per 100,000 pregnancies [2, 28]. While the adjusted relative risk (RR) of ischemic stroke was reported to be 0.7, the RR increased to 5.4 during the postpartum period [28]. Arterial events usually occur in the first and third trimesters, whereas venous events frequently occur postpartum. Cerebral infarctions cause less maternal mortality than cerebral hemorrhaging [9]. As for the symptoms of cerebral infarctions, they include motor deficits, speech deficits, and impaired consciousness. Cerebral infarctions are not always complicated with hypertension. Some of cerebral infarction develops hemorrhagic infarction which is caused by reperfusion in necrotic tissue as shown in Fig. 16.5f. Thrombolytic therapy using intravenous recombinant tissue plasminogen activator (rt-PA) has been established as a highly successful therapy for acute ischemic stroke and is one of the potential therapeutic choices for cerebral infarctions that occur during pregnancy [58]. While rt-PA does not transfer to the placenta, it might increase the risk of placental abruption and uterine hemorrhaging. So, the indications for thrombolytic therapy should be discussed among obstetricians, neurosurgeons, and neurologists.

16.3.2.4 Cerebral Venous Sinus Thrombosis (CVST)

Cerebral venous sinus thromboses (CVST) include cavernous sinus, lateral sinus, and sagittal sinus thromboses and lead to brain edema, venous infarctions, and cerebral hemorrhaging. They can cause headaches, impaired consciousness, and convulsions as symptoms [59]. The maternal prognosis of CVST is relatively good [60]. According to a report from the American Heart Association/ASA [61], CVST develop in 2% of all pregnancy-associated stroke cases, and 88% of CVST occur during the postpartum periods. Infection, cesarean section, and emergent delivery are risk factors for CVST. Since MRI and MR venography (MRV) are noninvasive and do not involve radiation, a combination of MRI and MRV is the best choice for investigating suspected CVST during pregnancy. In pregnant women with a history of CVST, the prevention of recurrent CVST with low-molecular-weight heparin might be useful [61].

16.3.2.5 Moyamoya Disease

Moyamoya disease is a chronic occlusive cerebrovascular disease with an unknown etiology that is characterized by bilateral occlusive changes in the terminal portion of the internal carotid artery [62]. The prevalence of moyamoya disease in Japan was reported to be 6.03 per 100,000 people in 2003 [63]. Since moyamoya disease is a rare entity, but particularly affects younger people and females, it is not uncommon to encounter pregnant patients with moyamoya disease. Cerebral ischemia due to hyperventilation and cerebral hemorrhaging caused by cerebrovascular damage are the main problems associated with moyamoya disease during pregnancy. Patients that are diagnosed with moyamoya disease before pregnancy are known to be at lower risk of cerebrovascular events. On the other hand, patients that suffer cerebrovascular events during late pregnancy and are newly diagnosed with moyamoya disease have a poor prognosis [64]. Pregnant women with moyamoya disease should be carefully managed with the collaboration of obstetricians and neurosurgeons. The delivery procedure, i.e., elected cesarean section or painless epidural-assisted vaginal delivery, should be selected with the aim of avoiding unfavorable sequelae caused by hyperventilation and blood pressure increases [64].

16.3.2.6 Arteriovenous Malformations (AVM)

AVM are rare congenital vascular malformations and are composed of an arterial feeder, nidus, and draining vein. The annual detection rate of symptomatic AVM is 1.1–1.2 per 100,000 people [65]. Regarding the symptoms of AVM, convulsions and headaches are seen in 27–38% and 7–48% of cases, respectively. In a recent report, the annual AVM hemorrhaging rate during pregnancy was 10.8%; the hemorrhaging rate per pregnancy was 8.1%, and the hazard ratio for intracranial hemorrhaging caused by the rupturing of an AVM during pregnancy was 7.91 [66]. A nationwide Japanese survey revealed that among all pre-existing cerebrovascular diseases, AVM is the most common cause of pregnancy-associated hemorrhagic strokes [45]. The maternal management of patients with ruptured AVM should be mainly based on neurosurgical indications rather than obstetric indications. When neurological deterioration occurs due to the rupturing of an AVM, emergent neurosurgery is necessary. In early pregnancy, AVM resection tends to be performed, and the mode of delivery is chosen based on obstetric indications. In late pregnancy, radical treatment for AVM tends to be performed after an emergent cesarean section [67]. The surgical treatments for AVM include removal of the hematoma, drainage, and complete resection of the AVM.

16.4 Case Presentation

16.4.1 Case 1: Eclampsia During Labor with Labor-Onset Hypertension

A 34-year-old Japanese primigravida woman was admitted to primary obstetric facility A due to labor onset at 40 weeks' gestation. On admission, her blood pressure was 124/80 mmHg. At 7 h after admission, she suddenly developed hypertonic

convulsions, which rendered her unconsciousness, and fetal bradycardia was also detected (Fig. 16.3), which were suggestive of eclampsia. Her blood pressure had increased to 210/120 mmHg, and she was immediately transported to intensive medical facility B. A live female baby was delivered (weight: 2979 g) by emergent cesarean section and displayed Apgar scores of 5 and 8 at 1 and 5 min, respectively. At 4 h after the operation, a second set of convulsions occurred (blood pressure: 133/45 mmHg). The convulsions were stopped via the administration of 5 mg diazepam and 125 mg phenytoin. A magnetic resonance imaging (MRI) scan obtained at 60 min after the second set of convulsions depicted vasogenic edema in the bilateral putamina and pontes (Fig. 16.4f–h). The patient's blood pressure fell to within the normal range without antihypertensive treatment. At 8 days postpartum, the patient and her baby were discharged without any neurological abnormalities. This case suggested that some pregnant women remain normotensive throughout pregnancy but then develop hypertension during labor.

16.4.2 Case 2: Antepartum Cerebral Hemorrhaging

A 39-year-old paragravida woman was managed at primary obstetric facility C. At 25 weeks' gestation, her blood pressure and urinary protein score on Tes-Tape were 96/58 mmHg and 1+, respectively. At 27 weeks' gestation, her blood pressure and urinary protein score were 146/80 mmHg and 3+, respectively. Since the following 2 days were holidays, the doctor diagnosed the patient with preeclampsia and advised her to go to an intensive medical facility 3 days later. At 2 days after the consultation, she suddenly developed convulsions, became drowsy, and started vomiting. She was transported to intensive medical facility D, where an emergency care doctor accepted her without the permission of an obstetrician. Computed tomography (CT) depicted intracerebral hemorrhages in the white matter of the bilateral occipital lobes (Fig. 16.5a, b). She was transported to university hospital E. By this point, she was unconscious, and her blood pressure had increased to 187/118 mmHg. Her laboratory data (platelets [PLT], 38,000/μL; aspartate aminotransferase [AST], 685 U/L; alanine aminotransferase [ALT], 21 U/L; lactate dehydrogenase [LDH], 3284 U/L; and fibrin degradation products, 37 μg/mL) indicated the presence of HELLP syndrome. A neurosurgeon examined her and advised that an emergent cesarean section should be performed. As a result, a live female baby was delivered (weight: 810 g) and displayed Apgar scores of 1 and 2 at 1 and 5 min, respectively. However, a postoperative CT scan of the mother's brain detected massive brain edema and multifocal intracerebral hemorrhages. She died the same day. This case suggested that gestational proteinuria without hypertension might lead to preeclampsia or eclampsia within a few weeks.

16.4.3 Case 3: Cerebral Hemorrhaging During Labor

A 35-year-old Japanese primigravida woman was moved to intensive medical facility F at 35 weeks' gestation. Her parents had a history of hypertension. During a routine prenatal examination performed at 35 weeks' gestation, her blood pressure and urinary

protein score were found to be 112/85 mmHg and 3+, respectively, whereas at 36 weeks' gestation, they were 187/120 mmHg and negative, respectively. She was misdiagnosed with white coat hypertension because she displayed blood pressure values of 130/100 mmHg at home. She was admitted due to labor onset at 40 weeks' gestation. While her blood pressure was 190/140 mmHg on admission, it was not monitored regularly over the following hours. By 11 h after admission, her blood pressure had increased to 223/139 mmHg, and nicardipine was not effective at reducing it. An emergent delivery was performed as the patient became drowsy and developed hypertension (263/176 mmHg). A live male baby was delivered (weight: 3174 g) and displayed Apgar scores of 9 and 10 at 1 and 5 min, respectively. The patient suddenly developed right hemiparesis during suturing of the perineum, and CT performed at 60 min after delivery detected cerebral hemorrhaging in the left caudate nucleus and ventricular release (Fig. 16.5c–e). An endoscopic hematoma evacuation was immediately performed. The patient's laboratory data (PLT, 331,000/μL; AST, 17 U/L; ALT, 13 U/L; and LDH, 226 U/L) indicated the absence of HELLP syndrome. At 57 days' postpartum, her condition had improved, and she was moved to a rehabilitation center. This case suggested that supporting medical staff should report the patient's elevated blood pressure data to a doctor immediately and discuss the necessity of medical intervention.

16.5 Challenges and Future Directions

Perinatal medical circumstances differ greatly from country to country. In countries in which many deliveries occur at primary medical facilities or outside medical facilities, the establishment of a maternal transport system that can be employed at the time of stroke onset is an urgent problem. It is necessary for both national and local government to work on the establishment of such a system. It is clear that cooperation between obstetricians and neurosurgeons is important when a stroke develops during pregnancy or puerperium. However, it is doubtful whether sufficient cooperation occurs at all hospitals. As well as cooperation between the obstetricians and neurosurgeons at each facility, cooperation between science societies is also important. The monitoring of office and home blood pressure during pregnancy and puerperium should be promoted proactively. It is assumed that neuroimaging-based screening of pregnant woman with a history of cerebrovascular disease or numerous births [55] would contribute to improving the prognosis of pregnancy-associated stroke.

Acknowledgments I warmly thank Dr. Kanji Tanaka (Hirosaki University), Dr. Katsumi Sakakibara (Okazaki Municipal Hospital), Dr. Michiyasu Kawai (Toyohashi Municipal Hospital), Dr. Shigehiko Morikawa (Komaki Municipal Hospital), and Dr. Shinichi Yamamoto (Kariya Toyota Hospital) for their support and case provision.

References

1. Douglas KA, Redman CW. Eclampsia in the United Kingdom. Br Med J. 1994;309:1395–400.
2. Kullberg G, Lindeberg S, Hanson U. Eclampsia in Sweden. Hypertens Pregnancy. 2002;21:13–21.

3. Sidorov EV, Caplan LR. Stroke in pregnancy and postpartum women. Expert Rev Cardiovasc Ther. 2011;9:1235–47.
4. Say L, Chou D, Gemmill A, Tuncalp O, Moller AB, Daniels J, et al. Global causes of maternal death: a WHO systematic analysis. Lancet Glob Health. 2014;2:e323–33.
5. Hasegawa J, Sekizawa A, Tanaka H, Katsuragi S, Osato K, Murakoshi T, et al. Current status of pregnancy-related maternal mortality in Japan: a report from the Maternal Death Exploratory Committee in Japan. BMJ Open. 2016. https://doi.org/10.1136/e010304.
6. Ohno Y, Terauchi M, Tamakoshi K, Shiozaki A, Saito S. The risk factors for labor onset hypertension. Hypertens Res. 2016;39:260–5.
7. Knight M. Eclampsia in the United Kingdom 2005. Br J Obstet Gynaecol. 2007;114:1072–8.
8. Ohno Y, Furuhashi M, Ishikawa K, Kaseki S, Kondo H, Kikkawa F. Results of a questionnaire survey on pregnancy-associated stroke from 2005 to 2012 in Aichi Prefecture, Japan. Hypertens Res Pregnancy. 2014;2:16–20.
9. Abalos E, Cuesta C, Carroli G. Pre-eclampsia, eclampsia, and adversed maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121:14–24.
10. Kanayama N, Nakajima A, Maehara K, Halin A, Kajiwara Y, Isoda H, et al. Magnetic resonance imaging angiography in a case of eclampsia. Gynecol Obstet Invest. 1993;36:56–8.
11. Trommer BL, Homer D, Milhael MA. Cerebral vasospasm and eclampsia. Stroke. 1988;19:326–9.
12. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, et al. A reversible posterior leukoencephalopathy syndrome. N Engl J Med. 1996;334:494–500.
13. Ohno Y, Iwanaga K, Itakura A, Mizutani S. Increased intracranial blood flow volume in a preeclamptic woman with postpartum photophobia. Obstet Gynecol. 2003;101:1082–4.
14. Ohno Y, Kawai M, Wakahara Y, Kitagaw M, Kakihara T, Arii Y. Ophthalmic artery velocimetry in normotensive and preeclamptic women with or without photophobia. Obstet Gynecol. 1999;94:361–13.
15. Ohno Y, Kawai M, Wakahara Y, Kitagaw M, Kakihara T, Arii Y. Cerebral hyperperfusion in patient with eclampsia. Acta Obstet Gynecol Scand. 1999;78:555–6.
16. Williams KP, Wilson S. Changes in cerebral perfusion pressure in puerperal women with preeclampsia. Obstet Gynecol. 1998;92:1016–9.
17. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. Cerebrovasc Brain Metab Rev. 1990;2:161–92.
18. Ohno Y, Kawai M, Morikawa S, Sakakibara K, Tanaka K, Ishikawa K, et al. Management of eclampsia and stroke during pregnancy. Neurol Med Chir. 2013;53:513–9.
19. Duley L, Henderson-Smart DJ, Walker GJ, Chou D, et al. Magnesium sulphate versus diazepam for eclampsia. Cochrane Database Syst Rev. 2010;(12):CD000127. https://doi.org/10.1002/1461858.
20. Duley L, Henderson-Smart DJ, Chou D: Magnesium sulphate versus phenytoin for eclampsia. Cochrane Database Syst Rev. 2010;(10):CD000128. https://doi.org/10.1002/14651858.
21. Cipolla MJ, Kraig RP. Seizures in women with preeclampsia: mechanisms and management. Fetal Matern Med Rev. 2011;22:91–108.
22. Sibai BM. Diagnosis, prevention, and management of eclampsia. Obstet Gynecol. 2005;105:402–10.
23. Staessen JA, Wang JG, Thijs L. Cardiovascular protection and blood pressure reduction: a meta-analysis. Lancet. 2001;358:1305–15.
24. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension. 2003;42:1206–52.
25. Guidelines Committee: 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens. 2003;21:1011–53.
26. Vaughan CJ, Delanty N. Hypertensive emergencies. Lancet. 2000;356:411–7.

27. Hamidou B, Aboa-Eboule C, Durier J, Jacquin A, Lemesle-Martin M, Giroud M, et al. Prognostic value of early epileptic seizures on mortality and functional disability in acute stroke: the Dijon Stroke Registry (1985-2010). J Neurol. 2013;260:1043–51.
28. Hurwitz AS, Brice JH, Overby BA, Evenson KR. Directed use of the Cincinnati prehospital stroke scale by laypersons. Prehosp Emerg Care. 2005;9:292–6.
29. Kothari RU, Panchioli A, Liu T, Brott T, Broderick J. Cincinnati prehospital stroke scale: reproducibility and validity. Ann Emerg Med. 1999;33:373–8.
30. Calabrese LH, Dodick DW, Schwedt TJ, Sinqhal AB. Narrative reversible cerebral vasoconstriction syndromes. Ann Intern Med. 2007;146:34–44.
31. Lanska DJ, Kryscio RJ. Risk factors for peripartum and postpartum stroke and intracranial venous thrombosis. Stroke. 2000;31:1274–82.
32. Lao TT, Chin RKH, Leung BFH. Labour related eclampsia. Eur J Obstet Gynecol. 1987;26:97–104.
33. Yamada T, Obata-Yasuoka M, Hamada H, Baba Y, Ohkuchi A, Yasuda S, et al. Isolated gestational proteinuria preceding the diagnosis of preeclampsia: an observational study. Acta Obstet Gynecol Scand. 2016;95:1048–54.
34. Parati G, Sterqiou GS, Asmar R, Bilo G, de Leeuw P, Imai Y, et al. European Society of Hypertension practice guidelines for home blood pressure monitoring. J Hum Hypertens. 2010;24:779–85.
35. Inoue R, Ohkubo T, Kikuya M, Metoki H, Asayama K, Obara T, et al. Stroke risk of blood pressure indices determined by home blood pressure measurement: the Ohasama study. Stroke. 2009;40:2859–61.
36. Awada A, AI Rajeh S, Duarte R, Russell N. Stroke and pregnancy. Int J Gynecol Obstet. 1995;48:157–61.
37. DiCarlo A, Lamassa M, Consoli D, Inzitari D, Gall SL, Donnan G, et al. Sex differences in presentation, severity, and management of stroke in a population based study. Neurology. 2010;75:670–1.
38. Sidorov EV, Caplan LR. Stroke in pregnant and postpartum women. Cardiovasc Ther. 2011;9:1235–47.
39. Benedetto C, Marozio L, Tavella AM, Salton L, Grivon S, Di Giampaolo T. Coagulation disorders in pregnancy: acquired and inherited thrombophilias. Ann N Y Acad Sci. 2010;1205:106–17.
40. Kittner SJ, Stern BJ, Feeser BR, Hebel R, Nagey DA, Buchholz DW, et al. Pregnancy and the risk of stroke. N Engl J Med. 1996;335:768–74.
41. Witlin AG, Friedman SA, Egerman RS, Franqieh AY, Sibai BM. Cerebrovascular disorders complicating pregnancy–beyond eclampsia. Am J Obstet Gynecol. 1997;176:1139–48.
42. Simolke GA, Cox SM, Cunningham FG. Cerebrovascular accidents complicating pregnancy and the puerperium. Obstet Gynecol. 1991;78:37–42.
43. Sharshar T, Lamy C, Mas JL. Incidence and causes of strokes associated with pregnancy and puerperium. A study in public hospitals of Ile de France. Stroke in Pregnancy Study Group. Stroke. 1995;26:930–6.
44. Jaigobin C, Silver FL. Stroke and pregnancy. Stroke. 2000;31:2948–51.
45. Kuklina EV, Tong X, Bansil P, George MG, Callaghan WM. Trends in pregnancy hospitalizations that included a stroke in the United States from 1994 to 2007: reasons for concern? Stroke. 2011;42:2564–70.
46. Liang CC, Chang SD, Lai SL, Hsieh CC, Chueh HY, Lee TH. Stroke complicating pregnancy and the puerperium. Eur J Neurol. 2006;13:1256–60.
47. Yoshida K, Takahashi JC, Takenobu Y, Suzuki N, Ogawa A, Miyamoto S. Strokes associated with pregnancy and puerperium: a nationwide study by the Japan Stroke Society. Stroke. 2017;48:276–82.
48. Takahashi JC, Iihara K, Ishii A, Watanabe E, Ikeda T, Miyamoto S. Pregnancy-associated intracranial hemorrhage: results of a survey of Neurosurgical Institutes across Japan. J Stroke Cerebrovasc Dis. 2014;23:e65–71.

49. James AH, Bushnell CD, Jamison MG, Myers ER. Incidence and risk factors for stroke in pregnancy and the puerperium. Obstet Gynecol. 2005;106:509–16.
50. Massaro AR. Clinical discriminators of lobar and deep hemorrhages: the Stroke Data Bank. Stroke. 1991;41:1881–5.
51. Broderick J. Lobar hemorrhage in the elderly: the undiminishing importance of hypertension. Stroke. 1993;24:49–51.
52. Tuttelman RM, Gleicher N. Central nervous system hemorrhage complicating pregnancy. Obstet Gynecol. 1981;58:651–6.
53. Dias MS, Sekhar LN. Intracranial hemorrhage from aneurysms and AVM during pregnancy and the puerperium. Neurosurgery. 1990;27:855–66.
54. Bateman BT, Olbrecht VA, Berman MF, Minehart RD, Schwamm LH, Leffert LR. Peripartum subarachnoid hemorrhage: nationwide data and institutional experience. Anesthesiology. 2012;116:324–33.
55. Kriplani O, Relan S, Misra NK, Mehta VS, Takkar D. Ruptured intracranial aneurysm complicating pregnancy. Int J Gynaecol Obstet. 1995;48:201–6.
56. Stoodley MA, Macdonald RL, Weir BK. Pregnancy and intracranial aneurysms. Neurosurg Clin N Ann. 1998;9:549–56.
57. Kataoka H, Miyoshi R, Neki R, Yoshimatsu J, Ishibashi-Ueda H, Iihara K. Subarachnoid hemorrhage from intracranial aneurysms during pregnancy and the puerperium. Neurol Med Chir. 2013;53:549–54.
58. Wardlaw J, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, et al. Recombinant tissue plasminogen activator for acute ischemic stroke: an updated systematic review and meta-analysis. Lancet. 2012;379:2364–72.
59. Stam J. Thrombosis of cerebral veins and sinuses. N Engl J Med. 2005;352:1791–8.
60. Cantu C, Barinagarrementeria F. Cerebral venous thrombosis associated with pregnancy and puerperium. Review of 67 cases. Stroke. 1993;24:1880–4.
61. Saposnik G, Barinagarrementeria F, Brown RD, Bushnell CD, Cucchiara B, Cushman M, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42:1158–92.
62. Suzuki J, Takaku A. Cerebrovascular 'moyamoya' disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20:288–99.
63. Baba T, Houkin K, Kuroda S. Novel epidemiological features of moyamoya disease. J Neurol Neurosurg Psychiatry. 2008;79:900–4.
64. Takahashi J, Ikeda T, Iihara K, Miyamoto S. Pregnancy and delivery in moyamoya disease: results of a nationwide survey in Japan. Neurol Med Chir. 2012;52:304–10.
65. Ogilvy CS, Stieg PE, Awad I, Brown RD, Kondziolka D, Rosenwasser R, et al. Recommendation for the management of intracranial arteriovenous malformation. Circulation. 2001;103:2644–57.
66. Gros BA, Du R. Hemorrhage from arteriovenous malformation during pregnancy. Neurosurgery. 2012;71:349–56.
67. Fukuda K, Hamano E, Nakajima N, Katsuragi S, Ikeda T, Takahashi J. Pregnancy and delivery management in patients with cerebral arteriovenous malformation. Neurol Med Chir. 2013;53:565–70.

Part VII

The Risk of Cardiovascular Events in Preeclamptic Cases

Cardiovascular Disease Following Hypertensive Pregnancy

17

Franziska Pettit, George Mangos, and Mark A. Brown

Abstract

Cardiovascular disease, including ischaemic heart disease and cerebrovascular disease, has become the number one cause of death in women worldwide. Having had a pregnancy complicated by pre-eclampsia may be more than just a risk factor for the development of cardiovascular disease later in life. Pre-eclampsia causes significant morbidity and mortality in the acute setting with multisystem involvement. We are now learning that while these acute effects resolve in the short to medium term, their legacy is long-lasting. In this chapter we explore the mechanisms involved in the development of long-term hypertension, chronic kidney disease, ischaemic heart and cerebrovascular disease, diabetes and venous thromboembolism. Ways in which the post-partum period can be used to improve the long-term health of these women are reviewed, including the difficulties in identifying subtle abnormalities in parameters such as post-partum blood pressure because normal values have been based on studies often done on middle-aged men. Unfortunately diet and exercise on their own appear to be ineffective in reducing the risk but are important first steps for new mothers to alter their longer-term risks and improve their journey through subsequent pregnancies.

Keywords

Pre-eclampsia • Ischemic heart disease • Stroke • Hypertension • Metabolic syndrome

F. Pettit • G. Mangos • M.A. Brown, M.D. (✉)
Department of Renal Medicine and St. George Hospital Clinical School, St. George Hospital and University of NSW, Sydney, NSW, Australia
e-mail: mbrown@unsw.edu.au

S. Saito (ed.), *Preeclampsia*, Comprehensive Gynecology and Obstetrics,
https://doi.org/10.1007/978-981-10-5891-2_17

17.1 Introduction

Cardiovascular disease (CVD), including ischaemic heart disease and cerebrovascular disease, has become the number one cause of death in women worldwide [1], greater than infection and cancer. Women now have a 30–40% lifetime risk of cardiovascular disease at 50 years of age [2], contributed to by increasing rates of underlying hypertension, obesity and diabetes, all of which are increasingly common in women undertaking pregnancy. Added to this is the proposal that not only is pre-eclampsia a marker for cardiovascular disease by unmasking underlying risk factors but it is also a true risk factor [3]. Controversy remains as to whether various subtypes of pre-eclampsia (e.g. early vs. late onset, small birth weight vs. normal) convey bigger risk, but for now pre-eclampsia per se should be considered a risk factor for later-life cardiovascular disease. Pregnancy in women of advanced maternal age is also becoming more common; not only do these women have greater pregnancy risks, but there is a higher risk of later-life stroke in women having children after age 40 [4] (Table 17.1).

Early studies suggested that women who had an uncomplicated pregnancy (devoid of hypertension) actually had a lower incidence of hypertension later in life when compared to women who have never had a pregnancy [5]. However, more recent studies have raised the possibility of an increased risk of ischaemic heart disease associated with an increasing number of healthy pregnancies [6]. Pregnancy has therefore been described as a 'stress test' for future cardiovascular health in women [7].

Traditionally, women who had pre-eclampsia or eclampsia were considered to have had a pregnancy-specific disorder that resolved soon after delivery and had no long-term cardiovascular consequences. It is peculiar how that original 'benign' notion developed. MacGillivray's classic text on pre-eclampsia published over 30 years ago [8] describes several studies addressing this issue, and he reports a study by Theobald in 1933, perhaps analysing the earliest registry data on long-term maternal outcomes, which showed no difference in cardiovascular or renal mortality between nulliparous and parous women and concluded that no pregnancy disorder could convey a significantly worse cardiovascular future. Chesley, a great observer and analyst of pre-eclampsia and its sequelae, felt that later-life hypertensive tendency could be revealed by pregnancy [9]. MacGillivray's group then studied women who had had proteinuric pre-eclampsia and those with gestational hypertension alone, between 35 and 50 years of age, well after their pregnancy. Both groups of women had higher blood pressures than women who had had normal

Table 17.1 Future cardiovascular risks following a pregnancy complicated by pre-eclampsia

Medical condition	Relative risk [95% CI]
Chronic hypertension	3.7 [2.7–5.05]
Ischaemic heart disease	2.16 [1.86–2.52]
Cerebrovascular disease	1.81 [1.45–2.27]
Deep vein thrombosis	1.79 [1.37–2.33]
End-stage renal disease	4.3 [3.3–5.6]
Type 2 diabetes	1.86 [1.22–2.84]

Adapted from SOMANZ guidelines 2014

pregnancies. Chesley, in 1976, also made the astute observation that women with eclampsia were more likely to develop diabetes in later life, though others felt this may not be extended to the pre-eclamptic group. We now consider hypertension in pregnancy as having lifelong implications, particularly for future hypertension and cardiovascular disease [10]; if we had paid better attention to the earlier studies of MacGillivray and Chesley, we may have always held this view.

Today we see pre-eclampsia as one of those few opportunities in life where we can glimpse into the future; pre-eclampsia (PE) and its association with future cardiovascular health have become a major worldwide issue. It is estimated to be a direct cause of death for about 76,000 women per year and half a million babies, a death rate equivalent to about 40% that of HIV worldwide [11]. If we were able to add the number of later-life cardiovascular deaths in women attributable to pre-eclampsia, we would be looking at an even greater health burden from pre-eclampsia.

17.2 Evidence for Long-Term Cardiovascular Risk

In the majority of cases of gestational hypertension or pre-eclampsia, hypertension resolves in the post-partum period, usually within the first week, but in some cases this can take months. Women whose blood pressure fails to normalise in this time frame may have underlying chronic hypertension that was not apparent early in pregnancy or misdiagnosed as gestational hypertension.

Early studies by Chesley and others suggested that once the hypertensive pregnancy was over, there was no increased risk of hypertension in the future [5, 12, 13] though others found an increased risk [14]. The risk of developing chronic hypertension following a hypertensive pregnancy is now thought to be about 25%, four times that of a woman who has not had a hypertensive pregnancy. This hypertension tends to occur earlier than traditionally expected with onset often preceding the menopause [15].

The renaissance for this thinking probably began in 1981 when Irgens and colleagues studied the outcomes of some 24,000 pre-eclamptic and 600,000 non-pre-eclamptic women in Norway at a median of just 13 years post-partum [16]. They found that having pre-eclampsia increased mortality risk by 1.2, even at this relatively early-stage post-partum. However, this risk was really only apparent in women who had pre-eclampsia preterm, with a death rate of 15.5/1000 women compared with 10.9/1000 women who also had preterm deliveries but without pre-eclampsia. Further analysis showed that death from cardiovascular disease was greater in women with a history of pre-eclampsia whether delivered preterm or not, but the risk was still greater in those delivered preterm.

This study was followed soon after by a pivotal analysis of about 3500 women who had had pre-eclampsia or gestational hypertension in Glasgow between 1950 and 1970, studied roughly at age 60 [17]. These authors found that both pre-eclampsia and gestational hypertension had higher risk of future hypertension (2.5- to 4-fold, significant for both groups), ischaemic heart disease death (1.2- to 2-fold, not statistically significant) and stroke death (2.9- to 3.6-fold, significant for the pre-eclampsia group). Of interest in this study was the observation that women with

pre-eclampsia or gestational hypertension already had higher early pregnancy and post-partum BMI, perhaps providing an early insight into the potential unmasking of pregnancy-related hypertension in women with metabolic syndrome and a subsequent link between this and later-life cardiovascular disease.

In 2007 Bellamy and colleagues undertook a meta-analysis of 25 studies relating pre-eclampsia to long-term outcomes that had been published between 1961 and 2006 [18]. Thirteen of these studies were related to the risk of future hypertension, and ten found an increased relative risk; the pooled analysis found a 3.7-fold relative risk for later-life hypertension at a mean follow-up of just 14 years, i.e. at a relatively young age. Statistically significant increased risks were also observed for the development of ischaemic heart disease, stroke and venous thromboembolism and all-cause mortality. Others have since also reported increased risk of stroke [19], cardiac ischaemia and death from coronary artery disease [20] and venous thromboembolism in the 10–20 years post pregnancy [21] as well as a fourfold risk of hypertension and threefold risk of type 2 diabetes mellitus [22]. Of note the risk of cerebrovascular mortality after pre-eclampsia complicated by a preterm delivery was higher, with a HR of 5.08 [16].

More recent studies seem to confirm the observations that future hypertension and cardiovascular disease are increased after pre-eclampsia [23]. A follow-up of the HYPITAT cohort in the Netherlands, a combined group of women who had either proteinuric pre-eclampsia or gestational hypertension at term, found that on average these women had a BP 124/82 compared with 110/72 mmHg in those with previously normotensive pregnancies. This cohort had a remarkably high 34% rate of hypertension just 2.5 years post-partum, commonly linked to metabolic syndrome features [24]. Another smaller Dutch study found a 17% likelihood of hypertension between 2 and 5 years post pre-eclampsia, and in this study that risk was heightened threefold in women with a low plasma volume (<1373 mL/m^2) measured between 3 and 30 months post-partum [25]. These incidence rates of hypertension so soon after pregnancy are probably higher than in other parts of the world but serve to highlight the potential of this risk.

Almost 16,000 women from Sweden who had at least one pregnancy (5% had pre-eclampsia) were assessed at age 40, and 9.6% had hypertension, though only 3% were using antihypertensives, reflecting the well-known under-recognition of hypertension in young women [26]. This rate of hypertension is much less than that in the Dutch studies above but remains clinically very significant at such a young age. Pre-eclampsia remained an independent risk factor for hypertension at age 40 in this group, along with early gestation at delivery <32 weeks and having a small for gestational age baby, the earlier gestation also being a factor associated with cardiovascular risk in another recent study [27].

A recent Australian study addressed mortality after a hypertensive pregnancy, examining records of 4387 women (31% pre-eclampsia, 54% gestational hypertension, 15% chronic hypertension) and finding that 1 in 50 had died by about age 46, the main listed causes of death being ischaemic heart disease, hypertension and type 2 diabetes [28]. The relative risk of death was similar to that of earlier studies, 1.56, but of interest this risk appeared confined to pre-eclamptic women; this may have been a function of statistical power as we would normally expect women with established chronic hypertension to also have an increased cardiovascular death risk.

Alternatively, this may highlight the problem of women with pre-eclampsia having elevated BP for many years that goes undetected and/or untreated.

In perhaps the largest registry study to date, from Utah, Theilen and colleagues found a 1.65 increased all-cause mortality risk amongst over 60,000 women who had formerly hypertensive pregnancies, the causes of death being ischaemic heart disease, diabetes, stroke and Alzheimer's dementia [29]. This study extended our knowledge further, showing the greatest risk was in fact amongst women who had had the apparently mild pregnancy condition of gestational hypertension. This same group has more recently extended their analyses to show that this mortality risk is increased further in women with recurrent hypertension during pregnancy [30]. This observation is relevant not only to heighten our awareness of cardiovascular disease in such women but also because this may imply that hypertensive pregnancy per se leads to the development of later-life cardiovascular disease rather than just unearthing a predisposing genotype or phenotype.

It appears that severity of maternal disease does impact on her future risk of developing complications. In a meta-analysis by McDonald et al. [21], the relative risk (RR) of cardiac disease following a pregnancy complicated by pre-eclampsia/eclampsia was 2.00 (1.83–2.19) for mild, 2.99 (2.51–3.58) for moderate and 5.36 (3.96–7.27) for severe disease ($p < 0.0001$). However defining severity of pre-eclampsia is difficult and often depends on local guidelines and may include a variety of parameters. It is also possible that gestation at onset plays a role in later-life risk with women suffering early-onset disease carrying a higher risk [31].

In summary, we should probably have known for the past 50 years that hypertensive pregnancy predisposes to later-life cardiovascular disease and increased mortality risk; for some reason these messages from early studies were lost until the resurgence of studies on this topic beginning in the 1980s. The message is now clear, that having a hypertensive pregnancy is associated with greater risk of later-life hypertension and cardiovascular disease and indeed a higher chance of dying early. Though the absolute rates of these events differ across different populations, and there remains minor controversy as to whether the risks are confined to women who have had only pre-eclampsia and the influence of gestation at delivery upon these findings, we should be considering any woman who has had a hypertensive pregnancy as being at increased cardiovascular risk.

17.3 Other Long-Term Risks of Hypertensive Pregnancy

Other risks after hypertensive pregnancy include cognitive dysfunction, the need for renal biopsy, end-stage kidney disease, diabetes, venous thromboembolism, cardiovascular disease in the offspring and, less likely, cancer.

17.3.1 Cognitive Dysfunction

Cognitive dysfunction was self-reported as being worse in 966 women who had pre-eclampsia compared with 342 women who had normotensive pregnancies; a history of migraine may have added to this finding [32]. This study is relevant to others that

have found self-reported cognitive dysfunction to be related to increased cerebral white matter lesions in women with a history of eclampsia or pre-eclampsia [33–35]. As these white matter lesions are generally considered a reflection of cerebral microvascular disease, it seems plausible that the increased blood pressure and other cardiovascular risk markers after pre-eclampsia could predispose to early and perhaps subtle cognitive dysfunction.

17.3.2 Chronic Kidney Disease

The typical lesion seen on renal biopsy in pre-eclampsia is glomerular endothelial swelling or endotheliosis, which may be a consequence of a reduction in local VEGF by anti-angiogenic factors and is said to disappear by 8 weeks post-partum. Although early studies suggested that this was a specific lesion of pre-eclampsia, this lesion may be present in normotensive pregnancies as well [36]. Acute kidney injury (AKI) can also affect about 2% of women with pre-eclampsia [37, 38], and although most recover after delivery, it is now known that any episode of AKI increases longer-term risks of renal and cardiovascular disease. A further confounder of long-term renal risk comes from case series from Chicago that suggested that about a quarter of women with pre-eclampsia, particularly multigravidas, had biopsy proven underlying renal diseases [39].

Evidence now exists for the longer-term risk of renal dysfunction. Vikse et al. have reported from Norwegian data that having had pre-eclampsia in a first pregnancy was a risk for needing a renal biopsy later in life [40]. Subsequent analysis of their registry data found that the adjusted relative risk of end-stage kidney disease (ESKD) after a pregnancy complicated by pre-eclampsia was 4.3 or 3.2 depending on whether pre-existing risk factors for renal disease were excluded. These risks increased to 4.7 and 10.9, respectively, if two or more pregnancies were complicated by pre-eclampsia. More recent registry data from Taiwan support these findings with a HR of between 9 and 12 for the development of CKD or ESKD following a hypertensive pregnancy [41]. This risk was greater in women who delivered over the age of 35 with further risks from chronic hypertension and diabetes, both features well recognised after hypertensive pregnancies. Not only was there an increased risk of ESKD but also microalbuminuria, an early marker of renal disease; this can go unnoticed for many years without appropriate follow-up and particularly in combination with other comorbidities can contribute to the development of established chronic kidney disease [42]. An important clinical perspective is that while the relative risk for needing dialysis or transplantation is high after a pre-eclamptic pregnancy, the absolute risk remains very small.

Suggested mechanisms for observed associations between pre-eclampsia and subsequent renal disease include both conditions having similar risk factors such as obesity, hypertension, insulin resistance and endothelial dysfunction. Angiogenic factors play a role both in pre-eclampsia and the progression of renal disease, and it

is possible that a pregnancy complicated by pre-eclampsia may thereby worsen subclinical renal disease not identified before the pregnancy.

17.3.3 Diabetes

A clear relationship exists between gestational diabetes and the risk of type 2 diabetes later in life [43, 44]. It appears that there is also a relationship between having had a hypertensive disorder of pregnancy and the development of type 2 diabetes [45], including a twofold increase in diabetes 21 years after a hypertensive pregnancy [46] and greater frequency of features of the metabolic syndrome later in life after pre-eclampsia [47–49]. Clearly this heightened risk for diabetes will be one potential factor contributing to the increased cardiovascular morbidity and mortality.

17.3.4 Venous Thromboembolism

In addition to an increased risk of arterial disease as outlined above, Hannaford et al. (Heart 1997) reported a 1.6-fold higher risk of venous thromboembolism (VTE) after pre-eclampsia; conversely thromboembolism preceding pregnancy has been associated with a higher risk of pre-eclampsia [50–52]. While this relative risk of later-life VTE was confirmed at 1.79 about 5 years post-partum in the meta-analysis of Bellamy et al. [18] where there were only 470 thromboembolic events amongst the 35,772 pre-eclamptic cases, an event rate of 1.3%, though significant, remains fairly low.

17.3.5 Cancer

Although some earlier studies had suggested an increased cancer risk in women who have had pre-eclampsia, these may have been population specific. The analysis of Bellamy included four studies of breast cancer risk and three studies of any cancer risk and found that neither was increased in women with a history of pre-eclampsia [18].

17.3.6 Cardiovascular Disease in the Offspring

The HUNT study followed three populations from Norway including 706 women who had de novo hypertension in pregnancy (370 pre-eclampsia, 306 gestational hypertension) [53]. Their offspring were examined at a mean age of 29 years. These offspring had small increases in blood pressure, BMI and waist circumference compared with offspring of 15,000 women who had normotensive pregnancies. The increments in blood pressure were accounted for by the higher BMI, and in a clever study

approach, the authors showed that there were no differences in BP or BMI in siblings born to the same mother where one was born after a hypertensive pregnancy and the other(s) after a normotensive pregnancy. In other words, the effects of maternal or paternal genetics, or else the family environment, may account for the higher BP in these offspring rather than any intrauterine cause. Regardless, the message is clear that offspring of mothers who have had pre-eclampsia or gestational hypertension should be monitored carefully for weight gain and hypertension as they get older.

17.4 Mechanisms by Which Pre-eclampsia Is Related to Cardiovascular Disease

Several mechanisms have been proposed to explain the link between pre-eclampsia or gestational hypertension and later-life cardiovascular disease. Broadly speaking, the question remains unanswered as to whether the development of hypertension in pregnancy provides the window of opportunity to recognise a woman already predestined for heart disease or whether the hypertensive pregnancy per se somehow induces change to the maternal heart and vasculature, specifically cerebral and coronary arteries. Most probably for many women, it is a combination of both. Pregnancy is a metabolically challenging event with insulin resistance, relative hyperglycaemia, hyperlipidaemia and increased tendency to clotting compared with the non-pregnant state. It is also an inflammatory challenging time, a factor known to be important to the development of atherosclerosis. Even normal pregnancy involves upregulation of the inflammatory cascade, and pre-eclampsia is considered a state of higher inflammation than normal pregnancy (Table 17.2).

17.4.1 Metabolic Syndrome

As well as changes in vasoactive factors, there are also abnormalities in the lipid profile of pre-eclamptic women that mirror that of non-pregnant women at cardiovascular risk. Normal pregnancy is associated appropriately with an increase in serum cholesterol, required for the production of steroid hormones such as progesterone. Cholesterol is also necessary for foetal brain development. The majority of this increase in cholesterol is from high-density lipoprotein (HDL) which is protective

Table 17.2 Putative mechanisms of long-term cardiovascular risk

	Increased	Reduced
Metabolic syndrome	BMI Fasting glucose HOMA scores LDL cholesterol 24 h BP	HDL cholesterol
Alterations in cardiac structure and function	Left ventricular mass index Diastolic BP	Left ventricular diastolic function
Imbalance of vasoactive components	Vasoconstrictor effects of RAAS	Vasodilator effects of A1-7 and MAS receptor
Endothelial dysfunction	Homocysteine levels	Nitric oxide availability

from a maternal cardiovascular point of view, but there is also a significant rise in LDL cholesterol, usually considered a poor prognostic indicator in the development of atheroma. This rise occurs early in the first trimester, peaks in the third trimester and normalises after delivery [54]. Women with hypertensive pregnancies have slightly different lipid profiles during their pregnancies with lower levels of HDL and much higher triglyceride levels when compared to their normotensive counterparts. A low-level high-density lipoprotein and high triglycerides are present in 40% and 20% of women who had early-onset pre-eclampsia [55], relevant as an unfavourable lipid profile is in and of itself a risk factor for ischaemic heart disease.

Amongst the most popular theories linking pre-eclampsia and cardiovascular disease is the high rate of underlying metabolic syndrome in these women. In the HYPITAT follow-up of women with late-onset pre-eclampsia, a staggering 25% had features of the metabolic syndrome just 2.5 years post-partum [24]. Another Dutch study found that 20% of women had metabolic syndrome 6 months post-partum and this more than doubled the risk of recurrent pre-eclampsia in the next pregnancy [56].

Our group has also addressed the issue of post-partum metabolic syndrome, studying women with a history of pre-eclampsia ($n = 39$), gestational hypertension ($n = 27$) and normal pregnancies ($n = 35$) between 2 and 12 years after delivery [15]. Laboratory measures included plasma fasting lipids, glucose, insulin, creatinine and urinary albumin-to-creatinine ratio. Blood pressure was measured by 24-h ambulatory blood pressure monitoring, endothelial function by flow-mediated dilatation and sympathetic activity by both head-up tilt test and cold pressor test, including the response of the circulating renin–angiotensin (RAA) system to tilt testing. We found no alterations in endothelial function or the RAA system or sympathetic nervous system function but noted higher 24-h BP, insulin resistance and BMI in women with a previously hypertensive pregnancy.

In a recent follow-up study from Norway 11 years after delivery, 5–7% of women who had pre-eclampsia had developed diabetes compared with 1.3% of women who had normotensive pregnancies [57]. It is difficult to know from this study how many women had fully diagnosed metabolic syndrome, but women with a pre-eclampsia history had slightly higher blood pressures, BMI, fasting glucose and HOMA score and lower HDL cholesterol. Surprisingly, these features were less pronounced in women who had had severe pre-eclampsia.

It is tempting to assert that these changes predated the hypertensive pregnancy and provide a common link between the maternal components of pre-eclampsia and later-life cardiovascular disease but further studies are still needed to confirm this.

17.4.2 Sympathetic Nervous System

Abnormal activity of the sympathetic nervous system is a well-recognised component of essential hypertension and a probable contributor to factors such as BP variability and the metabolic syndrome itself. Women with pre-eclampsia may have an overactive sympathetic nervous system during that pregnancy, which appears to revert to normal post-partum [58, 59]. We were unable to show increased stimulation post-partum using indirect measures of sympathetic activity [15]. Others have shown increased activity 40 years post-partum but only if the pre-eclamptic woman

had developed chronic essential hypertension by that stage [60]. Taken together the evidence suggests that although the sympathetic nervous system may be an amplifier of the process of pre-eclampsia during pregnancy, it is unlikely to play a significant role in the long-term cardiovascular outcomes.

17.4.3 Effects of Pre-eclampsia upon the Heart

Traditionally pre-eclampsia was not viewed as a cardiac disorder; although cases of pulmonary oedema occur, these were generally thought to be related to excessive fluid administration in a condition already characterised by capillary leak. In recent years careful echocardiogram studies have found changes in cardiac structure and function during pre-eclamptic pregnancies that may remain post-partum. In fact, it seems that even normal pregnancy is characterised by a tendency to diastolic dysfunction as the pregnancy progresses [61]; 7% of this normal pregnant cohort had shortness of breath at term, and most had diastolic dysfunction. One year later cardiac function had returned to normal in all women. On the other hand, there appears to be persistent changes in women who have had pre-eclampsia [62]. In one study at 1 year post-partum, asymptomatic left ventricular dysfunction/hypertrophy was significantly higher in preterm pre-eclampsia (56%) compared with term pre-eclampsia (14%) or matched controls (8%), and those with persistent changes were more likely to develop essential hypertension [63]. These findings were replicated by another group who studied 349 formerly pre-eclamptic women between 9 and 16 months post-partum and found that 36% went on to develop essential hypertension about 6 years later; this was preceded by increased left ventricular mass index and diastolic blood pressure at their initial post-partum screening [64].

Hemodynamic changes may also persist. At 12–18 months post pre-eclampsia in 75 women, there were greater left ventricular mass index and relative wall thickness. However, those with recurrent pre-eclampsia in their next pregnancy had maintained a low cardiac output/high vascular resistance pattern, whereas haemodynamics had normalised in those who did not develop pre-eclampsia in their next pregnancy [65].

Given the variability in these studies, i.e. many women with pre-eclampsia will have demonstrable changes in cardiac structure or function that revert to normal after delivery, while others remain abnormal, it seems likely that there is a subgroup who develop long-lasting, perhaps permanent, alterations in cardiac mass and diastolic function as a consequence of their pre-eclampsia which presumably goes undetected and has an adverse effect on their long-term outcome.

17.4.4 Changes in Vasculature

The maternal cardiovascular system goes through many changes while adapting to a normal pregnancy. There is expansion of plasma volume and extracellular fluid volume, and cardiac output increases significantly due to an increase in heart rate and stroke volume [66]. Despite this increase in cardiac output, the blood pressure decreases in normal pregnancy, a consequence of a profound reduction in systemic

vascular resistance perhaps due to the loss of responsiveness to vasoconstrictors such as angiotensin II (AII) [67]. Several mechanisms have been proposed to explain this resistance to AII including enhanced local vascular production of prostacyclin or nitric oxide, perhaps coupled with downregulation of vasoconstrictors such as thromboxane A2 and endothelin, or perhaps a vasodilatory effect of circulating progesterone or one of its metabolites. Failure of these mechanisms in pregnancy is possibly responsible for the development of pregnancy hypertension and if persistent post-partum could contribute to later hypertension. More recently, downregulation of the vasodilator arm of the RAAS, including A1-7 and its MAS receptor, has been implicated as cause for loss of vascular refractoriness to AII in pregnancy [68].

As all these protective mechanisms that maintain a healthy BP in normal pregnancy are also considered important in the non-pregnant state, and loss of these mechanisms often occurs in pre-eclampsia, it is possible that better understanding of this basic physiology would help us determine the reasons why pre-eclamptic women are at increased risk for later-life hypertension and cardiovascular disease.

Coronary calcification, a recognised marker of established coronary artery disease, is higher in women with a history of pre-eclampsia by about age 60, about 25% having a score considered clinically significant. Perhaps surprisingly, the highest scores were seen in normotensive formerly pre-eclamptics [69]. This is no doubt a result of many of the metabolic syndrome factors discussed above, but other factors may be at play. Visser and colleagues undertook a meta-analysis of studies addressing nonclassical features for cardiovascular risk in formerly pre-eclamptic women and concluded that homocysteine levels, but not markers of inflammation, thrombosis or angiogenesis, were higher in such women, presumably relevant to ongoing endothelial dysfunction [70]. This warrants further study as it is a modifiable factor through dietary means and perhaps supplemental folate.

Whether the cardiovascular risk is associated with abnormal endothelial function remains unclear. In a small study conducted 6–12 months post pre-eclampsia, there was evidence of abnormal flow-mediated dilatation [71] but others found no changes 10 years later [72] and our group did not find any abnormalities compared with formerly normotensive pregnant women on average 4.5 years postdelivery [15].

17.5 Opportunities to Prevent Cardiovascular Complications of Pre-eclampsia and Gestational Hypertension

See Table 17.3.

17.5.1 Recognition of Risk

The post-partum period has been described as an underused opportunity to improve women's health [73].

The American College of Cardiology now recommends that the evaluation of cardiac risk in women begins with a medical history, family history and *pregnancy complication history*, highlighting the importance of pre-eclampsia as a risk factor for coronary artery disease [1]. Looking at this from an obstetrics point of view, the

Table 17.3 Post-partum surveillance

Strategies	Challenges
Diagnosis and treatment of hypertension	Knowing what is 'normal' for young women and when to treat Use of non-pharmacological and pharmacological therapies to lower BP
Screening for albuminuria and investigation of possible underlying renal disease	Dealing with a well population who are busy with young children Healthcare providers who are unfamiliar with the risks
Screening for insulin resistance and overt diabetes	Done well for those women who have had gestational diabetes, less well recognised as being a consequence of PE
Reducing risk of IHD and stroke	Recognition of PE as an independent risk factor and using both non-pharmacological and pharmacological therapies to lower cholesterol
Weight management	Adherence to a diet and exercise programme is difficult and often fails Use of group education sessions and one on one motivation sessions to improve adherence
Smoking cessation	Use of motivational tools and nicotine replacement until habit is broken

simplistic approach is that every woman with a history of pre-eclampsia or gestational hypertension should be monitored periodically for traditional cardiac risk factors such as blood pressure, hyperlipidaemia, obesity, smoking, albuminuria and diabetes, possibly beginning as early as 6 months after delivery [74]. This approach is not working well. A large study from the USA found that women under age 50 with elevated blood pressure on two separate occasions were not diagnosed as hypertensive in more than half the cases [75]; in other words there is a great reluctance to label a young woman as hypertensive whether she is seen in an obstetric follow-up setting or a physician clinic.

Young women with type 2 diabetes are a well-known group at high risk of cardiovascular disease in later life. What is less well known is that these risks are not that dissimilar from those of a woman who have had a hypertensive pregnancy. Yet these women with diabetes have regular follow-up by specialist clinics including endocrinologists, specialist nurses, dieticians and sometimes even psychologists. They are actively encouraged to adopt a healthy lifestyle, and their compliance is monitored; the same level of follow-up does not occur for women with a history of hypertension in pregnancy.

Another approach to assessing post-partum risk is to use risk models such as the Framingham cardiac risk score. Women with prior (term) pre-eclampsia have higher 10- and 30-year risk scores for ischaemic heart disease when assessed 2.5 years post-partum, but only 5% had a 10-year event risk above 5% meaning that this approach probably underestimates the true incidence of this outcome [76]. Another Dutch study applied the Framingham score to 115 women with pre-eclampsia and

50 control women about 5–10 years post-partum. They concluded that women who are hypertensive after pre-eclampsia have a twofold risk of developing cardiovascular disease in the next 10–30 years, but formerly pre-eclamptic women who are normotensive in the first 10 years after their pregnancy have a comparable future cardiovascular risk to healthy controls [77]. It is hard to reconcile these findings, but these studies probably serve to suggest that relying on a cardiovascular risk score may not be accurate enough in this cohort. Nevertheless at least one analysis claims that post-partum screening is cost-effective [78], and it is at least a reasonable starting point in estimating risk.

One problem that probably explains under-recognition of risk is the limits used to define what is abnormal for parameters such as blood pressure when assessing women with prior hypertension in pregnancy. So-called 'normal' limits for comparison are generally derived from a broad section of the community and not related specifically to young women who have had a normal pregnancy. This led us to design the 'P4' study [79] which assesses post-partum blood pressure and other vascular risk parameters as well as psychology testing of normal pregnant women 6 months and 2 years later and paediatric follow-up of their babies. A major aim of this arm of the study is to define 'normal' for cardiovascular parameters such as blood pressure in young parous women. Only then can we recognise abnormally high blood pressure in formerly pre-eclamptic women.

17.5.2 Lifestyle, Exercise and Drug Therapy

Lifestyle interventions after pre-eclampsia seem to be effective in decreasing cardiovascular risk. Berks et al. estimated this decrease was in the order of 1–13%. However they do admit that this is dependent on long-term adherence to the interventions [3]. The interventions proposed include smoking cessation, weight management and lipid and blood pressure control. Smoking cessation can be done with or without the use of nicotine replacement and should include some form of cognitive behavioural therapy [80]. Dietary interventions should be guided by trained dieticians and should focus on a Mediterranean style diet [81]. Exercise seems a logical approach to preventing long-term complications after a hypertensive pregnancy, independent of weight loss strategies. In fact exercise should begin or be maintained during pregnancy as this is associated with reduced likelihood of developing pre-eclampsia in the first place [82]. Benefits of exercise post-partum include improvement in metabolic syndrome parameters and endothelial function [71], though not completely back to normal, and improvement in plasma volume and venous capacitance [83]. It has been estimated that a combination of exercise, improved diet and reduction in smoking would lower future cardiovascular risk in this group by only 4–13% [3]. Further prospective studies in this field, including identification of nontraditional cardiovascular risk factors will be very important.

Conclusions

It is apparent that women with pre-eclampsia or gestational hypertension have increased risk for later-life cardiovascular disease. The reasons for this remain poorly understood, but there are many changes in normal pregnancy which are exacerbated in pre-eclampsia and have potential relevance to the development of vascular disease. It is possible that subtle but permanent damage to the vasculature occurs in women during the pregnancy; most of these changes resolve post-partum, but a subgroup of women with hypertensive pregnancies will be at lifelong cardiovascular risk. The key to prevention is firstly public awareness of this problem then better recognition of abnormal blood pressure and other traditional risk factors post-partum. In the absence of better advice at present all women who have had a hypertensive pregnancy should receive targeted follow-up and adopt healthy lifestyle.

References

1. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women—2011 update: a guideline from the American Heart Association. Circulation. 2011;123(11):1243–62.
2. Lloyd-Jones DM, Tian L. Predicting cardiovascular risk: so what do we do now? Arch Intern Med. 2006;166(13):1342–4.
3. Berks D, Hoedjes M, Raat H, Duvekot JJ, Steegers EA, Habbema JD. Risk of cardiovascular disease after pre-eclampsia and the effect of lifestyle interventions: a literature-based study. BJOG. 2013;120(8):924–31.
4. Qureshi AI, Saeed O, Malik AA, Suri MFK. Pregnancy in advanced age and the risk of stroke in postmenopausal women: analysis of Womens Health Initiative Study. Am J Obstet Gynecol. 2017;216(4):409.e1–6.
5. Chesley LC. Remote prognosis after eclampsia. Perspect Nephrol Hypertens. 1976;5:31–40.
6. Catov JM, Newman AB, Sutton-Tyrrell K, Harris TB, Tylavsky F, Visser M, et al. Parity and cardiovascular disease risk among older women: how do pregnancy complications mediate the association? Ann Epidemiol. 2008;18(12):873–9.
7. Williams D. Pregnancy: a stress test for life. Curr Opin Obstet Gynecol. 2003;15(6):465–71.
8. MacGillivray I. Pre-eclampsia: the hypertensive disease of pregnancy. London: WB Saunders; 1983.
9. Chesley LC. Hypertensive disorders in pregnancy. New York: Appleton-Century-Crofts; 1978.
10. Brown MA. Pre-eclampsia: a life-long disorder. Med J Aust. 2003;179:182–4.
11. Brown MA. Pre-eclampsia in 2014: seven ways to make a difference. Pregnancy Hypertens. 2014;4(4):249–52.
12. Bryans CI Jr. The remote prognosis in toxemia of pregnancy. Clin Obstet Gynecol. 1966;9(4):973–90.
13. Fisher KA, Luger A, Spargo BH, Lindheimer MD. Hypertension in pregnancy: clinical-pathological correlations and remote prognosis. Medicine (Baltimore). 1981;60(4):267–76.
14. Sibai BM, el-Nazer A, Gonzalez-Ruiz A. Severe preeclampsia-eclampsia in young primigravid women: subsequent pregnancy outcome and remote prognosis. Am J Obstet Gynecol. 1986;155(5):1011–6.
15. Mangos GJ, Spaan JJ, Pirabhahar S, Brown MA. Markers of cardiovascular disease risk after hypertension in pregnancy. J Hypertens. 2012;30(2):351–8. https://doi.org/10.1097/HJH.0b013e32834e5ac7.
16. Irgens HU, Roberts JM, Reisæter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ. 2001;323(7323):1213–7.

17. Wilson BJ, Watson MS, Prescott GJ, Sunderland S, Campbell DM, Hannaford P, et al. Hypertensive diseases of pregnancy and risk of hypertension and stroke in later life: results from cohort study. BMJ. 2003;326(7394):845.
18. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007;335(7627):974.
19. Thorogood M, Mann J, Murphy M, Vessey M. Fatal stroke and use of oral contraceptives: findings from a case-control study. Am J Epidemiol. 1992;136(1):35–45.
20. Jonsdottir LS, Arngrimsson R, Geirsson RT, Sigvaldason H, Sigfusson N. Death rates from ischemic heart disease in women with a history of hypertension in pregnancy. Acta Obstet Gynecol Scand. 1995;74(10):772–6.
21. McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ. Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. Am Heart J. 2008;156(5):918–30.
22. Lykke JA, Paidas MJ, Langhoff-Roos J. Recurring complications in second pregnancy. Obstet Gynecol. 2009;113(6):1217–24.
23. Garovic VD, Bailey KR, Boerwinkle E, Hunt SC, Weder AB, Curb D, et al. Hypertension in pregnancy as a risk factor for cardiovascular disease later in life. J Hypertens. 2010;28(4):826–33.
24. Hermes W, Franx A, van Pampus MG, Bloemenkamp KW, Bots ML, van der Post JA, et al. Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study. Am J Obstet Gynecol. 2013;208(6):474.e1–8.
25. Scholten RR, Lotgering FK, Hopman MT, Van Dijk A, Van de Vlugt M, Janssen MCH, et al. Low plasma volume in normotensive formerly preeclamptic women predisposes to hypertension. Hypertension. 2015;66(5):1066–72.
26. Parikh NI, Norberg M, Ingelsson E, Cnattingius S, Vasan RS, Domellöf M, et al. Association of pregnancy complications and characteristics with future risk of elevated blood pressure. The Västerbotten Intervention Program. Hypertension. 2017;69(3):475–83.
27. Bokslag A, Teunissen PW, Franssen C, van Kesteren F, Kamp O, Ganzevoort W, et al. Effect of early-onset preeclampsia on cardiovascular risk in the fifth decade of life. Am J Obstet Gynecol. 2017;216(5):523.e1–7.
28. Tooher J, Thornton C, Makris A, Ogle R, Korda A, Horvath J, et al. Hypertension in pregnancy and long-term cardiovascular mortality: a retrospective cohort study. Am J Obstet Gynecol. 2016;214(6):722.e1–6.
29. Theilen LH, Fraser A, Hollingshaus MS, Schliep KC, Varner MW, Smith KR, et al. All-cause and cause-specific mortality after hypertensive disease of pregnancy. Obstet Gynecol. 2016;128(2):238–44.
30. Theilen L, Meeks H, Fraser A, Esplin MS, Smith KR, Varner M. Long-term mortality risk and life expectancy following recurrent hypertensive disease of pregnancy. Am J Obstet Gynecol. 2017;216(1 Suppl):S32–S3.
31. Gaugler-Senden IP, Berends AL, de Groot CJ, Steegers EA. Severe, very early onset pre-eclampsia: subsequent pregnancies and future parental cardiovascular health. Eur J Obstet Gynecol Reprod Biol. 2008;140(2):171–7.
32. Postma IR, Groen H, Easterling TR, Tsigas EZ, Wilson ML, Porcel J, et al. The brain study: cognition, quality of life and social functioning following preeclampsia; an observational study. Pregnancy Hypertens. 2013;3(4):227–34.
33. Postma IR, de Groot JC, Aukes AM, Aarnoudse JG, Zeeman GG. Cerebral white matter lesions and perceived cognitive dysfunction: the role of pregnancy. Am J Obstet Gynecol. 2014;211(3):257.e1–5.
34. Soma-Pillay P, Suleman FE, Makin JD, Pattinson RC. Cerebral white matter lesions after pre-eclampsia. Pregnancy Hypertens. 2017;8:15–20.
35. Aukes AM, de Groot JC, Aarnoudse JG, Zeeman GG. Brain lesions several years after eclampsia. Am J Obstet Gynecol. 2009;200(5):504.e1–5.
36. Strevens H, Wide-Swensson D, Hansen A, Horn T, Ingemarsson I, Larsen S, et al. Glomerular endotheliosis in normal pregnancy and pre-eclampsia. BJOG. 2003;110(9):831–6.
37. Sibai BM, Villar MA, Mabie BC. Acute renal failure in hypertensive disorders of pregnancy. Pregnancy outcome and remote prognosis in thirty-one consecutive cases. Am J Obstet Gynecol. 1990;162(3):777–83.

38. Drakeley AJ, Le Roux PA, Anthony J, Penny J. Acute renal failure complicating severe preeclampsia requiring admission to an obstetric intensive care unit. Am J Obstet Gynecol. 2002;186(2):253–6.
39. Lindheimer MD, Katz AI. Kidney function and disease in pregnancy. Philadelphia: Lea & Febiger; 1977.
40. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. Preeclampsia and the risk of end-stage renal disease. N Engl J Med. 2008;359(8):800–9.
41. Wang I-K, Muo C-H, Chang Y-C, Liang J-C, Chang C-T, Lin S-Y, et al. Association between hypertensive disorders during pregnancy and end-stage renal disease: a population-based study. Can Med Assoc J. 2013;185(3):207–13.
42. McDonald SD, Han Z, Walsh MW, Gerstein HC, Devereaux PJ. Kidney disease after pre-eclampsia: a systematic review and meta-analysis. Am J Kidney Dis. 2010;55(6):1026–39.
43. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care. 2002;25(10):1862–8.
44. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. Diabet Med. 2004;21(2):103–13.
45. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension. 2009;53(6):944–51.
46. Callaway LK, Lawlor DA, O'Callaghan M, Williams GM, Najman JM, McIntyre HD. Diabetes mellitus in the 21 years after a pregnancy that was complicated by hypertension: findings from a prospective cohort study. Am J Obstet Gynecol. 2007;197(5):492 e1–7.
47. Laivuori H, Tikkanen MJ, Ylikorkala O. Hyperinsulinemia 17 years after preeclamptic first pregnancy. J Clin Endocrinol Metab. 1996;81(8):2908–11.
48. Forest JC, Girouard J, Masse J, Moutquin JM, Kharfi A, Ness RB, et al. Early occurrence of metabolic syndrome after hypertension in pregnancy. Obstet Gynecol. 2005:105(6):1373–80.
49. Pouta A, Hartikainen AL, Sovio U, Gissler M, Laitinen J, McCarthy MI, et al. Manifestations of metabolic syndrome after hypertensive pregnancy. Hypertension. 2004;43(4):825–31.
50. Hannaford P, Ferry S, Hirsch S. Cardiovascular sequelae of toxaemia of pregnancy. Heart. 1997;77(2):154–8.
51. Pabinger I, Grafenhofer H, Kaider A, Ilic A, Eichinger S, Quehenberger P, et al. Preeclampsia and fetal loss in women with a history of venous thromboembolism. Arterioscler Thromb Vasc Biol. 2001;21(5):874–9.
52. Jacobsen AF, Skjeldestad FE, Sandset PM. Incidence and risk patterns of venous thromboembolism in pregnancy and puerperium—a register-based case-control study. Am J Obstet Gynecol. 2008;198(2):233 e1–7.
53. Alsnes IV, Vatten LJ, Fraser A, Bjørngaard JH, Rich-Edwards J, Romundstad PR, et al. Hypertension in pregnancy and offspring cardiovascular risk in young adulthood: prospective and sibling studies in the HUNT study (Nord-Trøndelag Health Study) in Norway. Hypertension. 2017;69(4):591–8.
54. Bartels A, Egan N, Broadhurst DI, Khashan AS, Joyce C, Stapleton M, et al. Maternal serum cholesterol levels are elevated from the 1st trimester of pregnancy: a cross-sectional study. J Obstet Gynaecol. 2012;32(8):747–52.
55. Stekkinger E, Zandstra M, Peeters LL, Spaanderman ME. Early-onset preeclampsia and the prevalence of postpartum metabolic syndrome. Obstet Gynecol. 2009;114(5):1076–84.
56. Stekkinger E, Scholten RR, van der Vlugt MJ, van Dijk APJ, Janssen MCH, Spaanderman MEA. Metabolic syndrome and the risk for recurrent pre-eclampsia: a retrospective cohort study. BJOG. 2013;120(8):979–86.
57. Alsnes IV, Janszky I, Forman MR, Vatten LJ, Økland I. A population-based study of associations between preeclampsia and later cardiovascular risk factors. Am J Obstet Gynecol. 2014;211(6):657.e1–7.
58. Schobel HP, Fischer T, Heuszer K, Geiger H, Schmieder RE. Preeclampsia—a state of sympathetic overactivity. N Engl J Med. 1996;335(20):1480–5.
59. Brown MA. Pre-eclampsia: a case of nerves? Lancet. 1997;349(9048):297–8.

60. Collén A-C, Manhem K, Sverrisdóttir YB. Sympathetic nerve activity in women 40 years after a hypertensive pregnancy. J Hypertens. 2012;30(6):1203–10. https://doi.org/10.1097/HJH.0b013e3283531ed2.
61. Melchiorre K, Sharma R, Khalil A, Thilaganathan B. Maternal cardiovascular function in normal pregnancy: evidence of maladaptation to chronic volume overload. Hypertension. 2016;67(4):754–62.
62. Melchiorre K, Sharma R, Thilaganathan B. Cardiovascular implications in preeclampsia. Circulation. 2014;130(8):703–14.
63. Melchiorre K, Sutherland GR, Liberati M, Thilaganathan B. Preeclampsia is associated with persistent postpartum cardiovascular impairment. Hypertension. 2011;58(4):709–15.
64. Ghossein-Doha C, Peeters L, van Heijster S, van Kuijk S, Spaan J, Delhaas T, et al. Hypertension after preeclampsia is preceded by changes in cardiac structure and function. Hypertension. 2013;62(2):382–90.
65. Valensise H, Lo Presti D, Gagliardi G, Tiralongo GM, Pisani I, Novelli GP, et al. Persistent maternal cardiac dysfunction after preeclampsia identifies patients at risk for recurrent preeclampsia. Hypertension. 2016;67(4):748–53.
66. Brown MA, Gallery EDM. Volume homeostasis in normal pregnancy and preeclampsia: physiology and clinical implications. Baillieres Clin Obstet Gynaecol. 1994;8(2):287–310.
67. Magness RR, Shaw CE, Phernetton TM, Zheng J, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. II. Pregnancy effects on NO synthase expression. Am J Physiol. 1997;272(4 Pt 2):H1730–40.
68. Irani RA, Xia Y. The functional role of the renin-angiotensin system in pregnancy and preeclampsia. Placenta. 2008;29(9):763–71.
69. White WM, Mielke MM, Araoz PA, Lahr BD, Bailey KR, Jayachandran M, et al. A history of preeclampsia is associated with a risk for coronary artery calcification 3 decades later. Am J Obstet Gynecol. 2016;214(4):519.e1–8.
70. Visser S, Hermes W, Ket JCF, Otten RHJ, van Pampus MG, Bloemenkamp KWM, et al. Systematic review and metaanalysis on nonclassic cardiovascular biomarkers after hypertensive pregnancy disorders. Am J Obstet Gynecol. 2014;211(4):373.e1–9.
71. Scholten RR, Thijssen DJH, Lotgering FK, Hopman MTE, Spaanderman MEA. Cardiovascular effects of aerobic exercise training in formerly preeclamptic women and healthy parous control subjects. Am J Obstet Gynecol. 2014;211(5):516.e1–e11.
72. Sandvik MK, Leirgul E, Nygård O, Ueland PM, Berg A, Svarstad E, et al. Preeclampsia in healthy women and endothelial dysfunction 10 years later. Am J Obstet Gynecol. 2013;209(6):569.e1–e10.
73. Rich-Edwards JW, Fraser A, Lawlor DA, Catov JM. Pregnancy characteristics and women's future cardiovascular health: an underused opportunity to improve women's health? Epidemiol Rev. 2014;36:57–70.
74. Spaan J, Peeters L, Spaanderman M, Brown M. Cardiovascular risk management after a hypertensive disorder of pregnancy. Hypertension. 2012;60(6):1368–73.
75. Schmittdiel J, Selby JV, Swain B, Daugherty SL, Leong TK, Ho M, et al. Missed opportunities in cardiovascular disease prevention? Low rates of hypertension recognition for women at medicine and obstetrics-gynecology clinics. Hypertension. 2011;57(4):717–22.
76. Hermes W, Tamsma JT, Grootendorst DC, Franx A, van der Post J, van Pampus MG, et al. Cardiovascular risk estimation in women with a history of hypertensive pregnancy disorders at term: a longitudinal follow-up study. BMC Pregnancy Childbirth. 2013;13:126.
77. Breetveld NM, Ghossein-Doha C, van Kuijk SMJ, van Dijk AP, van der Vlugt MJ, Heidema WM, et al. Cardiovascular disease risk is only elevated in hypertensive, formerly preeclamptic women. BJOG. 2015;122(8):1092–100.
78. van Baaren G-J, Hermes W, Franx A, van Pampus MG, Bloemenkamp KWM, van der Post JA, et al. Cost-effectiveness analysis of cardiovascular risk factor screening in women who experienced hypertensive pregnancy disorders at term. Pregnancy Hypertens. 2014;4(4):264–70.

79. Davis GK, Roberts L, Mangos G, Henry A, Pettit F, O'Sullivan A, et al. Postpartum physiology, psychology and paediatric follow up study (P4 study)—study protocol. Pregnancy Hypertens. 2016.
80. Raw M, McNeill A, West R. Smoking cessation: evidence based recommendations for the healthcare system. BMJ. 1999;318(7177):182–5.
81. Estruch R, Ros E, Martinez-Gonzalez MA. Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med. 2013;369(7):676–7.
82. Barakat R, Pelaez M, Cordero Y, Perales M, Lopez C, Coteron J, et al. Exercise during pregnancy protects against hypertension and macrosomia: randomized clinical trial. Am J Obstet Gynecol. 2016;214(5):649.e1–8.
83. Scholten RR, Hopman MTE, Lotgering FK, Spaanderman MEA. Aerobic exercise training in formerly preeclamptic women: effects on venous reserve. Hypertension. 2015;66(5):1058–65.