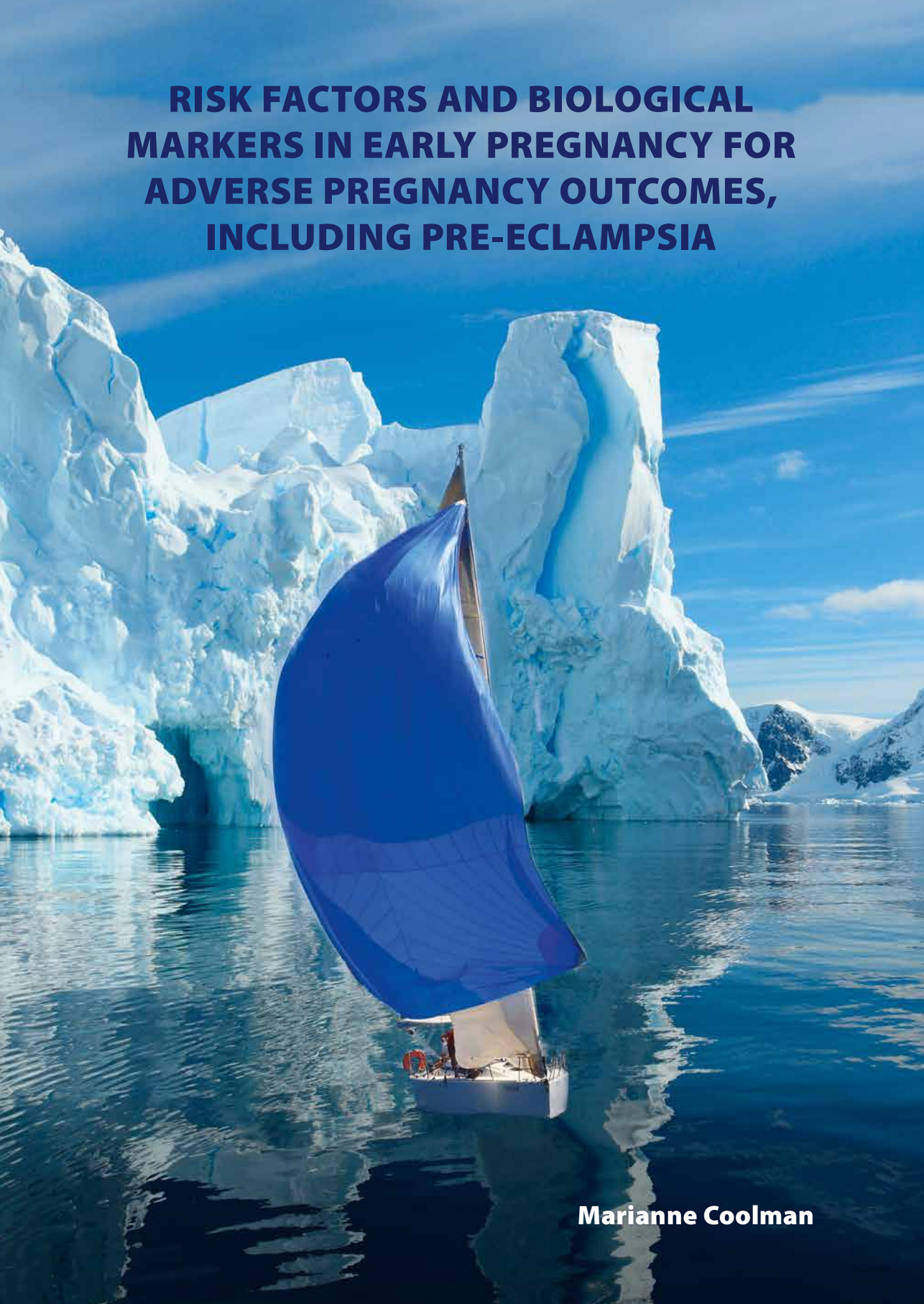
RISK FACTORS AND BIOLOGICAL MARKERS IN EARLY PREGNANCY FOR ADVERSE PREGNANCY OUTCOMES, INCLUDING PRE-ECLAMPSIA



Marianne Coolman

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RISK FACTORS AND BIOLOGICAL MARKERS IN EARLY PREGNANCY FOR ADVERSE PREGNANCY OUTCOMES, INCLUDING PRE-ECLAMPSIA

Risicofactoren en biologische markers vroeg in zwangerschap en slechte zwangerschapsuitkomsten, inclusief pre-eclampsie

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Chapter 1

General introduction

INTRODUCTION

Hypertensive disorders, intrauterine growth restriction (IUGR) and spontaneous preterm birth are pregnancy outcomes with a major impact on maternal and neonatal and child health with largely unknown causes. Tabel 1 shows the definitions of the different disorders. The leading hypotheses for hypertensive disorders and IUGR strongly rely on disturbed placentation in early pregnancy.

1.1 Hypertensive disorders

Hypertensive disorders, including pregnancy-induced hypertension (PIH) and pre-eclampsia, are common complications in pregnancy[1,2]. In particular pre-eclampsia and to a lesser extend PIH are associated with an increased maternal and fetal morbidity and mortality. PIH and pre-eclampsia are pregnancy-specific diseases. The prevalence of pre-eclampsia is reported between 2 and 8%.

The cause of pre-eclampsia remains largely unknown. Its pathogenesis is described as an abnormal placenta development[3]. The first (placental) stage of the disease might include an atypical maternal immune response to trophoblasts, resulting in a failure in trophoblast invasion. Episodes of placental hypoxia or reperfusion result in oxidative stress, subsequent apoptotic and necrotic disruption of syncytial architecture, and release of various components from the intervillous space into the maternal circulation. The second stage of the systemic maternal disease is associated with exaggerated endothelial activation and a generalised hyperinflammatory state compared to normal pregnancy.

1.2 Intrauterine growth restriction

IUGR is associated with perinatal morbidity and mortality rates are 4-8 times higher for infants with IUGR. IUGR affects 3-10% of pregnancy[4].

IUGR can be a manifestation of fetal, placental, or maternal determinants or combinations of those. Many of the cases of IUGR are the result of ischemic placental disease. This term refers to a disease process of the placenta that clinically manifests as IUGR isolated with the lack of clinical evident maternal disease e.g. systemic infection and neonatal disease e.g. chromosomal abnormalities. The involvement of abnormal placentation in IUGR is

clinically evident if complicated by pre-eclampsia as maternal manifestation, abruption, or a combination of these disorders[5,6]. All of these disorders may be associated with preterm birth or fetal loss and represent late manifestations of abnormal placental development dating from the earliest stages of pregnancy. Uteroplacental blood flow may be diminished by faulty development, acquired obstruction, or disruption of the uteroplacental vasculature. Obstetrical complications (eg, pre-eclampsia) associated with vasculopathy and/or reduced maternal blood volume or blood pressure diminish uteroplacental perfusion and result in IUGR[7,8].

1.3 Spontaneous preterm birth

Preterm birth is the leading cause of infant morbidity and mortality in the industrialized world. Given that low and very low birthweight newborns are at the highest risk of early death or disability, major focuses of obstetrical research include the pathogenic processes leading to preterm birth and development of preventive interventions. Approximately 70 percent of preterm deliveries occur spontaneously.

The cause for preterm birth is in many situations unknown. There is compelling clinical and research evidence to suggest that a number of pathogenic processes can lead to a final common pathway that results in spontaneous preterm birth. The four primary processes include premature activation of the maternal or fetal hypothalamic-pituitary-adrenal axis, exaggerated inflammatory response/infection, abruption (decidual hemorrhage) and pathological uterine distention. Spontaneously preterm birth is associated with histopathological evidence of abnormal placentation[9]. Premature fetal hypothalamic-pituitary-adrenal activation can result from the stress of uteroplacental vasculopathy. One study described that spontaneous preterm birth was associated with a lack of normal physiologic conversion of maternal spiral arteries[10].

Table 1 definition hypertensive disorders in pregnancy, intra-uterine growth restriction and spontaneous preterm birth

Definition / Disorder	RCOG 2011	ISSHP 2000	ACOG
Chronic or pre-existent hypertension	an absolute blood pressure ≥140/90 mmHg measured preconceptionally or before the 20th week of pregnancy	an absolute blood pressure ≥140/90 mmHg measured preconceptionally or before the 20th week of pregnancy	an absolute blood pressure ≥140/90 mmHg measured preconceptionally or before the 20th week of pregnancy
Pregnancy-induced hypertension	*de novo* hypertension (an absolute blood pressure ≥140/90 mmHg) without proteinuria, after the 20th gestational week	*de novo* hypertension (an absolute blood pressure ≥140/90 mmHg) without proteinuria, after the 20th gestational week	blood pressure of 140mmHg systolic or higher or 90mmHg or higher that occurs after 20 weeks of gestation in a woman with previously normal blood pressure
Pre-eclampsia	pregnancy-induced hypertension and proteinuria (≥ 0.3grams in a 24-hour urine specimen or ≥ 2 + [1 g/l] on a voided specimen, or ≥ 1+ [0.3 g/l] on a catheterised specimen)	*de novo* hypertension (an absolute blood pressure ≥140/90 mmHg), returning to normal postpartum AND proteinuria (≥ 0.3grams in a 24-hour urine specimen or protein/creatinine ratio≥ 30mg protein/mmol creatinine)	blood pressure of 140mmHg systolic or higher or 90mmHg or higher that occurs after 20 weeks of gestation in a woman with previously normal blood pressure AND proteinuria, defined as urinary excretion of 0.3g protein or higher in a 24-hour urine specimen
Severe hypertension	diastolic blood pressure ≥ 110mmHg on two occasions or systolic blood pressure ≥ 160mmHg on two occasions	diastolic blood pressure ≥ 110mmHg on two occasions or systolic blood pressure ≥ 160mmHg on two occasions	blood pressure of 160mmHg systolic or higher or 110mmHg diastolic or higher on two occasions at least 6 hours apart while the patient is on bed rest
Severe pre-eclampsia	pre-eclampsia with severe hypertension and/or with symptoms, and/or elevated liver enzymes and low platelet count	no consensus	pre-eclampsia with severe hypertension or and at least one of the severe criteria:
Early onset pre-eclampsia	pre-eclampsia before a gestational age of 34 weeks	pre-eclampsia before a gestational age of 34 weeks	
Late-onset pre-eclampsia	pre-eclampsia after a gestational age of 34 weeks	pre-eclampsia after a gestational age of 34 weeks	
HELLP syndrome	haemolysis, elevated liver enzymes and low platelet count	haemolysis, elevated liver enzymes and low platelet count	
Severe criteria		no consensus	
Symptoms	severe headache; visual disturbance; epigastric pain or vomiting		oliguria of less than 500ml in 24 hours cerbral or visual disturbances pulmonary edema or cyanosis epigastric or right upper-quadrant pain fetal growth restriction
Proteinuria	creatinine ratio is greater than 30mg/mmol or a validated 24-hour urine collection result shows greater than 300mg protein	≥ 0.3grams in a 24-hour urine specimen or protein/creatinine ratio≥ 30mg protein/mmol creatinine	5 g or higher in a 24-hour urine specimen or 3+ or greater on two random urine samples collected at least 4 hours apart
Biochemical and haematological impairment	platelet count < $100x10^9$/L, abnormal liver enzymes (ALT or AST rising to above 70U/L)		impaired liver function, thrombocytopenia
	general	Gardosi	Generation R
Intrauterine growth restriction (IUGR)	intrauterine fetal growth that deviates from expected norms	classification of birth weight using customized centiles adjusted for gestational age, parity, ethnicity and parental height and weight (www.gestation.net)	sex specific gestational age adjusted-birth weight below P2.3
Spontaneous preterm birth	a spontaneous delivery before a gestational age of 37 weeks		

Objective of the thesis

Defective deep placentation has been associated with a spectrum of complications of pregnancy including pre-eclampsia, intrauterine growth restriction, spontaneous preterm birth, late spontaneous abortion and abruption placentae[8]. The major obstetrical syndromes are associated with defective deep placentation, which may be associated with different degrees of restricted remodelling and obstructive lesions of spiral arteries in the junctional zone or inner myometrium[8].

Due to the unknown pathogenesis of placental related disorders prevention, prediction and rational treatment are lacking. Proper antenatal care and timely delivery is the most important part of preventing complications of placental related disorders in pregnancy. The identification of risk factors for these disorders at an early stage of pregnancy, could increase awareness and support timely detection. Moreover, new knowledge on risk factors might play a role in unravel the pathogenesis of placental related disorders in pregnancy.

Potential new risk factors, shared and different risk factors probably helps to elucidate the underlying pathogenetic mechanisms of placental related disorders. Generation R, a large prospective cohort study, has opened up these opportunities[11]. The existing literature suggests that socioeconomic status has its impact on health, which starts already in pregnancy. A low maternal socioeconomic status at time of pregnancy has been shown to increase the risk for low birth weight, prematurity and perinatal mortality. Socio-economic status as a risk factor of hypertensive disorders in pregnancy has been studied extensively in the Generation R Study. Models that can easily be used in a antenatal care setting will help to recognize those who are at risk. Pre-eclampsia in a previous pregnancy is an important risk factor for recurrent pre-eclampsia in subsequent pregnancies and pre-eclampsia is a risk for cardiovascular diseases in later life[12]. Risk assessments in pregnancy or later life are commonly determined by epidemiologic studies through subjects' self-report via personal interviews or mailed questionnaires self-administered questionnaires. Validation of self-reported pre-eclampsia will help to interpret results of studies, which examine associations between pre-eclampsia and different outcomes.

Abnormal placentation is associated with pre-eclampsia, intrauterine growth restriction and spontaneous preterm birth. These disorders of deep placentation are characterized by the

degree of restriction of physiologic transformation of the spiral arteries and the presence of arterial lesions in the junctional zone of the myometrium of the placental bed[8].

The mechanism(s) that triggers abnormal placentation and results in a subsequent progression into the systemic maternal response are intense areas of research. Numerous factors are proposed to be involved among which oxidative stress, thrombofilia, immunological factors and (anti-)angiogenic factors.

Haemostasis is a complex balance of activating and inhibitory pathways. Normal pregnancy is associated with hypercoagulation which is even more profound in complicated pregnancies[13]. It is necessary to describe the changes of components of the plasminogen-activator-system during normal pregnancy, to understand the involvement of the hemostatic imbalance in pregnancy-related complications. Placentation involves trophoblast invasion and angiogenesis and its success is dependent on vascular- and endothelial cell function[14]. An imbalance between circulating pro-angiogenic (PlGF) and anti-angiogenic (sFlt1) factors play a role in the etiology of different adverse outcomes of pregnancy. A series of studies showed a relationship between low PlGF and elevated sFlt1 concentrations in the maternal circulation and the risk of pre-eclampsia[15]. This anti-angiogenic profile has also been described in relation to growth restriction[16]. Circulating concentrations of especially PlGF and sFlt1 have been shown to be altered several weeks ahead of the onset of pre-eclampsia[17].

Imbalances of angiogenic (sFlt1 and PlGF) and hemostatic (PAI-2) factors as a mediating link between trophoblast failure and endothelial dysfunction might affect placentation, resulting in abnormal placentation and adverse pregnancy outcomes. The proteins could be a predictor for adverse pregnancy outcomes and might be a target for therapeutic intervention. Prerequisite for successful trophoblast invasion is degradation and remodelling of the uterine decidual extracellular matrix and apoptosis. Matrix metalloproteinases (MMP) play a crucial role in restructuring the extracellular matrix[18,19]. Several members of this enzyme family are present at the fetal-maternal interface. The inability to produce sufficient matrix metalloproteinases may be an early manifestation of abnormal placentation in pregnancy related complications. Programmed apoptosis is regulated through annexin A5, which is expressed in syncytiotrophoblasts and endothelial cells. Polymorphisms that are functional promoter polymorphisms may affect transcription rate and the amount of protein synthesised. Determination of circulating biomarkers,

including key proteins, and polymorphisms that affect the amount of proteins, which are specific for angiogenesis might give more insight in the trigger for abnormal placentation, responsible for adverse pregnancy outcomes.

Aims

The main objectives of this thesis can be summarized as follows:

A better understanding of the pathophysiologic mechanisms of placenta related disorders in pregnancy by;

1. Assessment of maternal and paternal risk factors, which play a role in development of placenta related pregnancy complications.
2. Investigation of early pregnancy circulating biomarkers in maternal blood (hemostatic and angiogenic factors) in relation to adverse pregnancy outcomes including pre-eclampsia.

Outline

This thesis is divided into two parts. Part 1 focuses on the assessment of maternal and paternal risk factors, which play a role in development of placental related pregnancy complications. Part 2 focuses on the investigation of circulating biomarkers in maternal blood (hemostatic and angiogenic factors) and adverse pregnancy outcomes.

Part 1

In chapter 2.1 we examine the maternal and paternal risk factors and development of pre-eclampsia and PIH. In chapter 2.2 and 2.3 the associations of low socio-economic status and, pre-eclampsia and gestational hypertension are assessed. In chapter 2.4 we present the validation of a prediction model for the development of hypertension in pregnancy in nulliparous women, based on clinical measurements obtained routinely at the first antenatal visit. In chapter 2.5 we evaluate the reliability of self-reported pre-eclampsia, often used in epidemiological studies.

Part 2

In chapter 3.1 we provide longitudinal data on activating and inhibitory components of the plasminogen-activator system during normal pregnancy. In chapter 3.2 we examined the

associations of determinant(s) of angiogenesis (sFlt1 and PlGF) and hemostasis (PAI-2) in maternal blood, and maternal and perinatal complications in pregnancy. In chapter 3.3 we focus on the determinants of angiogenesis (MMP9, MMP3 and annexin polymorphism) in maternal DNA and development of pre-eclampsia.

Studypopulation

The studies describing maternal and paternal risk factors, and the study describing sFlt1, PlGF and PAI-2 and adverse pregnancy outcomes were embedded within the framework of the Generation R Study. The Generation R Study is an ongoing population-based cohort study, designed to identify early environmental, biological and social determinants of growth, development and future health[11,20,21,22]. In total 9778 women enrolled in the Generation R Study, of whom 8880 women, 6384 partners and their children, of different ethnicities, and living in Rotterdam, the Netherlands enrolled prenatal. Enrolment was aimed in early pregnancy, but possible until birth of the child. Eighty% of the women was included before 18 weeks of gestation. Questionnaires, in early, mid and late pregnancy, were used to gather information about maternal and paternal socio-demografic, obstetrical, health-related and lifestyle determinants. Physical examinations, including maternal and paternal weight and height, and maternal blood pressure were obtained in early, mid and late pregnancy. Maternal blood samples were assessed in early and mid pregnancy. Several overlapping sources such as our own ultrasound facilities but also obstetric caregivers, and Municipal Health Services, provided information about maternal and perinatal outcomes, including gestational hypertensive disorders, intrauterine fetal growth, placental weight, birth weight, preterm birth and congenital anomalies.

All children were born between April 2002 and January 2006. The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. The Generation R Study follows the STROBE guidelines[23,24].

The studies describing balances in angiogenesis or hemostasis in pregnancy were performed in different cohorts. In chapter 3.2, the study describing plasminogen activator and their inhibitors was performed in samples from a subgroup from a multicentred cohort study, coordinated from the University Hospital St Radboud in Nijmegen, the Netherlands, between 1987 and 1990[25]. Maternal blood samples were assessed before pregnancy, throughout pregnancy and 6 weeks after pregnancy.

In chapter 3.3, the study describing "Matrix metalloproteinase-9 gene -1562C/T polymorphism" was performed in samples from a case control study, coordinated by the Leiden University Medical Centre, the Netherlands[26].

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Chapter 2

Parental risk factors for the development of gestational hypertensive disorders

Chapter 2.1

Parental risk factors in early pregnancy associated with gestational hypertensive disorders; The Generation R Study

Coolman M, de Groot CJM, Hofman A, Bonsel G, Raat H, Jaddoe VWV, Steegers EAP

Submitted

ABSTRACT

Introduction:

Timely identification of risk factors enables antenatal management tailored to that individual, which may contribute to the prevention of perinatal and maternal morbidity and mortality. Therefore, we studied maternal as well as paternal risk factors in nulliparous and multiparous women in *early* pregnancy.

Study design:

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. Information about maternal and paternal characteristics, such as sociodemografic status, lifestyle habits, and obstetrical history was obtained in early pregnancy from questionnaires, medical records and physical examinations. Associations between early pregnancy risk factors and pregnancy-induced hypertension and pre-eclampsia were evaluated in logistic regression models.

Results:

Of all pregnant women, 231 (4.5%) developed pregnancy-induced hypertension and 127 (2.5%) developed pre-eclampsia. For nulliparous women an increased BMI was associated with a three fold, European ethnicity with a two fold and family history of pre-eclampsia with a two fold increased risk for development of pregnancy-induced hypertension. For multiparous women an increased BMI and previous gestation hypertensive disorders were associated with an increased risk for developing pregnancy-induced hypertension (three and ten fold, respectively).

The most important risk factors for nulliparous women who developed pre-eclampsia included advanced maternal age (five fold increased risk), twin pregnancies (five fold increased risk), an increased BMI (three fold increased risk), a Surinamese ethnicity (two fold increased risk) and a maternal low birth weight (three fold increased risk). In multiparous women risk factors for pre-eclampsia were previous gestational hypertensive disorder, an increased BMI and headache (ten fold, four fold and three fold, respectively). Low education and Dutch ethnicity were associated a four fold decreased risk for developing pre-eclampsia. A history of family hypertension was associated with pre-eclampsia in neither nulliparous nor multiparous women. Paternal risk factors were not associated with development of pregnancy-induced hypertension nor pre-eclampsia. For

multiparous women paternal arthralgia showed a trend towards a risk for development of pre-eclampsia.

Conclusion:

Easily accessible risk factors in early pregnancy in nulliparous and multiparous women for pregnancy-induced hypertension and pre-eclampsia appear to be partly different. Suggested paternal risk factors could not be confirmed and new paternal risk factors were not identified.

INTRODUCTION

Pregnancy-induced hypertension and pre-eclampsia, are common complications in pregnancy[1]. In particular pre-eclampsia and to a lesser extent pregnancy-induced hypertension are associated with an increased perinatal and maternal morbidity and mortality[2,3]. The first (placental) stage of development of pre-eclampsia is suggested to be related to impaired trophoblast invasion. Episodes of placental hypoxia result in oxidative stress and release of various components into the maternal circulation. The second stage involves systemic exaggerated endothelial activation and a generalised hyperinflammatory state as compared to normal pregnancy[1].

Since the etiology remains largely unknown, rational prevention of these disorders is not possible. Detection, appropriate antenatal care and timely delivery are thus the most important tools to prevent maternal and fetal complications. Timely detection will be enhanced by the identification of risk factors for hypertensive disorders at an early stage of pregnancy, increasing awareness and focussed surveillance. Several risk factors for the development of pre-eclampsia, which may be present at antenatal booking, have been described in a systematic review[4,5]. Specific risk factors described for pre-eclampsia include nulliparity, older age, high body mass index (BMI), family history of pre-eclampsia, underlying renal disease or chronic hypertension, multiple pregnancy, more than 10 years between pregnancies, and a personal history of pre-eclampsia[6]. The expected rate of pre-eclampsia, when anyone of these risk factors is present, ranges from 3% to more than 30%, and many women have several risk factors[6]. Pre-pregnancy planning and health promotion even before conception offers the possibility to improve pregnancy outcomes as well. Modifiable risk factors including, obesity and smoking before conception, education, socioeconomic status and maternal diseases are important risk factors to consider known before pregnancy. More healthy women before pregnancy will result in more healthy pregnancies and less maternal morbidity and mortality. In addition, by studying risk factors for hypertensive disorders more knowledge will be gained in the insights of the etiology of hypertensive disorders.

Paternal risk factors for gestational hypertensive disorders were less well studied.

Therefore, within the Generation R study, which is a large prenatally recruited birth-cohort study with extensive assessments during pregnancy, we studied risk factors in early pregnancy for pregnancy-induced hypertension and pre-eclampsia, focussing potential new

maternal risk factors and paternal risk factors in addition to the factors already known, described earlier[6]. Those risks appear to be in part different for nulliparous and multiparous women[7]. As multiparous women with a history of uncomplicated pregnancies have a minimal risk for pre-eclampsia of only 1 % or less, we stratified according to parity[8].

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study examines early environment and genetic determinants of growth, development and health in fetal life, childhood and adulthood[9]. The cohort included 8880 mothers, 6384 fathers and their children, of different ethnicities, and living in Rotterdam, the Netherlands from 2003 till 2006. Enrolment was aimed in early pregnancy, but possible until birth of the child. Women and their partners were enrolled in the study at their routine ultrasound examination in pregnancy after written consent was obtained. Assessments in pregnancy, including anthropometrics of both parents and questionnaires were planned in early pregnancy. All children were born between April 2002 and January 2006. The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam.

Maternal risk factors

Information was obtained in one of the research centres by a physical examination of the pregnant woman and by questionnaire at enrolment. This self-reported home send questionnaire comprised information on maternal factors, included comorbidity, family history, lifestyle and pregnancy associated factors (about previous pregnancies and current pregnancy).

Maternal factors

Maternal age was categorized into two groups (<40 and ≥40 years)[5]. Height and weight of the mother were measured at enrolment. Body mass index was calculated as weight per height squared (kg/m^2) and categorized into two groups (≤25 and > 25kg/ m^2). Ethnicity was categorized into European, Surinamese, Turkish, Moroccan, Antillean, Cape Verdean

and other countries[7], a categorization reflecting the most prevalent migrant groups. Education (highest completed educational level of the mother) was categorized into three levels: low (no education or primary school), mid (secondary school) and high (high school or university).

Comorbidity

Maternal comorbidity was defined as a mother having a chronic disorder, including chronic hypertension, diabetes, migraine and arthralgia (yes, no or don't know).

Family history factors

The information provided on maternal birth weight was categorized as follows; less than 2000g, 2000-2500g, 2500-3000g, 3000-4000g, more than 4000g and not known. Maternal low birth weight was defined as a maternal birth weight less than 2500g. Diseases in family of the mother asked for included diabetes, hypertension, pregnancy-induced hypertension and pre-eclampsia (no, yes, don't know).

Lifestyle factors

Maternal smoking, alcohol, coffee and tea consumption habits were assessed by repeatedly applied questionnaires in pregnancy[10,11].

Pregnancy associated factors

The questionnaire also comprised questions regarding outcomes of previous pregnancies (pre-eclampsia or pregnancy-induced hypertension, no or yes) as well as specific symptoms that had occurred during the first trimester of the current pregnancy. Symptoms asked for were headache, nausea and oedema. The information obtained was categorized into 2 groups (having these symptoms a few days a week or more, and once a week or less).

Paternal risk factors

Information on paternal risk factors was obtained at enrolment from a physical examination of the father and a self-reported questionnaire. Paternal anthropometrics, including weight and height were measured and body mass index was calculated as weight

per height squared (kg/m2). Characteristics of the father studied included age, ethnicity and chronic diseases of the father (diabetes mellitus, hypertension and arthralgia). The questionnaire also contained questions about diseases in the family, including chronic hypertension and diabetes. The information provided on paternal birth weight was categorized as follows: less than 2000g, 2000-2500g, 2500-3000g, 3000-4000g, more than 4000g and not known. Paternal low birth weight was defined as a paternal birth weight less than 2500g. No questions on pregnancy-induced hypertension and pre-eclampsia in the family were included in the questionnaire for the father. Questions about lifestyle risk factors included smoking, alcohol, coffee and tea consuming and were categorized in yes or no.

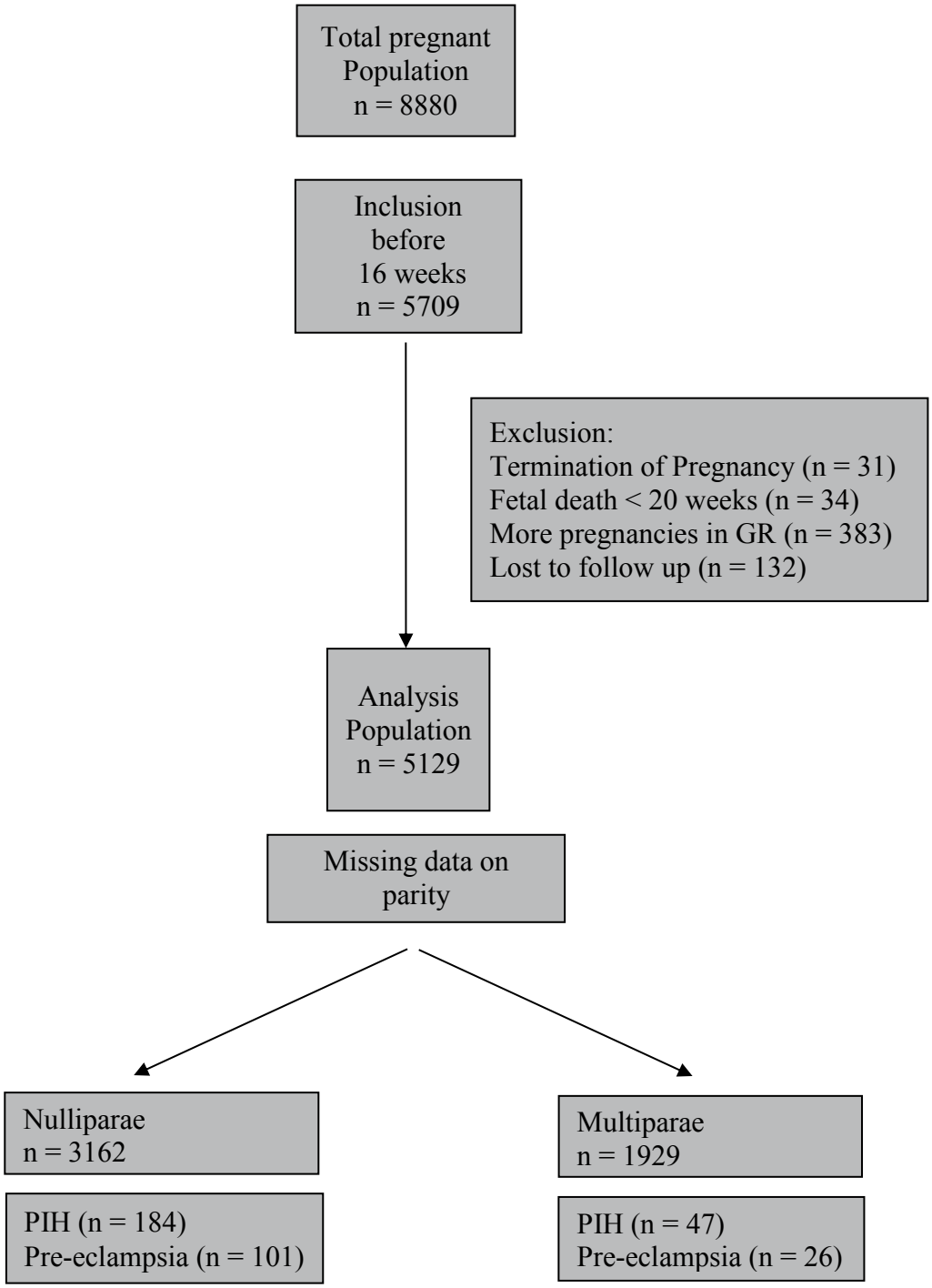
Maternal outcomes

According to Dutch standards of antenatal care, all women whose pregnancies are complicated by hypertension, should deliver in a hospital under medical supervision of an obstetrician. The delivery reports of all study participants who delivered under medical supervision were retrieved and these reports were screened by a trained medical record abstractor. Selection was based on the delivery report mentioning any kind of hypertensive complications, cases of chronic hypertension, pregnancy-induced hypertension, pre-eclampsia or HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets). To confirm the diagnosis of chronic hypertension, pregnancy-induced hypertension or pre-eclampsia, the same abstractor conducted, in addition, detailed reviews of the hospital charts of these women regarding blood pressure and laboratory analyses[12]. pregnancy-induced hypertension and pre-eclampsia were defined according to the International Society for the Study of Hypertension in Pregnancy ISSHP criteria[13]. HELLP syndrome was defined as thrombocytes $<100x10^9$/l, and both ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase) >70 U/l and LDH (lactate dehydrogenase) > 600 U/L.

Population for analysis

In total 8880 pregnant women were enrolled in the antenatal phase of Generation R Study. Figure 1 shows the study population flow chart. A subgroup enrolled before sixteen weeks of gestation (64%, n=5709). Women who had a termination of pregnancy, fetal death before twenty weeks of gestation or missing maternal pregnancy outcome were excluded

Figure 1 Study population flow chart



from the present study. When women had participated with more than one pregnancy in generation R, only the first pregnancy was included in this analysis. In 38 cases (0.7%) information on parity was missing. The associations of the different risk factors with pregnancy-induced hypertension or pre-eclampsia were analyzed for the remaining 5091 women. Different analyses were made for the subgroups of nulliparous women (62.1%, n=3162) and multiparous women (37.9%, n=1929). Paternal risk factors were analyzed in the subgroup of women that participated with their partner (79%, n= 4010).

Data analysis

The study assessed the associations of risk factors for pregnancy-induced hypertension and pre-eclampsia using logistic regression analysis. First crude, unadjusted odds ratios were calculated; thereafter different adjustments were made. Adjustment was resulting from an enter, forward and backward stepwise regression model. Adjustments were included in the analyses based on literature and on the condition that the effect in the forward and backward stepwise regression model changed more than 5%. For nulliparous women with pregnancy-induced hypertension we made adjustments for an increased body mass index and family hypertensive disorders. For multiparous women, adjustments were made for previous gestational hypertension. For nulliparous women with pre-eclampsia, adjustments were made for an increased body mass index and chronic hypertension, and for multiparous women adjustments were made in case of previous pre-eclampsia. Finally, for nulliparous and multiparous women with pregnancy-induced hypertension or pre-eclampsia, multiple adjustments (based on literature[5]) were calculated. Not all data are shown in the tables. For ethnicity we showed only the results for women with the European ethnicity compared to non-European ethnicity and the Surinamese ethnicity compared to non Surinamese ethnicity. For education we showed the results for women with the highest education compared women with the lowest and mid education.

We compared women with pregnancy-induced hypertension or pre-eclampsia to a reference group. The reference group was defined as all women in the cohort without pregnancy-induced hypertension, pre-eclampsia or HELLP syndrome. We considered "Don't know " answers as missing. Finally, we assessed the associations of risk factors in the subgroup of women who developed a hypertensive disorder in pregnancy and compared women with pregnancy-induced hypertension to women with pre-eclampsia.

The Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses.

Table 1. Maternal and paternal characteristics

	Nulliparous			Multiparous		
Maternal related	PIH N=184	Pre-eclampsia N=101	Reference N=2864	PIH N=47	Pre-eclampsia N=26	Reference N=1854
Age mean (sd)	29.1(4.8)	28.4(5.0)	28.7(4.9)	32.0(4.3)	30.3(5.4)	31.1(4.6)
Age>40years(%)	0.5	2	0.6	0	3.8	1.7
Missing(%)	0	0	0	0	0	0
BMI mean(sd)	26.8(5.2)	26.9(6.1)	23.8(4.1)	29.1(6.5)	28.3(5.2)	25.0(4.6)
BMI>25kg/m2(%)	52.2	51.5	28.7	70.2	65.4	42.4
Missing(%)	0.5	1	0.3	0	3.8	0.8
Ethnicity						
European(%)	73.9	54.5	62.3	68.1	26.9	53.6
Surinamese(%)	8.2	15.8	8.0	2.1	15.4	8.0
Missing(%)	3.3	5.0	4.2	2.1	15.4	6.7
Education						
High(%)	39.6	34.8	46.4	31.9	11.5	36.2
Mid(%)	51.1	55.4	43.1	44.6	34.6	42.5
Low(%)	4.3	5.0	5.5	17.0	34.6	12.6
Missing(%)	4.9	5.0	5.0	6.4	19.2	8.7
History						
PE in history(%)	-	-	-	6.4	11.5	1.6
Missing(%)				19.1	34.6	14.1
PIH in history(%)	-	-	-	38.3	30.8	6.3
Missing(%)				12.8	19.2	13.8
Maternal comorbidity						
Chronic hypertension (%)	-	1.1	-	-	1.2	-
Maternal diabetes(%)	0	2.0	0.3	0	0	0.5
Missing	10.9	7.9	12.3	10.6	30.8	14.0
Maternal birth weight <2500g	6.5	16.8	6.4	2.1	3.8	5.6
Missing(%)	16.8	15.8	18.4	27.7	34.6	19.4
Family history factors						
Hypertension in	41.3	38.6	30.4	44.7	34.6	30.3
Missing	13.6	13.9	15.4	10.6	38.5	16.4

Table1 Continued

	Nulliparous			Multiparous		
Characteristics	PIH N=184	Pre-eclampsia N=101	Reference N=2864	PIH N=47	Pre-eclampsia N=26	Reference N=1854
Diabetes in family(%)	19.0	17.8	15.0	17.0	19.2	13.2
Missing	12.0	12.9	12.0	14.9	38.5	12.0
Lifestyle factors in first trimester						
Smoking(%)	8.7	8.9	10.5	10.6	7.7	12.0
Missing(%)	9.8	7.9	10.6	8.5	30.8	12.7
Alcoholics(%)	8.7	9.9	13.9	8.5	0	14.8
Missing(%)	9.2	5.9	10.3	8.5	26.9	12.2
Coffee(%)	38.6	43.6	44.3	48.9	38.5	49.5
Missing(%)	8.7	6.9	10.2	10.6	26.9	12.7
Tea(%)	85.9	87.1	82.4	80.9	65.4	79.7
Missing(%)	8.7	6.9	10.2	10.6	26.9	12.6
Pregnancy-associated factors						
Twin pregnancy(%)	0.5	3.0	0.7	2.1	3.8	1.2
Missing(%)	20.7	24.8	16.6	34.0	34.6	18.7
Headache(%)[#]	25.0	36.6	27.7	29.8	42.3	30.6
Missing(%)	13.6	8.9	11.6	17.0	26.9	16.1
Paternal factors						
Participation(%)	88.6	77.2	83.6	83.0	50.0	70.4
Hypertension(%)	2.2	4.0	2.1	2.1	0	1.3
Missing(%)	24.5	41.6	31.3	34.0	65.4	45.8
Diabetes(%)	0	1.0	0.3	0	0	0.7
Missing(%)	19.0	39.6	29.0	31.9	65.4	43.9
Arthralgia(%)	0	1.0	0.3	0	3.8	0.2
Missing(%)	20.1	61.4	28.9	29.8	61.5	44.4
Birth weight < 2500g(%)	4.3	2.0	2.6	0	0	1.8
Missing(%)	40.8	57.4	52.4	53.2	88.5	63.9
BMI>25 kg/m2(%)	48.4	32.7	38.3	51.1	34.6	36.1
Missing(%)	12.0	22.8	16.5	17.0	50.0	29.7

[#] Headache a few days a week or more

RESULTS

Maternal and paternal characteristics of the studypopulation are described in table 1.

In the subgroup of 5129 women who were included before 16 weeks gestational age, 4.5% developed pregnancy-induced hypertension and 2.5% developed pre-eclampsia (figure 1).

Nulliparous women had a higher risk to develop pregnancy-induced hypertension and pre-eclampsia (crude odds ratio with 95% confidence interval, 2.56, 1.81 – 3.70 and 4.54, 2.56 – 7.69, respectively).

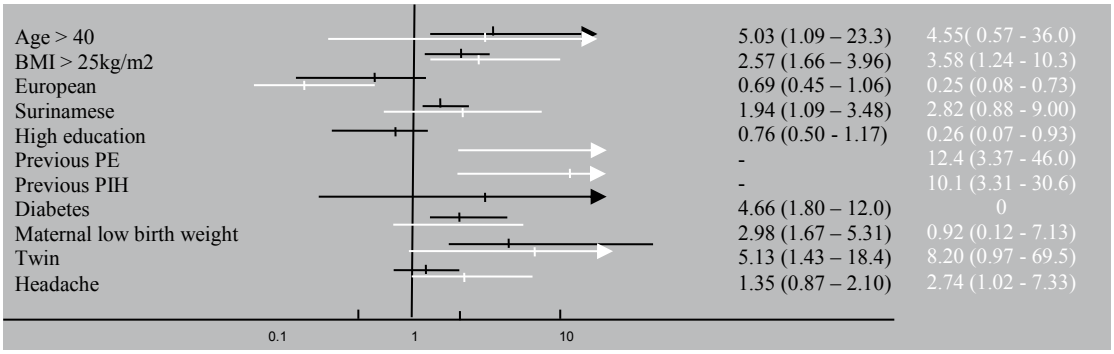
Risk factors for pregnancy induced hypertension

Table 2 and 3 show the associations between risk factors and pregnancy-induced hypertension. An increased BMI, European ethnicity and a history of family hypertension were associated with pregnancy-induced hypertension in nulliparous women. Previous gestational hypertension and an increased BMI were associated with pregnancy-induced hypertension in multiparous women. Advanced maternal age, Surinamese ethnicity, maternal low birth weight, twin pregnancies, headache and paternal factors were neither associated with pregnancy-induced hypertension in nulliparous nor multiparous women. The consumption of alcoholics showed a trend to protect for development of pregnancy-induced hypertension in the first pregnancy. We did not find any significant associations with regards to smoking, and drinking coffee or tea in early pregnancy.

Risk factors for pre-eclampsia

Table 4 and 5 show the associations between risk factors and pre-eclampsia. All potential risk factors for pre-eclampsia are summarized in figure 2.

Figure 2. Multivariable analysis of potential significant risk factors for pre-eclampsia for nulliparous and multiparous women



Results from multiple logistic regression . Values are odds ratios (95% Confidence Interval).

Risk factors for pre-eclampsia are presented in black for nulliparous women and in white for multiparous women.

Advanced maternal age, maternal low birth weight and twin pregnancy were associated with pre-eclampsia in nulliparous women after adjustments made for an increased BMI and hypertension. In addition, an increased BMI and chronic hypertension appeared to be independently associated with pre-eclampsia. Chronic hypertension and headache were associated with an increased risk for pre-eclampsia in multiparous women after adjustments for previous gestational hypertension. Moreover, previous pre-eclampsia and previous pregnancy-induced hypertension were associated with an increased risk of pre-eclampsia. Women from Dutch or European ethnicity and those with a high education exhibit a lower risk to develop pre-eclampsia. Family history of hypertension, lifestyle factors and paternal factors were associated with pre-eclampsia in neither nulliparous nor multiparous women.

Risk factors for pre-eclampsia or pregnancy-induced hypertension

Table 6 shows potential different risk factors for pre-eclampsia versus pregnancy-induced hypertension in the subgroup of women who developed a hypertensive disorder in pregnancy. Nulliparous women of Surinamese ethnicity, low maternal birth weight or complaints of headache in early pregnancy had a higher risk to develop a pre-eclampsia. Multiparous women with a high education were more prone to develop pregnancy-induced hypertension.

Table 2. Potential risk factors of pregnancy-induced hypertension for nulliparous women, n=3048.

	Crude‡	Adjusted†	Adjusted#
Risk factor			
Maternal related factor			
Age > 40years	1.02 (0.99-1.05)	1.75 (0.22-14.2)	1.70 (0.21-13.6
BMI > 25kg/m2	2.73 (2.02-3.69)*	2.70 (1.93-3.78)*	2.64 (1.91-3.66)*
European ethnicity	1.74 (1.22-2.48)*	1.94 (1.28-2.94)*	1.94 (1.29-2.93)*
Surinamese ethnicity	1.02 (0.59-1.75)	0.85 (0.44-1.62)	0.92 (0.51-1.66)
High education	0.68 (0.50-0.93)*	0.77 (0.55-1.09)	0.79 (0.56-1.10)
Maternal comorbidity			
Diabetes	0	0	0
Maternal birth weight	1.00 (0.55-1.84)	1.17 (0.62-2.18)	1.14 (0.61-2.11)
Family history			
Hypertensive disorder in family	1.90 (1.35-2.69)*	-	1.54 (1.11-2.14)*
Diabetes in family	1.57 (1.06-2.31)*	1.25 (0.82-1.91)	1.32 (0.88-1.98)
Lifestyle factors			
Smoking	0.93 (0.65-1.33)	0.79 (0.53-1.18)	0.63 (0.34-1.15)
Alcohol	0.58 (0.34-0.98)*	0.67 (0.39-1.15)	0.65 (0.38-1.11)
Coffee	0.75 (0.55-1.03)	0.76 (0.54-1.07)	0.76 (0.54-1.06)
Tea	1.41 (0.73-2.70)	1.39 (0.69-2.79)	1.39 (0.71-2.72)
Pregnancy-associated factors			
Twin pregnancy	0.86 (0.11-6.47)	0.73 (0.09-5.69)	0.74 (0.10-5.67)
Headache in first trimester	0.89 (0.63-1.27)	0.81 (0.55-1.20)	0.86 (0.59-1.25)
Paternal related factors			
Paternal hypertension	0.93(0.33-2.58)	0.84 (0.26-2.78)	0.84 (0.26-2.75)
Diabetes	0	0	
Arthralgia	0	0	
Paternal birth weight	1.38 (0.65-2.94)	1.39 (0.57-3.37)	1.28 (0.53-3.06)
Hypertension family	1.19 (0.83-1.71)	1.13 (0.76-1.68)	1.16 (0.79-1.71)
BMI > 25kg/m2	1.44 (1.05-1.98)*	1.13 (0.79-1.62)	1.17 (0.82-1.65)

Results from simple and multiple logistic regression analyses. Data are odds ratios (95% Confidence Interval)
‡ Crude: unadjusted
†Adjusted: adjusted for body mass >25kg/m2 and hypertensive disorders in family
#Adjusted for age>40, body mass>25, hypertensive disorders in family
*P<0.05.

Table 3. Potential risk factors of pregnancy-induced hypertension for multiparous women, n=1901.

Risk factor	Crude‡	Adjusted†	Adjusted #	Adjusted^
Maternal related factor				
Age > 40years	0	0	0	0
BMI > 25kg/m2	3.15 (1.68-5.93)*	2.96 (1.48-5.93)*	3.52 (1.73-7.16)*	2.68 (1.32-5.42)*
European ethnicity	1.69 (0.90-3.19)	1.51 (0.75-3.02)	1.46 (0.73-2.92)	1.79 (0.86-3.71))
Surinamese ethnicity	0.24 (0.03-1.73)	0	0	0
High education	0.79 (0.42-1.48)	0.75 (0.38-1.49)	0.74 (0.37-1.50)	0.86 (0.41-1.79)
Previous pre-eclampsia	4.62 (1.34-15.9)*	0.84 (0.22-3.22)	-	0.99 (0.19-5.11)
Previous pregnancy-induced hypertension	10.0 (5.25-19.1)*	-	9.43 (4.54-19.6)*	7.41 (3.24-17.0)*
Hypertensive disorder in pregnancy in history	9.55 (5.02-18.2)*	-	-	8.30 (4.16-16.5)*
Maternal comorbidity				
Diabetes	0	0	0	
Maternal birth weight	0.41 (0.06-3.02)	0.34 (0.05-2.57)	0.45 (0.06-3.36)	0.42 (0.05-3.22)
Family history				
Hypertensive disorder in family	1.87 (0.95-3.66)	1.05 (0.50-2.19)	1.35 (0.66-2.78)	1.11 (0.56-2.20)
Diabetes in family	1.11 (0.51-2.44)	0.97 (0.41-2.27)	0.97 (0.40-2.36)	0.79 (0.32-1.99)
Pregnancy-associated factors				
Twin pregnancy	2.15 (0.28-16.4)	3.27 (0.38-28.3)	2.87 (0.37-22.3)	3.45 (0.36-33.2)
Headache in first trimester	0.97 (0.50-1.89)	1.02 (0.50-2.06)	1.21 (0.59-2.45)	0.91 (0.45-1.86)
Paternal risk factors				
Paternal hypertension	1.36 (0.18-10.4)	1.05 (0.12-8.92)	1.40 (0.17-11.2)	0.99 (0.11-8.70)
Diabetes	0	0	0	0
Arthralgia	0	0	0	0
Paternal birth weight	0	0	0	0
Hypertension family	0.87 (0.38-2.00)	0.98 (0.41-2.35)	1.17 (0.49-2.82)	1.03 (0.41-2.54)
BMI > 25kg/m2	1.51 (0.79-2.91)	1.47 (0.74-2.92)	1.25 (0.63-2.49)	1.14 (0.56-2.31)

Results from simple and multiple logistic regression analyses. Data are odds ratios (95% Confidence Interval)
‡ Crude:unadjusted
†Adjusted: adjusted for PIH in a previous pregnancy
#Adjusted: adjusted for pre-eclampsia in a previous pregnancy
^Adjusted for age>40, body mass>25, hypertensive disorders in family and PIH or pre-eclampsia in a previous pregnancy
*P<0.05.

Table 4. Potential risk factors of pre-eclampsia for nulliparous women, n=2965.

Risk factor	Crude‡	Adjusted†	Adjusted#	Adjusted^
Maternal related factor				
Age > 40 years	3.38 (0.77-14.8)	3.84 (0.86-17.1)	5.03 (1.09-23.3)*	4.27 (0.80-22.9)
BMI > 25kg/m2	2.84 (1.22-6.61)*	-	2.57 (1.66-3.96)*	2.18 (1.40-3.41)*
European ethnicity	0.72 (0.48-1.09)	0.78 (0.51-1.17)	0.69 (0.45-1.06)	0.68 (0.44-1.06)
Surinamese ethnicity	2.21 (1.27-3.84)*	1.96 (1.12-3.43)*	1.94 (1.09-3.48)*	1.82 (0.97-3.40)*
High education	0.60 (0.39-0.92)	0.78 (0.51-1.18)	0.76 (0.50-1.17)	0.73 (0.47-1.15)
Maternal comorbidity				
Diabetes	6.88 (1.44-32.8)*	5.21 (1.06-25.6)*	3.79 (0.66-21.7)	5.66 (1.01-31.9)*
Hypertension	13.9 (6.63-29.1)*	4.66 (1.80-12.0)*	-	7.86 (3.35-18.4)*
Maternal birth weight	2.94 (1.69-5.11)*	3.01 (1.72-5.26)*	2.98 (1.67-5.31)*	2.61 (1.42-4.78)*
Family history				
Hypertensive disorder in family	1.46 (0.91-2.33)	1.38 (0.86-2.21)	1.24 (0.76-2.02)	1.24 (0.79-1.93)
Diabetes in family	1.38 (0.77-2.44)	1.29 (0.76-2.20)	1.28 (0.73-2.24)	1.23 (0.70-2.18)
Pregnancy-associated factors				
Twin pregnancy	5.13 (1.48-17.7)*	4.55 (1.29-16.0)*	5.13 (1.43-18.4)*	5.22 (1.46-18.7)*
Headache in first trimester	1.47 (0.96-2.25)	1.38 (0.90-2.11)	1.35 (0.87-2.10)	1.28 (0.81-2.03)
Paternal factors				
Paternal hypertension	2.66 (0.93-7.62)	2.15 (0.75-6.15)	2.30 (0.79-6.71)	2.44 (0.81-7.41)
Diabetes	4.05 (0.51-32.2)	3.12 (0.38-25.3)	3.80 (0.46-31.1)	2.66 (0.25-28.0)
Arthralgia	4.97 (0.61-40.5)	3.57 (0.43-29.7)	4.43 (0.51-38.4)	6.69 (0.74-60.8)
Paternal birth weight	1.03 (0.24-4.35)	0.86 (0.20-3.64)	0.58 (0.08-4.34)	1.00 (0.23-4.38)
Hypertension family	1.15 (0.63-2.08)	1.08 (0.63-1.88)	1.15 (0.65-2.03)	1.11 (0.62-1.97)
BMI > 25kg/m2	0.79 (0.48-1.30)	0.72 (0.45-1.15)	0.72 (0.44-1.18)	0.75 (0.45-1.25)

Results from simple and multiple logistic regression analyses. Data are odds ratios (95% Confidence Interval)
‡ Crude: unadjusted
†Adjusted: adjusted for body mass index
#Adjusted: adjusted for body mass index and chronic hypertension
^Adjusted for age>40, body mass>25, hypertensive disorders in family and chronic hypertension
*P<0.05.

Table 5. Potential risk factors of pre-eclampsia (1.4%) for multiparous women, n=1880.

Risk factor	Crude‡	Adjusted†	Adjusted^
Maternal related factor			
Age > 40years	2.37 (0.31-18.08)	4.55 (0.57-36.0)	3.43 (0.34-34.7)
BMI > 25kg/m2	2.67 (1.14-6.28)*	3.58 (1.24-10.3)*	2.74 (0.90-8.33)
European ethnicity	0.30 (0.11-0.77)*	0.25 (0.08-0.73)*	0.27 (0.09-0.85)*
Surinamese ethnicity	2.51 (0.84-7.57)	2.82 (0.88-9.00)	2.34 (0.62-8.77)
High education	0.23 (0.07-0.79)*	0.26 (0.07-0.93)*	0.28 (0.06-1.30)
Previous pre-eclampsia	11.6 (3.15-42.4)*	12.4 (3.31-46.0)*	0
Previous pregnancy-induced hypertension	9.94 (3.64-27.2)*	10.1 *(3.31-30.6)	6.89 (1.97-24.1)*
Hypertensive disorder in pregnancy in history	12.2 (4.75-31.3)*	-	7.51 (2.45-23.0)*
Maternal comorbidity			
Diabetes	0	0	0
Hypertension	15.0 (4.00-55.9)*	9.54 (2.11-43.1)*	6.33 (1.31-30.5)*
Maternal birth weight	0.90 (0.11-6.88)	0.92 (0.12-7.13)	0.96 (0.12-7.87)
Family history			
Hypertensive disorder in family	5.11 (1.10-23.7)*	4.18 (0.88-19.9)	1.49 (0.52-4.26)
Diabetes in family	2.22(0.76-6.56)	1.63(0.52-5.21)	1.78(0.58-5.44)
Pregnancy-associated factors			
Twin pregnancy	4.03 (0.51-31.7)	8.20 (0.97-69.5)	7.01 (0.66-74.4)
Headache in first trimester	2.29 (0.96-5.98)	2.74 (1.02-7.33)*	1.54 (0.54-4.37)
Paternal related factors			
Paternal hypertension	0	0	
Diabetes	0	0	
Arthralgia	32.1 (3.22-319)*	32.2 (3.18-325)*	10.0 (0.64-156)
Paternal birth weight	0	0	
Hypertension family	0.26 (0.03-2.16)	0.30 (0.04-2.61)	0
BMI > 25	1.89 (0.57-6.31)	2.34 (0.60-9.19)	1.84 (0.40-8.41)

Results from simple and multiple logistic regression analyses. Data are odds ratios (95% Confidence Interval).
‡ Crude: unadjusted
†Adjusted: adjusted for pre-eclampsia in a previous pregnancy
^Adjusted for age>40, body mass>25, hypertensive disorders in family and chronic hypertension and hypertensive disorder in previous pregnancy
*P<0.05.

Table 6. Potential risk factors for pre-eclampsia versus pregnancy-induced hypertension for nulliparous and multiparous.

Risk factor	Nulliparous N=285	Multiparous N=73
Maternal related factors		
Age > 40years	3.70 (0.33-41.3)	0
BMI > 25kg/m2	0.98 (0.60-1.60)	0.90 (0.32-2.57)
European ethnicity	0.41 (0.24-0.71)*	0.20 (0.07-0.61)*
Surinamese ethnicity	2.17 (1.02-4.62)*	10.0 (1.05-95.7)*
High education	1.01 (0.61-1.67)	0.29 (0.07-1.14)
Previous pre-eclampsia	-	2.50 (0.45-13.9)
Previous pregnancy-induced hypertension	-	1.14 (0.37-3.53)
Hypertensive disorder in pregnancy in history	-	1.28 (0.42-3.88)
Maternal comorbidity		
Diabetes	0	0
Hypertension	2.20 (0.65-7.42)	
Family history		
Maternal birth weight	2.94 (1.32-6.50)*	2.06 (0.12-35.1)
Hypertensive disorder in family	0.77 (0.43-1.36)	2.74 (0.52-14.6)
Diabetes in familiy	0.93 (0.49-1.77)	1.82 (0.49-6.74)
Pregnancy-associated factors		
Twin pregnancy	5.96 (0.61-58.3)	1.87 (0.11-32.0)
Headache in first trimester	1.65 (0.96-2.84)*	2.46 (0.80-7.54)
Paternal related factors		
Paternal hypertension	2.46 (0.59-10.2)	0
Diabetes	0	
Arthralgia	0	0
Paternal birth weight	0.62 (0.13-3.02)	
Hypertension family	0.91 (0.48-1.74)	0.30 (0.03-2.87)
BMI > 25kg/m2	0.60 (0.35-1.04)	1.41 (0.37-5.39)

Results from simple logistic regression analyses. Data are odds ratios (95% Confidence Interval).

*P<0.05.

DISCUSSION

An increased BMI was associated with development of pregnancy-induced hypertension and pre-eclampsia in nulliparous and multiparous women as described in tables 1-4. Maternal low birth weight was associated with an increased risk for pre-eclampsia in

nulliparous women. Previous hypertensive disorders were associated with pregnancy-induced hypertension and pre-eclampsia in multiparous women. Headache in early pregnancy was associated with development of pre-eclampsia in multiparous women. Determination of risk factors for gestational hypertensive disorders is important to further unravel the pathophysiology and for risk selection to provide adequate antenatal care tailored to the individual. Modifiable risk factors known before conception include obesity and advanced maternal age. More healthy women before pregnancy will result in more healthy pregnancies and less maternal morbidity and mortality.

The findings of our study confirm the association between an increased BMI and pregnancy-induced hypertension and pre-eclampsia[14,15]. With respect to the association of an increased BMI and pre-eclampsia, Athukorala et al. demonstrated an increased risk of pre-eclampsia amongst obese women defined by a BMI greater than 30kg/m^2, but just a trend towards increasing risk in the overweight group versus a normal weight[16]. Another previous study described that an increased BMI is one of the strongest risk factors for pre-eclampsia and Catov et al. described a linear association between an increasing BMI and pre-eclampsia[117,18,19]. The mechanisms explaining that an increased BMI predispose to pregnancy-induced hypertension and pre-eclampsia have not been well studied. An increased BMI is characterized by a systemic inflammatory response[20]. Several of the metabolic and inflammatory disorders that characterise an increased BMI have been documented in pregnant women[21]. An inflammatory response is amplified in obese individuals because of the pro-inflammatory output from adipose tissue and this may account for the increased susceptibility of obese pregnant women to fail the stress test of pregnancy, clinically manifested by pre-eclampsia. Other important mediators include oxidative stress, insulin resistance, reduced immune function, reduced endothelial dysfunction, other markers of dyslipidemia, or lifestyle factors such as poor prenatal diet and prenatal physical inactivity. Bodmar et al. described that pathways may also vary by ethnicity for development of pregnancy-induced hypertension or pre-eclampsia[22].

Maternal low birth weight associated with pre-eclampsia supports the hypothesis of fetal origin of an adult disease. There is evidence to link low birth weight with endothelial dysfunction in adults especially in hunger winter studies[23]. Low birth weight is associated with cardiovascular diseases in later life as well[24]. Maternal predisposition for cardiovascular disease might contribute to malplacentation and subsequent subclinical

maternal endothelial dysfunction might predispose to the development of pre-eclampsia. With respect to pregnancy-induced hypertension, Rasmussen described in a population-based study an association between maternal low birth weight and both pregnancy-induced hypertension or pre-eclampsia[25]. Wikstrom et al. showed an increased risk of developing cardiovascular disease after gestational hypertension[26]. The absolute risk for pregnancy-induced hypertension (odds ratio 1.6 (95%CI 1.3-2.0)), however, was small compared to risks in women after severe gestational hypertensive disorders (odds ratio 2.8 (95%CI 2.3-3.7)) or recurrent hypertensive disorders (odds ratio 2.8 (95%CI 2.0-3.9)). Secondly, maternal low birth weight and pre-eclampsia may share a genetic origin[27], in view of the evidence of intergenerational recurrence and increased risk of recurrence in subsequent pregnancies of placental dysfunction related disorders[25].

We demonstrated a strong association between previous gestational hypertension and pre-eclampsia, and the risk of pregnancy induced hypertension and pre-eclampsia in multiparous women up to a ten fold increased risk for pre-eclampsia. This supports the idea that the first pregnancy is a stress-test for later life: women who fail the test, i.e. develop hypertension during pregnancy are at increased risk for development of hypertensive diseases in a subsequent pregnancy and of cardiovascular events later in life due to an unfavorable risk factor profile[28,29].

European ethnicity was associated with an increased risk for pregnancy-induced hypertension in nulliparous women and a lower risk for pre-eclampsia in multiparous women. In addition, Bouthoorn et al. demonstrated substantial differences in blood pressure levels and pregnancy hypertensive disorders in a multiethnic society[30]. These differenties could not be explained bij education or lifestyle. These results suggest different mechanisms in development of pregnancy-induced hypertension and pre-eclamspia.

High education is suggested to reflect a protective effect for the development of pre-eclampsia in multiparous women. Education has been the strongest and most consistent socioeconomic predictor of cardiovascular disease[31]. Because low socio-economic status is a marked risk factor for an increased BMI, metabolic syndrome, hypertension and cardiovascular disease[32-34], socio-economic status is also likely to contribute to the prevalence of hypertensive disorders in pregnancy[35,36].

Headache in early pregnancy appeared to be associated with pre-eclampsia in multiparous women. This association could not be explained by maternal age, migraine,

consuming of coffee or an increased BMI (results not shown). Migraine and pre-eclampsia may represent a common pathological link due to abnormal vascular reactivity, increased platelet agreeability, and high underlying cardiovascular risk profile[37]. A positive association between headaches and pregnancy-induced hypertension or pre-eclampsia was reported in 14 out of 16 studies[38]. Facchinetti et al. described in a prospective cohort study a three fold increased risk of gestational hypertensive disorders in case of migraine[39].

The association of paternal arthralgia with pre-eclampsia might be coincidence. We only found two cases with men with arthralgia who fathered a pregnancy complicated by pre-eclampsia. However, others reported that men who had fathered a pre-eclamptic pregnancy in one woman had an increased risk of fathering a pre-eclamptic pregnancy in another woman[40]. Paternal genes could be passed to the fetus and increase the risk of pre-eclampsia[41,42]. Both maternal and paternal contributions to fetal genes may have a role in defective placentation. Women with rheumatologic diseases are more likely to have pre-eclampsia than women without rheumatologic disease (8.8% and 2.3% respectively)[43].

Both nulliparous and multiparous women who develop pregnancy-induced hypertension partly share risk factors with women who develop pre-eclampsia. In women with an underlying predisposition such as chronic hypertension, an increased BMI, type II diabetes or metabolic syndrome a pregnancy may cause a maternal syndrome in a highly susceptible individual. The origins of the pre-eclampsia lie in events in very early pregnancy[44]. Ethnicity and maternal low birth weight may be a sign for such underlying predisposition[44]. This is in line with previous literature describing several phenotypes of pre-eclampsia as a result of different linking mechanisms between placental stage and the second stage of maternal systemic disease[1]. It is apparent that changes relevant to pre-eclampsia can be detected in the first trimester, long before the failed vascular remodelling necessary to reduce placental perfusion is completed[45]. Furthermore, it is evident that linkage is not likely to be one factor but several, different for different women, resulting in several phenotypes of the disease.

Methodological considerations

The strength of the Generation R study is that it is a population-based cohort with a large number of women prospectively included. The study examines these women and their children from early pregnancy onwards. The diagnoses of gestational hypertensive disorders in the Generation R Study were verified. All women were living in a specific

area, enabling to manually check all medical reports of women with hypertension disorders in pregnancy in systematic way[12]. However, a limitation of this study is that some data were collected using questionnaires. Although commonly used in epidemiological studies, self-reported information on complications in (previous) pregnancies, chronic diseases, disorders in the family and lifestyle determinants may have introduced some misclassifications[11]. However, some limitations should be addressed such as The Generation R‘ response rate of 61% implicating that selective participating may have influenced the observed associations[46]. There was some selection towards a relatively high educated, and healthier study population[47]. Secondly, even though we were able to control for a large number of potential confounders residual confounding is always reason of concern and should be taken into account.

Conclusion

Several easily accessible risk factors in early pregnancy for the development of subsequent pregnancy-induced hypertension or pre-eclampsia were identified. Some risk factors in early pregnancy seem to apply to both pregnancy-induced hypertension and pre-eclampsia whereas others differ between these two gestational complications, as well as between nulliparous and multiparous women. This may point at differences in the pathophysiology of the two disorders.

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Chapter 2.2

Low socioeconomic status is a risk factor for pre-eclampsia.

Chapter 2.2

Low socioeconomic status is a risk factor for pre-eclampsia: The Generation R Study.

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ABSTRACT

Objectives: To examine whether maternal socioeconomic status, as indicated by maternal educational level, is associated with pre-eclampsia, and if so, to what extent known risk factors for pre-eclampsia mediate the effect of educational level.

Methods: In the Generation R Study, a population-based cohort study, we examined data of 3547 pregnant women. Odds ratios (OR) of pre-eclampsia for low, mid-low and mid-high educational level compared to high educational level were calculated after adjustment for confounders and additional adjustment for a selection of potential mediators (family history, material factors, psychosocial factors, substance use, working conditions, pre-existing medical conditions, maternal arthropometrics and blood pressure at enrollment) that individually caused more than 10% change in the OR for low education.

Results: Adjusted for the confounding effects of age, gravidity and multiple pregnancy, women with low educational level were more likely to develop pre-eclampsia (OR 5.12; 95% CI: 2.20,11.93) than women with high educational level. After additional adjustment for financial difficulties, smoking in pregnancy, working conditions, body mass index and blood pressure at enrollment, the OR 4.91; 95% CI: 1.93,12.52).

Conclusions: Low maternal socioeconomic status is a strong risk factor for pre-eclampsia. Only a small part of this association can be explained by the mediating effects of established risk factors for pre-eclampsia. Further research is needed to disentangle the pathway from low socioeconomic status to pre-eclampsia.

INTRODUCTION

Pre-eclampsia, marked by hypertension and proteinuria, is a leading cause of perinatal and maternal morbidity and mortality and complicates 5-7% of first pregnancies and 1-3% of all pregnancies [1-4]. The exact pathogenesis is unknown, but it has been suggested that pre-eclampsia may be an early adult manifestation of the metabolic syndrome[5]. This is based on observations that the metabolic abnormalities in pre-eclampsia resemble those in the metabolic syndome[6] and that women with a history of pre-eclampsia have an increased risk for development of cardiovascular disease later in life[7,8].

The known risk factors for pre-eclampsia are age above 35 years, nulliparity, history of pre-eclampsia in previous pregnancies, family history of pre-eclampsia, multiple pregnancy, pre-existing medical conditions such as diabetes, gestational diabetes, time between pregnancies, high body mass index and high blood pressure (BP) in early pregnancy[9,10]. Psychosocial stressors and strenuous working conditions have also been associated with an increased risk for pre-eclampsia[11,12]. Surprisingly, smoking has been shown to reduce the risk for pre-eclampsia[13]; the underlying mechanism is unknown. Low socioeconomic status is a marked risk factor for obesity, high BP, the metabolic syndrome and cardiovascular disease[14-17], and may also be associated with an increased risk for pre-eclampsia. However, only few studies of pre-eclampsia have evaluated its association with maternal socioeconomic status, and showed inconsistent results[10, 18-23]: some have found socioeconomic circumstances tob e negatively associated with pre-eclampsia[18-20], others have found no association[10, 21-23].

Within the framework of the Generation R Study, a large prenatally recruited birth cohort study with extensive assessments during pregnancy[24], we examined the association between socioeconomic status and pre-eclampsia. We used maternal education as an indicator of socioeconomic status as it has been described as the most consistent socioeconomic predictor of cardiovascular disease risk factors[25]. The present study was restricted to an ethnic homogeneous population, as literature indicates that prevalence of pre-eclampsia and its risk factors[26], as well as socioeconomic disparities in pre-eclampsia[20] may differ by ethnic groups.

We also evaluated whether a possible association can be explained by the mediating effect of known risk factors for pre-eclampsia, including family history of hypertensive complications in pregnancy, material factors, psychosocial factors, substance

use, working conditions, pre-existenting medical conditions, maternal anthropometrics and BP at enrollment.

METHODS

Design

The present study was embedded in The Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study was designed to identify early environmental and genetic determinants of growth, development and health, and has been described previously in detail[24-27]. Briefly, the cohort includes 9778 mothers and their children (response rate 61%) of different ethnicities living in Rotterdam, the Netherlands[27]. Enrollment was aimed in early pregnancy, but was possible until birth of the child. Assessments in pregnancy, including physical examinations, ultrasound assessments and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam. Written consent was obtained from all participants.

Study population

All pregnant women who were resident in the study area at their delivery date from April 2002 until January 2006 were invited to participate. Of the total of 9778 enrolled women, 91% (n=8880) were enrolled in pregnancy[27]. Women with a Dutch ethnicity (n=1457, 45.7%) comprised the largest ethnic subgroup and were selected for present analyses. A women was of Dutch ethnicity, when she reported that both her parents were born in the Netherlands[28]. Of the women who participated with more than one pregnancy in this study (8.3%), data on the second (n=332) or third pregnancy (n=5) were excluded from analyses to avoid clustering. We excluded women with missing information on their educational level (n=21), cases of induced abortions (n=14), fetal death before 20 weeks of gestation (n=7), women lost to follow-up (n=3), and women without information on of pre-

eclampsia (n=72), gravidity (n=5), anthropometrics (n=17), or BP at enrollment (n=34), leaving 3547 women for analyses.

Socioeconomic status

The highest educational level achieved by mother was used as an indicator of maternal socioeconomic status. Maternal education was assessed by questionnaire at enrollment, according to the Dutch standard classification[29], and was categorized into four educational levels: high (university or PhD degree), mid-high (higher vocational training), mid-low (more than 3 years general secondary school, intermediate vocational training or first year of higher vocational training), and low education (no education, primary school, lower vocational training or intermediate general school or 3 years or less general secondary school).

Pre-eclampsia

After each delivery, the present community midwife or obstetrician completed a delivery report. According to the Dutch standards of antenatal care, all women whose pregnancies are complicated by pre-eclampsia should deliver in a hospital under medical supervision of an obstetrician. The delivery reports of study participants who delivered under medical supervision were retrieved and screened by a trained medical record abstractor. Based on the documentation of any kind of hypertensive complications or fetal growth retardation on the delivery report, 398 women were suspected to have pre-eclampsia. To confirm presence of pre-eclampsia, the same abstractor conducted detailed reviews of hospital charts of these women. Pre-eclampsia was defined according to the criteria described by the International Society for the Study of Hypertension in Pregnancy (ISSHP): development of systolic BP at least 140 mmHg and/or diastolic BP at leat 90 mmHg after 20 weeks of gestation in a previously normotensive woman and proteinuria (defined as two or more dipstick readings of 2+ or greater, one cathether sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein)[30]. Neither women with eclampsia nor women with hemolysis, elevated liver enzyme and low platelet syndrome were defined as cases.

Potential confounders and mediators

Information on all factors was collected during pregnancy. Categories are indicated in parentheses.

Potential confounders

The following risk factors were considered to potentially confound the effect of maternal education on pre-eclampsia.

General characteristics. Maternal age was assessed at enrollment in one of the research centres and categorized into three groups (<30 years, 30-35 years, ≥35 years). Gravidity (primigravida, multigravida) was obtained by questionnaire. Presence of multiple pregnancy (singleton pregnancy, twin pregnancy) was determined by fetal ultrasound in early pregnancy.

Potential mediators

The known risk factors for pre-eclampsia may be in the pathway from socioeconomic status to pre-eclampsia were considered potential mediators.

Family history. Information about history of gestational hypertension (no, yes, do not know) and pre-eclampsia (no, yes, do not know) in a first-degree relative was retrieved from questionnaire.

Material factors. Employment status (not employed, part-time employed, fulltime employed), and presence of financial difficulties in the preceding year (no, yes) were assessed by questionnaire.

Psychosocial factors. Presence of long-lasting difficulties (score in tertiles) was measured by questionnaire with a 12 item-checklist covering financial problems, social deprivation, neighbourhood problems and problems in relationships[31]. Maternal psychopathology was assessed by questionnaire using the Global Severity Index (score in tertiles) of the Brief Symptom Inventory[32].

Substance use. Smoking and alcohol consumption (never, before pregnancy, until pregnancy known, continued in pregnancy) were assessed by questionnaire.

Working conditions during pregnancy. Through the questionnaire in mid-pregnancy, participants were asked whether (yes, no) they had been exposed to the following working conditions in the preceding three months: prolonged sitting, prolonged working behind a monitor screen, these two were defined as sedentary working conditions, prolonged standing, prolonged walking, prolonged working in a warm environment, lifting or carrying loads of 5 kilograms or more, lifting or carrying loads of 25 kilograms or more, these were defined as physically demanding working conditions, and prolonged vehicle driving and nightshifts.

Medical conditions at enrollment. Presence of pre-existent diabetes and raised cholesterol (no, yes, do not know) were assessed by questionnaire at enrollment.

Anthropometrics at enrollment. Maternal anthropometrics were assessed in one of the research centres at enrollment. Height and weight were measured without shoes and heavy clothing. Body mass index (BMI) was calculated from height and weight (weight/height^2) and categorized into normal weight (<25 kg/m^2), overweight (25-30 kg/m^2), and obese (≥30 kg/m^2) according to WHO standards. Systolic and diastolic blood pressure were measured using an Omron 907® Automated Blood Pressure Monitor (Omron Healthcare, Europe B.V. Hoofddorp, The Neteherlands). BMI and BP values were adjusted for gestational age at time of measurement.

Statistical analyses

We assessed the frequency distributions of pre-eclampsia and risk factors for pre-eclampsia according to educational level. To test the trend across educational level, chi-square tests for trend were used for categorical factors and one-way analysis of variance for continuous factors.

Multivariate logistic regression was used to calculate the odds ratios (OR) of pre-eclampsia and their 95% confidence intervals (CI) for levels of education, adjusted for the potential confounding effects of age, gravidity and multiple pregnancy, and additionally adjusted for potential mediators. The highest educational level was set as reference. Missing data on categorical factors were included in the analyses as a separate category.

The conceptual hierarchical framework

To take into account the interrelations between maternal education and potential mediators, a conceptual hierarchical framework was developed[35].

(1) Hierachical levels of maternal education and potential mediators:
   (a) Hierarchical level 1: Maternal education
   (b) Hierarchical level 2: Family history of hypertensive disorders in pregnancy
   (c) Hierarchical level 3: Material and psychosocial factors
   (d) Hierarchical level 4a: Substance use and working conditions during pregnancy
   (e) Hierarchical level 4b: Medical conditions, anthrpometrics and bloed pressure at enrollment
(2) Outcome: pre-eclampsia

We hypothesized maternal education (as seen in listed above) to be the most distal factor that may directly or indirectly determine all proposed mediators. The next hierarchical level (hierarchical level 2) comprises family history, which is partly determined by socioeconomic status. Hierarchical level 3 included material and psychosocial factors, which are partly determined by maternal education. Hierarchical level 4 included substance use, working conditions during pregnancy, medical conditions, anthropometrics and BP at enrollment, which are all partly determined by maternal education, psychosocial and material factors. As substance use and working conditions may affect BP[36, 37], hierarchical level 4 was divided into 2 sublevels: hierarchical level 4a (substance use and working conditions during pregnancy) and hierarchical level 4b (medical conditions and anthropometrics at enrollment).

Hierarchical logistic models

We started with model 1, which represented the overall effect of maternal education. To evaluate the individual mediating effects of all potential mediators, these factors were added separately to model 1. For each adjustment, the percentage change in OR for the educational levels with an increased risk for pre-eclampsia was calculated ($100x[OR_{model\ 1} - OR_{+mediator}]/[OR_{model\ 1} - 1]$). We defined factors that caused an attenuation of the OR as

mediator, and factors that caused an increase of the OR as suppressor in the association between maternal education and preeclampsia[38].

Next, hierarchical logistic models were built. Starting with model 1, factors from the next hierarchical levels were added stepwise. Only those factors that individually produced at least 10 percent change[39] in the OR for the educational level with the highest risk were included. Because BMI may affect pre-eclampsia risk through an increased BP[40], BP was added to the logistic models in a separate step.

All analyses were performed using Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Of the 3547 women in this study, mean age was 31.2 years (sd 4.6); 34.7% were younger than 30 years and 18.0% were 35 years or older. Of these women, 54.4% were primigravida. The median gestational age at enrollment was 13.8 weeks (90% range: 10.9 - 21.9). The median gestational age at delivery was 40.1 weeks (90% range: 36.7 - 42.1); the newborns had a mean birth weight of 3471 grams (sd 563.4).

Of all women, 17.6% were low educated and 31.5% were high educated (Table 1). Fifty-one women (1.5%) developed pre-eclampsia; this percentage was 0.8%, 0.8%, 2.1% and 2.9% for women with high, mid-high, mid-low and low education respectively (p for trend < 0.001, table 1).

Age, employment status, family history of hypertension in pregnancy, alcohol consumption during pregnancy, sedentary working conditions, prolonged vehicle driving (p for trend < 0.001) and night shifts (p for trend < 0.05) were positively associated with level of education (see also table 1). Gravidity, family history of pre-eclampsia, financial difficulties, long lasting difficulties, psychopathology, smoking during pregnancy, physically demanding working conditions, BMI and BP (p for trend < 0.001) and pre-existent diabetes (p for trend < 0.05), were negatively associated with the level of education (see also table 1).

Table 1 Distribution of pre-eclampsia and a selection of risk factors by maternal educational level (n = 3547)

	Level of maternal education					
	Total n=3547	High n=1118 (31.5%)	Mid-high n=885 (25.0%)	Mid-low n=918 (25.9%)	Low n=626 (17.6%)	P for trend[a]
Pre-eclampsia (%)	1.5	0.8	0.8	2.1	2.9	<0.001
General characteristics						
Age						
<30 years (%)	34.7	16.3	30.2	46.8	56.2	
30-35 years (%)	47.3	61.6	49.8	38.9	30.7	<0.001
≥ 35 years (%)	18.0	22.1	20.0	14.3	13.1	
Gravidity						
Primigravidity	54.4	56.7	56.5	56.6	43.9	<0.001
Multiple pregnancy						
Twin pregnancy	1.4	1.3	1.7	1.6	1.0	0.69
Material factors						
Financial difficulties						
Yes (%)	10.6	4.2	8.0	12.7	22.7	<0.001
Missing (%)	12.2	6.8	6.4	13.8	27.8	
Substance use						
Smoking						
Never (%)	49.0	59.7	52.9	45.1	30.0	
Before pregnancy (%)	19.4	20.2	21.1	19.1	15.8	
Until pregnancy known (%)	8.1	7.5	9.1	9.0	6.4	<0.001
Continued in pregnancy (%)	17.1	5.2	10.3	20.7	42.5	
Missing (%)	6.5	7.4	6.6	6.1	5.3	
Working conditions						
Prolonged sitting						
Yes (%)	69.3	86.2	76.5	62.9	38.7	<0.001
Missing (%)	11.0	6.4	6.3	12.6	23.2	
Prolonged working behind a monitor screen						
Yes	60.6	82.0	62.9	53.5	29.4	<0.001
Missing (%)	11.1	6.6	6.6	12.6	23.0	
Prolonged walking (%)						
Yes (%)	41.1	30.1	44.3	47.1	47.4	<0.001
Missing (%)	11.0	6.7	6.1	12.5	23.2	
Prolonged vehicle driving						
Yes (%)	13.5	19.3	15.0	9.4	6.9	<0.001
Missing (%)	10.9	6.4	6.0	12.7	23.0	
Anthropometrics and BP at enrollment						
BMI[b]						
Normal weight (%)	67.4	76.9	73.1	60.2	52.6	
Overweight (%)	23.5	19.6	21.7	26.2	29.2	<0.001
Obese (%)	9.1	3.5	5.2	13.6	18.2	
Systolic BP[b] in mmHg (mean, sd)	117.8 (12.3)	116.1 (11.3)	117.1 (11.9)	119.6 (12.9)	119.4 (12.9)	<0.001
Diastolic BP[b] in mmHg (mean, sd)	68.8 (9.5)	68.0 (8.7)	68.4 (9.3)	69.9 (10.0)	69.1 (10.3)	<0.001

BMI, body mass index; BP, blood pressure; SD, standard deviation. [a] P values are derived from χ^2 tests for trend across educational levels (categorical factors) and for (linear) trend component of one-way analysis of variance (continuous factors). [b] Values of body mass index, systolic and diastolic blood pressure at enrolment are adjusted for gestational age at enrolment.

Compared to women with high education, women with low and mid-low education had an increased risk for pre-eclampsia after adjustment for age, gravidity and mutiple pregnancy (model 1, tables 2 and 3), with the highest risk in the lowest educational level (OR 5.12; 95% CI: 2.20,11.93).

Table 2 Change in odds ratios of pre-eclampsia for levels of education after individual adjustment for potential mediators (n=3547).

Level of matenal education	High (ref) (n=1118) OR	Mid-high (n=885) OR (95% CI)	Mid-low (n=918) OR (95% CI)	Change[a] 1	Low (n=626) OR (95% CI)	Change[a] 2
Model 1. (includes material education, age, gravidity and multiple pregnancy)	1.00	1.05 (0.39,2.84)	3.01 (1.34-6.81)		5.12 (2.20,11.93)	
Family history						
Model 1 + family history of hypertension in pregnancy	1.00	1.06 (0.39,2.86)	3.02 (1.33,6.83)	+0.5%	5.23 (1.33,6.83)	+2.7%
Model 1 + family history of pre-eclampsia	1.00	1.05 (0.39,2.84)	2.99 (1.32,6.76)	-1.0%	5.14 (2.20,12.01)	+0.5%
Material factors						
Model 1 + employment status	1.00	1.00 (0.37,2.71)	2.88 (1.26,6.56)	-6.5%	4.96 (2.07,11.89)	-3.9%
Model 1 + financial difficulties	1.00	1.04 (0.38,2.81)	2.91 (1.28,6.60)	-5.0%	4.55 (1.90,10.89)	-13.8%
Psychosocial factors						
Model 1 + long lasting difficulties	1.00	1.04 (0.38,2.82)	2.96 (1.31,6.69)	-2.5%	4.95 (2.11,11.59)	-4.1%
Model 1 + maternal psychopathology	1.00	1.08 (0.40,2.93)	3.12 (1.38,7.08)	+5.5%	5.19 (2.21,12.19)	+1.7%
Substance use						
Model 1 + smoking	1.00	1.06 (0.39,2.88)	3.27 (1.45,7.41)	+12.9%	6.56 (2.77,15.54)	+35.0%
Model 1 + alcohol consumption	1.00	1.02 (0.38,2.78)	2.88 (1.26,6.61)	-6.5%	4.84 (2.10,11.65)	-6.8%
Working conditions						
Model 1 + prolonged sitting	1.00	1.08 (0.40,2.94)	3.21 (1.42,7.29)	+10.0%	5.73 (2.39,13.76)	+14.8%
Model 1 + prolonged working behind a monitor screen	1.00	1.15 (0.42,3.11)	3.33 (1.46,7.57)	+15.9%	6.00 (1.46,7.57)	+21.4%
Model 1 + prolonged standing	1.00	1.12 (0.41,3.04)	3.14 (1.38,7.15)	+6.5%	5.28 (2.22,12.55)	+3.9%
Model 1 + prolonged walking	1.00	1.00 (0.37,2.71)	2.77 (1.22,6.32)	-11.9%	4.42 (1.86,10.50)	-17.0%
Model 1 + prolonged working in a warm environment	1.00	1.11 (0.41,3.01)	3.20 (1.41,7.27)	+9.5%	5,21 (2.20,12.31)	+2.2%

Table 2 continued

Level of matenal education	High (ref) (n=1118) OR	Mid-high (n=885) OR (95% CI)	Mid-low (n=918) OR (95% CI)	Change[a] 1	Low (n=626) OR (95% CI)	Change[a] 2
Working conditions						
Model 1 + lifting or carrying weights > 5 kilograms	1.00	1.04 (0.38,2.81)	2.92 (1.29,6.62)	-4.5%	4.77 (2.02,11.23)	-8.5%
Model 1 + lifting or carrying weights > 25 kilograms	1.00	1.05 (0.39,2.84)	2.95 (1.30, 6.70)	-3.0%	4.79 (2.03,11.30)	-8.0%
Model 1 + prolonged vehicle driving	1.00	1.02 (0.38,2.75)	2.80 (1.23,6.34)	-10.4%	4.51 (1.92,10.64)	-14.8%
Model 1 + night shifts	1.00	1.04 (0.39,2.83)	2.94 (1.30,6.65)	-3.5%	4.72 (2.01,11.12)	-9.7%
Pre-existing medical conditions						
Model 1 + pre-existing diabetes	1.00	1.06 (0.39,2.86)	3.03 (1.34,6.84)	+1.0%	4.96 (2.13,11.59)	-3.9%
Model 1 + pre-existing cholesterol	1.00	1.05 (0.39, 2.83)	3.03 (1.35,6.91)	+2.5%	5.24 (2.25,12.19)	+2.9%
Anthropometrics and BP at enrollment						
Model 1 + BMI	1.00	1.01 (0.37,2.73)	2.54 (1.11,5.82)	-23.4%	4.06 (1.71,9.65)	-25.7%
Model 1 + systolic BP	1.00	1.01 (0.37,2.74)	2.73 (1.20,6.21)	-13.9%	4.68 (2.00,10.96)	-10.7%
Model 1 + diastolic BP	1.00	1.01 (0.37,2.74)	2.71 (1.19,6.15)	-14.9%	4.68 (1.99,11.00)	-10.7%

Abbreviations: ref, reference category: OR, odds ratio, CI confidence interval, BMI, body mass index; BP, blood pressure.
* Change 1 and change 2 represent the change in odds ratio relative to model 1 for mid-low and low education respectively, after individual adjustment for potential mediators (100 x [ORmodel 1 -OR+mediator]/[ORmodel 1 -1]). Changes in odds ratio for mid-high education are not presented, as there was no increased risk for preeclampsia in the subgroup with mid-high education compared with the subgroup with high education.

Table 3 Hierarchical logistic regression models fitted on pre-eclampsia (n=3547)

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4a OR (95% CI)	Model 4b OR (95% CI)
Maternal education					
High (ref)	1.00	1.00	1.00	1.00	1.00
Mid-high	1.05 (0.39,2.84)	1.04 (0.38,2.81)	1.06 (0.39,2.89)	1.02 (0.37,2.80)	1.02 (0.37,2.80)
Mid-low	3.01 (1.34,6.81)	2.91 (1.28,6.60)	3.19 (1.39,2.89)	2.69 (1.15,6.27)	2.61,1.12,6.08)
Low	5.12 (2.20,11.93)	4.55 (1.90,10.89)	6.32 (2.53,15.74)	5.00 (1.97,12.68)	4.91 (1.93,12.52)
Material factors					
Financial difficulties					
No (ref)		1.00	1.00	1.00	1.00
Yes		1.26 (0.54,2.98)	1.58 (0.66,3.81)	1.46 (0.61,3.54)	1.52 (0.62,3.71)
Missing		1.60 (0.78,3.29)	1.60 (0.32,7.98)	1.40 (0.29,6.82)	1.37 (0.29,6.56)
Substance use					
Smoking					
Never (ref)			1.00	1.00	1.00
Before pregnancy			0.80 (0.37,1.72)	0.81 (0.38,1.76)	0.83 (0.38,1.81)
Until pregnancy known			1.37 (0.58,3.24)	1.44 (0.61,3.42)	1.60 (0.67,3.82)
Continued in pregnancy			0.37 (0.15,0.95)	0.40 (0.16,1.03)	0.45 (0.18,1.16)
Missing			1.21 (0.45,3.27)	1.26 (0.47,3.39)	1.26 (0.46,3.42)
Working conditions					
Prolonged sitting					
No (ref)			1.00	1.00	1.00
Yes			1.32 (0.46,3.78)	1.31 (0.45,3.82)	1.21 (0.41,3.58)
Missing*			-	-	-
Prolonged working behind a monitor screen					
No (ref)			1.00	1.00	1.00
Yes			2.13 (0.83,5.51	2.12 (0.81,5.53)	2.15 (0.81,5.70)
Missing*			-	-	-
Prolonged walking					
No (ref)			1.00	1.00	1.00
Yes			1.65 (0.87,3.13)	1.65 (0.87,3.12)	1.70 (0.90,3.23)
Missing*			-	-	-
Prolonged vehicle driving					
No (ref)			1.00	1.00	1.00
Yes			0.43 (0.13,1.43)	0.43 (0.13,1.42)	0.44 (0.13,1.44)
Missing*			-	-	-

Table 3 continued

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4a OR (95% CI)	Model 4b OR (95% CI)
Anthropometrics and BP at enrollment					
BMI					
Normal weight (ref)				1.00	1.00
Overweight				1.64 (0.86,3.12)	1.32 (0.68,2.58)
Obese				2.71 (1.29,5.68)	1.64 (0.72,3.74)
Systolic BP†					1.00 (0.97,1.02)
Diastolic BP†					1.05 (1.01,1.09)

BMI, body mass index; BP, blood pressure; CI, confidence interval; OR, odds ratio; ref, reference category.
* Due to small or zero cells, results for these categories were invalid. As these effects were not of primary interest they are not presented.
† Values of body mass index, systolic and diastolic blood pressure at enrolment are adjusted for gestational age at enrolment.
Model 1: Maternal education, age, gravidity and multiple pregnancy.
Model 2: Model 1 + financial difficulties.
Model 3: Model 2 + smoking, prolonged sitting, prolonged working behind a monitor screen, prolonged walking, prolonged vehicle driving.
Model 4a: Model 3 + body mass index at enrolment.
Model 4b: Model 3 + body mass index, systolic and diastolic blood pressure at enrolment.

Individual adjustment for financial difficulties, prolonged walking, prolonged vehicle driving, BMI, systolic and diastolic BP at enrollment attenuated the OR for low education by more than 10%, where as adjustment for smoking, prolonged sitting and prolonged working behind a monitor screen increased the OR for low education also by more than 10% (Table 2). These factors were included in the hierarchical logistic models. Financial difficulties, when added to model 1 (model 2, table 3), mediated 14% of the effect of low education (adjusted OR: 4.55; 95% CI:1.90,10.89). The addition of smoking and the selected working conditions in model 3 resulted in an increase of the OR for low education (adjusted OR 6.32; 95% CI: 2.53,15.74), which was mostly due to the effect of smoking; women who continued smoking in pregnancy had a reduced risk for pre-eclampsia (OR 0.37, 95% CI: 0.15, 0.95) compared to never smokers.

In model 4a, BMI at enrollment was added, which mediated 25% of the effect of low education (adjusted OR: 5.00; 95% CI: 1.97,12.68). Adjusted for the other factors in this model, obesity was associated with an increased risk for pre-eclampsia (OR: 2.71; 95% CI: 1.29,5.68). Additional adjustment for systolic and diastolic BP at enrollment in the final model (model 4b) resulted in further mediation, but not elimination, of the effect of low education (OR: 4.91; 95% CI: 1.93,12.52), and partial mediation of the effect of obesity. Diastolic BP at enrollment was significantly associated with pre-eclampsia risk in

this model (OR per mmHg increase: 1.05; 95% CI: 1.01,1.09). The effect of smoking was no longer significant due to additional adjustment for BMI and BP.

DISCUSSION

The present study showed that low-educated pregnant women had a five-fold increased risk for pre-eclampsia compared with high-educated women. Although the effect of low education was in part mediated by financial difficulties, occupational exposure to prolonged walking, prolonged vehicle driving, and BMI and BP at enrollment, the association between education level and pre-eclampsia remained largely unexplained.

Methodological considerations

Present results were based on a population-based prospective cohort study in which a large number of women were enrolled early in pregnancy, and information on numerous potential confounders and mediaters was available. We used medical chart review and applied standard international criteria for a consistent pre-eclampsia definition.

Socioeconomic status refers to the 'social and economicfactors that influence what positions individuals or groups hold whitin the structure of society'[42]. It is a complex and multifactor construct. The most frequently used indicators of socioeconomic status are educational level, income level and occupational class[42,43]. In this study, we used educational level as single indicator of maternal socioeconomic status. Education is an important determinant of employment and economic circumstances, and thus reflects material resources but also noneconomic social characteristics, such as general and health-related knowledge which influences health behaviour, literacy, problem-solving skills and prestige[43,44]. It has been shown to be the strongest and most consistent socio economic predictor of cardiovascular disease risk factors[25]. Additionally, level of education as socioeconomic indicator can be applied to teenage and unemployed mothers, unlike for example occupational class. However, educational level does not entirely capture the material and financial aspects of socioeconomic status[43,44].

Information on maternal education and many of the evaluated risk factors was derived from questionnaires, which may have induced some misclassification. Misclassification of

potential mediating risk factors may have contributed to the lack of explanation of the observed association between maternal education and pre-eclampsia.

Comparison with other studies

The incidence of pre-eclampsia in this cohort was 1.5%, which is lower than that reported in some other studies. A Danish birth cohort study[1], for example, reported an incidence of 3%. This may be due to regional differences in pre-eclampsia incidence, but may also be due to difference in case definition and data collection[9]. For our study, we conducted detailed analyses of hospital charts of all participants with suspected pre-eclampsia, with regard to the strict criteria of hypertension and proteinuria. In contrast, many studies were based on self-reported diagnoses of pre-eclampsia or hospital registries[1].

Our study supports others that found a comparable association between measures of socioeconomic status and pre-eclampsia[18-20]. Haelterman et al.[18] found an OR of pre-eclampsia of 2.3 (95% CI: 1.2,4.4) for women with primary education compared with women with education higher than primary school. The lower magnitude of effect compared with our results is probably due to the difference in the educational composition of the reference category. When we repeated our analyses, after categorizing maternal education into two levels similar to Haelterman et al.[18], we found a comparable effect (OR: 2.47, 95% CI: 0.86, 7.08).

Our study challenged those studies[10,21-23] that did not find an association between socioeconomic status and development of pre-eclampsia. This discrepancy may be attributable do differences in exposure definition or case definition. Lawlor et al.[21] used occupation of the women's partners as indicator of maternal socioeconomic status, which may influence risk for pre-eclampsia differently than maternal education. Parazzini et al.[23] and Savitz and Zhang et al.[22] not only included pre-eclampsia, but also pregnancy-induced hypertension without proteinuria in the outcome definition, leading to a more heterogeneous group.

Mediating and suppressing mechanisms

Part of the observed effect of low education on pre-eclampsia was mediated by higher rates of financial difficulties, occupational exposure to prolonged walking and obesity, higher BP levels at enrollment and lower rates of occupational exposure to prolonged vehicle

driving among low educated women. The effect of vehicle driving on pre-eclampsia has been poorly studied, but emotional stress, of which financial difficulties may be a source[45] and occupational exposure to prolonged walking have been associated with an increased risk for pre-eclampsia[12]. Overactivation of the sympathetic nervous system may be involved in this association[45,46]. However, the effects of these factors on pre-eclampsia were not statistically significant in our study, and further research is necessary to elucidate the underlying mechanism from low socioeconomic status through emotional and physical stress to pre-eclampsia.

BMI at enrollement had the highest mediating effect. Obesity was a significant risk factor for pre-eclampsia, and in trun, more than half the effect of obesity was mediated through BP early in pregnancy. These findings are in line with current hypotheses on the underlying mechanism of how obesity leads to pre-eclampsia; it may act through raised triglyceride levels, increased systemic inflammation and increase in BP from early pregnancy[9, 47]. Even within the normal range of BP, the risk for pre-eclampsia is known to increase with increased BP in early pregnancy[10].
In contrast, part of the effect of low education on pre-eclampsia was suppressed by lower rates of sedentary working conditions and higher rates of continued smoking in pregnancy among low-educated women. These factors partly masked the vulnerability of low-educated women to develop pre-eclampsia. Although the increased risk for pre-eclampsia associated with sedentary working conditions was not significant in our study, our results were comparable with results of a recent study by Saftlas et al[48]. They suggest that women who spend a lot of their work time sitting have a higher risk for pre-eclampsia compared to women who spend less time sitting. Regular physical activity may reduce the risk for pre-eclampsia.

Smoking in pregnancy had the largest suppressing effect on the risk for pre-eclampsia in low-educated women. As described in previous studies[13], we found continued smoking in pregnancy to be protective of pre-eclampsia. The underlying mechanism is unclear, but our findings suggest that the effect of smoking acts partly through changes in BP.

Conclusions and perspectives for future research

We conclude that low socioeconomic status, as indicated by low level of education, is a strong risk factor for pre-eclampsia. Remarkably, this association remains largely unexplained, athough we included a wide range of known risk factors for pre-eclampsia in oru study. This implies that the established risk factors for pre-eclampsia included in this study do not fully capture the underlying pathway by which socioeconomic circumstances affect pre-eclampsia risk. Other potential determinants of pre-eclampsia that were not available for the current study, such as leisure time, physical activity, dietary factors, periodontal health, metabolic factors (e.g. cholesterol and fatty acid levels), parameters of endothelial function and factors related to vascular inflammation (e.g. C-reactive protein), currently unknown risk factors may also contribute to the explanation[6,49-52].

As pre-eclampsia is considered an early adult predictor of cardiovascular disease, our findings extend the literature on socioeconomic inequalities in cardiovascular disease[14] by demonstrating that low socioeconomic status is also associated with pre-eclampsia. The observed socioeconomic gap in pre-eclampsia may represent the emergence of socioeconomic inequalities in cardiovascular disease morbidity and mortality in women. Given the short and long-term adverse health consequences associated with pre-eclampsia, further research is needed to disentangle the pathway from low socioeconomic status to pre-eclampsia. Understanding this association may contribute to an earlier diagnosis and development of effective interventions and may reduce morbidity and mortality from this disease.

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Chapter 2.3

Maternal educational level and risk of gestational hypertension.

Chapter 2.3

Maternal educational level and risk of gestational hypertension; The Generation R Study

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ABSTRACT

We examined wheter maternal educational level as an indicator of socioeconomic status is associated with gestational hypertension. We also examined the extent to which the effect of education is mediated by maternal substance use (i.e., smoking, alcohol consumption and illegal drug use), pre-existing diabetes, anthropometrics (i.e., height and body mass index (BMI)), and blood pressure at enrollment.

This was studied in 3262 Dutch pregnant women participating in the Generation R Study, a population-based cohort study. Level of maternal education was established by questionnaire at enrollment, and categorized into high, mid-high, mid-low and low. Diagnosis of gestational hypertension was retrieved from medical records using standard criteria. Odds ratios (OR) of gestational hypertension for educational levels were calculated, adjusted for potential confounders, and additionally adjusted for potential mediators.

Adjusted for age and gravidity, women with mid-low (OR: 1.52; 95% CI: 1.02,2.27) andlow education (OR: 1.30; 95% CI: 0.80, 2,12) had a higher risk of gestational hypertension than women with high education . Additional adjustment for substance use, pre-existing diabetes, athropometrics and blood pressure at enrollement attenuated these ORs to 1.09 (95% CI: 0.70, 1.69) and 0.89 (95% CI: 0.50, 1.58) respectively. These attenuations were largely due to the effects of BMI and blood pressure at enrollment.

Women with relatively low educational levels have a higher risk of gestational hypertension, which is largely due to higher BMI and blood pressure levels from early pregnancy. The higher risk of gestational hypertension in these women is probably caused by pre-existing hypertensive tendencies that manifested themselves during pregnancy.

INTRODUCTION

Gestational hypertension is associated with perinatal morbidity, including preterm birth and foetal growth retardation[1,2]. It is characterized by de novo hypertension after the twentieth week of pregnancy without proteinuria, and complicates about 7–18% of first pregnancies and 4–9% of all pregnancies[1–5]. Although little is known about the pathophysiology of gestational hypertension, studies have shown that it is associated with features of the metabolic

syndrome[6] and with later development of essential hypertension and cardiovascular disease[7,8]. This suggests that these conditions may have similar pathological mechanisms. Known risk factors for gestational hypertension are high maternal age, twin pregnancy, pre-existing diabetes, obesity and high-normal blood pressure in early pregnancy[2,9]. In some studies, smoking during pregnancy has been associated with a lower risk of gestational hypertension[10,11]. Because low socioeconomic status is a marked risk factor for obesity, metabolic syndrome, hypertension and cardiovascular disease[12–14], socioeconomic status is also likely to be associated with gestational hypertension. As early as the 1950s, researchers described associations between measures of socioeconomic status and hypertension during pregnancy[15–19]. However, most earlier studies focused primarily on pre-eclampsia, which is characterized by hypertension and proteinuria, and which is thought to have a different aetiology than gestational hypertension[20]. The results of these studies also conflict. For example, in 1955 Nelson studied maternal social class as measured by the husband's occupation in relation to the incidence of pre-eclampsia, and found no association[17]. In contrast, Davies et al.[15], and, more recently, Haelterman et al.[16] found that, relative to women with a higher educational level, those with a low educational level had a higher risk of pre-eclampsia. We found only two studies that evaluated socioeconomic status in relation to isolated gestational hypertension[18,19]. Surprisingly, these found no associations, but this may have been due to the study design or to the chosen measures of socioeconomic status. For example, while these two studies used occupation of the woman's partner[18] and the woman's area of residence[19] as measures of socioeconomic status, such measures may not reflect all aspects of a pregnant woman's individual socioeconomic circumstances. Given the adverse health consequences for the offspring of mothers with gestational hypertension, it is important for clinical practice and for public health policy to know whether socioeconomically disadvantaged women run a

higher risk of gestational hypertension[20]. Studying the association between socioeconomic status and gestational hypertension might also improve our insight into the causes of socioeconomic inequalities in women's cardiovascular health. Working within the framework of the Generation R Study, a large birth-cohort study recruited prenatally[21], we studied the association between maternal educational level as an indicator of maternal socioeconomic status and gestational hypertension. We also examined whether such an association can be explained by the mediating effects of substance use (that is smoking, alcohol consumption and illegal drug use), pre-existing diabetes, and maternal anthropometrics and blood pressure at enrolment. We used level of maternal education as it has been found to be the strongest and most consistent socioeconomic predictor of cardiovascular health[22]. As the literature indicates that socioeconomic disparities in hypertensive complications of pregnancy may differ between ethnic groups, the present study was restricted to an ethnically homogeneous population[23].

MATERIAL AND METHODS

The Generation R Study

The present study was embedded within the Generation R Study, a population-based prospective cohort study from foetal life until young adulthood. The Generation R Study has previously been described in detail[21,24]. Briefly, the cohort includes
9778 (response rate 61%) mothers and children of various ethnicities living in Rotterdam, the Netherlands[24]. Although enrolment ideally took place in early pregnancy, it was possible until the birth of the child. All children were born between April 2002 and January 2006. Assessments during pregnancy included physical examinations, ultrasound assessments and questionnaires, and took place in early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18–25 weeks) and late pregnancy (gestational age ≥25 weeks). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki, and has been approved by the Medical Ethical Committee at the Erasmus MC, University Medical Centre Rotterdam (Erasmus MC). Written consent was obtained from all participating parents.

Study population

Of the 9778 women, 91% (n=8880) were enrolled during pregnancy[24]. Women of Dutch ethnicity (n=4057) comprised the largest ethnic subgroup, and were selected for the analyses described below. Awoman was classified as Dutch if she reported that both her parents had been born in the Netherlands[25]. Of the women who participated in this study with more than one pregnancy (8.3%), data on the second (n=332) or third pregnancy (n=5) were excluded from analyses to avoid clustering. Women who had been included after 25 weeks of gestation (n=77) were also excluded, because we were mainly interested in the effects of maternal anthropometrics and blood pressure early in pregnancy. To restrict the study to adult pregnant women, women younger than 20 years of age (n=63) were excluded. We also excluded twin pregnancies (n=51), cases of induced abortion, foetal deaths before 20 weeks of gestation and women lost to follow-up (n=23), and women lacking information on their educational level (n=20), diagnosis of gestational hypertension (n=65), gravidity (n=5), anthropometrics (n=17) or blood pressure at enrolment (n=29). Finally, as this study focused on de novo and isolated hypertension in pregnancy, we excluded women with pre-existing hypertension and those who developed pre-eclampsia, eclampsia or haemolysis, elevated liver enzyme and low platelet syndrome (n=108). This left 3262 women for analysis.

Educational level

On the basis of a questionnaire used at enrolment, we established the highest education achieved by each mother. This was categorized into four levels: (1) high (university degree), (2) mid-high (higher vocational training), (3) mid-low (more than 3 years general secondary school, intermediate vocational training or first year of higher vocational training) and (4) low (no education, primary school, lower vocational training, intermediate general school or 3 years or less at general secondary school) [26].

Gestational hypertension

After each participant had given birth, the attending community midwife or obstetrician completed a delivery report. The reports on those participants who had given birth under the medical supervision of an obstetrician were selected and screened by a trained medical-record abstractor. On the basis of documentation on the delivery report of any kind of

hypertensive complication or foetal growth retardation, 398 women were suspected of having gestational hypertension. To confirm the presence of gestational hypertension, the same abstractor conducted detailed reviews of these women's hospital charts. Gestational hypertension was defined according to the criteria described by the International Society for the Study of Hypertension in Pregnancy[27]: development of systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg without proteinuria after 20 weeks of gestation in previous normotensive women.

Potential mediators and confounders

Level of maternal education cannot directly affect the risk of gestational hypertension, but is likely to act through more proximal risk factors, so-called mediators[28]. We considered the following factors to be potential mediators in the pathway between maternal education and gestational hypertension: factors involving substance use, that is smoking, alcohol consumption and illegal drug use; preexisting diabetes; maternal anthropometrics and blood pressure at enrolment (Figure 1). Categories are indicated below in parentheses.

Substance use

Smoking, alcohol consumption and illegal drug use, including marijuana, hashish, cocaine, heroin and ecstasy (never, before conception, until pregnancy was known, continued in pregnancy) were established using questionnaires in early, mid and late pregnancy.

Pre-existing diabetes

Presence of pre-existing diabetes (no, yes, unknown) was established by questionnaire at enrolment. Because we could not assume that women who answered 'no' to this question had actually been tested for diabetes, we recoded 'no' into 'unknown'.

Anthropometrics and blood pressure at enrolment

Maternal anthropometrics and blood pressure were measured at enrolment in one of the research centres. Height and weight were measured without shoes and heavy clothing, and body mass index (BMI) was calculated from height and weight (weight/height2). BMI was categorized according to WHO standards into normal weight (<25kgm2), overweight (25–30kg/m^2) and obese (≥30kg/m^2). Systolic and diastolic blood pressure were measured

using an Omron 907 Automated Blood Pressure Monitor (OMRON Healthcare Europe BV, Hoofddorp, the Netherlands) [29]. Gestational age at enrolment varied from 5.1 to 24.9 weeks, and was correlated with level of education. We therefore adjusted BMI and blood pressure values for gestational age at time of measurement. First, we performed a separate linear regression analysis with gestational age at time of enrolment as predictor and BMI/blood pressure as outcome. Next, per woman, we added the difference between the fitted BMI/blood pressure value at the individual's gestational age at enrolment and the actual BMI/blood pressure observation to the fitted value at the population median gestational age at enrolment (14 weeks). All models were adjusted for age and gravidity, treating them as potential confounders, because the effects of these factors in the association between maternal education and gestational hypertension were not of primary interest in this study, and as they cannot be considered indisputable mediators (Figure 1). Maternal age was assessed at enrolment in one of the research centres and categorized into four groups (20–25, 25–30, 30–35 and ≥35 years). Gravidity (first pregnancy, ≥second pregnancy) was obtained through questionnaires at enrolment in the study.

Statistical analyses

We assessed the frequency distribution of potential confounders and mediators according to educational level. Chi-squared tests for trend were used for categorical factors, and Spearman's correlation coefficients were used for continuous factors. Multivariate logistic regression was used to calculate the odds ratios (OR) of gestational hypertension and their 95% confidence intervals (CI) for levels of education after adjustment for the potential confounders (model 1), and after additional adjustment for potential mediators. The highest educational level was set as reference. Missing data on categorical factors were included as separate categories. First, to evaluate the individual mediating effects of all potential mediators, these factors were added separately to model 1. For each adjustment, we calculated the percentage change in OR for the educational levels with a higher risk of gestational hypertension compared to the reference ($100x\{(OR_{model1} - OR_{+mediator}) \}/\{ (OR_{model} - 1\})$. When the OR attenuated to lower than 1, the change was set at 100%. Factors that caused an attenuation of the OR were defined as mediators in the association between maternal education and gestational hypertension[30]. In the subsequent analyses, hierarchical logistic models[31] were built for two reasons: (1) to evaluate the mediating effects of substance use, pre-existing diabetes, anthropometrics and blood pressure at

enrolment in the association between maternal education and gestational hypertension and (2) their own effects on gestational hypertension, taking due account of the conceptual hierarchical relationships between these factors. We hypothesized that, as an indicator of socioeconomic status, maternal education was the factor most distal to gestational hypertension that might influence risk of gestational hypertension through substance use, pre-existing diabetes, anthropometrics and blood pressure at enrolment. In turn, substance use might influence gestational hypertension risk directly, or indirectly through diabetes[32] or changes in anthropometrics[33]. Finally, we hypothesized that pre-existing diabetes, height and BMI at enrolment might influence gestational hypertension risk directly, or indirectly through blood pressure changes.9 For the logistic hierarchical models, we started with model 1, then added smoking, alcohol consumption and illegal drug use (model 2). To this model, we then added pre-existent diabetes, height and BMI at enrolment (model 3). In the final model (model 4), additional adjustment was made for systolic and diastolic blood pressure at enrolment. All analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 3262 women in the study, mean age was 31.3 years (s.d. 4.3), 8.9% were between 20 and 25 years old, 17.6% were 35 years or older and 53.6% were primigravida. The median gestational age at enrolment was 13.6 weeks (90% range: 10.9, 21.2). Participants gave birth at a median gestational age of 40.3 weeks (90% range: 37.1, 42.1); their children had a mean birth weight of 3492g (s.d. 547.9).

Of all women, 16.3% had a low educational level and 32.6% had a high educational level (Table 1). Gestational hypertension developed in 180 women (5.5%); the respective percentages for women with high, mid-high, mid-low and low education were 5.1, 4.4, 7.2 and 5.6% (w2: 6.77; degrees of freedom: 3; P-value: 0.08).

Age, alcohol consumption in pregnancy and height were positively associated with level of education (P for trend <0.001). Gravidity, smoking and illegal drug use during pregnancy, BMI, systolic and diastolic blood pressure at enrolment were negatively associated with level of education (P for trend <0.05). Women with a mid-low educational level had the highest systolic and diastolic blood pressure values at enrolment (Table 1).

Table 1 Distribution of general characteristics, substance use, pre-existing diabetes, anthropometrics and blood pressure at enrollment in the total study population and by educational level

	Level of maternal education					
	Total n=3262	**High n=1063 (32.6%)**	**Mid-high n=843 (25.8%)**	**Mid-low n=823 (25.2%)**	**Low n=533 (16.3%)**	**P for trend***
General characteristics						
Age, in years mean (s.d.)	31.3 (4.3)	32.9 (3.2)	31.9 (3.8)	30.0 (4.5)	29.2 (5.0)	<0.001
Age, categorical						
20–25 years (%)	8.9	0.1	3.3	15.9	24.2	
25–30 years (%)	25.1	16.2	27.5	31.2	29.6	<0.001
30–35 years (%)	48.4	62.1	49.3	39.9	33.2	
≥35 years (%)	17.6	21.6	19.9	13.0	13.0	
Gravidity First pregnancy (%)	53.6	56.4	56.1	55.3	41.3	<0.001
Parity Nulliparous (%)	64.6	64.9	67.9	67.1	55.0	0.004
Substance use						
Smoking						
Never (%)	49.4	59.7	52.9	45.8	29.1	
Before conception (%)	19.4	20.1	21.1	19.1	15.8	
Until pregnancy was known (%)	8.3	7.7	8.9	9.5	6.5	<0.001
Continued in pregnancy (%)	16.4	5.1	10.3	19.9	43.3	
Missing (%)	6.5	7.4	6.8	5.7	5.3	
Alcohol consumption						
Never (%)	13.1	3.4	9.9	17.8	30.0	
Before conception (%)	19.0	13.9	15.9	23.6	27.0	
Until pregnancy was known (%)	15.2	13.0	16.1	17.9	14.1	<0.001
Continued in pregnancy (%)	49.4	67.3	54.8	36.2	25.7	
Missing (%)	3.3	2.4	3.3	4.5	3.2	
Illegal drug use						
Never (%)	86.7	90.5	86.7	85.0	81.8	
Before conception (%)	4.4	1.8	5.0	5.8	6.7	
Until pregnancy was known (%)	2.1	0.6	1.8	1.7	6.2	<0.001
Continued in pregnancy (%)	0.8	0.1	0.3	1.3	1.9	
Missing (%)	6.0	7.0	6.2	6.2	3.4	

Table 1 Continued

	Total n=3262	High n=1063 (32.6%)	Mid-high n=843 (25.8%)	Mid-low n=823 (25.2%)	Low n=533 (16.3%)	P for trend*
Pre-existing diabetes						
Unknown (%)	92.4	91.6	92.1	92.4	94.7	
Yes (%)	0.2	0.1	0	0.4	0.4	0.097
Missing (%)	7.4	8.3	7.9	7.2	4.9	
Anthropometrics and BP at enrolment						
Height, in cm mean (s.d.)	170.7 (6.4)	171.4 (6.0)	171.3 (6.3)	170.6 (6.5)	168.9 (6.7)	<0.001
BMI†, in kgm^{-2} mean (s.d.)	24.2 (4.0)	23.3 (3.1)	23.5 (3.3)	24.9 (4.5)	25.7 (5.0)	<0.001
BMI†, categorical						
Normal weight (%)	68.2	77.6	73.8	60.8	52.4	
Overweight (%)	23.3	18.8	21.9	26.1	29.8	<0.001
Obese (%)	8.5	3.6	4.3	13.1	17.8	
SBP†, in mmHg mean (s.d.)	117.4 (11.9)	116.0 (11.2)	116.3 (9.1)	119.1 (12.5)	118.6 (12.3)	<0.001
DBP†, in mmHg mean (s.d.)	68.5 (9.2)	68.0 (8.6)	68.3 (9.1)	69.4 (9.8)	68.5 (9.5)	0.017

Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure.
*P-values are for w2-test for trend (categorical factors) or Spearman's correlation coefficient (continuous factors).
†Values of BMI and SBP and DBP at enrolment are adjusted for gestational age at enrolment.

Compared with women with high education, those with a mid-low and low education had a higher risk of gestational hypertension after adjustment for age and gravidity; those with a mid-low education had the highest risk (OR: 1.52; 95% CI: 1.02, 2.27; model 1; Tables 2 and 3). The OR for women with a low educational level did not reach statistical significance (OR: 1.30; 95% CI: 0.80, 2.12).

Individual adjustment for each potential mediator resulted in +2 to -71% changes in the OR for midlow education and +10 to1-100% change in the OR for low education (Table 2). The largest attenuations were caused by BMI, systolic and diastolic blood pressure at enrolment.

Table 3 presents the hierarchical logistic models fitted on gestational hypertension. Part of the effect of a mid-low and low educational level on gestational hypertension was mediated by substance use. When added to model 1, substance use, in particular alcohol consumption, attenuated the ORs by 21 and 63% to 1.39 (95% CI: 0.92, 2.11) and 1.11 (95% CI: 0.64, 1.92), respectively (model 2). Although alcohol consumption tended to reduce the risk of gestational hypertension in this model, this effect was not significant. In

contrast, smoking before conception was associated with a higher risk of gestational hypertension than never smoking was (OR: 1.68; 95% CI: 1.14, 2.46).

Pre-existing diabetes, height and BMI at enrolment further mediated more than half the effect of mid-low education (OR: 1.12; 95% CI: 0.73, 1.71; model 3) and all of the remaining effect of low education (OR: 0.83; 95%: 0.48, 1.44). This mediation was due mainly to BMI at enrolment. After adjustment for the other factors in model 3, overweight (OR: 2.43; 95% CI: 1.70, 3.46) and obesity (OR: 5.15; 95% CI: 3.34, 7.95) were significant risk factors for gestational hypertension. Systolic and diastolic blood pressure at enrolment, when added in model 4, further mediated the effect of mid-low education with 25% (in relation to model 3) to an OR of 1.09 (95% CI: 0.70, 1.69). This final OR for mid-low education corresponded with a total attenuation of 83% relative to model 1.

Additionally, blood pressure mediated half the effect of overweight (OR: 1.70; 95% CI: 1.17, 2.45) and 72% of the effect of obesity (OR: 2.13; 95% CI: 1.31, 3.47) on gestational hypertension risk. Adjusted for all other factors in model 4, the risk of gestational hypertension increased with increasing systolic (OR per mmHg increase: 1.02; 95% CI: 1.00, 1.04) and diastolic blood pressure (OR per mmHg increase: 1.07; 95% CI: 1.04, 1.09). The effect of smoking hardly changed after adjustment for BMI and blood pressure at enrolment.

Table 2 Odds ratios, and change in odds ratios of gestational hypertension for the different levels of maternal education

Maternal education	High n=1063 OR (95% CI)	Mid-high n=843 OR (95% CI)	Mid-low n=823 OR (95% CI)	Change a*	Low n=533 OR 95% CI	Change b*
Model 1 (includes maternal education, age and gravidity)	1.00	0.87 (0.56,1.34)	1.52 (1.02,2.27)		1.30 (0.80,2.12)	
Substance use						
Model 1 + smoking	1.00	0.86 (0.55,1.32)	1.51 (1.01,2.25)	-2%	1.26 (0.76,2.11)	-13%
Model 1 + alcohol consumption	1.00	0.85 (0.55,1.31)	1.44 (0.95,2.16)	-15%	1.19 (0.71,1.98)	-37%
Model 1 + illegal drug use	1.00	0.87 (0.56,1.34)	1.52 (1.02,2.27)	0%	1.33 (0.81,2.18)	+10%
Pre-existing diabetes						
Model 1 + pre-existing diabetes	1.00	0.87 (0.56,1.34)	1.52 (1.02,2.27)	0%	1.30 (0.79,2.11)	0%
Anthropometrics and BP at enrolment						
Model 1+height	1.00	0.87 (0.56,1.34)	1.53 (1.02,2.27)	+2%	1.31 (0.80.2.14)	+3%
Model 1+BMI (categorical)	1.00	0.83 (0.54,1.28)	1.15 (0.76,1.74)	-71%	0.87 (0.53,1.45)	-100%
Model 1+SBP	1.00	0.81 (0.52,1.26)	1.26 (0.84,1.90)	-50%	1.10 (0.66,1.81)	-67%
Model 1+DBP	1.00	0.83 (0.53,1.29)	1.31 (0.87,1.98)	-40%	1.18 (0.70,1.96)	-40%

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; OR, odds ratio; SBP, systolic blood pressure.
*Change a and change b represent the respective changes in OR for mid-low and low education relative to model 1 after individual adjustment for potential mediators (100 X (ORmodel 1-OR+mediator)/(ORmodel 1-1)). Since the subgroup with mid-high education did not have a higher risk of gestational hypertension than the subgroup with high education, changes in OR for mid-high education are not presented.

Table 3 Hierarchical logistic regression models fitted on gestational hypertension

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 3 OR (95% CI)
Maternal education				
High (reference)	1.00	1.00	1.00	1.00
Mid-high	0.87 (0.56,1.34)	0.83 (0.54,1.29)	0.81 (0.52,1.25)	0.79 (0.50,1.24)
Mid-low	1.52 (1.02,2.27)	1.39 (0.92,2.11)	1.12 (0.73,1.71)	1.09 (0.70,1.69)
Low	1.30 (0.80,2.12)	1.11 (0.64,1.92)	0.83 (0.48,1.44)	0.89 (0.50,1.58)
Substance use				
Smoking				
Never (reference)		1.00	1.00	1.00
Before conception		1.68 (1.14,2.46)	1.63 (1.10,2.40)	1.70(1.14,2.53
Until pregnancy was known		1.20 (0.67,2.16)	1.20 (0.66,2.16)	1.41 (0.77,2.58)
Continued in pregnancy		1.28 (0.79,2.09)	1.21 (0.74,1.97)	1.35 (0.81,2.24)
Missing		1.41 (0.48,4.11)	1.53 (0.48,4.85)	1.58 (0.46,5.48)
Alcohol consumption				
Never (reference)		1.00	1.00	1.00
Before conception		0.89 (0.53,1.49)	1.01 (0.59,1.70)	1.02 (0.601.76)
Until pregnancy was known		0.85 (0.49,1.48)	1.00 (0.56,1.76)	1.07 (0.501.91)
Continued in pregnancy		0.68 (0.41,1.13)	0.86 (0.52,1.45)	0.97 (0.57,1.64)
Missing		0.50 (0.15,1.70)	0.59 (0.17,2.08)	0.71 (0.202.54)
Illegal drug use				
Never (reference)		1.00	1.00	1.00
Before conception		1.11 (0.56,2.20)	1.36 (0.68,2.72)	1.39 (0.68,2.81)
Until pregnancy was known		0.48 (0.11,2.01)	0.59 (0.14,2.52)	0.67 (0.16,2.91)
Continued in pregnancy		0.66 (0.09,5.06)	0.59 (0.07,4.68)	0.68 (0.08,5.47)
Missing		1.13 (0.37,3.47)	1.45 (0.39,5.43)	1.54 (0.37,6.35)
Pre-existing diabetes				
Unknown (reference)			1.00	1.00
Yes			1.49 (0.16,14.13)	1.27 (0.13,12.67)
Missing			0.69 (0.20, 2.34)	0.60 (0.17,2.19)

Table 3 Continued

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 3 OR (95% CI)
Anthropometrics and BP at enrolment				
Height			1.01 (0.99,1.04)	1.00 (0.98,1.03)
BMI				
Normal weight (reference)			1.00	1.00
Overweight			2.43 (1.70,3.46)	1.70 (1.17,2.45)
Obese			5.15 (3.34,7.95)	2.13 (1.31,3.47)
SBP				1.02 (1.00,1.04)
DBP				1.07 (1.04,1.09)

Abbreviations: BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Model 1: Adjusted for age and gravidity

Model 2: Model 1 + smoking, alcohol consumption and illegal drug use

Model 3: Model 2 + pre-existing diabetes, height and BMI at enrolment

Model 4: Model 3 + SBP and DBP at enrolment (full mode)

DISCUSSION

This study showed that women with relatively low levels of education had a higher risk of gestational hypertension than women with a high level. This higher risk was explained by unequal distributions of known risk factors for gestational hypertension across educational levels, particularly by the higher rates of overweight and obesity and the relatively high blood pressure levels at enrolment found in lower educated women.

Methodological considerations

The main strength of this study lies in its population-based prospective design, in which a large number of women were enrolled early in pregnancy. The detailed information available on known risk factors for gestational hypertension enabled us to explain much of the association we observed between maternal education and gestational hypertension. Furthermore, the use of a conceptual hierarchical framework afforded insight into the interrelationships between maternal education and mediators, and their combined effects on gestational hypertension. An additional strength was the use of medical chart review and applied standard international criteria for a consistent definition of gestational hypertension. Although other measures of socioeconomic status exist, such as income level and occupational class[34]; for our study we selected maternal educational level as a main indicator of socioeconomic status. We did this for two reasons: (1) not only does

educational level partly reflect material resources because it structures occupation and income, it also reflects non-economic social characteristics, such as general and health-related knowledge, literacy, problemsolving skills and prestige[35,36] and (2) educational level has also been shown to be the strongest and most consistent socioeconomic predictor of cardiovascular health[22]. To various extents, our results may have been influenced by the following limitations. First, the response rate among pregnant Dutch women in the Generation R Study was relatively high (68%)[37], but there was some selection towards a relatively high educated and healthier study population[24]. Second, review of delivery reports and hospital charts was restricted to women who had been referred for delivery under medical care. However, in Dutch practice, community midwives often remain responsible for the care of women with a diastolic blood pressure between 90 and 100mmHg, provided that proteinuria does not develop. In the event of a diastolic blood pressure between 95 and 100mmHg, they are required to consult an obstetrician. All women with gestational hypertension with a diastolic blood pressure over 100mmHg should receive antenatal care and give birth in the hospital under the supervision of an obstetrician. Our study may therefore have missed mild cases of gestational hypertension with a diastolic blood pressure up to 100mmHg. Third, in all logistic models, we adjusted for gravidity, to take account of the protective effect of a previous pregnancy, including those that ended in spontaneous abortions. Although a woman's risk of gestational hypertension is highest during her first pregnancy, the literature indicates that a change of partner between pregnancies may cause the risk to revert towards the same level as a primigravida[38]. Unfortunately, in this study we had no information on change of partners between pregnancies. Finally, our study may have been vulnerable to misclassification, particularly with regard to substance-use factors, which were measured using questionnaires. Similarly, in accordance with the Dutch Standard Classification[25], we assigned a Dutch ethnicity to a participant if both her parents had been born in the Netherlands. However, when identifying immigrant descent in Dutch residents, this classification goes no further than the second generation. The number of third-generation immigrants is nonetheless likely to have been very small and not to have affected our conclusions.

Comparison with other studies

Socioeconomic differences in blood pressure and prevalence of hypertension have been consistently reported among the general, adult population[14,39].

According to a review by Colhoun et al.[39] most studies performed in developed countries associate indicators of low socioeconomic status with higher blood pressures; these associations are stronger in women than in men, and are largely explained by socioeconomic differences in BMI.

Hypertension during pregnancy, particularly pre-eclampsia, has also been associated with level of education as a measure of socioeconomic status[15,16]. However, two studies that evaluated the association between indicators of socioeconomic status and isolated gestational hypertension[18,19] did not find an association. Although this contrasts with our own findings, the discrepancy in both cases is probably due to differences in study design or in exposure definition. One study[18] depended on retrospective data and had to deal with a large amount of missing data. The same study also primarily used occupation of the women's partners as an indicator of maternal socioeconomic status - which, because it reflects other aspects of socioeconomic status, may therefore influence risk of gestational hypertension differently than maternal education does. The second study[19] examined an area-based measure of socioeconomic status in relation to occurrence of gestational hypertension. However, an area-based measure of socioeconomic status is unlikely to fully capture health risks that are associated with socioeconomic status at an individual level.

Educational level and risk of gestational hypertension

Relative to women with a high educational level, those with a low educational level and those with a mid-low educational level had, respectively, a 30 and 52% higher risk of gestational hypertension. The finding that the highest risk was not found in women with the lowest educational level somewhat weakens the evidence for a firm conclusion that maternal education level is negatively associated with gestational hypertension risk. However, this finding was probably attributed to chance; women with low education comprised the smallest subgroup, and the difference in gestational hypertension incidence between mid-low and low-educated women was not statistically significant (7.2 versus 5.6%; chi-squared: 1.25; degrees of freedom: 1; P-value: 0.263).

Another hypothetical explanation for this finding is that women with a low education received better medical care, due for example to their coverage under social medicine schemes. However, this is unlikely: in the Netherlands, obligatory health insurance ensures equal primary prenatal care for everyone.

Referral bias is a third possible explanation. As previously discussed, mild cases of gestational hypertension were not necessarily referred to an obstetrician. If women with a low education with gestational hypertension were more likely to remain under a midwife's care, these cases may have been selectively missed in our study.

The last possible explanation is the selection bias that would have resulted if low-educated women who did not participate in this study had a higher risk of gestational hypertension than low-educated women who did participate. However, among the participants we found a clear linear trend across educational levels in a variety of other factors, such as smoking, alcohol consumption and BMI. This makes selection bias less likely.

Mediating mechanisms

Most of the higher risk of gestational hypertension in women with mid-low and low education was mediated by relatively high rates of overweight and obesity at enrolment in these subgroups. Although obesity is an important risk factor for gestational hypertension, the underlying biological mechanism is not completely clear. A recent study suggested that obesity mostly increases the risk of gestational hypertension through higher blood pressure levels[9]. Our results indeed suggest that at least half the effect of overweight and obesity acts through relative increases in blood pressure early in pregnancy. In women with a mid-low education, relatively high blood pressure levels at enrolment further contributed independently of BMI to the explanation of their increased risk of developing gestational hypertension.

Blood pressure in early pregnancy has been shown to be positively associated with the risk of gestational hypertension, even when it is within the normal range[9]. Normal pregnancy is characterized by haemodynamic changes, which cause a steady decrease in blood pressure in the first half of pregnancy, followed by a rise in blood pressure in the second half until delivery[40]. It is plausible that the higher the blood pressure is at the start of pregnancy, the higher the blood pressure will be when haemodynamic demands increase

in the second half of pregnancy, and the sooner blood pressure will cross the threshold level of hypertension.

The higher risk of gestational hypertension in women with mid-low and low education was explained to a lesser extent by lower rates of alcohol consumption before and during pregnancy. This was due to a trend shown in our data towards a protective effect on gestational hypertension of alcohol consumption, which seemed to act through changes in BMI and blood pressure. Moderate alcohol consumption is known to lower blood pressure and to reduce the risk of development of essential hypertension in the non-pregnant population[41]. It is unknown whether moderate alcohol consumption during pregnancy has a similar effect on gestational hypertension.

Maternal smoking and illegal drug use did not contribute an explanation of the effects of a mid-low and low educational level. Remarkably, we observed that smoking before conception and during pregnancy tended to increase the risk of gestational hypertension, significantly so for smoking before conception. This is in contrast with many other studies which reported that women who smoke during pregnancy have a lower risk of gestational hypertension than women who have never smoked[11]. However, with regard to the effect of smoking before conception, studies have shown conflicting results. Zhang et al.[42] found that past smoking was associated with a lower risk of gestational hypertension, whereas a more recent study by England et al.[10] showed that women who smoked before pregnancy did not have a lower risk.

In non-pregnant women, cessation of smoking has been associated with a higher risk of hypertension than continued smoking or never smoking,[43] a finding that appears to support our results. Further study is needed to confirm a similar association between cessation of smoking and gestational hypertension.

Implications and conclusions

It has been postulated that gestational hypertension is a 'sign of latent hypertension unmasked by pregnancy'[44]. The present study supports this hypothesis. The educational subgroups with the highest risk of gestational hypertension had the highest blood pressure values at enrolment, and their increased risk of gestational hypertension was almost entirely explained by factors that are also associated with essential hypertension[45]. These

findings suggest that the relatively high risk of gestational hypertension in women with relatively low levels of education may reflect pre-existing hypertensive tendencies that are disclosed by the physiological stress of pregnancy.

We conclude that a relatively low educational level is associated with a higher risk of gestational hypertension. The educational inequalities observed in gestational hypertension may represent an early manifestation of the socioeconomic differences in morbidity and mortality from cardiovascular disease in women[13]. Strategies to reduce educational inequalities in gestational hypertension should be aimed primarily at reducing the burden of overweight and obesity in lower socioeconomic groups.

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Chapter 2.4

Validation of a prediction model for hypertension in pregnancy in nulliparous women.

Chapter 2.4

Validation of a prediction model for hypertension in pregnancy in nulliparous women. The Generation R Study

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Submitted.

ABSTRACT

Objective

To validate the accuracy of a previously published clinical prediction model for the identification of women who develop hypertension before 36 weeks gestation in an independent population sample.

Methods

The prediction model includes three easy to measure variables: systolic blood pressure, diastolic blood pressure and weight. The performance of this prediction model was studied in 988 healthy nulliparous women recruited from 7 midwifery practices in Rotterdam, The Netherlands. We assessed its discrimination and calibration and calculated sensitivity, specificity and negative predictive value for different risk categories.

Results

58 of 988 women (5.9%) developed hypertension before 36 weeks of gestation. The AUC of the prediction model was 0.73 compared to 0.78 in the original study. Of the women with a very low score (13.4% of the population), none developed hypertension before 36 weeks of gestation. In those with a high score (12.8% of the population) the risk was 15.9%.

Conclusion

This validation study confirms the predictive ability of the model, which may be of use to reduce the number of frequent antenatal visits in healthy nulliparous women.

INTRODUCTION

Hypertension is one of the most common complications of pregnancy and is a major cause of maternal and perinatal morbidity and mortality, in particular when hypertension is combined with proteinuria (pre-eclampsia).[1] Because gestational hypertension can shortly progress to pre-eclampsia, de novo hypertension occurring after mid-gestation in a nullipara always requires intensified antenatal surveillance, and often more interventions, such as antihypertensive medication, hospital admission and, finally, induction of labor.

Although there is no effective prevention strategy and the only cure is by delivery, identification of women at a high risk of developing hypertension already early in pregnancy is important. These women might benefit from intensified antenatal surveillance and timely diagnosis, improving pregnancy outcome. On the other hand, identifying women at very low risk of the disease may offer a possibility to reduce the number of antenatal visits in this group. Results from at least ten randomized trials suggest that in healthy nullipara, reduction of the number of antenatal visits to as few as four to eight could be implemented without any increase in adverse maternal and perinatal outcomes[2]. The organisation of antenatal care could be much more efficient, targeting health care strategies to those women who might really benefit.

In healthy normotensive nulliparous women adequate risk classification of hypertensive diseases has proven to be very difficult. Recent studies focus mainly on combinations of biomarkers to predict a high risk of developing pre-eclampsia[3,4]. Although some show promising results, prediction models should preferably be based on parameters that are widely available and obtained at no or minimal cost. In a previous study, we developed and internally validated a model for prediction of hypertension in pregnancy before 36 weeks of gestation using simple clinical variables routinely obtained at the first antenatal visit, namely systolic blood pressure, diastolic blood pressure and weight[5]. The model accurately identified those women at very low risk of developing hypertension before 36 weeks gestation, as well as those at high risk. Its predictive accuracy was confirmed by an area under the receiver operating characteristic (ROC) curve of 0.78. Geographical validation showed that the model was stable across populations in two different cities in The Netherlands.

Although the prediction model was internally and geographically validated in the derivation study, it has not been externally validated in a separate sample. Specifically, the

model was derived from data that was collected between 1990 and 1994, raising concerns about the model performance in contemporary populations. In this study, we investigated the accuracy of the prediction model in a subpopulation of the Generation R study, a recent, independent population-based cohort.

METHODS

Study population

The Generation R study is a population-based prospective cohort study to investigate early environment and genetic determinants of growth, development and health in fetal life, childhood and adulthood. The study is conducted in Rotterdam, the second largest city in the Netherlands. Rationale and details of the Generation R study have been previously published[6]. In total 9778 pregnant women with a delivery date between April 2002 and January 2006 were enrolled at their routine ultrasound examination in pregnancy after written consent was obtained. For this validation study only healthy, nulliparous women with a singleton pregnancy, who entered antenatal care at a midwife practicebefore 16 weeks of gestation were included (n=1038). This is the only group for which all necessary data were available.The women had an expected date of delivery between April 2002 and December 2006 and had their forst control of blood pressure before 16 weeks of gestational age. Gestational age was determined from the best estimate according to menstrual history or ultrasound measurement early in pregnancy. Blood pressure was measured by auscultatory sphygmomanometry. Only women who were normotensive (blood pressure <140/90 mmHg) at booking were included in our analysis (n = 988).
Hypertension before 36 weeks of pregnancy was defined as SBP ≥140 mmHg and/or DBP ≥ 90 mmHg measured twice with an interval of at least 6 hours, based on blood pressure measurements from midwives during regular routine antenatal visits.

Statistical analyses

The published prediction model uses SBP, DBP, and maternal weight for calculating the risk of hypertension (see Appendix)[5]. Missing values in the validation set on these variables (2.9%) were imputed with single regression techniques[7-9].

To quantify external validity of the prediction model, we assessed its discrimination and calibration. Discrimination is the ability of the model to discriminate

between patients with and without hypertension, and is quantified by the area under the receiver operating characteristic curve (AUC). An AUC ranges from 0.5 (no discrimination; same as flipping a coin) to 1.0 (perfect discrimination). Calibration refers to the agreement between the predicted probabilities and observed frequencies of hypertension.

To further explore predictive ability, all patients were assigned to 1 of 4 risk categories (very low risk, low risk, moderate risk and high risk), based on the score chart derived from the previous published prediction model (see Appendix). For the thresholds, we calculated corresponding sensitivity, specificity and negative predictive value.

The data were analyzed using the statistical software package SPSS for Windows (version 17.0, SPSS Inc., Chicago, Illinois, USA) and R (version 2.7.0, http://www.r-project.org/).

RESULTS

There were 988 participants who were normotensive at booking. Measurements were performed at 12.0 ± 1.8 weeks of pregnancy, compared to 12.2 ± 2.1 weeks in the derivation study.

Maternal characteristics and pregnancy outcome are given in Table 1. There were more women of Non-Caucasian ethnic origin in the validation sample (64% vs 76%). In the validation study 5.9% (N=58) of the women developed hypertension in pregnancy before 36 weeks of gestation, compared to 6.0% in the original study.

The area under the curve of the model was 0.73 (95% CI 0.67-0.80), compared 0.78 (95% CI 0.75-0.82) in the original study. The calibration plot is depicted in Figure 1. This figure shows that the mean predicted probability to develop hypertension before 36 weeks of gestation equals the mean observed proportion of women who did develop hypertension, up till a predicted probability of 18%. After this percentage, the predicted risks are too high.

After applying the score chart, 132 out of 988 women were categorized as having a very low risk (Table 2). None of these women developed hypertension before 36 weeks of gestation. Application of the model using this threshold could thus reduce the number women needing frequent antenatal visits by 13% (compared to 19% in the original study), with a sensitivity of 100%, and a corresponding specificity of 14%. By increasing the

threshold of less frequent antenatal visits specificity increases, but at the cost of lower sensitivity (missing women who do develop hypertension before 36 weeks of gestation). In the high risk category 16% of the women developed hypertension, compared to 23% in the original study.

Figure 1 Calibration plot of observed and predicted probability of developing hypertension after applying the prediction model to the Generation R data.

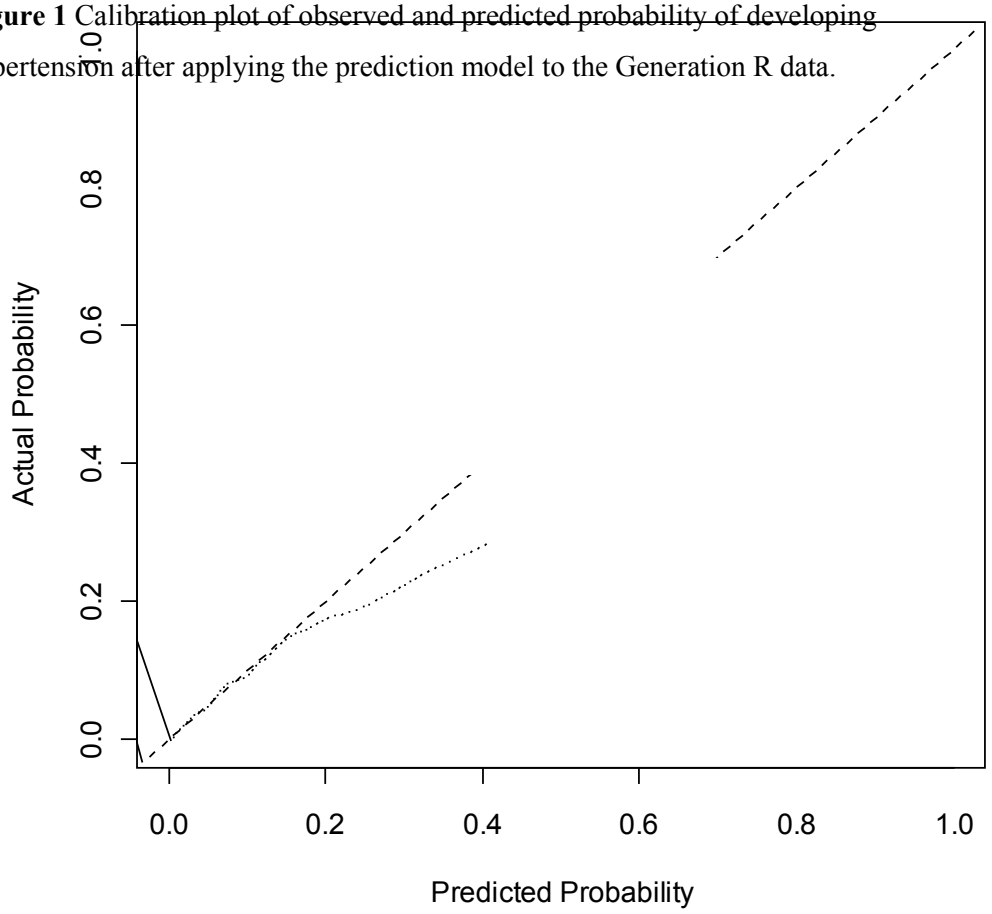

Table 1 Maternal characteristics and pregnancy outcome in the validation and derivation study.

	Validation study (N=988)	Derivation study (N=2334)
Characteristics		
Age (years)	28.0± 5.2	27.6 ± 4.7
Weight (kg)	67.9 ± 12.3	65.2 ± 10.9
Height (cm)	168.4 ± 7.4	167.7 ± 7.0
Caucasian, *N* (%)	628 (63.6)	1768 (75.7)
SBP (mmHg)	114.0 ± 9.7	113.3 ± 12.6
DBP (mmHg)	67.0 ± 7.4	70.1 ± 8.2
Pregnancy Outcome		
Gestational age at delivery	39.9 ± 1.9	39.6 ± 2.9
Birth weight (gram)	3339.4 ± 564.1	3279.0 ± 635.3
Hypertension before 36 weeks gestation, *N* (%)	58 (5.9)	141 (6.0)

Data expressed as mean±SD or numbers(%). SBP; systolic blood pressure, DBP;diastolic blood pressure.

Definition gestational hypertension according to definition of ISSHP.

Table 2 Classification in risk categories in the validation and derivation study.

	Women developing HT N (%)	Women without HT N (%)	SE(%)	SP(%)	NPV(%)	Women needing less frequent visits N (%)
Validation study (N=988)						
Very low risk (N=132)	0 (0.0)	132 (100.0)	100.0	14.2	100.0	132 (13.4)
Low risk (N=445)	15 (3.4)	430 (96.6)	74.1	60.4	97.4	577 (58.4)
Moderate risk (N=285)	23 (8.1)	262 (91.9)	34.5	88.6	95.6	862 (87.2)
High risk (N=126)	20 (15.9)	106 (84.1)				
Derivation study (N=2334)						
Very low risk (N=438)	2 (0.5)	436 (99.5)	98.6	19.9	99.5	438 (18.8)
Low risk (N=982)	27 (2.7)	955 (97.3)	79.4	63.4	98.0	1420 (60.8)
Moderate risk (N=599)	40 (6.7)	559 (93.3)	51.1	88.9	96.6	2019 (86.5)
High risk (N=315)	72 (22.9)	243 (77.1)				

HT: hypertension; SE: sensitivity; SP: specificity; NPV: negative predictive value

Very Low risk = Sum score ≤4.5; Low risk = Sum score 5-6; Moderate risk = Sum score 6.5-7; High risk = Sum score ≥7.5. See score chart in Appendix.

DISCUSSION

In this study we validated a previously derived prediction model for hypertension before 36 weeks gestation in healthy nulliparous women by using recent data (2002-2006) of a separate sample of women receiving routine prenatal care in a primary health care setting. The data of this independent cohort confirm adequate predictive ability of the model and support its potential use to classify women into subgroups at very low, low, moderate and high risk for developing hypertension. Although the model performed more accurately in the data from which it was derived (AUC = .78) compared to the validation data (AUC = .73), this difference was not statistically significant. The calibration plot showed that after a predicted probability of 18%, the predicted risks were too high. This is not clinically relevant however; a predicted risk of 18% is high enough to classify a women as having a high risk anyway. More important is that low risks were adequately predicted, to classify women correctly as having a very low or low risk.

Previous studies have also attempted to predict development of hypertensive disorders in pregnancy. Results of a large systematic review in 2004, conducted by the WHO failed to identify clinically useful "single" screening tests to predict the development of preeclampsia[10]. In 2008, authors of another systematic review concluded that no test,

nor combinations of tests had emerged with high enough sensitivity and specificity to be of clinical use[11]. The importance of continuing research to identify useful markers was stressed. The authors of a recent literature review concluded that specific angiogenic, antiangiogenic and proteomic markers are the most promising biomarkers of pre-eclampsia[12]. In a recent case-control study, a consistent discriminatory metabolite signature in early pregnancy plasma was discovered, consisting of a combination of 14 metabolomic biomarkers[3]. These kind of studies offer insight into disease pathogenesis and generate hope that ultimately the screening test for pre-eclampsia will be developed. In the development of our model we specifically focused on commonly available clinical variables, easy to use in routine antenatal care in midwifery practices. After risk classification based on our model, validated screening tests based on biomarkers may help classify risk further. The cost-effectiveness of using these more expensive tests in all, or a selection of women needs further study.

Some aspects of our study need to be addressed to appreciate the results. First, a validation sample should include preferably a 100 events to detect substantial changes in accuracy with 80% power, e.g. a 0.1 change in c-statistic[13]. In our validation sample, 58 women developed hypertension before 36 weeks of gestation. This affects the precision of our estimates. Second, in the derivation study, blood pressure was measured by an automated device (BOSO). Based on the validation results of the BOSO equipment, measurements on average are slightly higher than what a standard mercury device would have measured. BOSO on average overestimates diastolic pressure by 1 mmHg and systolic pressure by 2 mmHg, as compared to standard auscultatory mercury sphygmomanometry by trained observers[14]. In the validation sample, blood pressure was measured routinely by auscultatory shygmomanometry. The model however performed adequately despite this different method. Third, the validation sample included less Caucasian women. Although our model does not include ethnicity as a predictor and performed well in the validation sample, we recognise the importance of ethnicity in the risk of developing hypertensive disorders.

A strength of the present study is that the subgroup of the Generation R population that received routine health care in multiple midwifery practices provided recent data, ideally suited for external validation of the prediction model. Furthermore, our model is

very easy to use in clinical practice because it uses predictive variables that are obtained as part of routine care.

The clinical implications of our study are that risk of hypertension before 36 weeks of gestation can be established early in pregnancy and patient management tailored to risk levels. The model could serve as a tool to identify women who might require intensified surveillance in pregnancy, in the future maybe after further screening based on biomarkers, while the antenatal visits in very low or low risk women might potentially be reduced. This would obviously have implications in terms of more efficient utilisation of health-care resources. It is unknown whether such strategies based on this prediction model would be of benefit; this should be the focus of future research.

In summary, a clinical prediction model consisting of three variables easily available to midwives, demonstrated adequate performance for classifying women according to their risk of developing hypertension before 36 weeks of gestation.

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Appendix

Prediction model for the development of hypertension before 36 weeks gestation[5].

Variable	Regression Coefficient **	Odds ratio	95% CI
Systolic blood pressure (category)*	0.33	1.39	1.26-1.53
Diastolic blood pressure (category)*	0.29	1.34	1.19-1.51
Weight (kg)	0.02	1.02	1.01-1.03
Intercept	-9.25		
Area under the ROC curve***	0.78		0.75-0.82
Formula to calculate the risk of hypertension: $1 / (1+\exp^{-(\text{intercept} + 0.334 \times \text{category SBP} + 0.291 \times \text{category DBP} + 0.023 \times \text{weight})})$			

SBP: systolic blood pressure; DBP: diastolic blood pressure; ROC: receiver operating characteristic

*Systolic blood pressure categorized per 5 mmHg: <80 (category 1); 80-84 (2); 85-89 (3); 90-94 (4); 95-99 (5); 100-104 (6); 105-109 (7); 110-114 (8); 115-119 (9); 120-124 (10); 125-129 (11); 130-134 (12); ≥135 (13).

Diastolic blood pressure categorized per 5 mmHg: <50 (category 1); 50-54 (2); 55-59 (3); 60-64 (4); 65-69 (5); 70-74 (6); 75-79 (7); 80-84 (8); ≥85 (9).

Score chart for the risk of developing hypertension before 36 weeks of gestation[5].

Predictor	Score	
Systolic blood pressure (mmHg)		…
	< 85 0.5	
	85-89	1
	90-99 1.5	
	100-104	2
	105-114 2.5	
	115-119	3
	120-129 3.5	
	≥ 130	4
Diastolic blood pressure (mmHg)		…
	< 55 0.5	
	55-64	1
	65-74 1.5	
	75-79	2
	≥ 80 2.5	
Weight (kg)		…
	< 55	1
	55-79 1.5	
	80-99	2
	≥100 2.5	
		…
		-------+
Total sum score		…

Chapter 2.5

Medical record validation of maternally reported history of pre-eclampsia: The Generation R study

Chapter 2.5

Medical record validation of maternally reported history of pre-eclampsia: The Generation R study

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ABSTRACT

Objective:

In this study, we assessed the validity of maternally, self-reported history of preeclampsia.

Study Design and Setting:

This study was embedded in the Generation R Study, a population-based prospective cohort study. Data were obtained from prenatal questionnaires and one questionnaire 2 months postpartum from the mother. All women who delivered in hospital and returned a 2 month postpartum questionnaire (n=4330) were selected.

Results:

Of the 4330 women, 76 out of 152 (50 %) women who self reported preeclampsia appeared not to have had the disease according to the definition (International Society for the Study of Hypertension in Pregnancy). From the women who self reported not to have experienced preeclampsia, 11 out of 4178 (0.3%) had suffered from preeclampsia. Sensitivity and specificity were 0.87 and 0.98, respectively. Higher maternal educational level and parity were associated with a better self reported diagnosis of preeclampsia.

Conclusion:

The validity of maternal-recall self-reported preeclampsia is moderate. The reduced self-reported preeclampsia might suggest a lack of patient-doctor communication. Therefore, doctors have to pay attention to make sure that women understand the nature of preeclampsia.

INTRODUCTION

Pre-eclampsia is still a major cause of fetal and maternal morbidity and mortality[1,2]. Its pathogenesis is unknown, and as a consequence, rational therapy is lacking. In clinical management, it is important to act on known risk factors at antenatal booking. Recently, Milne et al. developed a community guideline, which provided an evidence based risk assessment as a framework for appropriate care for the best outcome for the mothers and their babies[3]. This guideline provides a risk assessment for individual care using known risk factors for pre-eclampsia. The authors recommend referral before 20 weeks' gestation if women have risk factors, including a history of pre-eclampsia and chronic hypertension. These risk factors have mainly been determined by epidemiologic studies through subjects' self-report by means of personal interviews or mailed questionnaires. Besides using pre-eclampsia as a risk factor for subsequent pregnancies, recent reports in epidemiologic studies have suggested an association of pre-eclampsia as a risk factor for cardiovascular disease in later life[4]. This implies a necessity for adequate information on the correct diagnosis of pre-eclampsia. In this study, we assessed the agreement of a self-reported history of pre-eclampsia with the information on diagnostic criteria for pre-eclampsia in medical files.

Study Design and Setting

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study examines early environmental and genetic determinants of growth, development, and health in fetal life, childhood and adulthood[5,6]. Briefly, the cohort includes 9778 women, of whom 8880 were enrolled during pregnancy, of different ethnicities living in Rotterdam, The Netherlands. Enrollment was aimed in early pregnancy but possible until birth of the child. Women and their partners were enrolled in the study at their first routine ultrasound examination in pregnancy after written consent was obtained. Data from three different questionnaires during pregnancy, data about the delivery, and data from a questionnaire 2 months post partum were analyzed. All children were born between April 2002 and January 2006. The Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, has approved the study.

Medical charts and maternal outcome

Women within the Generation R Study, who delivered in hospital and had chronic hypertension or were reported to have experienced pregnancy-induced-hypertension (≥ 140/90 mmHg) or hypertension related complications (pre-eclampsia, proteinuria, eclampsia and/ or HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets)) were selected from hospital registries. Their individual medical records were subsequently studied. Pregnancy-induced-hypertension, pre-eclampsia and eclampsia were defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria[7] and according to those of the American College of Obstetricians and Gynaecologists (ACOG)[8]. HELLP syndrome was defined as thrombocytes <100x10^9/l, and both ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase) >70 U/l and LDH (lactate dehydrogenase) > 600 U/L. Severe pre-eclampsia was defined according to the criteria described by Sibai[9].

Self-reporting of complications of pregnancy

Information on the index pregnancy was obtained from the questionnaire for the mother, 2 months postpartum. Pre-eclampsia was asked for as being hypertension in combination with proteinuria (no, yes or don't know). Pre-eclampsia was explained in a lay term (translated in Dutch as "zwangerschapsvergiftiging") as a high blood pressure in combination with loss of proteins in urine. Women who answered don't know were classified as missing (n=37, 0.8%).

Covariates

Information about maternal characteristics (age, parity (≥ 1), ethnicity, education, netto income per household (> € 2200), chronic hypertension), family diseases (chronic hypertension, pregnancy-induced hypertension or pre-eclampsia) and quality of language was obtained from the prenatal questionnaire in early pregnancy at enrolment. The highest educational level achieved by the mother was categorized into three educational levels: high (high school or university), mid (secondary school) and low (no education or primary school). Quality of language was asked for as how well are you able to speak, read and write your language (not at all, a little, reasonable, quite well, very well). Information

about satisfaction of prenatal care was obtained from the prenatal questionnaire in the third trimester.

Population for analysis

In total 8880 women were enrolled in pregnancy in the Generation R Study. Of these women, 6897 participated in the postnatal phase of the study. Data of women who did not continue the study were excluded. Women who had missing information on pregnancy outcome (1.2%, n=77) were excluded from present study. Some women had contributed with more than one pregnancy in the Generation R Study. In total, 468 pregnancies (6.9%) were a second pregnancy, and four pregnancies (0.05%) were a third pregnancy within the study. Data on only the first ongoing pregnancy were used for analysis to avoid clustering. The response for the cohort of this questionnaire was 82%[10]. A subgroup of the study population did not receive or return the questionnaire 2 month postpartum (30%, n = 1938). As we started to distribute the questionnaire at the postnatal age of 2 months in a later stage of the study, not all women received this questionnaire (9%). This group of women did not differ from the others and language problems were no issue. A second group was not sent a questionnaire, because no postnatal consent was given (6%). Sixty three women who did not answer specific question about pre-eclampsia (0.8%, n=63) were classified as missing. Four (6.3%) of them had had pre-eclampsia, according to their medical record. The results of thirty-seven women (0.3%) who answered 'do not know', were classified as missing. Therefore, 4330 subjects remained for further analyses.

Data analysis

We treated the diagnosis pre-eclampsia according to the medical record as "gold standard" (i.e., true pre-eclampsia; true no pre-eclampsia, defined by the ISSHP criteria) and calculated sensitivity (percent true pre-eclampsia reported as pre-eclampsia), specificity (percent true no pre-eclampsia reported as no pre-eclampsia), positive predictive value (PPV; percent of reported as pre-eclampsia that is true pre-eclampsia), negative predictive value (NPV; percent of reported as no pre-eclampsia that is true no pre-eclampsia)[11]. Both the definition of the ISSHP and ACOG were used to calculate sensitivity and specificity. Furthermore, calculations were made for the diagnosis severe pre-eclampsia according to Sibai[9]. The associations of specific determinants and validity were assessed using logistic

regression analysis. A new variable for validity was computed, categorized as yes (self-reported diagnosis and the diagnosis from medical record are similar) and no (self-reported diagnosis and the diagnosis from medical record are dissimilar). All regression analyses were adjusted for age, parity and ethnicity. The analyses were adjusted for age and parity, because older women or women with more pregnancies could be more experienced with complications and, therefore, could be confounding variables. Different ethnicity could have an effect on education, by different language or culture.

The Statistical Package of Social Sciences version 12.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses.

Results

Table 1 shows the characteristics of the women who received and returned a questionnaire versus who were not sent a questionnaire or the questionnaire was not returned. As no data were available from women to whom no questionnaire was sent and from women to whom one was sent but who did not return, they are described as one group in the manuscript. In the group who returned a questionnaire, we found less pre-eclampsia, more first pregnancies, older women, higher educated, more Dutch ethnicity, and a higher income. Figure 1 and table 2 present the data from the questionnaire and the medical records. Of the 4330 women 4178 (96.5%) answered that they had not experienced pre-eclampsia, and 152 (3.5%) answered that they had experienced pre-eclampsia (table 2). In total, 152 women who answered to have suffered from pre-eclampsia, had, according to their records, suffered in 43 cases from hypertension without proteinuria and in 5 cases from HELLP syndrome without proteinuria (ISSHP criteria). Table 3 shows sensitivity, specificity, PPV and NPV calculated for the different definitions of pre-eclampsia. Similar results were found for sensitivity and specificity using ACOG criteria. However, using the definition of severe pre-eclampsia of Sibai[9], a sensitivity of 0.92 could be calculated. Table 4 shows the crude and adjusted odd ratios for potential risk factors and a correct diagnosis of self-reported pre-eclampsia.

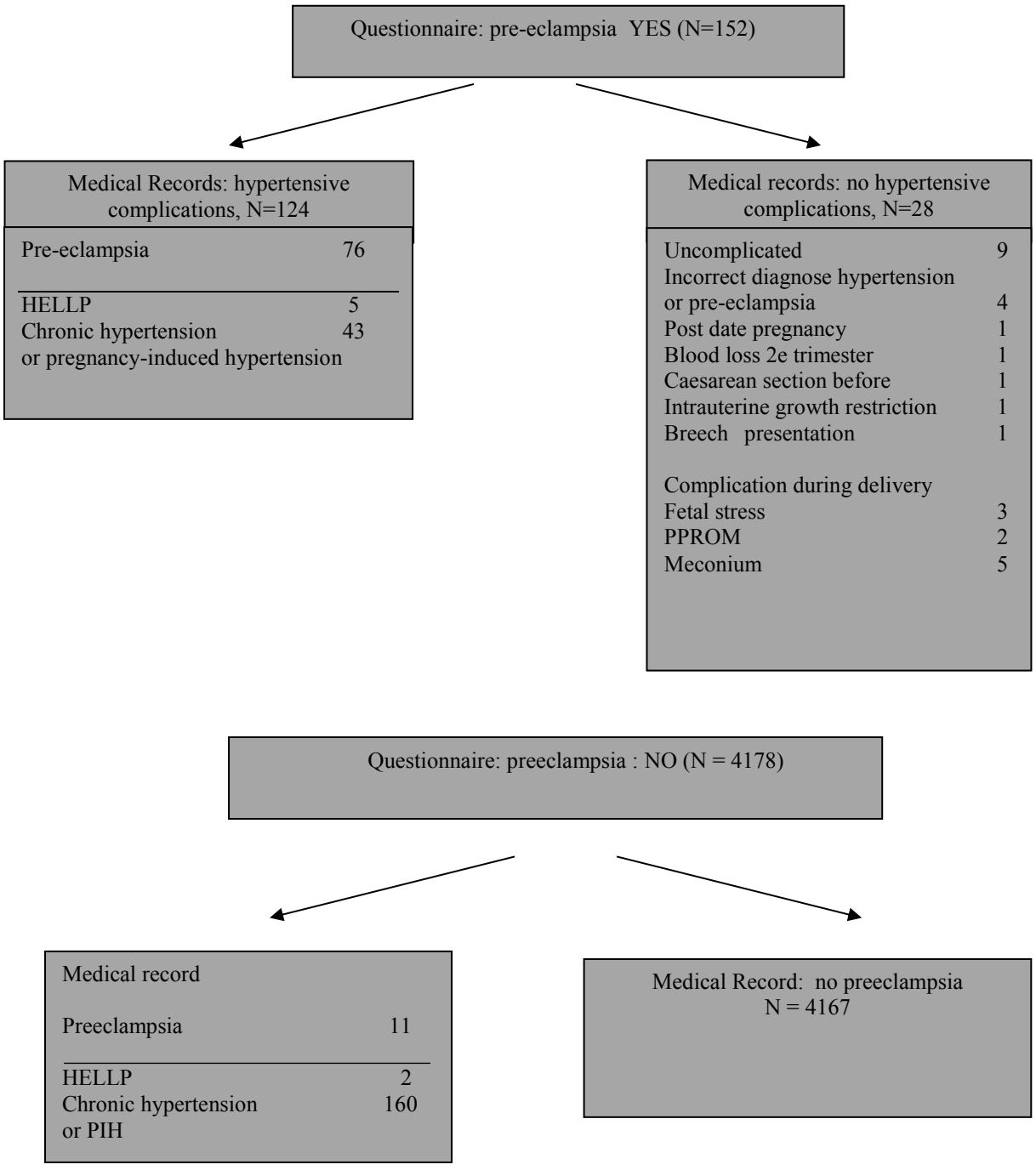
Multiparity was associated with the correct self-reported diagnosis of pre-eclampsia (odds ratio with 95% confidence interval, 1.39, 1.00 – 1.94). For ethnicity, no associations could be found. Higher maternal education level was associated with the correct self-reported diagnosis of pre-eclampsia or its absence (primary school (odds ratio with 95% confidence

Table 1. Characteristics of women who were returned a questionnaire versus not sent or not returned.

	Received N = 4330	Not sent or not returned N = 1918
Characteristics		
Pre-eclampsia (in %)	2.1	2.8*
Age (mean in year)	30.6	28.6*
Parity ≥1 (in %)	35.8.	43.2*
Missing	2.7	7.0
Ethnicity (in %)		
Dutch and other European	63.1	39.5*
Surinamese	6.0	10.8*
Turkish	7.5	12.9*
Moroccan	3.8	9.8*
Antillean	2.2	2.8
Cape Verdian	2.6	6.1*
Other	9.0	7.9
Missing	7.5	16.6*
Education (in %)		
Low	3.2	9.2*
Mid	31.7	43.2*
High	55.0	27.6*
Missing	10.1	20.0
Income per household (in %)		
Less	45.8	77.6*
High	54.2	22.4*
Missing		
Quality of language		
Speaking low	4.1	9.4*
Speaking high	85.2	71.9*
Missing	10.7	18.7
Reading bad	3.8	8.5*
Reading well	85.2	71.6*
Missing	11.0	19.9
Writing bad	5.0	10.0*
Writing well	84.0	70.0*
Missing	11.1	20.0

* $p < 0.05$

Figure 1 Data about self-reporting of pre-eclampsia, obtained from the questionnaire compared to the medical records (PIH = pregnancy induced hypertension, HELLP = hemolysis, elevated liverenzymes and low platelets, PPROM = preterm premature rupture of the outer membranes. Total n=4330.



interval, 0.39, 0.18 – 0.84), secondary school (0.70, 0.51 – 0.96), and high education (1.63, 1.19 – 2.24) (trend analysis for education $P < 0.05$)).

Income per household was not associated with the validity of the diagnosis. Chronic hypertension and pre-eclampsia in a preceding pregnancy were inversely associated with the correct self-reported diagnosis of pre-eclampsia or its absence (odds ratio with 95% confidence, 0.05, 0.03 – 0.10 and 0.17, 0.06 – 0.47, respectively).

No association was found between quality of language of the women and the validity of the self-reported diagnosis. An association between the validity of the diagnosis of pre-eclampsia and satisfaction of prenatal care asked for in the last trimester of pregnancy was not found (odds ratio with 95% confidence interval 1.00, 0.77 – 1.32).

Table 2 Data about self-reporting of pre-eclampsia, obtained from the questionnaire compared to the medical records.

Medical record / **Self-reported**	Preeclampsia	No preeclampsia	Total
Pre-eclampsia	76	76	152
No pre-eclampsia	11	4167	4178
Total	87	4243	4330

Table 3. Accuracy of maternal recall of the diagnosis pre-eclampsia, using different definitions for pre-eclampsia.

	Pre-eclampsia according to ISSHP criteria	Pre-eclampsia according to ACOG criteria	Severe pre-eclampsia
Sensitivity (%)	87	84	92
Specifity (%)	98	98	
Positive predictive value (%)	50	57	
Negative predictive value (%)	99	99	99

Table 4 Risk factors for a correct diagnosis of pre-eclampsia, odds ratios with 95% confidence are shown.

Risk factor	Crude OR	Adjusted OR
Age >35 years	0.78 (0.55 – 1.11)	0.70 (0.48 – 1.02)
Parity>1	1.39 (1.01 – 1.90)	1.39 (1.00 – 1.94)
Ethnicity		
European	0.82 (0.59 – 1.13)	0.83 (0.49 – 1.41)
Surinamese	0.70 (0.42 – 1.17)	0.62 (0.31 – 1.25)
Turkish	1.79 (0.83 – 3.84)	1.64 (0.63 – 4.27)
Marroccan	1.56 (0.63 – 3.85)	1.25 (0.45 – 3.49)
Cape verdian	2.72 (0.67 – 11.1)	2.17 (0.49 – 9.60)
Antillian	0.89 (0.36 – 2.21)	0.75 (0.27- 2.10)
Other	1.15 (0.68 – 1.94)	1.34 (0.48 – 3.77)
Education		
Primary	0.59 (0.41 – 1.15)	0.39 (0.18 – 0.84)
Secondary	0.79 (0.58 – 1.06)	0.70 (0.51 – 0.96)
Tertiary	1.38 (1.03 – 1.85)	1.63 (1.19 – 2.24)
Quality of language		
Speaking	0.96 (0.47 – 1.99)	0.74 (0.33 – 1.70)
Reading	1.01 (0.46 – 2.18)	0.82 (0.34 – 1.96)
Writing	1.19 (0.58 – 2.46)	1.00 (0.45 – 2.23)
Income > €2200	0.99 (0.75 – 1.31)	1.23 (0.90 – 1.70)
Satisfaction of prenatal care	1.04 (0.80 – 1.35)	1.00 (0.77 – 1.32)
Chronic hypertension	0.06 (0.03 – 0.11)	0.05 (0.03 – 0.10)
Previous PE	0.17 (0.06 – 0.46)	0.17 (0.06 – 0.47)
Family history of PE	0.53 (0.27 – 1.03)	0.54 (0.28 – 1.06)
Family history of PIH	0.57 (0.38 – 0.84)	0.57 (0.38 – 0.85)
Family history of chronic hypertension	0.50 (0.37 – 0.67)	0.52 (0.38 – 0.71)

CONCLUSION

Personal interviews and self-reported questionnaires are commonly used for risk assessment in pregnancy or later life and in epidemiologic studies as a sole source of exposure information. Our study showed that the validity of maternal self-reported pre-eclampsia is moderate.

An explanation for the reduced maternal self-reported pre-eclampsia is that our study has not tested the validity of maternal recall of what they wre told by medical professionals but

rather the agreement of their recall with the diagnostic cirteria for pre-eclampsia. Therefore, these results might suggest an inaccurate use of the definition of pre-eclampsia in the communication between doctor and the patient. Actually, the agreement between pre-eclampsia according to the criteria and pre-eclampsia according to the questionnaire is reduced.

We found that parity, low maternal education, chronic hypertension, and pre-eclampsia in a preceding pregnancy were associated with the validity of self-reported pre-eclampsia. Quality of language of the woman herself and ethnicity did not seem to influence the validity of maternal self-reported pre-eclampsia. This again suggests that maternal knowledge gaps on the characteristics of the disease pre-eclampsia might be responsible for the inadequate understanding of information. In contrast to the expectation that chronic hypertension and/or pre-eclampsia in a preceding pregnancy were positively associated with the correct diagnosis because of their knowledge, we found that both factors were found more often in women with the incorrect recall. This may imply a similar problem in patient-doctor communication as described earlier. The low number of women with pre-eclampsia in a preceding pregnancy could also explain this finding.

In a study by Yawn et al., pre-eclampsia defined from medical records and mother's report 10-15 year after delivery had high levels of negative agreement (agreement that the event did not occur) but had low level of positive agreement (51.6%)[12]. In our study, we found a higher sensitivity (87% and 84%) of mothers' self-report of pre-eclampsia using the ISSHP and ACOG definition, as described earlier (66%), but the same high specificity (99%)[13]. In addition, Sou et al. described that the symptoms of pre-eclampsia, such as proteinuria, are recalled worse than the diagnosis itself[13]. Recently, Klemmensen showed that the information on the diagnosis pre-eclampsia either being recorded in a registry or obtained by detailed interview by telephone had acceptable validity (sensitivity: 71% and 73%, respectively, and kappa: 0.74 and 0.64, respectively)[14]. As in this study, written questionnaires were used, and results cannot be compared with those of our study.

The validity of maternal data, in general, obtained by questionnaire, varies. Self-reported information on having had an infant delivered by caesarean section, maternal diabetes before pregnancy, smoking habits, and having had amniocentesis have a high level of agreement[12,13,15]. These items, however, are easy to define, asked by direct questions and actual events at one or more points in time[13]. The moderate recall of pre-eclampsia might

reflect a lack of patient-physician communication rather than a time gap bias, because the questionnaire in our study was sent 2 months post partum.

Diehl et al. recently demonstrated an 80% sensitivity and 96% specificity in verifying a history of pre-eclampsia, with a PPV of 51%[16]. This study tested the accuracy of a questionnaire used to screen a history of pre-eclampsia 24.5 years after the index pregnancies in 144 women who had a pregnancy complicated by pre-eclampsia, eclampsia, or a toxaemia, and in 158 women who had had a normotensive pregnancy. They conclude that their validated questionnaire may be a useful tool in identifying women with a previous history of pre-eclampsia. Our results indicate that inadequate maternal recall of pre-eclampsia may lead to substantial bias in studies on estimates of potential associations of complications in subsequent pregnancies and maternal cardiovascular disease in later life. However, withsuch a high specifity, it is unlikely that women without a history of pre-eclampsia would self-report positive history.Therefore, for studies considering associations between pre-eclampsia and future cardiovascular disease, the currently reported sensitivities and specificities of maternal recall are sufficient to use self-reported pre-eclampsia.

It is of clinical interest that PPV of maternal recall increased from 50% to 82% by including all hypertensive complications in our study. Different studies showed an increased risk of pre-eclampsia in a subsequent pregnancy if a pregnancy is complicated by a hypertensive complication, including pre-eclampsia, HELLP syndrome, and chronic hypertesnion[3,17].

Harlow and Brown demonstrated a diversity of diagnoses of pre-eclampsia as a result of using different definitions of this condition[18]. As comparison of results among studies is fundamental to the correct elucidation of knowledge about pre-eclampsia, standardization of the classification and diagnostic criteria of the hypertensive disorders of pregnancy should be a priority[17].

We used the definition according to the ISSHP because of its international agreement and ability to compare different studies in the literature. A definition that includes even more women with pre-eclampsia, using the ACOG criteria, revealed similar sensitivity and specificity. The sensitivity using the definition for severe pre-eclampsia (according to the Sibai criteria) was higher, as expected. Women who have been severely ill during pregnancy may experienced more detailed and repeated patient-physician communication.

Women with severe pre-eclampsia are more likely to have complications of pregnancy, including preterm delivery and caesarean section.

This study has a number of strengths. Firstly, the data collection in the Generation R Study are very detailed, which implies that we were able to study covariates. Secondly, all data were abstracted in a methodologically consistent manner from the medical records.

However, several limitations to our report need to be discussed as well. Firstly, the medical record was assumed to be complete and correct, which might not be always the case. Secondly, not all data were available from the questionnaires. Third, a subgroup had not been sent a questionnaire postpartum.

To assess possible bias introduced by this, data in both groups were compared for the presence of pregnancy complications. In the group that had been sent and returned a questionnaire, less women had had pre-eclampsia, and women were more frequently nulliparous, older, and higher educated. Furthermore, women were more often of Dutch native origin and had higher incomes. In the analyzed group, parity and education were shown to be associated with the validity. This means that the effect of education and parity on the validity of self-reported pre-eclampsia may have been stronger than that suggested in this study.

In conclusion, our study found that the validity of maternally self-reported pre-eclampsia is moderate. A clear diagnosis based on the criteria of hypertension and proteinuria, which is clear for the doctor and the patient, is essential for the management of a subsequent pregnancy, preconception care, and possible prevention of cardiovascular disease in later life. For studies studying associations between pre-eclampsia and future cardiovascular disease, the currently reported sensitivities and specifities of maternal recall are sufficient to use self-reported pre-eclampsia.

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CHAPTER 3

Hemostatic and angiogenic factors in pregnancy and adverse outcome

Chapter 3.1

Concentrations of plasminogen activators and their inhibitors in blood preconceptionally, during and after pregnancy.

Chapter 3.1

Concentrations of plasminogen activators and their inhibitors in blood preconceptionally, during and after pregnancy.

Coolman M, de Groot CJ, Steegers EA, Geurts-Moespot A, Thomas CM, Steegers-Theunissen RP, Sweep FC

Eur J Obstet Gynecol Reprod Biol. 2006;128(1-2):22-8

ABSTRACT

Background

Haemostasis is a complex balance of activating and inhibitory pathways resulting in coagulation and lysis. Normal pregnancy is associated with hypercoagulation that is even more profound in complicated pregnancies.

Objective

To study the role of the plasminogen-activator system in complicated pregnancy with regard to haemostasis, it is essential to have reference values of components of this system during uneventful pregnancy. In this study we investigated the concentrations of six different components of the plasminogen-activator system preconceptionally, during and after uncomplicated pregnancies.

Material and methods

Tissue-type and urokinase-type plasminogen activator (tPA and uPA), plasminogen inhibitor type-1 and -2 (PAI-1 and-2), and the complexes between tPA and PAI-1, and between uPA and PAI-1 (tPA-PAI-1, uPA-PAI-1) were measured by ELISAs in blood obtained preconceptionally, at 6, 10, 20, 32 weeks of gestation, and 6 weeks after delivery in uncomplicated pregnancies (n=41; all six parameters n=22).

Results

tPA and uPA concentrations decreased in the first 10 weeks of pregnancy and subsequently increased in the third trimester. PAI-1 concentrations increased in the third trimester and PAI-2 concentrations increased throughout pregnancy (preconception versus 32 weeks of gestation; 38.73 versus 102.23ng/ml, and 0.024 versus 151.06ng/ml, respectively). tPA-PAI-1 and uPA-PAI-1 complex concentrations decreased in the first trimester, followed by an increase in the third trimester. The concentrations of all components returned to the preconception values 6 weeks after delivery.

Conclusion

This study provides longitudinal data on activating and inhibitory components of the plasminogen-activator system during pregnancy. Insight in the longitudinal changes in these concentrations may be of help in the understanding of the thrombotic tendency in pregnancy complications such as pre-eclampsia.

INTRODUCTION

Haemostasis is regulated by a complex system of activating and inhibitory pathways. Vascular endothelial cells, circulating platelets and plasma proteins play a central role in this system. The fibrinolytic components, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) promote the conversion of plasminogen to plasmin, which is responsible for fibrin degradation. Plasminogenactivatorinhibitor-type1(PAI-1) is a potent inhibitor of tPA and uPA. Vascular endothelial cells are primarily responsible for tPA synthesis and secretion into the blood. Furthermore, vascular smooth muscle cells, platelets and the liver also produce PAI-1. Plasminogen activator inhibitor-type 2 (PAI-2) is mainly
produced by trophoblasts and is reported to play an important role in counteracting uPA during pregnancy[1]. An excess of inhibitors of fibrinolysis could lead to thrombosis.

Endothelial cells present a confluent, non-thrombogenic surface to which platelets do not adhere. However, when insults to endothelial cells expose the subendothelium, platelets rapidly cover these areas and initiate the formation of a haemostatic plug[2]. The viability of these plugs is related to the balance between endogenous fibrinolytic components and inhibitors of fibrinolysis.

Uncomplicated pregnancy is associated with a reduction
of fibrinolytic activity[3] and an increase of PAI-1[4]concentrations in blood, contributing to a state of hypercoagulation. Reduced fibrinolytic activity is even more profound in pregnancies complicated by abnormal placentation as in the case of pre-eclampsia, abruption
placenta and intrauterine growth restriction[5].

In order to gain insight in the possible involvement of abnormal fibrinolytic activity in the pathophysiology of major complications of pregnancy in the future, we studied the concentrations of plasminogen activators and inhibitors and the complexes between these components in blood from women with uneventful pregnancy throughout gestation.

Material and methods

Patients

Between 1987 and 1990, 41 women were selected by strict criteria from a larger cohort described previously[6-8]. These exclusion criteria included risk factors of adverse pregnancy outcomes, first-degree relatives with a genetic disorder known to cause major congenital malformations and treatment for infectious, metabolic, endocrine or malignant diseases. Women were recruited before conception. All selected women were Caucasian, nulliparous and had a singleton and uncomplicated pregnancy. The study was a multi-centred cohort study. The Medical Ethical Committee of the Radboud University Nijmegen Medical Centre, The Netherlands, approved the protocol. After informed consent a researchnurse visited the participants at home to obtain a medical history and to collect blood samples (P0). The research nurse revisited the women if they were not pregnant within 6 months after first blood sample collection for obtaining a new preconceptional blood sample. Pregnancy was confirmed by a monoclonal antibody-based pregnancy test on the 17th day of conception and menstrual history. One or two transvaginal ultrasonographic examinations before the 8th week of gestation were performed in order to ascertain the duration of pregnancy. Subsequent blood sampling took place at weeks 6 (P1), 10 (P2), 20 (P3) and 32 (P4) of gestation as well as at 6 weeks after delivery (P5). Of all women included in the study (n = 41), from 22 women the complete set of six samples from preconception till 6 weeks after delivery were available for analysis. In the remaining samples of 19 women one or more of the longitudinal samples were missing.

Methods

From all women blood was drawn from a brachial vein in sterile tubes containing EDTA. Within 1 h of collection, blood was centrifuged at 1500 x g for 10 min, and plasma was stored at -30 °C until assayed. Plasma concentrations of tPA, uPA, PAI-1, PAI-2, tPA-PAI-1 complex and uPA-PAI-1 complex were assayed with enzyme-linked immunosorbent assay procedures as described before[9,10]. The samples were analyzed in 2002. We performed the

measurements in duplicate.

In each run, an international reference sample developed by the Department of Chemical Endocrinology (the Quality Assurance Center for the Receptor and Biomarker Group of the European Organisation for Research and Treatment of Cancer) was run to check between-assay variability and to monitor overall performance. Therefore, lyophilized control preparations were used. For tPA thewithin-run coefficient of variability (CV) and between-run CVare found to be 3.3% and 10.7%, respectively, and for uPA these values amount to 4.0% and 9.2%, respectively. For PAI-1 the within-run CV and between-run CV are found to be 2.0% and 6.2%, respectively, and for PAI-2 5.3% and 11.2%, respectively. Within-run and between-run CVs for uPA-PAI-1 are 4.2% and 11.6%, and for tPA-PAI PAI-1-1 complex 5.9% and 12.9%, respectively.

Hematocrit and creatinine were also measured.

Statistical analysis

Friedman test with repeated measurements was used to analyse differences between the different time points of each component of the plasminogen-activator system since the data appear to be non-parametric. Simple linear regression analysis was used to determine associations between the concentrations of each component of the plasminogen-activator system and hematocrit and creatinine at each specific time point. Simple linear regression analysis was also used to determine associations between the concentrations of each component of the plasminogen-activator system and maternal body mass index and smoking habits.

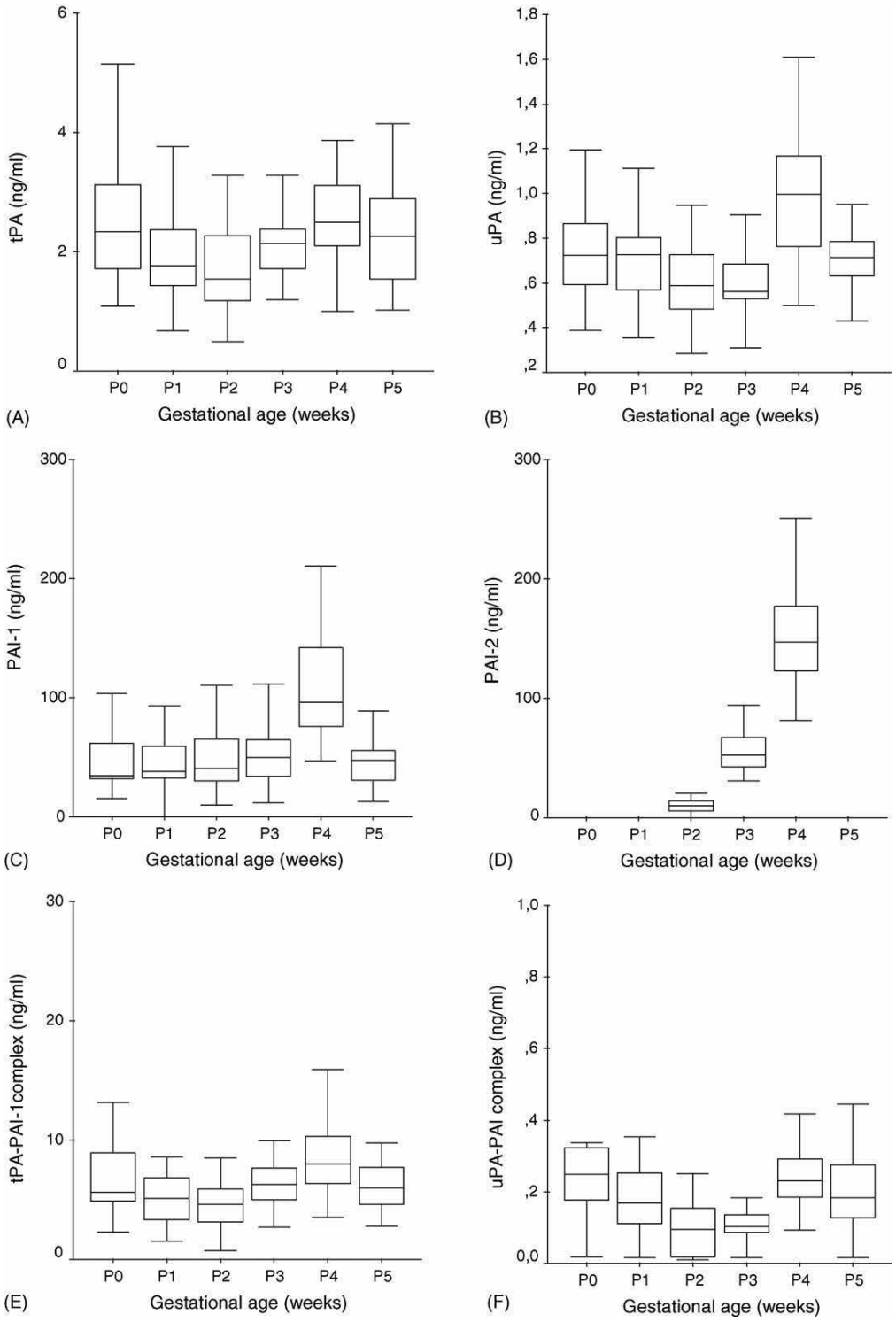

Fig. 1. Data are shown as median concentrations with 5th–95th percentile of tPA and uPA, their inhibitors, PAI-1 and PAI-2, and the complexes tPA-PAI-1 and uPA-PAI-1 inplasmaof41women.TheX-axis represents time of bloodsampling, preconceptional(P0), week6(P1), week10(P2), week20(P3), week32(P4) and 6 weeks after delivery (P5).

RESULTS

Demographic and clinical data of all participants

The mean maternal age at 6 weeks of gestation was 29.7 ± 0.51 (S.D.) years. The gestational age at delivery ranged between 37 and 42 weeks. The mean birth weight was 3377 ± 61 (S.D.) grams (range 2700–4200 g).

Laboratory data

Plasma concentrations of tPA and uPA, their inhibitors, PAI-1 and PAI-2, as well as the complexes tPA-PAI-1 and uPA-PAI-1in blood obtained before, during and after pregnancy are shown in the Fig. 1A–F. The 22 women with all six samples throughout pregnancy available are shown in Fig. 2A–F (for all parameters $p < 0.01$ using Friedman test). The median concentrations of tPA and uPA significantly decreased in the first trimester (preconception versus 10 weeks of gestation; 2.45 versus 1.66 ng/ml, and 0.68 versus 0.52 ng/ml, respectively, Fig. 1A and B). This was followed by a gradual increase towards term (preconception versus 32 weeks of gestation, tPA: 2.45 versus 2.75 ng/ml, and uPA: 0.68 versus 0.98 ng/ml, respectively). PAI-1 concentrations increased in the third trimester and PAI-2 concentrations increased throughout pregnancy (preconception versus 32 weeks of gestation; 38.73 versus 102.23 ng/ml, and 0.024 versus 151.06 ng/ml, respectively, Fig. 1C and D). In particular, blood concentrations of PAI-2 were very low during the first trimester and dramatically increased in the second half of pregnancy. The tPA-PAI-1 and the uPA-PAI-1 complex concentrations showed a decrease in the first half of pregnancy (preconception versus 10 weeks of gestation; 6.58 versus 4.74 ng/ml, and 0.21 versus 0.086 ng/ml, respectively, Fig.1E and F)and then gradually increased to preconception concentrations towards term. The plasma concentrations of all six components of the plasminogen-activator system 6 weeks after pregnancy were similar to those before conception.

No correlations were found between the concentrations of the components of the plasminogen-activator system and hematocrit or creatinine at the various time points (R^2 between 0 and 0.342), suggesting that the concentrations of these factors were not influenced by hemodilution. No correlations were found between the concentrations of the components of the plasminogen-activator system and body mass index or smoking at the various time points (R^2 between 0 and 0.342).

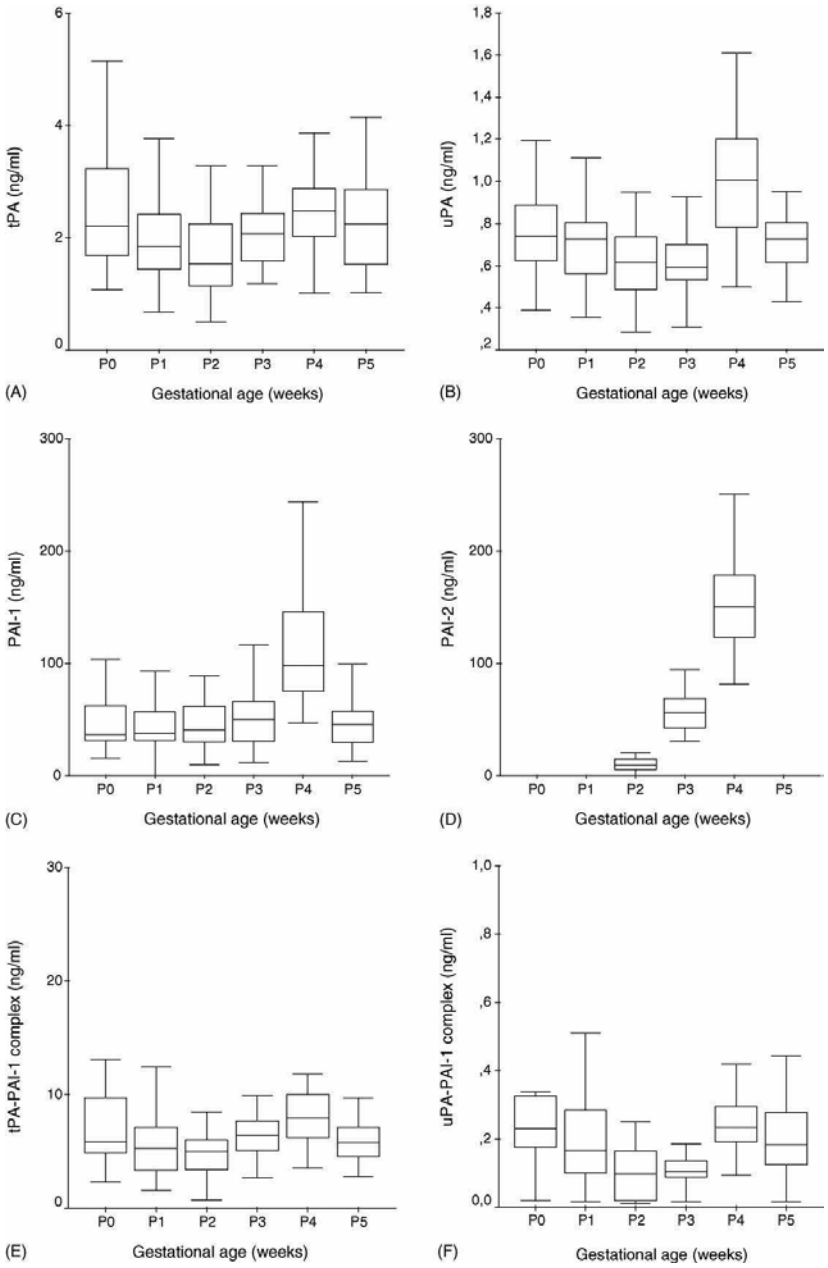

Fig. 2. Data shown are median concentrations with 5th–95th percentile of tPA and uPA, their inhibitors, PAI-1 and PAI-2, and the complexes tPA-PAI-1 and uPA-PAI-1inplasmaof22womencollectedlongitudinal.TheX- axis represents time of bloodsampling, preconceptional(P0), week6(P1), week10(P2) ,week 20 (P3), week 32 (P4) and 6 weeks after delivery (P5).

Discussion

We observed profound changes in the concentrations of components of the plasminogen-activator system in blood of women with uneventful pregnancy indicating the presence of a delicate balance between activators and inhibitors of fibrinolysis during pregnancy. All components of the plasminogen-activator system returned to preconceptional concentrations after pregnancy indicating that all changes are induced by pregnancy. It is suggested that these transient changes are explained by hormonal influences[11].

In conjunction with our finding is the study of Koh et al. which described similar changes of haemostasis in normal pregnancy during gestational periods, labour and after placental separation and reported similar concentrations of a portion of the fibrinolytic components (tPA, uPA, PAI-1 and PAI-2)[12]. The primary fibrinolytic components, tPA and uPA were transiently decreased during the first and second trimester of pregnancy, while in the third trimester the concentrations of uPA were significantly increased compared to preconceptional concentrations.The decrease in the concentrations of these fibrinolytic components cannot be explained by hemodilution during pregnancy, since we found no association between the concentrations of these factors and the hematocrit.

The concentration of particularly that of PAI-2, increased substantially during pregnancy, which is in line with earlier observations[13,14]. The undetectable concentrations of PAI-2 in the first trimester indicate that this compound is produced by the trophoblastic cells that proliferate during pregnancy leading to higher synthesis and release into the bloodstream. These inhibitors form stable complexes with the endogenous thrombolytic entities uPA and tPA in a one-to-one fashion. Interestingly, the patterns of the uPA-PAI-1 and tPA-PAI-1 complex concentrations as observed throughout pregnancy, strongly resemble those of uPA and tPA concentrations, respectively. This despite the concentrations of the two inhibitors increased profoundly during pregnancy. This indicates that the formation of the complexes in blood of pregnant woman strongly depends on the concentrations of uPA and tPA in blood, which indeed are much lower than those of their inhibitors. The decrease in uPA and tPA concentrations and in the levels of their complexes with PAI-1 suggest a decreased synthesis or an increased turnover rate of uPA and tPA during pregnancy.

It has to be noted, however, that we only measured tPA and uPA complexed to PAI-1 and not the complexes of these two compounds with PAI-2. As the concentrations

of PAI-2 are sharply increased during pregnancy, it is likely that the free tPA and uPA will be predominantly complexed byPAI-2 and not by PAI-1. Our complex ELISA does not detect these complexes. Consequently, the concentrations of tPA-PAI-1 and uPA-PAI-1 in blood would decrease during pregnancy, which is in line with our observations. Following this line of reasoning the decreased concentrations of the uPA-PAI-1 and tPA-PAI-1 complexes would not necessarily implicate a decreased synthesis of tPA and uPA.

An alternative explanation for the decrease in tPA and uPA concentrations in first 10 weeks of gestation might be found in the detection efficiency of the complexes in the ELISAs. The tPA and the uPA ELISAs detect free as well as complexed tPA and uPA, albeit that the complexedforms are most probably less efficiently detected because of hindrance of antibody binding sites by the inhibitors. Therefore, the concentrations of total tPA and uPA immunoreactivity (i.e. free and complex components) detected by ELISA may decrease in blood, while the tPA and uPA production is not affected or even might be slightly increased.

Although we cannot conclude whether the decrease in plasma uPA and tPA concentrations observed is due to decrease synthesis,increased turnover,or enhanced complex (with PAI-2) formation, it is reasonable to assume that the concentrations of free tPA and free uPA, which are the biologically active forms, are decreased in blood during the first 10 weeks of pregnancy. This is in line with the observations of Van Wersch et al. who reporteda decrease of tPA concentration during pregnancy, whereas all the other investigated fibrinolytic parameters (PAI, plasminogen, a2-plasmin and D -dimer) showed a progressive increase[15]. The reduced fibrinolytic potential of the plasminogen-activator system in early pregnancy may result in degradation and proteolysis of the decidua to secure adequate trophoblast invasion. This process may also play a role to dissolve the trophoblastic plugs at the end of the first trimester in order to establish adequate perfusion[16].

The increased uPA concentrations in blood observed in week 32 of pregnancy suggests an increased synthesis at this phase of pregnancy. Other authors previously reported a relatively slight increase of tPA during normal pregnancy compared with the rise in PAI-1 [12,17]. Belo et al. reported that the rise in PAI-1 concentration was higher than that of tPA which may explain the reduced fibrinolytic potential during pregnancy[17]. Moreover, they reported an additional significant rise in the levels of D -dimers, assayed as

fibrin degradation products, which can explain the compensated state of low-grade intravascular coagulation, showing that the fibrinolytic system remains functionally active. Choi and Pai[18] reported that PAI-1 levels were strongly associated with tPA as well as D - dimer levels, suggesting that fibrinolysis protects the pregnant woman from hypercoagulation. Interestingly, Kruithof et al. [13] measured by a radioiodinated fibrin plate assay the overall fibrinolytic activity in plasma during pregnancy and showed that despite large variations in the levels of tPA, uPA, PAI-1 and PAI-2 concentrations, the fibrinolytic activity did not change.

It should be noted, that the EDTA plasma is stored for a long period before analysis. Although, we did not test the stability of the proteins during this period of storage, the department of Chemical Endocrinology the Quality Assurance reference laboratory for biomarkers within the European Organisation for Research and Treatment of Cancer has a longstanding experience with uPA and PAI-1 measurements. Within their Quality Assurance studies reference preparations of pooled materials are used and appeared to be very stable for more than 8 years. Nilsson et al. recently described only marginally higher values of the tPA/PAI-1 complex after long storage of 8–11 years[19]. Since we collected and stored all the samples in the same way, we expect a similar effect on the protein concentrations.

Endothelial dysfunction, and subsequently changes in haemostasis, is considered a key factor in the aetiology of pre-eclampsia. To understand the involvement of the plasminogen-activator system in pregnancy-related-complications, particularly of pre-eclampsia and intrauterine growth restriction[20,14], it is necessary to describe the changes of components of the plasminogen-activator system during normal pregnancy. To our knowledge this is the first study describing six components of the fibrinolytic systems longitudinally from the preconceptional period onwards, throughout pregnancy up to 6 weeks after pregnancy. The present study offers the opportunity to gain further insight in coagulation in normal pregnancy and is conditional to study complicated pregnancies with a thrombotic tendency, such as pre-eclampsia, in the future.

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Chapter 3.2

Angiogenic and Fibrinolytic Factors in Blood during first half of Pregnancy and Pregnancy Outcomes

Chapter 3.2

Angiogenic and Fibrinolytic Factors in Blood during first half of Pregnancy and Pregnancy Outcomes

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ABSTRACT

Objective

The imbalance of angiogenic factors (soluble fms-like tyrosine kinase 1 (sFlt1), placental growth factor (PlGF)) and fibrinolytic factors (plasminogen activator inhibitor-2 (PAI-2)) might affect placentation in early pregnancy.

Methods

We studied the associations of maternal sFlt1, PlGF and PAI-2 concentrations in the first trimester (<18 weeks), and sFlt1 and PlGF concentrations in the second trimester (18-25 weeks) with placental function and adverse pregnancy outcomes. This study was embedded in a population-based prospective cohort study. Data were used from 7519 women. Biomarkers concentrations were divided in deciles and evaluated in multivariable linear and logistic regression models.

Results

First trimester high sFlt1 was associated with a 5.2% lower uterine artery index in second trimester and a 1.6% higher birth weight (55 grams, CI95% 15 - 95). Neither in the first, nor in the second trimester were sFlt1 concentrations significantly associated with pre-eclampsia. First trimester low PlGF was associated with a 6.1% higher uterine artery index and a 3.4% lower birth weight (-115 grams, CI95% -157 - -74). First trimester low PlGF was associated with fetal growth restriction (OR 2.62, CI95% 1.68 - 4.08) and pre-eclampsia (OR 2.46, CI95% 1.49 - 4.08). First trimester low PAI-2 was associated with a 1.9% higher uterine artery index and a 2.7% lower birth weight (-94 grams, CI95% -136 - -51). First trimester low PAI-2 was associated with a higher risk of fetal growth restriction (OR 2.22, CI 95% 1.39 - 3.55).

Conclusion

First half of pregnancy concentrations of sFlt1, PlGF and PAI-2 are associated with uteroplacental vascular resistance, placental weight and birth weight. Moreover, first trimester PlGF and PAI-2 are associated with an increased risk of adverse pregnancy outcomes.

INTRODUCTION

First trimester abnormal placentation may result in adverse pregnancy outcomes including preterm birth, fetal growth restriction and pre-eclampsia[1,2]. In placenta development and functioning growth factors including vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) play a key role in the remodelling process of the maternal endothelium in the spiral arteries. Both VEGF and PlGF bind to soluble fms-like tyrosine kinase 1 (sFlt1), a splice variant of the VEGF receptor 1 and primarily localized to the syncytiotrophoblasts[3,4]. sFlt1 reduces free circulating levels of the proangiogenic factors, VEGF and PlGF, and thereby blunts the beneficial effects of these factors.

A second important cascade in placental development and functioning is the fibrinolytic system. Fibrinolysis is activated by conversion of plasminogen to plasmin which is responsible for fibrin degradation. Uncomplicated pregnancy is associated with a reduction of fibrinolytic activity contributing to a state of hypercoagulation[5]. Reduced fibrinolytic activity is even more profound in pregnancies complicated by abnormal placentation as in the case of pre-eclampsia and intrauterine fetal growth restriction[6]. Plasmin depends on a balance between plasminogen activators and plasminogen activator inhibitors (PAI). PAI-2 is produced by the trophoblast. It increases with gestational age until term in plasma of pregnant women with uncomplicated pregnancies and declines to undetectable levels 6 weeks after delivery[7,8]. A decreased production of PAI-2 is thought to be a result of impaired placental function[9].

Previously, an association between higher sFlt1 and lower PlGF circulating blood concentrations in women with pre-eclampsia was described[10]. Similarly (anti-) angiogenic profiles have been described in women with pregnancies complicated by fetal growth restriction[11]. The pathogenesis of pre-eclampsia is described as abnormal placenta development. The first (placental) stage of the disease might result in a failure in trophoblastinvasion. Episodes of placental hypoxia result in oxidative stress and release of various components into the maternal circulation. The second stage of the systemic maternal disease is associated with exaggerated endothelial activation and a generalised hyperinflammatory state compared to normal pregnancy[1]. We hypothesized that high sFlt1, low PlGF and low PAI-2 concentrations in blood act as a sign of the first placental stage, resulting in the second stage of systemic maternal disease or placental related diseases, including preterm birth, fetal growth restriction and pre-eclampsia. We therefore

investigated the associations of sFlt1, PlGF and low PAI-2 concentrations in first and second trimester of pregnancy with the uterine artery resistance index in second trimester, placental weight and birth weight. Secondly, we studied the associations of these factors with the risks for complications including preterm birth, fetal growth restriction and pre-eclampsia.

MATERIALS AND METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in the city of Rotterdam, the Netherlands[12]. The Generation R Study examines early environment and genetic determinants of growth, development and health in fetal life, childhood and adulthood. Enrolment was aimed in the first trimester, but possible until birth of the child. Women were enrolled in the study at their routine ultrasound examination in pregnancy after written consent was obtained. Assessments in pregnancy, including anthropometrics and questionnaires were planned in first, second and third trimester. All children were born between April 2002 and January 2006. Of all eligible children in the study area, 61% participated at birth in the study. The Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, approved the study.

Blood sFlt1, PlGF and PAI-2 concentrations

Maternal non-fasting venous blood samples were drawn in first trimester (weeks in median with 90% range, 13.4, 10.5 - 17.2)) and second trimester (weeks in median with 90% range, 20.4, 18.8 - 22.9). Details of processing procedures have been described previously[13]. Blood samples were stored at -80°C. Plasma sFlt1 and PlGF concentrations were analyzed by the department of Clinical Chemistry of the Erasmus Medical Centre. sFlt1 and PlGF concentrations were analyzed using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands). The between-run coefficients of variation for plasma sFlt-1 were 2.8 % at 5.5 ng/ml and 2.3% at 34.0 ng/ml. The coefficients for plasma PlGF were 4.7 % at 24 pg/ml, and 3.8% at 113 pg/ml. Plasma PAI-2 concentrations, analyzed by the Department of Laboratory Medicine of the Radboud University Nijmegen Medical Centre, were determined by

ELISA with the same experimental setup as described previously[14,15]. For calibration, recombinant PAI-2 generously provided by Biotech Australia (Roseville, Australia) was used. The analytical sensitivity, defined as the amount of PAI-2 giving a signal in the ELISA greater than two standard deviations above blank values, was 11 pg/ml, whereas the functional sensitivity was 32 pg/ml. For estimation of the accuracy of the method a reference preparation was used in each microtiter plate. The mean PAI-2 concentration in this preparation was 88.4 ng/ml, while the intra-assay variation, the between-plates variation and the inter-assay variation amounted to 3.4%, 2.9% and 8.4%, respectively.

Maternal and fetal complications in pregnancy

Maternal and child outcomes including hypertensive disorders in pregnancy (pregnancy-induced hypertension and pre-eclampsia), gestational age at birth, birth weight and placental weight were obtained from medical records, completed by community midwives and obstetricians. The occurrence of hypertension and hypertension-related complications (including pre-eclampsia, proteinuria, eclampsia, and/or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets)) were cross-validated using hospital registries[16]. Pre-eclampsia was defined after completion of the pregnancy according to the International Society for the Study of Hypertension in Pregnancy criteria[17]. Pre-eclampsia was defined as a *de novo* hypertension (an absolute blood pressure ≥140/90 mmHg) after the 20th gestational week with concurrent proteinuria (≥ 0.3grams in a 24-hour urine specimen or ≥ 2 + [1 g/l] on a voided specimen, or ≥ 1+ [0.3 g/l] on a catheterised specimen). Superimposed pre-eclampsia was defined as a chronic hypertension (an absolute blood pressure ≥140/90 mmHg preconceptionally or before the 20th week of pregnancy) and new-onset proteinuria. Early-onset pre-eclampsia was defined as pre-eclampsia with a delivery before a gestational age of 34 weeks[18]. Pre-eclampsia was diagnosed in 167 women (2.2%) and superimposed pre-eclampsia in 25 women (0.3%). Early-onset pre-eclampsia was diagnosed in 23 women (0.3%). Preterm birth was defined as a delivery started spontaneously before a gestational age of 37 weeks. Fetal growth restriction was defined a sex specific gestational age adjusted birth weight below 2.3th percentile of the study cohort.

Uteroplacental vascular resistance measurements

Ultrasound examination to assess uterine artery resistance index (UtA-RI) was performed in mid-pregnancy (20.5 weeks in median with 90% range, 19.4 – 22.1). For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound and the mean was used for further analyses[19]. Doppler measurements were available in 54% of the women. The latter was because Doppler measurements could only be performed within one of the research centres. Characteristics of these women did not differ from those without a Doppler measurement.

Covariables

Information on maternal characteristics was obtained directly in one of the research centres or by a self-administered questionnaire at enrolment. In this questionnaire ethnic background was extracted from information from country of birth of the woman herself and her parents and classified as follows: European and non-European. Education (highest completed educational level of the mother) was categorized into three levels: low (no education or primary school), mid (secondary school) and high (college or university). Maternal comorbidity was defined as a mother having a chronic disorder, including chronic hypertension, chronic heart disease, diabetes, hypercholesterolemia, thyreoid disease and systemic lupus erythematosus. Maternal smoking and alcohol consumption habits were assessed by repeatedly applied questionnaires in pregnancy[20,21]. Maternal height and weight were measured at enrolment. Body mass index (BMI) was calculated as weight per height squared (kg/m^2).

Study population

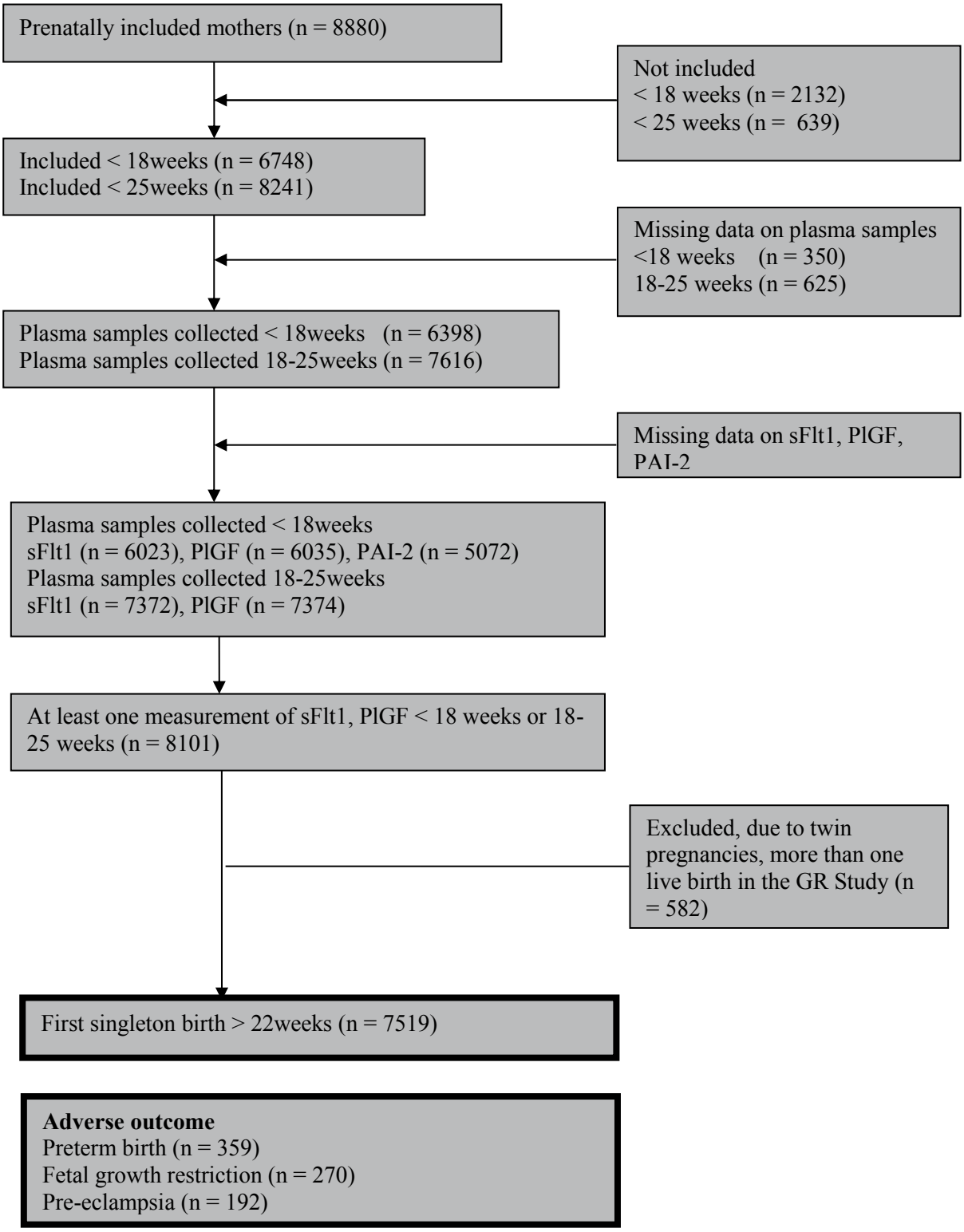
Of the total of 8880 mothers who were enrolled during pregnancy, 76% (n = 6748) were enrolled before a gestational age of 18 weeks (first trimester)[12]. Blood samples from 95% of these mothers (n = 6398) were collected before 18 weeks. Before a gestational age of 25 weeks 93% (n = 8241) mothers were enrolled. Blood samples between 18 and 25 weeks (second trimester) were collected in 92% (n = 7616) of the women. With respect to mothers with multiple pregnancies in the Generation R Study, we only included the first pregnancy to avoid clustering. Women with a stillbirth were also excluded. Consequently the present analysis was limited to singleton birth, delivered at a gestational age of more

than 22 weeks. sFlt1, PlGF and/or PAI-2 were analyzed in the remaining 7519 women who had at least one measurement of sFlt1 or PlGF in <18 weeks or between 18-25 weeks gestation (Figure 1).

Statistical analysis

Power calculations in the Generation R Study are based on 7000 subjects in the whole cohort. For a normally distributed continuous outcome it is possible to detect with a type I error of 5% and a type II error of 20% (power 80%) a difference of 0.11 SD in the whole cohort if 10% of all subjects has the relevant exposure. For dichotomous outcomes with the same type I and II errors, it is possible to detect a relative risk of 1.39 in the whole cohort if 10% of all subjects has the relevant exposure and the one year incidence of the outcome of interest is 10%. Rates of most dichotomous environmental and genetic exposures in the Generation R Study are expected to vary generally between 10 and 20%[12]. Standard deviation scores (SDS) were created for each biomarker. A linear regression model was used to assess the associations of the maternal characteristics with the biomarker. To enable comparisons of the effect estimates between biomarkers and risk factors, we analyze our results as change per standard deviation score. These scores enable adjustment for gestational age (GA) avoiding the inclusion of non-linear functions of GA in models. Our approach for developing reference models for constructing SDS was based on the LMS model of Cole and Green[22], as implemented in the GAMLSS software of Rigby and Stasinopoulos[23], and previously applied and reported by us[24]. Subsequently, all factors associated with the biomarkers ($P<0.05$) were included in the multivariable model to assess the associations between the biomarkers and, placental function and adverse pregnancy outcomes. We assessed individual changes in sFlt1 en PlGF (delta sFlt and delta PlGF, n = 4849 and 4868, respectively) and we assessed sFlt1-PlGF ratios (first trimester and second trimester, n = 5495 and n = 6768 respectively). Since sFlt1 and PlGF concentrations were not normally distributed (evaluated in a histogram), we applied a logarithmic transformation for these analyses. To assess the associations of biomarkers concentrations, deltas and ratios, with maternal and fetal outcomes, we created deciles of each biomarker, the delta and ratio.

Figure 1 Flow chart of Study participants



Furthermore, because high sFlt1 and low PlGF or PAI-2 concentration may represent potential risk factors for adverse outcomes, we additionally used for sFlt1 the tenth deciles and for PlGF and PAI-2 the first deciles as cut-off values. For sFlt1 in first trimester, concentrations ≤9.83ng/ml and >9.83ng/ml and in second trimester, concentrations ≤11.12ng/ml and >11.12ng/ml were considered as low (reference) and as high (tenth deciles), respectively. For PlGF in first trimester, concentrations ≤21.30pg/ml and >21.30pg/ml, and in second trimester, concentrations ≤108.09pg/ml and >108.09pg/ml were considered as low (first deciles) and as high (reference), respectively. For PAI-2 in first trimester, concentrations ≤23.61ng/ml and ≥23.61ng/ml were considered as low (first deciles) and as high (reference), respectively. A multivariable linear regression model was used to assess the associations between sFlt1, PlGF and PAI-2 concentrations, and placental vascular resistance, placental weight and birth weight. Likewise, multivariable logistic regression models were used to study the associations between sFlt1, PlGF and PAI-2 and preterm birth, fetal growth restriction and pre-eclampsia. The consideration of confounding variables was based on literature[25-29]. These possible confounding factors included maternal age, BMI, parity, maternal ethnicity, maternal education, smoking, the use of alcohol and folic acid use as well as gender of the newborn. In the multivariable models missing data were completed using multiple imputation (missing: maternal ethnicity 7%, maternal education 14%, maternal comorbidity 12%, parity 1%, BMI 1%, smoking 13%, alcohol use 13% and folic acid use 25%). Data were imputed according to the Markov Chain Monte Carlo method assuming no monotone missing pattern. Five imputed data sets were created. Subsequently, multiple regression analyses were performed on each imputated dataset and thereafter combined to one pooled estimate[30-32]. Since there were no major differences in the observed results between analyses with imputed missing data or complete cases only, only results including imputed data are presented.

RESULTS

Characteristics

Baseline characteristics are depicted in table 1. Maternal age, BMI, multiparity, European ethnicity, smoking and the male sex of the baby were associated with lower sFlt1 concentrations (P<0.05).

Table 1 Characteristics of participants

Characteristic	Total	Outcome of pregnancy			
		Normal	Preterm birth	Intrauterine growth restriction	Pre-eclampsia
	N = 7519	n = 6782	n = 359	n = 270	n = 192
Gestational age in weeks at intake, median (90% range)	14.4 (10.9 – 21.8)	14.2 (11.6 - 20.5)	14.8 (11.6 - 20.8)	14.4 (11.4 - 20.8)	14.4 (11.5 - 20.6)
Age in years mean (sd)	29.5 (5.2)	29.6 (5.2)	29.1 (5.6)	29.2 (5.7)	29.3 (5.4)
Age (%)					
<25	1605 (21.3)	1388 (21.0)	87 (24.2)	71 (26.3)	39 (20.3)
25-34	4856 (64.6)	4292 (64.8)	228 (63.5)	158 (58.5)	126 (65.6)
≥35	1058 (14.1)	944 (14.3)	44 (12.3)	41 (15.2)	27 (14.1)
Ethnicity (%)					
European	3990 (53.1)	3568 (53.9)	173 (48.2)	119 (44.1)	90 (46.9)
Non-European	2993 (39.8)	2598 (39.2)	153 (42.6)	129 (47.8)	87 (45.3)
Missing	536 (7.1)	458 (6.9)	33 (9.2)	22 (8.1)	15 (7.8)
Education level (%)					
Primary or no school	397 (5.3)	356 (5.4)	19 (5.3)	13 (4.8)	12 (6.3)
Secondary	2916 (38.8)	2511 (37.9)	162 (45.1)	125 (46.3)	87 (45.3)
University or college	3177 (42.3)	2866 (43.3)	118 (32.9)	90 (33.3)	68 (35.4)
Missing	1029 (13.7)	891 (13.5)	60 (16.7)	42 (15.6)	25 (13.0)
Comorbidity (%)					
No	6232 (82.9)	5532 (83.5)	275 (76.6)	219 (81.1)	139 (72.4)
Yes	359 (4.8)	280 (4.2)	34 (9.5)	16 (5.9)	35 (18.2)
Missing	928 (12.3)	812 (12.3)	50 (13.9)	35 (13.0)	18 (9.4)
Parity (%)					
Primiparity	4402 (58.5)	3797 (57.3)	245 (68.2)	192 (71.1)	151 (78.6)
Multiparity	3038 (40.4)	2767 (41.8)	110 (30.6)	77 28.5)	40 (20.8)
Missing	79 (1.1)	60 (0.9)	4 (1.1)	1 (0.4)	1 (0.5)
Body mass index in kg/m2 in median (90% range)	23.8 (19.3 - 33.7)	23.9 (20.2 - 30.8)	24.2 (19.9 - 31.6)	22.7 (19.2 - 29.2)	25.6 (20.1 - 35.7)
Body mass index (%)					
<20	676 (9.0)	576 (8.7)	38 (10.6)	42 (15.6)	16 (8.3)
20-24.9	3870 (51.5)	3442 (52.0)	159 (44.3)	147 (54.4)	66 (34.4)
25-29.9	1978 (26.3)	1745 (26.3)	112 (31.2)	55 (20.4)	63 (32.8)
≥30	939 (12.5)	812 (12.3)	47 (13.1)	23 (8.5)	45 (23.4)
Missing	56 (0.7)	49 (0.7)	3 (0.8)	3 (1.1)	2 (1.0)
Smoking (%)					
No	4802 (63.9)	4278 (64.6)	204 (56.8)	140 (51.9)	122 (63.5)
Until pregnancy known	569 (7.6)	503 (7.6)	25 (7.0)	18 (6.7)	19 (9.9)
Continued	1147 (15.3)	975 (14.7)	71 (19.8)	73 (27.0)	25 (13.0)
Missing	1001 (13.3)	868 (13.1)	59 (16.4)	39 (14.4)	26 (13.5)
Alcohol (%)					
No	3250 (43.2)	2851 (43.0)	156 (43.5)	117 (43.3)	94 (49.0)
Until pregnancy known	907 (12.1)	782 (11.8)	52 (14.5)	36 (13.3)	26 (13.5)
Continued	2400 (31.9)	2153 (32.5)	97 (27.0)	80 (29.6)	50 (26.0)
Missing	962 (12.8)	838 (12.7)	54 (15.0)	37 (13.7)	22 (11.5)

Values are mean (sd), median (90% range) or percentages (%).

Table 1 Continued

Characteristic	Total	Outcome of pregnancy: Normal	Preterm birth	Intrauterine growth restriction	Pre-eclampsia
Folic acid supplements (%)					
No	1634 (21.7)	1413 (21.3)	88 (24.5)	76 (28.1)	43 (22.4)
Yes	3989 (53.1)	3556 (53.7)	175 (48.8)	112 (45.2)	102 (53.1)
Missing	1896 (25.2)	1655 (25.0)	96 (26.7)	72 (26.7)	47 (24.5)
Infant sex (%)					
Male	3790 (50.4)	3332 (50.3)	185 (51.5)	142 (52.6)	93 (48.4)
Female	3729 (49.6)	3292 (49.7)	174 (48.5)	128 (47.4)	99 (51.6)
UtA-RI					
mean (sd)	0.54 (0.09)	0.54 (0.09)	0.59 (0.10)	0.58 (0.10)	0.60 (0.12)
Missing	3485 (46.3)	3005 (45.5)	196 (55.0)	133 (49.2)	98 (51.0)
Birth weight in grams mean (sd)	3402 (562)	3502 (465)	2330 (645)	2390 (397)	2776 (544)
Missing	46 (0.7)	8 (0.1)	12 (3.3)	0	3 (1.6)
Placental weight in grams mean (sd)	634 (144)	647 (140)	510 (150)	464 (118)	575 (164)
Missing	2065 (27.5)	1740 (26.4)	103 (28.7)	60 (22.2)	42 (21.9)
Gestational age at birth in weeks median (90% range)	39.8 (36.9 - 42.1)	40.3 (38.4 - 41.7)	35.4 (30.4 - 36.7)	39.7 (37.0 - 41.4)	38.3 (32.9 - 40.9)
Missing	0	0	0	0	0

Maternal age, BMI and the use of alcohol were associated with lower PlGF concentrations (P<0.05). Multiparity, low education, non-European ethnicity, smoking and no use of folic acid supplements were associated with higher PlGF concentrations (P<0.05). BMI, multiparity, smoking and the male sex of the baby were associated with lower PAI-2 concentrations (P<0.05).

Uterine artery resistance index, placental weight and birth weight

Placental weight and birth weight were significantly correlated (r=0.63, P=0.01).

High sFlt1 concentrations in both the first (>9.83ng/ml) and second trimester (>11.1ng/ml) were associated with a lower uterine artery resistance index in second trimester (5.2% and 3.1%, respectively), an increased placental weight (5.8% and 4.5%, respectively) and an increased birth weight (1.6% and 3.1%, respectively). Women with low first trimester and second trimester PIGF concentrations (<21.3pg/ml and <108pg/ml, respectively) had an increased uterine artery resistance index in second trimester (6.1% and 3.9%, respectively), a decreased placental weight (4.6% and 5.4%, respectively) and a decreased birth weight (3.4% and 4.1%, respectively). Lastly women with low concentrations of PAI-2

(≤23.61ng/ml) in the first trimester had and increased uterine artery resistance index in second trimester (1.9%), a decreased placental weight (3.6%) and a decreased birth weight (2.7%). Data are shown in table 2

Adverse pregnancy outcomes

In table 3 the concentrations of sFlt, PlGF and PAI-2, the delta's of sFlt1 and PlGF between the first and second trimester, and the ratio's of sFlt1 and PlGF in first and second trimester are presented. Table 4 shows the results of the associations between the biomarkers and adverse pregnancy outcomes. A high delta sFlt1 (difference between first and second trimester concentration, >0.096ng/ml) was associated with an almost two fold increased risk of preterm birth (OR 1.86, CI 95% 1.27, 2.74). A trend, though not significant, towards a lower risk of fetal growth resistriction was observed for high second trimester sFlt1 (>11.12ng/ml) concentrations (OR 0.62, CI95% 0.38, 1.02). First and second trimester sFlt1 concentrations were not associated with the occurrence of subsequent pre-eclampsia. Low PlGF (≤108.1pg/ml) concentrations in second trimester were associated with a two to almost 4 times increased risk of preterm birth (OR 1.64, CI95% 1.16, 2.31), fetal growth restriction (OR 2.55, CI95% 2.55, 4.10) and pre-eclampsia (OR 3.71, CI95% 2.55, 5.40). In addition, low PlGF (≤108.1pg/ml) concentrations in second trimester were considerably associated with early-onset pre-eclampsia with an almost 12 times increased risk (OR 11.9, CI 95% 4.20 – 33.3). Likewise, the delta of PlGF between first and second trimester was associated with a substantially increased risk of early-onset pre-eclampsia (OR 9.35, CI 95% 3.12, 28.0). Lastly, low first trimester PAI-2 (≤23.6ng/ml) was associated with higher risk of fetal growth restriction (OR 2.22, CI 95% 1.39, 3.55).

Table 2 Associations between sFlt1, PlGF and PAI 2, and uteroplacental vascular resistance, placental weight and birth weight.

	UtA-RI mid	*Adjusted* [#]	Placental weight	*Adjusted* [F]	Birth weight	*Adjusted* [F]
Biomarker	Mean (sd)		Mean (sd) in grams		Mean (sd) in grams	
sFlt1 < 18wk						
≤9.83ng/ml	0.54 (0.089)	0 (reference)	630 ± 145	0 (reference)	3407 ± 567	0 (reference)
>9.83ng/ml	0.51 (0.082)	-0.028 (0.039, '-0.017)*	661 ± 145	36.8 (22.0, 51.1)*	3425 ± 550	55.1 (15.1, 95.2)*
sFlt1 18-25wk						
≤ 11.12ng/ml	0.54 (0.090)	0 (reference)	634 ± 146	0 (reference)	3410 ± 557	0 (reference)
>11.12ng/ml	0.52 (0.088)	-0.017 (-0.027, -0.008)*	646 ± 154	28.8 (15.2, 42.5)*	3363 ± 615	48.2 (12.2, 84.1)*
Delta sFlt1						
≤0.096ng/ml	0.54 (0.088)	0 (reference)	637 ± 145	0 (reference)	3429 ± 556	0 (reference)
>0.096ng/ml	0.54 (0.094)	0.00 (-0.010, 0.011)	616 ± 159	-9.9 (-25.5, 5.6)	3267 ± 646	-61.6 (-103.7, -19.4)*
PlGF < 18wk						
≤21.29pg/ml	0.57 (0.096)	0.033 (0.022, 0.044)*	599 ± 136	-29.5 (-44.7, -14.3)*	3272 ± 639	-115.3 (-156.8, -74.0)*
>21.29pg/ml	0.54 (0.088)	0 (reference)	637 ± 146	0 (reference)	3420 ± 554	0 (reference)
PlGF 18-25wk						
≤108.09pg/ml	0.56 (0.099)	0.021 (0.010, 0.032)*	606 ± 147	-34.4 (-47.8, -20.8)*	3258 ± 694	-139.5 (-175.2, -103.8)*
>108.09pg/ml	0.54 (0.088)	0 (reference)	638 ± 146	0 (reference)	3421 ± 545	0 (reference)
Delta PlGF						
≤46.4pg/ml	0.54 (0.085)	0.00 (-0.011, 0.011)	618 ± 156	-17.7 (-33.4, -14.3)*	3265 ± 703	-85.3 (-126.4, -44.3)*
>46.4pg/ml	0.54 (0.089)	0 (reference)	636 ± 146	0 (reference)	3424 ± 556	0 (reference)
sFlt1/PlGF <18wk						
≤0.00015	0.54 (0.088)	0 (reference)	631 ± 146	0 (reference)	3407 ± 563	0 (reference)
>0.00015	0.54 (0.095)	0.003 (-0.007, 0.014)	648 ± 146	22.6 (7.8, 37.3)*	3313 ± 588	34.4 (-4.86, 73.6)
sFlt1/PlGF 18-25wk						
≤0.000027	0.54(0.089)	0 (reference)	634 ± 145	0 (reference)	3412 ± 550	0 (reference)
>0.000027	0.54 (0.098)	-0.002 (-0.011, 0.008)	643 ± 163	22.6 (9.2, 36.0)*	3349 ± 656	19.2 (-16.4, 54.6)
PAI 2						
≤23.61ng/ml	0.55 (0.090)	0.010 (0.001, 0.18)*	623 ± 146	-22.8 (-38.5, -7.0)*	3385 ± 585	-93.6 (-136.1, -51.2)*
>23.61ng/ml	0.54 (0.88)	0 (reference)	634 ± 146	0 (reference)	3408 ± 563	0 (reference)

Results of multiple linear regression analyses. Values are means with standard deviation scores (sd) and regression coefficients (95% Confidence Interval). The regression coefficients reflect the difference in weight in grams for birth weight compared to the biomarkers concentrations: sFlt1, PlGF and PAI-2 in the first or second trimester, their ratios and deltas.

UtA-RI mid = Uterine artery resistance index measured in second trimester.

[#] Adjusted for gestational age of sampling/ ultrasound measurement, maternal age, ethnicity, education, comorbidity, body mass index, parity, smoking, use of alcohol, use of folic acid supplements.

F Adjusted for gestational age of sampling, gestational age at birth, maternal age, ethnicity, education, comorbidity, body mass index, parity, smoking, use of alcohol, use of folic acid supplements and gender.

*P<0.05

Table 3 Concentrations of biomarkers, delta's between second and first trimester and ratio's of sFlt and PlGF.

	Pregnancy outcome			
Biomarkers	**Normal**	**Preterm birth**	**Fetal growth restriction**	**Pre-eclampsia**
	n = 7117	n = 359	n = 270	n = 192
sFlt < 18wk (ng/ml)	5.14, 2.75 - 9.90	4.67, 2.58 - 9.38	4.18, 2.26 - 8.99*	4.94, 2.45 - 9.82
sFlt 18 - 25 wk (ng/ml)	5.03, 2.31 - 11.3	5.16, 2.35 - 12.6	4.46, 2.00 - 11.2*	5.20, 2.37 - 12.3
Delta sFlt (ng/ml)	-0.032, -0.35 - 0.43	0.04, -0.30 - 0.68*	0.036, -0.30 - 0.42	0.011, -0.27 - 0.48*
PlGF < 18wk (pg/ml)	43.5, 21.9 – 120	39.0, 17.7 - 120	38.3, 17.7 - 109	36.3, 17.4 – 111.6
PlGF 18 - 25wk (pg/ml)	203, 111 – 415	199, 10.2 - 440	196, 71.7 - 424	149, 64.8 – 341*
Delta PlGF (pg/ml)	21.4, 10.1 - 47.2	20.9, 5.12 - 44.2	20.5, 5.46 - 50.6	14.7, 4.69 - 35.5*
sFlt/PlGF <18wk	0.00012, 0.00004 - 0.00028	0.00012, 0.00003 - 0.00034*	0.00010, 0.00003 - 0.00030	0.00012, 0.000033 -0.00041*
sFlt/PlGF 18-25wk	0.000025, 0.000009 - 0.000063	0.000025, 0.000009 - 0.000096*	0.000023, 0.000008 - 0.000084*	0.000031, 0.000011 - 0.00013*
PAI 2 (ng/ml)	39.8, 23.7 - 63.5	38.8, 24.4 - 68.9	38.8, 20.2 - 58.4	39.8, 23.7 - 65.6

Results are shown in median (90 % range)

*P<0.05

Table 4 Associations between sFlt1, PlGF and PAI 2 and adverse pregnancy outcomes.

	Pregnancy outcome					
	Preterm birth		Fetal growth restriction		Pre-eclampsia	
Biomarkers	%	Adjusted#	%	Adjusted#	%	Adjusted#
sFlt1 < 18wk						
≤9.83ng/ml	4.7	1 (reference)	3.5	0 (reference)	2.6	1 (reference)
>9.83ng/ml	4.0	0.76 (0.46, 1.26)	3.2	0.79 (0.47, 1.35)	2.7	0.97 (0.94, 0.99)
sFlt1 18-25wk						
≤ 11.12ng/ml	4.6	1 (reference)	3.8	1 (reference)	2.5	1 (reference)
>11.12ng/ml	6.4	1.21 (0.84, 1.74)	3.4	0.62 (0.38, 1.02)	2.7	0.92 (0.53, 1.60)
Delta sFlt1						
≤0.096ng/ml	4.1	1 (reference)	3.7	1 (reference)	2.6	1 (reference)
>0.096ng/ml	8.1	1.86 (1.27, 2.74)*	3.1	1.14 (0.70, 1.85)	2.7	0.96 (0.51, 1.78)
PlGF < 18wk						
≤21.29pg/ml	6.6	1.39 (0.90, 2.15)	6.2	2.62 (1.68, 4.08)*	5.1	2.46 (1.49, 4.08)*
>21.29pg/ml	4.4	1 (reference)	3.2	1 (reference)	2.4	1 (reference)
PlGF 18-25wk						
≤108.09pg/ml	7.1	1.64 (1.16, 2.31)*	7.6	3.61 (2.55, 4.10)*	7.0	3.71 (2,55, 5.40)*
>108.09pg/ml	4.5	1 (reference)	3.3	1 (reference)		1 (reference)
Delta PlGF						
≤46.4pg/ml	4.2	1.63 (1.09, 2.44)*	8.2	1.46 (0.93, 2.30)	6.7	1.96 (1.21, 3.18)*
>46.4pg/ml	6.8	1 (reference)	3.2	1 (reference)	2.2	1 (reference)
sFlt1/PlGF <18wk						
≤0.00015	4.6	1 (reference)	3.4	1 (reference)	2.4	1 (reference)
>0.00015	5.3	1.11 (0.72, 1.74)	4.0	0.92 (0.55, 1.55)	5.2	2.14 (1.32, 3.47)*
sFlt1/PlGF 18-25wk						
≤0.000027	4.4	1 (reference)	3.6	1 (reference)	2.3	1 (reference)
>0.000027	7.5	1.70 (1.14, 2.54)*	5.2	1.15 (0.76, 1.74)	4.7	1.30 (0.80, 2.11)
PAI 2						
≤23.61ng/ml	3.8	0.60 (0.34, 1.06)	5.4	2.22 (1.39, 3.55)*	2.6	1.03 (0.54, 1.95)
>23.61ng/ml	4.6	1 (reference)	3.4	1 (reference)	2.7	1 (reference)

Results from simple and multiple logistic regression. Values are odds ratios (95% Confidence Interval).

[#] Adjusted for gestational age of sampling, maternal age, ethnicity, education, comorbidity, body mass index, parity, smoking, use of alcohol, use of folic acid supplements and gender.

*P<0.05.

DISCUSSION

In this large observational study we show strong associations between angiogenic, placental growth and fibrinolytic factors, and placental development and function with subsequent risks for significant pregnancy outcomes.

sFlt1

High sFlt1 concentrations were associated with lower placental vascular resistance, with subsequently higher placental weight and birth weight. Secondly, a high delta of sFlt1 between first and second trimester was associated with a decreased risk of preterm births. These results are in line with earlier studies showing increased sFlt1 concentrations in twin pregnancies compared to singletons, suggesting a positive association between trofoblastic mass and sFlt1 concentrations. Each trophoblast does not seem to be programmed to produce more of the anti-angiogenic protein sFlt1, however more sFlt1 is produced because of a greater number of trophoblastic cells. From this we hypothesize that in women with higher sFlt1 blood concentrations these elevations might be due to a greater amount of trophoblastic mass.

With respect to preterm birth, Smith et al. described a high sFlt1 in the first trimester associated with a decreased risk of preterm births[34]. Previously expression of VEGF has been demonstrated in human myometrium and VEGF peaks have been associated with cervical ripening in rats. High sFlt1 could result in a decreased VEGF expression and hence protect for a preterm birth.

Previously, increased sFlt1 concentrations were associated with pre-eclampsia, but only up to 5 weeks before onset of pre-eclampsia[10]. Other studies, however, presented elevated sFlt1 in first and second trimester as a risk factor for pre-eclampsia[35]. In contrast to this, Kusanovic and Smith showed no association between first trimester sFlt-1 and pre-eclampsia[34,36]. Previous studies showing an association between a high sFlt1 and pre-eclampsia were relatively small and did not always adjust for the gestational age at time of sampling. We, like other larger studies, were also unable to confirm a positive association between sFlt-concentrations in early pregnancy and pre-eclampsia[36].

The up-regulation of sFlt1 release has been described as a result of a hypoxic environment. sFlt1 is highly expressed in the first trimester and it seems plausible that low first trimester reflects low angiogenic activity and thereby impaired placental development.

One consequence of the placental impairment may be fetoplacental hypoxia followed by a strong subsequent angiogenic activity. High concentrations of sFlt1 may be a marker of this activity[37]. High sFlt1 concentrations appearing later in pregnancy, lead to endothelial dysfunction and mediate a pre-eclampsia[38]. In the presence of a placenta with an appropriate size for gestational age, predisposing cardiovascular and metabolic syndrome-like disorders might also set off a cascade of placental oxidative stress, resulting in late onset pre-eclampsia[39]. Finally, sFlt1 is mainly produced by placenta, although other sources have been described as well such as peripheral blood monocytes and vascular endothelial cells[40].

PlGF

Low PlGF was associated with a higher uteroplacental vascular resistance in mid-pregnancy, a lower placental weight and birth weight, and considerable high risks for preterm births, fetal growth restriction and pre-eclampsia. The significant associations between low first trimester concentrations and high uteroplacental vascular resistance in mid-pregnancy may indicate early placental insufficiency. Specific binding of sFlt1 to PlGF has been suggested as explanation of decreased PlGF in pregnancies with adverse outcomes. However, in our study the sFlt1-PlGF ratio was not associated with placental weight and birth weight, suggesting another underlying mechanism[41]. Our first to second trimester delta of PlGF concentrations showed an association with a low placental weight and birth weight. It has been suggested that remodelling of the spiral arteries probably begins in late first trimester after which it is completed by 18-20 weeks of gestation[42,43]. This may imply that the second trimester placental development is also important in relation to adverse pregnancy outcome.

With respect to pre-eclampsia, a decrease of PlGF concentrations nine to eleven weeks before the development of pre-eclampsia with a considerable decrease five weeks before the actual onset, has been described[10]. We demonstrated strong associations between low PlGF concentrations in the first and second trimester and a low delta of PlGF and the risk of (early-onset) pre-eclampsia up to 10%. An explanation for development of pre-eclampsia would be a primary unknown trigger resulting in an abnormal placentation with a decreased release of PlGF, starting in early and second trimester, and with less effect on the sFlt1 concentration in first and second trimester. Furthermore, placental

insufficiency with low PlGF concentrations in early pregnancy is even more profound in early-onset pre-eclampsia.

PAI-2

The associations between PAI-2 and birth weight are in agreement with earlier studies[44,45]. Because villous cells are the source of PAI-2, its concentrations may reflect trophoblastic mass and therefore explain the association with birth weight. The association between PAI-2 concentrations and the risk for preterm birth has not been described before. Only one study assessed the association between PAI-2 and preterm birth through an association with PAI-2 polymorphism[46]. In accordance with our results, Estelles et al. showed that low placental expression of PAI-2 was shown to be associated with fetal growth restriction[44]. Finally, low PAI-2 concentrations in third trimester have been associated with pre-eclampsia[9]. However, it is uncertain whether the altered concentrations of PAI-2 precede the clinical onset. Clausen et al. reported increased PAI-2 concentrations at 18 weeks in women who developed pre-eclampsia[47]. As in our study, Akolekar et al. found no association between PAI-2 concentrations in the first trimester and pre-eclampsia[9].

Methodological issues

This was a large population-based prospective cohort study with an extensive data collection. Though participation rates for were relatively high and the ethnic distribution differs only moderately from that of the eligible population in the study area, The Generation R Study is characterized by a rather highly educated and healthy study population compared to available city data[12]. In cohort studies missing data analysis is always a critical issue. We attempted to deal with this by using multiple imputation for missing covariables. However, with repect to some outcomes, such as here placental weight complete information was missing in almost 27% of the women. Characteristics of these women did not differ from those with a known placental weight. For this reason, we do not expect that this has signifcantly influenced our results. Though we are aware that bias can not be fully excluded.

Conclusion

First trimester sFlt1 concentrations do not seem to be a valuable predictor for adverse pregnancy outcomes. Lower PlGF concentrations, however, are associated with increased risks of preterm birth, fetal growth restriction and pre-eclampsia. Lower PAI-2 concentrations in the first trimester are associated with a higher risk of fetal growth restriction, but not pre-eclampsia.

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Chapter 3.3

Matrix metalloproteinase-9 gene -1562C/T polymorphism mitigates pre-eclampsia

Chapter 3.3

Matrix metalloproteinase-9 gene -1562C/T polymorphism mitigates pre-eclampsia

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ABSTRACT

Introduction. Although the aetiology of pre-eclampsia is unknown, there is substantial evidence that it finds its roots in abnormal placentation. Prerequisites for successful placentation include trophoblast invasion, degradation and remodelling of the uterine decidual extracellular matrix, and apoptosis without thrombosis. We tested this hypothesis by analyzing the effect of functional polymorphisms in the genes coding for MMP9, MMP3 and annexin A5 on the risk of pre-eclampsia using a case-control design.

Materials and Methods. In 163 women with pre-eclampsia and 163 controls we studied the association with polymorphisms in the MMP9 (–1562 C/T), MMP3 (–1612 5A/6A) and annexin A5 (–1 C/T) genes using logistic regression analysis.

Results. A lower prevalence of the rare T-allele of the MMP9 (–1562 C/T) polymorphism in women with pre-eclampsia was found (odds ratio 0.48, 95% confidence interval 0.25 – 0.90). The distribution of the MMP3 (–1612 5A/6A) and annexin A5 (–1 C/T) gene polymorphisms were similar in cases and controls.

Conclusion. Our results suggest that the MMP9-1562T allele is associated with a reduced risk of pre-eclampsia and therefore may protect against maladaptation of the spiral arteries and decreased decidual degradation. The elevated MMP9 concentrations reported to be associated with the –1562T allele might be essential for the development of an adeqaute maternal-fetal interface early in pregnancy by facilitating trophoblast apoptosis and degradation.

INTRODUCTION

Pre-eclampsia is a multiorgan disorder of pregnancy characterized by hypertension and proteinuria. Although its aetiology is unknown, there is substantial evidence that pre-eclampsia finds its roots in abnormal placentation[1]. Early placentation is determined by genes inherited from both parents and maternal environmental factors[2]. Placentation involves trophoblast invasion and angiogenesis and its success is dependent on vascular-, and endothelial cell function[3]. In normal early pregnancy, trophoblast invasion is characterized by cytotrophoblast migration invading the decidua and replacing the endothelium of decidual and myometrial vessels[4]. Prerequisite for successful trophoblast invasion is degradation and remodelling of the uterine decidual extracellular matrix. Matrix metalloproteinases (MMP's) play a crucial role in restructuring the extracellular matrix by activating the secretion of gelatinases, collegenases and proteolytic enzymes[5,6]. Several members of this enzyme family are present at the foetal- maternal interface. Cytotrophoblast MMP9 activity has been shown to be increased in human placental tissue[7]. A second matrix metalloproteinase important for placenta development is matrix metalloproteinase 3. MMP3 is produced by first trimester decidual cells and cytotrophoblast cells[8]. MMP3 activates MMP9 and MMP2 and is implicated in connective tissue destruction and associated with endothelial cell dysfunction. The inability to produce sufficient matrix metalloproteinases may be an early manifestation of abnormal placentation such as in pre-eclampsia[9].

Apoptosis has also been implicated to play a role in the failure of extravillous trophoblast invasion observed in placentas from women with pregnancies complicated by pre-eclampsia[10]. Programmed apoptosis is regulated through annexin A5, which is expressed in syncytotrophoblasts and endothelial cells. Placentas of patients with pre-eclampsia and the antiphospholid syndrome demonstrate diminished annexin A5 coverage on the placental villi. Annexin A5 is a strong anticoagulant protein due to the shielding of negatively charged phospholipids. Exposure of negatively charged phospholipids accelerates coagulation[11].

Given the strictly regulated and co-ordinated actions of matrix metalloproteinases, apoptosis and coagulation on normal placentation we studied the effect of functional polymorphisms in the genes coding for MMP9 (–1562 C/T), MMP3 (–1612 5A/6A) and annexin A5 (–1 C/T) on the risk of pre-eclampsia using a case-control design. We selected

these polymorphisms because they are functional promoter polymorphisms and may affect transcription rate and amount of protein synthesized[12,13,14].

MATERIAL AND METHODS

Study group

Women for the case-control study were recruited from the obstetric clinics of the Leiden University Medical Centre (n=99 per group) and the St. Joseph Hospital Veldhoven, The Netherlands (n=64 per group) as has previously been described[15]. Women who had developed pre-eclampsia during their first pregnancy (n=163) and control subjects (n=163) were selected from a computer database and patient charts. The study protocol was approved by the local Ethics Review Board. Data were analyzed anonymously.

Pre-eclampsia was defined by means of strict criteria: as a rise of blood pressure (≥30 mm Hg systolic or ≥15 mm Hg diastolic over values in the first 20 weeks or, if blood pressure was unknown before 20 weeks gestation); late-pregnancy hypertension (defined as an absolute blood pressure ≥140/90 mm Hg); and proteinuria (≥2+ [1 g/l] on a voided specimen or ≥1+ [0.3 g/l] on a catheterized specimen). Severe pre-eclampsia was defined as an absolute diastolic blood pressure of ≥110 mm Hg and proteinuria (≥2+ [1 g/l]) on a catheterized specimen on admission. HELLP (haemolysis, elevated liver enzymes, and low platelets) was defined as thrombocytes $<100x10^9$/l, and both ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase) >70 U/l and LDH (lactate dehydrogenase) > 600 U/L.

Control subjects were selected according to the following criteria: first pregnancy, no rise in blood pressure, no hypertension or proteinuria, similar age (±5 years) and a delivery date as close as possible to the delivery date of the case subject. Both cases and controls that had multiple pregnancies, chronic hypertension, renal disease, diabetes, collagen vascular diseases, cancer, or thrombosis before their first pregnancy were excluded from the study. Clinical characteristics of both groups have been described before[15]. Data concerning smoking were explored in the medical record, which were noted during the first antenatal visit.

Genotyping of polymorphisms in MMP9, MMP3 promoter region and annexin A5

Blood was collected from the antecubital vein into Monovette tubes (Sarstedt, Nümbrecht, Germany) containing 0.106 mol/L trisodium citrate. High-molecular-weight deoxyribonucleic acid (DNA) was isolated from leukocytes and stored at -20^{o}C.

The –1562 C/T polymorphism (rs3918242) in the promoter of the *MMP9* gene was analyzed using polymerase chain reaction (PCR). The reaction mixture contained 6.25 pmol of each primer (forward primer: 5'-GCC TGG CAC ATA GTA GGC CC-3', reverse primer: 5'-CTT CCT AGC CAG CCG GCA TC-3'), 1 * 10^4 pmol of each nucleotide, 1.3 U of Taq DNA polymerase (Amersham Biosciences) and 5 ng of genomic DNA. The PCR started with 4 min denaturation at 96°C, followed by 33 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72°C. Subsequently, the 434 bp PCR product was digested with the restriction enzyme SphI (Roche), the digested products separated on a 1.5% agarose gel and visualized using ethidiumbromide. The C allele remains undigested (434 bp) while the T allele is digested in fragments of 193 and 241 bp.

The –1612 5A/6A polymorphism (rs3025058) in the *MMP3* gene was analyzed using the Taqman allelic discrimination assay (Applied Biosystems, Foster City, Calif). Primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems (for details, see http://store.appliedbiosystems.com). Reactions were performed with the Taqman Prism 7900HT 384 wells format and started with 10 min denaturation at 95ºC, followed by 40 cycles of 15 sec at 92°C, and 1 min at 60°C for annealing/extension. The results were analyzed with the Taqman Analyser and its special software.

The –1 C/T polymorphism in the *annexin A5* gene (rs11575945) was analyzed by RFLP*:* Exon 2 of the annexin A5 gene was amplified out of genomic DNA by PCR. The PCR reaction was carried out in a final volume of 50 µl reaction mixture containing 50 ng genomic DNA, 1.5 U Taq DNA polymerase (Pharmacia), 5 µl PCR reaction buffer (10x) containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 10 pmol of forward (5'-CGC TAA GCC CGA GGT TTC T-3') and reverse (5'-CGC AGC ATA CAA AGT TGT GG-3') primer. The PCR reactions were performed in a Perkin Elmer 9700 thermocycler (PE Applied Biosystems). Cycling conditions for PCR were as follows, an initial denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s and elongation at 72 °C for 30 s. The last cycle was followed by an additional

elongation step of 72°C for 7 min and a cooling step to 4 °C. The amplicons were subsequently digested with the restriction enzyme NcoI, and analysed by PAGE (7%), after staining by ethidium bromide. The T allele remains undigested (130 bp) and the C allele was digested in fragments of 64 and 66 bp. Impaired cleavage occurs in the presence of the -1 C/T polymorphism. Laboratory personnel were blinded to case-control status. In each PCR run samples with known genotypes were included. We also re-analyzed 10% of the samples (at random), and the results were confirmed.

Genotyping was unsuccessful in 30/326 subjects for MMP9 (–1562 C/T), in 4/326 for MMP3 (–1612 5A/6A) and in 3/326 subjects for annexin A5 (–1 C/T). At least three attempts were made to genotype the samples. In the analysis, positive controls were added and confirmed as well as negative controls.

Statistical analysis

In this case-control group, we calculated based on a population frequency of the T allele of the MMP9 (-1562C/T) polymorphism of 13% that with a power 80% and □=0.05, an Odds Ratio (OR) of 2.2 would be detected for carriers of the T allele. The power calculations were not performed for the subgroups (eclampsia, HELLP). Odds ratios are calculated by logistic regression and are not affected by the correction made for the total number of cases and / or controls and successfully genotyped cases and / or controls. The odds ratios were calculated as estimates of the relative risks by simple cross-tabulation for the –1562 C/T polymorphism in the promoter of the MMP9 gene, the -1612 5A/6A polymorphism in the promoter of the MMP3 gene and the –1 C/T polymorphism of the annexin A5 gene. We analyzed the association between carriership of the rare alleles (-1562 T) MMP9, (-1612 5A) MMP3 and (-1T) annexin A5 with pre-eclampsia using unconditional logistic regression techniques that allowed adjustments for other risk factors for pre-eclampsia like a family history of hypertension, smoking, a high body mass index (BMI>25). The odds ratios are not affected by the correction made for the total number of cases and / or controls and successful genotyped cases and / or controls. The statistical analysis has been calculated by the SPSS package program (SPSS 12).

RESULTS

The frequency of the *MMP 9* **(**-1562 T) allele was 0.06 in women with pre-eclampsia and 0.12 in controls. Women carrying the T-allele were less likely to develop pre-eclampsia when compared with women with the CC genotype (Table 1). Adjustment in the logistic analysis for clinical parameters previously associated with pre-eclampsia like familial hypertension, obesity and smoking did not influence the association of the MMP9 (–1562 T) allele and the risk of pre-eclampsia (Table 2). As expected a trend was observed towards an increased risk for pre-eclampsia in women with a history of hypertension in the family and in women with a high body mass index, whereas a trend towards a lower risk was observed for smoking. In this study we did not observe an association between the presence of the –1562 T allele (CT+TT genotypes) with *severe* pre-eclampsia, eclampsia or HELLP.

Table 1 Genotype frequencies of the MMP9 (–1562 C/T) polymorphism in women with pre-eclampsia and control subjects

	Women with pre-eclampsia (n=145)		**Control subjects** (n=151)		**Odds ratio#** (95% CI)
Genotype MMP 9	No.	%	No.	%	
CC	128	88.3	118	78.1	1
CT	16	11.0	31	20.5	0.48 (0.25 – 0.91)
TT	1	0.7	2	1.3	0.46 (0.04 – 5.16)
CT+TT	17	11.7	33	21.9	0.48 (0.25 – 0.90)

odds ratios were determined using logistic regression analysis

CI = confidence interval

Table 2 Ratios and odds ratios for different genotypes after adjustment in the logistic analysis for clinical parameters previously associated with pre-eclampsia

Variable	Ratio cases versus controls (number 145/151)	Odds ratio# (95% CI)
MMP 9 –1562 CT+TT	17/33	0.51 (0.28 – 0.92)
Hypertension in family	8/12	1.53 (0.95 – 2.48)
High body mass index	8/11	1.48 (0.91 – 2.42)
Smoking	1/10	0.48 (0.24 – 0.96)

odds ratios were determined using logistic regression analysis

High body mass is defined as a BMI > 25

The observed frequency of the *MMP3* 5A allele was 0.49 in women with pre-eclampsia and 0.54 in controls (Table 3). There was no significant difference in the frequency of the three genotypes of the MMP 3 (5A5A, 5A6A, 6A6A) gene or the presence of the 5A allele (5A5A, 5A6A) between women who developed pre-eclampsia and control subjects. The presence of the 5A allele (5A5A, 5A6A genotypes) was not associated with *severe* pre-eclampsia, eclampsia or HELLP.

The observed frequency of the *annexin A5* T allele was 0.14 in women with pre-eclampsia and 0.13 for controls. There was no significant difference in the frequency of the three genotypes of the *annexin A5* gene (CC,CT,TT) gene or the presence of the T allele (CT,TT) between women who developed pre-eclampsia and control subjects. The presence of the T allele (CT,TT genotypes) was not associated with *severe* pre-eclampsia, eclampsia or HELLP.

Table 3 Genotype frequencies in the MMP3 (-1612 5A/6A) gene in women with pre-eclampsia and control subjects

	Women with pre-eclampsia (n=159)		Control subjects (n=163)		Odds ratio# (95% CI)
Genotype MMP 3	No.	%	No.	%	
5A/5A	38	23.9	48	29.4	1
5A/6A	79	49.7	80	49.1	0.80 (0.47 – 1.36)
6A/6A	42	26.4	35	21.5	0.66 (0.36 – 1.22)
5A/5A + 5A/6A	117	73.6	128	78.5	1.12 (0.86 – 1.45)

odds ratios were determined using multiple logistic regression analysis

CI = confidence interval

No synergistic effects were found for the various combinations of the polymorphisms in the matrix metalloproteinase 9 (–1562 C/T), matrix metalloproteinase 3 (–1612 5A/6A) and annexin A5 (–1 C/T) gene were found (0.48 (CI 95% 0.26 – 0.91), 1.03 (CI 95% 0.78 – 1.37), 0.93 (CI 95% 0.65 – 1.76), respectively).

For all three polymorphisms the genotypic distributions were in agreement with Hardy Weinberg equilibrium.

Table 4 Genotype frequencies in the annexin A5 (–1 C/T) polymorphism in women with pre-eclampsia and control subjects

	Women with pre-eclampsia (n=162)		Control subjects (n=161)		Odds ratio# (95% CI)
Genotype annexin A5	No.	%	No.	%	
CC	117	72.2	120	74.5	1
CT	44	27.2	39	24.2	0.86 (0.52 – 1.43)
TT	1	0.6	2	1.2	1.95 (0.17 – 21.8)
CT+TT	45	27.8	41	25.5	0.88 (0.54 – 1.46)

odds ratios were determined using logistic regression analysis

CI = confidence interval

DISCUSSION

In women with pregnancies complicated by pre-eclampsia abnormal placentation can be partly explained by the inadequate trophoblast invasion and maladaptation of the spiral arteries *early* in pregnancy. The rare T-allele of this polymorphism has been previously associated with increased MMP9 levels[16]. Our findings of a lower prevalence of the rare T allele of the MMP9 polymorphism in women with pre-eclampsia suggest that women with uncomplicated pregnancies have more often increased MMP9 levels and may therefore have an increased capacity of restructuring the extracellular matrix at the foetal-maternal interface. In addition, the decreased MMP9 activity has been reported in primary cultures of cytotrophoblasts isolated from placentas obtained from women with pre-eclampsia (assuming that the maternal genotype is inherited)[17]. However, no differences in MMP9 expression in early pregnancy in chorion villous biopsies were described between women who later developed pre-eclampsia and women who had an uncomplicated pregnancy[18]. Differences in sample size, definition of pre-eclampsia and methodology of metalloproteinase detection (protein or RNA expression) may explain the differences[19]. The frequencies of the MMP9 (–1562 C/T) genotypes in the control subjects were similar to those reported previously in a population of the United Kingdom and France[16,20].

MMP9 (-1562 C/T) has also been described as a predictor of cardiovascular disease[20-24]. Data from epidemiological studies suggest that cardiovascular disease and pre-eclampsia share common biological pathways, being endothelial cell dysfunction and risk factors for atherosclerosis. Moreover, epidemiological studies have found an association of pre-eclampsia and cardiovascular disease in later life and therefore pregnancy may be considered as a test for risk of atherosclerosis later in life[25]. Surprisingly, we found a lower frequency of the T allele of the MMP9(–1562 C/T) polymorphism in women with pre-eclampsia. In order to speculate on the underlying mechanism of this finding, we suggest that the MMP9 induction has a different role at the fetal-maternal interface than in the vascular remodelling during atherogenesis. Apoptosis and degradation of the cytotrophoblastcells are normal, physiologic constituents of trophoblast turnover[26]. High MMP9 concentrations, as found in cytotrophoblasts in human placental tissue[7], might in fact be essential for the trophoblast apoptosis and degradation, which is needed for the development of an adequate fetal-maternal interface early in pregnancy. In contrast, in pre-eclampsia normal apoptotic release is reduced resulting in an inadequate fetal-maternal

interface and finally endothelium dysfunction due to aberrant gene-environment interaction including low MMP9 levels. In this respect it is of interest to note that smoking, which is associated with increased plasma MMP9 levels[20] is a risk factor for cardiovascular disease but protects against preeclamspia[27]. Moreover, MMP3 and annexin A5 have been reported to play a role in both abnormal placentation and cardiovascular disease in later life[28,14]. MMP3 and MMP9 activity was found to be decreased in extracts of umbilical cord artery taken from control newborns compared to those taken from newborns delivered by mothers with pre-eclampsia[29].

Recently Pöllänen et al. observed a concerted action of the MMP3 and MMP9 gene promoter polymorphisms as risk factors for the development of complicated coronary artery plaques[30]. Therefore we analyzed the effects of the simultaneous presence of polymorphisms in MMP9 (–1562 C/T), MMP3 (–1612 5A/6A) and annexin A5 (–1 C/T) on the risk of pre-eclampsia. In atherosclerosis the balance between proliferation and cell death is highly dependent on matrix remodelling by growth factors, cytokines and MMPs leading to proliferation or differentiation as well as to apoptosis. The absence of such an association between the co-expressed alleles of MMP3, MMP9 and annexin A5 and pre-eclampsia in our study suggests that environmental and other genetic factors may be involved in abnormal placentation and the development of atherosclerosis in later life. However, this will need to be confirmed in larger studies.

In conclusion, we found a lower prevalence of the rare T-allele of the MMP9 (–1562C/T) polymorphism in women with pre-eclampsia suggesting a genetically determined mechanism, which protects women against pre-eclampsia.

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Chapter 4

General discussion

INTRODUCTION

The aim of the present thesis was to elucidate risk factors in early pregnancy and markers for placental related disorders, in particular pre-eclampsia, and in line with these findings gain more knowledge about the etiology of placental related pregnancy disorders. We will summarize the main findings, followed by an outline of interpretations of the findings. First, definitions of several placental disorders are discussed followed by risk factors, markers and biophysical tests, and the associations with placental disorders. Second, implications for pathogenesis are discussed. Finally, we discuss implications of our results for clinical practice and future research.

SUMMARY OF FINDINGS

Risk factors

Several easily accessible risk factors in early pregnancy for development of pregnancy-induced hypertension or pre-eclampsia were confirmed. Some risk factors seem to apply to both complications of pregnancy whereas others differ between pregnancy-induced hypertension and pre-eclampsia. This may point at differences in the pathophysiology of the two disorders. In addition, we added the question whether risk factors are different not only for both disorders but also for nulliparous and multiparous women. Table 1 shows the risk factors for pregnancy-induced hypertension and pre-eclampsia for nulliparous and multiparous women (chapter 2.1). Interestingly, we found that headache, which is not often described before, especially in the first trimester is associated with pregnancy-induced hypertension and pre-eclampsia in multiparous women. In chapter 2.2 and 2.3 we described more into detail that low educated pregnant women had an increased risk for pregnancy-induced hypertension and pre-eclampsia compared with high-educated women. A clinical prediction model consisting of three variables (maternal weight, diastolic blood pressure and systolic blood pressure) easily available to midwives and obstetricians demonstrated adequate performance for classifying women according to their risk of developing hypertension before 36 weeks of gestation. We described in chapter 2.4 the validation study of this prediction model. In our study the predictive ability of the model, that can accurately identify women at very low (<1%) and very high risk (>16%) of

becoming hypertensive during pregnancy before 36 weeks of gestation was confirmed. We could identify using this model 13.4 % women at very low risk and 12.8% women at very high risk. In daily practice we use personal interviews for the risk assessment in pregnancy as a sole source of exposure information. Our study showed that the validity of the maternal self-reported pre-eclampsia per se is moderate (50%), however, for hypertension in pregnancy relative adequate (81%)(chapter 2.5).

Table 1 Risk factors for pregnancy-induced hypertension and/ or pre-eclampsia

Disorder / Risk factor	Pregnancy-induced hypertension		Pre-eclampsia	
	nulli	multi	nulli	multi
Age > 40 years	0	0	+	0
Increased body mass	+	+	+	+
European ethnicity	+	0	0	-
Surinamese ethnicity	0	0	+	0
History of hypertensive disorders	-	+	-	+
Low maternal birth weight	0	0	+	0
Twin pregnancy	0	0	+	0
Chronic hypertension			0	+
Headache	0	+	0	+

0 no association, + positive association (higher risk), - negative association (lower risk)

Markers

Six components of the fibrinolytic systems (tissue-type plasminogen activator (tPA) urokinase-type plasminogen activator (uPA), plasminogen-activator-inhibitor 1 (PAI-1), plasminogen-activator-inhibitor 2 (PAI-2), tPA/PAI-1 and uPA/PAI-1) were described longitudinally from the preconception period onwards throughout pregnancy up to 6 weeks after pregnancy (chapter 3.1). Profound changes in concentrations of the plasminogenactivator system in blood of women with uneventful pregnancy indicates the presence of a delicate gestational balance between activators and inhibitors of fibrinolysis. PAI-2, soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF) concentrations in early pregnancy in association with pregnancy outcomes were subsequently studied (chapter 3.2). Table 2 shows the associations of PAI-2, sFlt-1 and PlGF concentrations in first and second trimester and the risk of pre-eclampsia,

intrauterine growth restriction or spontaneous preterm birth. Lower PAI-2 concentrations in the first trimester are associated with a higher risk of intrauterine growth restriction, but not with pre-eclampsia. We found that first trimester sFlt-1 concentrations do not seem to be a valuable predictor for adverse pregnancy outcomes. Lower PlGF concentrations are associated with increased risk of pre-eclampsia, intrauterine growth restriction and preterm birth (in second trimester). Finally, in chapter 3.3, we described a lower prevalence of the rare T allele of the matrix metalloproteinase 9 polymorphism (MMP9 (-1562C/T)) in women with pre-eclampsia which is suggested to protect against maladaptation of the spiral arteries and decreased decidual degradation. This finding suggests a genetically determined mechanism, which protects women against pre-eclampsia.

Table 2 Associations of PAI-2, sFlt-1, PlGF, sFlt-1/PlGF ratio and MMP9 and pre-eclampsia, intrauterine growth restriction or spontaneous preterm birth

Disorder / **Markers**	**Pre-eclampsia**		**Intrauterine growth restriction**		**Spontaneous preterm birth**	
	<18 weeks	18-25 weeks	<18 weeks	18-25 weeks	<18 weeks	18-25 weeks
PAI-2	0		+		0	
sFlt-1	0	0	0	0	0	0
PlGF	+	+	+	+	0	+
sFlt-1/PlGF ratio	+	0	0	0	+	0
MMP9 (-1562C/T)	-					

0 no association, + positive association (higher risk), - negative association (lower risk)

INTERPRETATION OF FINDINGS

Placental related disorders

The two stages model of pre-eclampsia is widely accepted[1]. The first stage, poor placentation, occurs in the first half of pregnancy when there are no clinical features of the disorder. The second stage, arises from factors released by the placenta. Defective deep placentation has been associated with a spectrum of complications of pregnancy, including pre-eclampsia, intrauterine growth restriction and spontaneous preterm birth[2]. By what exact mechanism the placenta is disturbed in women who develop adverse placental related pregnancy disorders is unknown. Identification of risk factors for placental related

disorders may help us to elucidate the etiology of placental related disorders. In addition, the identification of risk factors for placental related disorders at an early stage of pregnancy, increasing awareness and focused surveillance.

Pre-eclampsia is a common complex disease, an important cause of maternal death worldwide. All definitions of pre-eclampsia are attempts to describe what is observed in clinical terms. Pre-eclampsia is better defined as a syndrome[3]. A syndrome cannot be precisely defined in terms based on its etiology or defined pathogenesis. Pre-eclampsia is a multi-system disorder characterized by the new onset of hypertension and either proteinuria or end-organ dysfunction in the last half of pregnancy[4]. It is possible that within the syndrome there may be different phenotypes with pathogenic pathways that differ between the subtypes (pregnancy-induced hypertension, pre-eclampsia, HELLP and eclampsia). The capacity to recognize and to exploit different subtypes is of obvious importance for prediction, prevention, and treatment.

Intrauterine growth restriction is a second, complex disease which is also defined by clinical terms. Studies have been hampered by the widespread practice of using the terms intrauterine growth restriction and small for gestational age synonymously[4]. However, small for gestational age refers to a birth weight below a given threshold for its gestation, but a significant proportion of smallness due to constitutional or physiological causes is not pathological[5]. This means that the association between pathological smallness and adverse outcome is blurred; According to the standards mentioned before pathological smallness is not always pathological and normal could be pathological.

For a long time spontaneous preterm birth (70 % of all premature births) was viewed as an unpredictable and inevitable fact of life. Medical efforts thus focused on improving the consequences of prematurity rather than preventing its occurrence. Neonatal outcomes has been improved, but it remains one of the biggest problems in obstetrics in terms of both suffering infants and their families and the economic burden on society[7]. In addition, the world's preterm birth rate has been consistently rising[8]. Therefore, identification early in pregnancy and subsequent prevention is needed in order to improve health of the next generation and decrease costs related to long term effects of prematurity.

Risk factors

Redman described different stages of pregnancy[9]. This thesis describes risk factors for hypertensive disorders in pregnancy which can be identified in different stages of pregnancy (figure 1)[9]. Stage 1 and 2 of pre-eclampsia are generally accepted. Earlier stages are hypothetical. As suggested by Redman, stage -1 comprises maternal toleration to paternal antigens expressed in semen or on sperm. Stage 0 comprises preconception early recognition by uterine decidual immune cells of paternal antigens expressed on trophoblast immediately after implantation extending to the time that uteroplacental circulation opens into the intervillous space. It is proposed that poor recognition leads to reduced trophoblast and placental growth[9].

Figure 1 Risk factors or symptoms for hypertensive disorders in the different stages[9]

	Stages of pregnancy				
	Preconception (stage -1)	Conception (stage 0)	Poor placentation (Stage 1)	Systemic phase (stage 2)	
				No symptoms	Symptoms
Risk factors					
Maternal factor	-advanced maternal age -parity -obesity -ethnicity -education -socioeconomic status -disease[#] -history of hypertension in pregnancy -maternal birth weight -familial disease	-disease [#] -smoking -alcoholics	-headache -multiple gestation -nutrition -smoking -alcoholics		-headache -nausea -abdominal pain -changes vision
Paternal factor	-genetics	-smoking -alcoholics			

[#] Maternal diseases include chronic hypertension, diabetes, renal disease, antiphosphfolipid antibodies

Advanced or low maternal age, obesity, maternal disease, non-Caucasian ethnicity, low socio economic factors, history of pre-eclampsia, intrauterine growth restriction or preterm birth and familial history are risk factors associated with different placental related

disorders known before conception. Among women considered as high risk (including women with a history of pre-eclampsia, multiple pregnancy, family history, nulliparity, a raised body mass, maternal age > 40 years) approximately 25% will develop pre-eclampsia compared with the 5% in the general population[10]. Advanced maternal age and obesity suggest endothelial dysfunction involved in placental related disorders in pregnancy[11,12].

Almost half of the overall occurrence of pre-eclampsia may be related to nulliparity. In terms of public health, nulliparity is therefore the most important risk factor for pre-eclampsia[13]. In terms of pathophysiology, it is often suggested that mechanisms may differ between nulliparous and multiparous women[14]. Differences in immunological responses, angiogenic factor profile or reactivity to insulin resistance between first pregnancies and subsequent pregnancies are described[15]. Simon et al. showed in a systematic review that parity is largely ignored in randomized controlled trials concerning the prevention of pre-eclampsia, which raises difficulties in interpreting results[16]. However, increased awareness of parity will gain more insight in the different mechanisms of placental related diseases (nulliparous versus multiparous hypertensive complications in pregnancy). In the following paragraph we will discuss potential risk factors to identify high risk pregnancies for pre-eclampsia in an assessment in early in pregnancy.

Firstly, there is a concern that a higher maternal age (>40years) may be associated with an increase in obstetric complications secondary to a revealing of underlying medical disease or diminished ability to adapt to physical stress that may accompany ageing[17,18]. The increased incidence of chronic hypertension and pregnancy-induced hypertension have been confirmed in prior studies and mostly reflects these age-related changes[19]. Therefore, obstetric complications associated with advanced maternal age are not associated only with advanced maternal age per se but also with the concurrently maternal underlying diseases.

Secondly, obesity is characterized by a systemic inflammatory response in non-pregnant individuals[20]. Several of the metabolic and inflammatory disorders that characterize an increased body mass index have also been documented in pregnant women[21]. An inflammatory response is amplified in obese individuals because of the pro-inflammatory output from adipose tissue and this may account for the increased susceptibility of obese

pregnant women to fail the stress test of pregnancy, contributing to the clinical manifestation of pre-eclampsia. Other important mediators of obesity may be oxidative stress, insulin resistance, reduced immune function, other markers of dyslipidemia, or lifestyle factors such as poor prenatal diet and prenatal physical inactivity. Therefore, in addition to advanced maternal age, obesity is an important risk factor for complicating a pregnancy by pre-eclampsia and adds its unfavorable outcome by several pathologic pathways.

Further, ethnicity and familial history of pre-eclampsia imply a genetic component in the development of placental related disorders. A woman who becomes pregnant by a man whose previous partner had pre-eclampsia is also at a higher risk of developing pre-eclampsia[22]. From an epidemiologic point of view paternal risk factors should be associated with pregnancy-induced hypertension or pre-eclampsia. In this thesis we were, however, not able to confirm this. Recently, Boyd et al. described in a large study with almost 1.4 million pregnancies limited associations between paternal family history of pre-eclampsia and the risk of pre-eclampsia[23]. The association between maternal genetic polymorphisms and development of diseases in pregnancy is an active area of investigation. Studies of candidate genes have been on a small scale and have not yielded clinical reliable results[24]. Genetics plays an important role in the development of placental related disorders. Interesting is theoretically that the paternal genes are likely to play a role in pre-eclampsia since the fetus consists of 50% foreign to the mother, however research is still searching for the proven role of paternal genes.

Fourth, women with a low socioeconomic status seem to have a lower chance of uncomplicated pregnancy. They are more likely to develop hypertension disorders in pregnancy as described in this thesis. There is evidence that women with hypertension in pregnancy have a higher risk for future hypertension and cardiovascular diseases. Hypertensive disorders in pregnancy and cardiovascular diseases share risk factors as well as underlying metabolic abnormalities suggesting similarities in etiology. Both hypertensive disorders in pregnancy and cardiovascular diseases in later life have been proposed to be associated with the presence of endothelial dysfunction prior to pregnancy.

Further, we demonstrated a strong association between previous gestational hypertension and pre-eclampsia, and the risk of pregnancy induced hypertension and pre-eclampsia in a subsequent pregnancy (up to a tenfold increased risk for pre-eclampsia). This supports the idea that the first pregnancy is a stress-test for later life: women who fail the test, i.e. develop hypertension during pregnancy are at increased risk for development of hypertensive diseases in a subsequent pregnancy and of cardiovascular events later in life due to an unfavorable risk factor profile[25,26]. A large study from Denmark suggested that the timing of pre-eclampsia onset should be taken into consideration if pre-eclampsia heritability and etiology are to be unraveled. In their study previous early-, intermediate-, or late-onset pre-eclampsia increased the risk of recurrent pre-eclampsia with the same time of onset 25.2 times, 19.7 times and 10.3 times, respectively, compared with having no such history[23]. A history of pre-eclampsia or pregnancy-induced hypertension gives information about the etiology of placental related disorders but also about future risks of the woman and should be added to the early assessment in pregnancy for high-risk pregnancy.

Six, maternal low birth weight associated with pre-eclampsia supports the hypothesis of fetal origin of an adult disease. There is evidence to link low birth weight with endothelial dysfunction in adults especially in hunger winter studies[27]. Low birth weight is associated with cardiovascular diseases in later life as well[28]. Maternal predisposition for cardiovascular disease might contribute to impaired placentation and subsequent subclinical maternal endothelial dysfunction might predispose to the development of pre-eclampsia and therefore should be added to the early assessment in pregnancy for high-risk pregnancy.

Further, headache in early pregnancy appeared to be associated with pre-eclampsia later in pregnancy. The association of headache in early pregnancy and pre-eclampsia has not been described before. Headache can be diagnosed as a primary headache or secondary headache[29]. Most headaches seen in women are primary headaches (migraine, tension-type headache, and occasionally cluster headache). A secondary headache is secondary to a pregnancy complication or condition that presents during pregnancy (eg, eclampsia). Several studies have explored a possible association between migraine and pre-eclampsia

with contradictory results. Adverse perinatal outcomes, characterized by ischemic placental disorders, are noted to be increased among pregnant women with migraine[30]. Observed associations of migraine and pre-eclampsia are biologically plausible. Abnormal vascular reactivity, increased platelet aggregation, and a high underlying ischemic stroke or cardiovascular risk profile may account for observed associations. Williams et al. described elevated blood pressures among pregnant women with migraine compared to pregnant women without migraine[31]. Low birth weight and preterm birth are recognized to be more common among pregnant women with migraines as well[32,33]. In our study the association of headache in early pregnancy and pre-eclampsia could not be explained by maternal age, migraine, consuming of coffee or an increased body mass index in our study. The relationship between pre-eclampsia and headache in early pregnancy is interesting. Headache in early pregnancy could be an early sign of placental related diseases or migraine.

Finally, the negative effect of smoking on fetal health is well established. Cigarette smoking has been associated with intrauterine growth restriction and preterm birth. While the pathophysiology is not completely understood, several possible mechanisms related to impaired gas exchange, direct toxicity, and sympathetic activation have been proposed[34]. Surprisingly, a meta-analysis has shown that maternal cigarette smoking is associated with a significant reduction in the risk of pre-eclampsia[35]. However, possible benefits of a reduced risk of pre-eclampsia does not outweigh the multiple medical and obstetrical risks associated with smoking during pregnancy both for the mother, her fetus and her environment. In addition, evidence on whether quitting smoking before or in early pregnancy reduces the risk remains inconclusive[35]. Based on an in vitro experiment, one of the mechanisms involved may be that cigarette smoke reduces fms-like tyrosine kinase-1 (sFlt-1), which is the opposite of the changes observed in women who develop pre-eclampsia[36].

In this thesis we showed that pregnancy-induced hypertension and pre-eclampsia share some risk factors, but not all. In women with an underlying disease, including chronic hypertension, a higher body mass index, diabetes or a metabolic syndrome, a pregnancy could provoke a maternal syndrome, including pre-eclampsia in the clinical phase during

pregnancy. This maternal syndrome starts in early pregnancy or even is existing before pregnancy and can be reflected in different phenotypes of hypertension in pregnancy. This may indicate different pathophysiological mechanisms leading to another disease as a result of different linking mechanisms between placental stage and systemic stage (symptomatic or non symptomatic). Or both diseases have the same underlying mechanism, where maternal susceptibility associated with genetic and environmental factors leads to different phenotypes. Poon et al. showed that certain risk factors with different subsets of factors performed better in the prediction of early pre-eclampsia (previous history of pre-eclampsia, ethnicity, chronic hypertension and ovulation inductors) than in late pre-eclampsia[37]. Their findings showed that screening for pre-eclampsia by maternal characteristics and previous history to define the intensity of antenatal care is potentially useful only when the various factors are incorporated into a combined algorithm derived by multivariate analysis. In such case, the estimated detection rates for early-PE, late-PE and GH are about 37, 29 and 21%, respectively, at a 5% false positive rate. The alternative strategy of treating each of the risk factors as a separate screening test would have falsely classified two thirds of the obstetric population as high risk and in need of intensive monitoring.

In this thesis we did not investigate risk factors for intrauterine growth restriction or preterm birth. However, from literature we know that hypertensive disorders in pregnancy, intrauterine growth restriction and preterm birth share similar placenta pathology and share some risk factors in all stages of pregnancy. In addition, risk factors described for intrauterine growth restriction can be classified according to similar stages of pregnancy as in pre-eclampsia (figure 2). Intrauterine growth restriction may be caused by fetal, placental and maternal factors[38]. Fetal factors include structural or chromosomal anomalies, inborn errors of metabolism and fetal infection. Maternal factors can affect placental transfer of nutrients such as low pre-pregnancy weight, under nutrition, substance abuse or severe anemia. Medical conditions can also affect placental implantation and vasculature and hence transfer such as pre-eclampsia, autoimmune disease, thrombophilias, renal disease, diabetes and hypertension.

Many maternal factors have been associated with an increased risk of spontaneous preterm birth, including previous preterm birth, smoking, alcohol consumption, low maternal body mass index, advanced maternal age and a short interval between pregnancies[39].

Figure 2 Risk factors or symptoms for intrauterine growth restriction according to different stages, described for hypertension by Redman[9]

	Stages of pregnancy			
	Preconception (stage -1)	Conception (stage 0)	Poor placentation (Stage 1)	Systemic phase (stage 2)
Risk factors				
Maternal factor	-*teenage* -obesity -ethnicity -education -socioeconomic status -maternal disease -history IUGR, -stillbirth, pre-eclampsia -*uterine anomalies*[*] -family history IUGR -genetics	-*malnutrition* -smoking -substance abuse -medication -*environmental pollution* -*maternal infection* -maternal history of pre-eclampsia	-*infection* -multiple gestation -*malnutrition* -smoking -alcoholics -substance abuse -*medication*	-hypertensive disorders
Paternal factor	genetics			
Fetal factor			-*chromosomal abnormality* -*malnutrition*	

In *italics* risk factors are shown that differ from those in hypertensive disorders
[*]large myomata, septa, synechia

Markers

The plasminogen activator/inhibitor pathway and the matrix metalloproteinases play a key role in cellular invasion by degrading the extracellular matrix. In addition to its well-researched action of stimulating endothelial cell proliferation and migration, vascular endothelial growth factor-A is known to stimulate metalloproteinase activity of the endothelial cells. The vascular endothelial growth factor family of angiogenic growth factors are important molecules regulating early placental vascular changes (placental angiogenesis and maternal spiral artery remodeling). These pathways with different markers play a role in placental development and placental disorders. For recognizing,

prevention and treatment of placental disorders it is important to find these markers as early as possible. Our results imply that pre-eclampsia and intrauterine growth restriction are both diseases which have an impaired placental development but with different biological determinants.

PAI-2

Low concentrations of PAI-2 in the third trimester have been associated with pre-eclampsia[40]. However, it is uncertain whether the altered concentrations of PAI-2 precede the clinical onset. Clausen et al reported higher concentrations of PAI-2 at 18 weeks gestation in women who developed pre-eclampsia[41]. In line with Akolekar, we found no associations between PAI-2 concentration in early pregnancy and development of pre-eclampsia[42].

Decreased PAI-2 concentrations seem to indicate decreased placental function and intrauterine growth retardation as a result[43,44]. These results are consistent with recent findings of a positive association between PAI-2 concentrations in early pregnancy and intrauterine growth at a mean of 12.4 weeks gestation by Crown Rump Length (CRL)[45].

The association between PAI-2 and spontaneous preterm birth has not been described before. Gibson et al. described an association between PAI-2 and a PAI-2-polymorphism[46]. However, recently a major susceptibility locus for preterm birth has been localized on chromosome 18q21.33-q23. A strong positional candidate gene in the 18q linked region is PAI-2[47]. In our study, we found a trend for a negative association between a low PAI-2 concentration and the risk for preterm birth.

Decreased PAI-2 concentration in early pregnancy acts as a marker of impaired placental function and could be used as a predictor for intrauterine growth restriction and preterm birth, but not pre-eclampsia. These data suggest a different pathway for development of pre-eclampsia and intrauterine growth restriction or preterm birth.

sFlt-1

Prospective cohort studies have demonstrated that serum/plasma concentrations of PlGF, sFlt-1 and the sFlt-1/PlGF ratio may be useful biomarkers in the prediction of pre-eclampsia. However, due to its limited sensitivity, these biomarkers are still not useful early in gestation to permit intervention[48]. A meta-analysis of such studies concluded that the test accuracies of serum PlGF and sFlt-1 before 30 weeks' gestation are too poor for accurate prediction of pre-eclampsia in clinical practice[49]. The deviation of the plasma/serum concentrations of these biomarkers from the normal reference ranges are more pronounced in women with earlier onset pre-eclampsia. Okhuchi et al. suggest thresholds for sFlt-1/PlGF ratio measured between 26 and 31 weeks of gestation may be useful for detecting pre-eclampsia with onset before 36 weeks of gestation[50]. Most cases of intrauterine growth restriction are associated with placental insufficiency. Recently Heraiz showed in a case-control-study that pregnancies with fetal growth restriction or pre-eclampsia as well as pregnancies with pre-eclampsia and fetal growth restriction had higher values of sFlt-1/PlGF ratio than control pregnancies[51]. However, the differences among the case subgroups were not statistically different. We, like other large studies, were unable to confirm an association between a high sFlt-1 concentration in early pregnancy and pre-eclampsia.

High sFlt-1 concentration in early pregnancy was not associated with intrauterine growth restriction. High sFlt-1 concentration was positively associated with birth weight and placental weight. These results were in line with the positive association of sFlt-1 concentration in early pregnancy and CRL described recently[45]. Bouwland-Both et al. suggest in early pregnancy higher sFlt-1 concentration may potentially reflect a low and stable environment needed for early placental and embryonic development. Research has shown that low oxygen concentration increases sFlt-1 concentration in cytotrophoblasts. Whereas higher sFlt-1 concentration in second and third trimester may reflect the response to fetal-placental hypoxia, which is associated with placental impairment.

In early pregnancy, high sFlt-1 concentration is not associated with placental related disorders. sFlt-1 concentration in early pregnancy reflects a different pathway than in second or third trimester.

PlGF

PlGF is expressed during early embryonic development. Transcripts encoding mouse PlGF were abundant in trophoblastic giant cells associated with the parietal yolk sac at early stages of embryogenesis suggesting a role to coordinate vascularization in the deciduum and placenta during early embryogenesis[52]. In addition, PlGF is expressed at a low level in several other organs including the heart, lung, thyroid, skeletal muscle, and adipose tissue under normal physiological conditions[52]. We demonstrated an association between a low PlGF concentration in first and second trimester and development of pre-eclampsia. Previous studies have reported that in pre-eclampsia and intrauterine growth restriction reduced concentrations of PlGF are apparent from the first trimester of pregnancy. The underlying cause for this decrease has been attributed to impaired placentation leading to early and persisting placental hypoxia.

A low PlGF concentration in early pregnancy was in our study associated with a higher uteroploacental vascular resistance in midpregnancy, a lower placental weight and birth weight, and considerably higher risks for intrauterine growth restriction. These significant associations between low early pregnancy PlGF concentrations and high uteroplacental vascular resistance in midpregnancy may indicate early placental insufficiency. Bouwland-Both showed that low PlGF concentration in early pregnancy was associated with a low CRL as well[45]. These data may imply that vascular placental development can influence early fetal development, which can have implications for subsequent development and outcome. Low PlGF concentration in early pregnancy is an early predictor for placental related disorders, reflecting early placental impairment, with higher uteroplacental vascular resistance in midpregnancy and finally placental disorders.

Although early onset pre-eclampsia and intrauterine growth restriction are often considered together as consequences of placental insufficiency, Gosh et al. noted that women with low concentrations of PlGF at 20-22 weeks of gestation were more prone to develop intrauterine growth restriction than pre-eclampsia (34% developed early pre-eclampsia and intrauterine growth restriction versus 66% developed early intrauterine growth restriction only)[53]. They showed no significant difference in median PlGF concentrations between the different groups. In other words they suggest utero-placental insufficiency at the same

level in both groups, but the eventual outcome is different. However, Burton suggested that there are many common features in the placental changes seen in pre-eclampsia and intrauterine growth restriction and, isolated intrauterine growth restriction and that the differences are mostly a matter of degree of placental changes[54]. The two conditions may represent different points along a spectrum of placental pathologies secondary to deficit spiral artery conversion. Thus maternal and fetal constitutional factors may modulate how the placenta responds to the maternal vascular insult, and how the mother is affected by the placental factors released.

Recently cross-reactivity of the PlGF assay has been described[55]. PlGF exists in at least 4 isoforms due to alternative mRNA splicing of the PlGF primary transcript, but the major ones are thought to be PlGF-1 and PlGF-2[52]. The main difference between the 4 isoforms is that PlGF-1 and PlGF-3 are non-heparin binding and can potentially affect targets in a paracrine manner, whereas PlGF-2 and PlGF-4 have additional heparin-binding domains and most likely work in an autocrine way[52] . The first stage, at 11-13 weeks' gestation, should be primarily aimed at the effective prediction and prevention of severe early-onset disease. Although in the first trimester a high cross-reactivity of the PlGF assay to PlGF-2 may be detrimental to the performance of the screening, Nucci et al. suggest that this may not be the case in screening during the third trimester[56].

MMP 9

Matrix metalloproteinases (MMPs) play a crucial role in restructuring the extracellular matrix by activating the secretion of gelatinases, collegenases and proteolytic enzymes. Cytotrophoblast MMP9 activity has been shown to be increased in human placental tissue[57]. A second matrix metalloproteinase important for placenta development is matrix metalloproteinase 3 (MMP3). MMP3 is produced by first trimester decidual cells and cytotrophoblast cells. MMP3 activates MMP9 and MMP2, and is implicated in connective tissue destruction and associated with endothelial cell dysfunction. Our findings of a lower prevalence of the rare T allele of the MMP9 polymorphism in women with pre-eclampsia suggest that women with uncomplicated pregnancies have more often increased MMP9 concentrations and may therefore have an increased capacity of restructuring the extracellular matrix at the fetal maternal interface. MMP9 (-1562 C/T) has also been

described as a predictor of cardiovascular disease[59]. We suggest that the MMP9 induction has a different role at the fetal maternal interface than in the vascular remodeling during atherogenesis. Apoptosis and degradation of the cytotrophoblast cells are normal, physiologic constituents of trophoblast turnover. In addition, MMP activity is associated with preterm birth as well[60]. When contractions begin or the membranes rupture, MMP activity in the amnion and chorion increases and levels of interstitial and basement membrane collagens decrease. Regional activation of MMP's near delivery may trigger a cascade of events that reduce fetal membrane integrity and promote rupture at specific sites. Therefore, we suggest a potential role for MMP and development of placental related disorders.

Biophysical tests

Abnormal early placentation can lead to higher uterine and umbilical artery resistance patterns, which can be measured by Doppler wave forms[61]. Umbilical, and to a lesser extent uterine, artery Doppler are used as markers to evaluate placental dysfunction[62]. Several studies have demonstrated that mean arterial blood pressure (MAP) and increased uterine artery pulsatility index (Ut-Pi), measured between 11-13+6 weeks of gestation, 20-24 weeks of gestation or 30-33 weeks of gestation can predict women who later develop pre-eclampsia[63]. Recently, a prospective longitudinal study described the temporal changes in MAP and Ut-Pi in women with singleton, high risk pregnancies who developed hypertension or not. MAP and Ut-Pi decreased with gestational age between 12 and 24 weeks and then increased in women with a normal outcome[64]. MAP and Ut-Pi were shown to be higher from early pregnancy and the difference for both increased in women who developed preterm pre-eclampsia compared to women with a normal outcome. MAP was shown to be increased from 12 weeks and Ut-Pi is significantly increased from 33 weeks onwards in women who developed term pre-eclampsia[64]. Our study showed that sFlt-1, PlGF and PAI-2 concentrations in early pregnancy were associated with uterine artery pulsatility index. High sFlt-1 concentration in early pregnancy was associated with lower uterine artery pulsatility index in the second trimester. High sFlt-1 concentration was not associated with uterine artery pulsatility indexand placental disorders. Low PlGF concentration was associated with higher uterine artery pulsatility index in the second trimester. And finally, low PAI-2 was associated with higher uterine artery pulsatiliy index

in the second trimester. These results imply a different role for sFlt-1 in early pregnancy than in second or third trimester in relation to development of placental related disorders.

IMPLICATIONS FOR PATHOGENESIS

Placental disorders are complex with different pathways leading to several subtypes. Our data showed that different risk factors and markers in early pregnancy are associated with placental disorders or subtypes. Therefore in each stage of pregnancy different pathways can lead to impaired placental development. Impaired placental development is associated with defects in endovascular extravillous trophoblast invasion, where some arteries are not invaded at all and some are superficially invaded, leading to a lack of the normal physiological adaptation of spiral arteries to pregnancy, reduced blood flow into the intervillous space, and relative hypoxia/ ischemia. Environmental, immunological, inflammatory and genetic factors all appear to play a role in this process.

Development of placenta starts after fertilization. Defective trophoblast differentiation and accompanying ischemia are thought to be the primary events. Hypoperfusion appears to be both a cause and a consequence of abnormal placental development. Immunologic abnormalities, similar to those observed in organ rejection graft versus host disease have been observed in pre-eclamptic women[65]. A conflict between maternal and paternal genes is believed to induce abnormal placental implantation through increased natural killer cell activity. Both maternal and paternal contributions to fetal genes may have a role in defective placentation.

The clinical features of pre-eclampsia can be explained as responses to generalized endothelial dysfunction. Placentation requires extensive angiogenesis for the establishment of a suitable vascular network to supply oxygen and nutrients to the fetus. Proangiogenic and antiangiogenic factors are elaborated by the developing placenta. A disturbed balance of production of angiogenic factors results in the systemic endothelial dysfunction The vascular endothelial growth factor family of angiogenic factors are important molecules regulating early placental vascular changes. The key molecules VEGF-A and PlGF and the receptors, sFlt-1 and VEGF-2 are expressed in the human placenta throughout gestation.

The VEGF family is known to regulate placental angiogenesis and maternal spiral artery remodeling. Pre-existing endothelial damage due to pre-existing vascular disease may contribute to development of the clinically disease as well.

Further the correlation between placental volume and the risk of pre-eclampsia is also confirmed[66]. Effendi showed that first-trimester placental volume is strongly associated with fetal and placental growth[67]. There was a correlation between placental volume in early pregnancy and placental volume at birth and birth weight. The extent and the degree of defective deep placentation may explain the different clinical presentations[68]. Placental vascular lesions are different in pregnancies complicated by pre-eclampsia (predominant maternal vascular supply lesions), by intrauterine growth restriction (predominant fetal vascular supply lesions) and by pre-eclampsia with intrauterine growth restriction (both maternal and fetal compartments are involved). The levels of angiogenic factors in maternal blood reflect different biological processes and stages of placentation and maternal adaptation during pregnancy. The elevated maternal sFlt-1 levels in early pregnancy may reflect the low and stable oxygen environment needed for early placental development. The elevated sFlt-1 levels in later stages of pregnancy may reflect the response of fetoplacental hypoxia, which is associated with placental impairment.

Maternal endothelial dysfunction is mainly attributed to defective placentation. However, there is increasing evidence to suggest abnormal placentation may not be the sole reason for altered endothelial function. The shared risk factors for both pre-eclampsia and later-life vascular diseases, as well as the familial segregation of all these disorders suggest that these diseases share a common genetic predisposition that interacts with the environment and may predispose individuals to vascular disorders which manifest at different time points throughout the life course[69]. The timing of pre-eclampsia onset should be taken into consideration if pre-eclampsia heritability and etiology are to be unraveled. Early pre-eclampsia appears to have a large genetic component, whereas environmental factors likely contribute most to late onset pre-eclampsia[23].

IMPLICATIONS FOR CLINICAL PRACTISE AND FUTURE RESEARCH

In this thesis we used the model in which different stages in pregnancy are described. In each stage different risk factors and markers for placental related disorders could be identified. Pre-pregnancy planning and health promotion offers the possibility to improve pregnancy outcomes. Modifiable risk factors known before conception include obesity, smoking, advanced maternal age and multiple embryo transfer with artificial reproductive technology. Important non modifiable risk factors known before pregnancy to consider include education, socioeconomic status and maternal diseases. More healthy women before pregnancy will result in more healthy pregnancies and less maternal morbidity and mortality. In addition, identifying risk factors at the different points in pregnancy gives the opportunity to unravel the etiology of placental complications of pregnancy.

Perspectives for prevention

Until now research on prediction models focus on specific pregnancy outcomes. And so far first-trimester screening tests for pre-eclampsia have low positive predictive value, and there are no data demonstrating that they lead to improved outcomes[70]. However, ideally a prediction model will focus on different adverse outcomes, including hypertensive disorders, intrauterine growth restriction and preterm birth. A prediction model for placental related disorders in early pregnancy would ideally consist of maternal risk factors, markers of defective placentation and markers of abnormal vascular adaptation to pregnancy. Maternal risk factors (even modifiable) should include eg smoking, alcoholics, body mass index, maternal low birth weight, history of hypertensive disorders, maternal diseases, maternal age and headache. Markers of defective placentation in early pregnancy should include PlGF and PAI-2. Markers of abnormal vascular adaptation to pregnancy include uterine artery Doppler velocimetry. After selecting women with high risk pregnancies, at different points in pregnancy ongoing risk stratification may allow for early detection of adverse outcomes.

Low-dose aspirin has been shown to reduce severe pre-eclampsia and other complications in women who started taking it before 16 weeks, but these studies used clinical risk factors to identify the women who could benefit from taking it[70]. Velauthar showed in a meta analysis that first-trimester uterine artery Doppler is a highly specific test for predicting

early-onset pre-eclampsia with moderate sensitivity[71].The specificity for predicting pre-eclampsia and fetal growth restriction at any gestational age is high, but the sensitivity is low. The number needed to treat with aspirin to prevent early-onset pre-eclampsia after uterine artery Doppler screening is comparable to that based on the 'high-risk' clinical factors currently being used[71]. Until now this test is not recommended because of the quite high false positive rate of this test, leading to excessive patiente anxiety and health care costs.

Future research

For a first-trimester risk assessment for pre-eclampsia to be useful in clinical practice, future screening tests will need to have sensitivities and positive predictive values, high enough to accurately identify women who will develop pre-eclampsia, and interventions will need to be available that improve clinical outcome in women who test positive[70]. Research should focus on a composition of tests in early pregnancy to detect the risk for developing placental related pregnancy disorders in early pregnancy, combined with the currently routine screening tests for fetal abnormalities. Identification of adverse outcomes in early pregnancy will give the opportunity to facilitate targeted antenatal surveillance and possibly intervention.

Furthermore research should focus on development of prediction models, incorporating the clinical characteristics with uterine artery Doppler to increase the accuracy of risk assessment. An individual patient data meta-analysis will allow development of optimal testing strategies for prediction of maternal and fetal complications[72].

Prospective cohort studies with the aim of collecting data on relevant clinical, environmental and lifestyle risk factors coupled with longitudinal measurement of angiogenic factors, endothelial function and genetic variations in candidate gene pathways will be beneficial in establishing interactions which predispose to placental related disorders of pregnancy in line with the hypothesis of several stages of placental disorders and possibilities of interactions at different stages.

CONCLUSION

The findings from this thesis suggest that in the different stages of pregnancy risk factors and markers of placental related disorders will help us to predict hypertensive diseases of

pregnancy, intrauterine growth restriction and preterm birth, and unravel the pathology of these diseases (figure 3). Placental related disorders start in early pregnancy or even is existing before pregnancy and can be reflected in different phenotypes of hypertension in pregnancy. This may indicate different pathophysiological mechanisms leading to another disease as a result of different linking mechanisms between placental stage and systemic stage (symptomatic or non symptomatic). Or both diseases have the same underlying mechanism, where maternal susceptibility associated with genetic and environmental factors leads to different phenotypes.

Figure 3 Risk factors, markers and biophysical tests to predict placental related complications of pregnancy in different stages of pregnancy

	Stages of pregnancy				
	Preconception (stage -1)	Conception (stage 0)	Poor placentation (Stage 1)	Systemic phase (stage 2)	
				No symptoms	Symptoms
Risk factors					
Maternal factor	-age -parity -obesity -ethnicity -education -socioeconomic status -disease -history of hypertension and/or IUGR in pregnancy	-disease -nutrition -smoking -alcoholics	-mutiple gestation -nutrition -smoking -alcoholics		
Biophysical test			-MAP -uterine artery Pi		
Marker			-PlGF -PAI-2	-PlGF -sFlt-1 -PAI-2	
Paternal factor					

[#] Maternal diseases include chronic hypertension, diabetes, renal disease, antiphosphfolipid antibodies

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CHAPTER 5

Summary/Samenvatting

SUMMARY OF THIS THESIS

Hypertensive disorders, intrauterine growth restriction and preterm birth are complications of pregnancy with unknown origins. These complications of pregnancy are important causes of maternal and perinatal diseases and death. Previous studies showed that placental development plays (an important) role in the origin of hypertensive disorders and intrauterine growth restriction, as well as preterm birth.

The most important aim of the present thesis is to gain more knowledge about the etiology of placental related complications. A general introduction and the aims have been described in chapter 1. We describe in chapter 2 the analysis of associations between risk factors (maternal, paternal, pregnancy related and family factors, and maternal and paternal comorbidity) and placental related pregnancy disorders. In chapter 3 we describe the associations between biomarkers (components of blood clotting and extracellular matrix degradation, and angiogenesis) measured in blood taken in early pregnancy, and placental related complications.

All studies in chapter 2 were embedded in The Generation R Study, a prospective population-based study from early pregnancy onwards in Rotterdam, The Netherlands. It aims to gain more insight into environmental, social and nutritional determinants of growth, development, and health in a contemporary population based multi-ethnic cohort of children from fetal life until young adulthood. Eventually, these results from The Generation R Study will contribute to the development of strategies for optimising health and healthcare for both pregnant women and their children.

In **chapter 2.1** we focused on maternal and paternal risk factors in early pregnancy for hypertensive disorders. Women who did not develop hypertension in their first pregnancy have a low risk for pregnancy-induced hypertension or pre-eclampsia. Therefore, we studied similarities and differences in risk factors for pregnancy-induced hypertension and pre-eclampsia in nulliparous as well as multiparous women (table 1). An increased BMI (>25kg/m2) was associated with development of pregnancy-induced hypertension and pre-eclampsia in nulliparous and multiparous women. Previous hypertensive disorders in pregnancy were associated with a higher risk for pregnancy-induced hypertension and pre-eclampsia in multiparous women. Women with development of hypertension in their first pregnancy had an increased risk for hypertension in the next

pregnancies (pregnancy-induced hypertension as well as pre-eclampsia). However, women with a normotensive first pregnancy had a low risk for hypertensive disorders in their subsequent pregnancies (<1%). The first pregnancy seems to be a stress test for next pregnancies. A low maternal birth weight was associated with pre-eclampsia in nulliparous women. Headache in early pregnancy was associated with pregnancy-induced hypertension and pre-eclampsia in nulliparous and multiparous women. The risk factors for pregnancy-induced hypertension and pre-eclampsia in nulliparous and multiparous women are presented in table 1. Some risk factors in early pregnancy seem to differ between pregnancy-induced hypertension and pre-eclampsia, as well as between nulliparous and multiparous women, exclusively an increased body mass index.

Table 1. Risk factors for pregnancy-induced hypertension and pre-eclampsia

Disease / Risk factor	Pregnancy-induced hypertension		Pre-eclampsia	
	nulli	multi	nulli	multi
Age>40years	0	0	+	0
Increased body mass (>25kg/m2)	+	+	+	+
European ethnicity	+	0	0	-
Surinamese ethnicity	0	0	+	0
Previous hyertensive disorders		+		+
Low maternal birth weight	0	0	+	0
Twin pregnancy	0	0	+	0
Chronic hypertension			0	+
Headache	0	+	0	+
Paternal arthralgia	0	0	0	+

0 geen associatie, + positieve associatie (hoger risico), - negatieve associatie (lager risico)

In **chapter 2.2** we described the associations between maternal education level (as measurement of socioeconomic status) and the development of pre-eclampsia. Women with a lower education level had an increased risk for pre-eclampsia. We found a strong

educational gradient, where the lowest educational subgroup of pregnant women had five times higher odds compared with the highest educational subgroup to develop pre-eclampsia. We did not find factors that could explain this association, including financial problems, smoking, work related problems and body mass index. So, the association between women with a lower educational level and a higher risk for pre-eclampsia is mainly unexplained.

As described in **chapter 2.3**, women with relatively low levels of education had a 30 to 50% higher risk for pregnancy-induced hypertension than women with a high educational level. Obesity and the relatively high blood pressure at enrolment were associated with a higher risk for pregnancy-induced hypertension in lower educated women. These findings suggest that pregnancy-induced hypertension in women with a low educational level could reflect to a predisposition for chronic hypertension, emerged by physiological stress of pregnancy.

Prediction of hypertensive disorder in pregnancy at intake is difficult, especially in a low risk population of nulliparous women. A prediction model usable in daily clinical practise is needed to select women at intake with a high risk for development of hypertensive disorder in pregnancy. Recently, a prediction model for development of hypertension (≥140 and/or 90mmHg) determined before 36 weeks gestation has been described. This model uses clinical variables collected for routine control at intake of pregnancy before 16 weeks of gestation. Nulliparous women from Tilburg and Amsterdam have been studied. The prediction model has been derived from a regression model and transformed to a scorecard usable for daily practise. A score can be calculated using maternal weight, diastolic and systolic blood pressure (measured before 16 weeks of gestation). Women with a low score had a low risk for pregnancy-induced hypertension (<1%) and women with a high score had a high risk for pregnancy-induced hypertension (>16%).

In **chapter 2.4** we investigated the accuracy of a prediction model in the Generation R population. The incidence of hypertension (≥ 140 and/or 90mmHg) was 5.9%. Of the women with a low score (13.4% of the population), none developed hypertension before 36 weeks of gestation. In those with a high score (12.8% of the population) the risk was 15.9%. This validation study confirms the predictive ability of the previous model, that can accurately identify women at very low and very high-risk of becoming a hypertension in

pregnancy before 36 weeks of gestation. Application of the model may lead to a reduction in frequency of antenatal visits for low-risk and increased surveillance for high-risk women.

In general, large epidemiological studies use questionnaires to collect their data. **Chapter 2.5** provides the validation of pre-eclampsia which is necessary for interpretation of self-reported pre-eclampsia. We investigated 4330 women, 2% developed a pre-eclampsia according to the ISSHP definition. From 152 women who reported a pre-eclampsia, 50% did not have a pre-eclampsia. From 4178 women who reported no pre-eclampsia, actually 0.3% had a pre-eclampsia. The sensitivity and specificity of self-reported pre-eclampsia was 0.87 and 0.98, respectively. Higher education and parity are associated with a better self-reported diagnosis of self-reported pre-eclampsia. A clear diagnosis based on criteria of hypertension and proteinuria, which is clear for the doctor and patient, is essential for preconception care, the management of a subsequent pregnancy, and possible prevention of cardiovascular disease in later life. In our study the validity of the maternal recall of self-reported pre-eclampsia was moderate.

In **chapter 3** of this thesis, we investigated the involvement of the hemostasis (components of blood clotting), fibrinolysis (extracellular matrix degradation) and angiogenesis, and development of placental related complications in pregnancy. The associations between biomarkers and pre-eclampsia, intrauterine growth restriction and spontaneous preterm birth are presented in table 2.

Hemostasis is a complex balance between inhibitory and activating pathways resulting in coagulation and lysis. Normal pregnancy contributes to a state of relative hypercoagulation, while in complicated pregnancies hypercoagulation is even more profound. **Chapter 3.1** describes the concentrations of the activator (tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA)) and inhibitor (plasminogenactivator-inhibitor type-1 (PAI-1) and plasmingenactivator-inhibitor type-2 (PAI-2)) components of the plasmingen activator-inhibitory system to gain more insight in longitudinal data during pregnancy until 6 weeks postpartum. For this study, we used blood samples from a subgroup of 41 women, from a multicentre study coordinated by the University Medical Centre of Nijmegen. The longitudinal changes in concentrations of this system might be helpful in the understanding of pregnancy complications, including

pre-eclampsia. The concentration of tPA and uPA decreased in the first 10 weeks of gestation, followed by an increase in the third trimester and finally a decrease 6 weeks postpartum to the preconceptional concentration. PAI-2 concentration increased with gestational age until term and declined to undetectable levels 6 weeks after delivery. A decreased production of PAI-2 is thought to be a result of impaired placental function. A relative hypercoagulation appear to be in normal pregnancy as described before: in the third trimester a decrease of activating tissue-type plasminogen-activator (tPA) and urokinase-type plasminogen-activator (uPA) and an increase of inactivating plasminogen-activator-inhibitor type 2 (PAI-2) components of the plasminogen-activator-inhibitor system.

In **chapter 3.2**, we described PAI-2 concentration <18 weeks gestation in relation to development of placental related pregnancy complications, spontaneous preterm delivery, intrauterine growth restriction and pre-eclampsia. This study was embedded in the Generation R study. Low PAI-2 concentration was associated with a lower birth weight and a lower placental weight. Since PAI-2 has been produced by trophoblastcells, we suggest that a defective placentation will result in a smaller placenta with less trophoblastcells. In addition, low PAI-2 concentration was associated with a higher risk of preterm birth and intrauterine growth restriction, but not pre-eclampsia.

Many studies have been focused on the angiogenesis and development of pre-eclampsia. Especially soluble fms-like tyrosine kinase-1 (sFlt-1) and placenta growth factor (PlGF) might be potential factors in the early placental stage in association with defective development of placenta. High sFlt-1 concentration was associated with pre-eclampsia, even 5 weeks before the clinical onset. And low PlGF concentration was associated with pre-eclampsia as well as intrauterine growth restriction. These results suggest placental impairment in early pregnancy.

Chapter 3.2 describes besides PAI-2 associations also the associations between sFlt-1 and PlGF concentration measured before 18 weeks of gestation and between 18 and 25 weeks of gestation, and uterine artery resistance index, placental weight, birth weight, preterm birth, intrauterine growth restriction and pre-eclampsia. High sFlt-1 concentration (measured before 18 weeks and between 18 and 25 weeks of gestation) was associated with a lower uterine artery resistance index in second trimester and a higher birth weight. In our study, high sFlt-1 concentration was not associated with preterm birth, intrauterine

growth restriction or pre-eclampsia. sFlt-1 do not seem to be a valuable predictor in early pregnancy of placental related complications. Low sFlt-1 concentration in first trimester seems to be an expression of low angiogenesis as a result of placental impairment. Fetal-placental hypoxia reflects a response of abnormal development of the placenta, with an elevation of angiogenesis subsequently in pregnancy. Low PlGF concentration (measured <18 weeks and between 18 and 25 weeks of gestation) was associated with a higher uterine artery resistance index and a lower birth weight. Lower PlGF concentration before 18 weeks of gestation was associated with preterm birth, intrauterine growth restriction and pre-eclampsia. PlGF seems to be a potential marker usable in early pregnancy to predict placental related complications.

Table2 Biomarkers for preterm birth, intrauterine growth restriction and pre-eclampsia

Disease / Biomarker	Preterm birth		Intra-uterine growth restriction		Pre-eclampsia	
	<18 weeks	18-25 weeks	<18 weeks	18-25 weeks	<18 weeks	18-25 weeks
PAI-2	0		+-		0	
sFlt-1	0	0	0	0	0	0
PlGF	0	+	+	+	+	+
sFlt-1/PlGF ratio	+	0	0	0	+	0

0 no association, + positive association (higher risk), - negative association (lower risk)

In **chapter 3.3** we described the functional polymorphisms of MMP3 (-1562 C/T), MMP3 (-1612 5A/6A) and annexin A5(-1 C/T) and the risk of pre-eclampsia. These polymorphisms are functional promoter polymorphisms and influence the amount of protein production. Matrix metallopreinases (MMP's) play an important role in early pregnancy by influencing the degradation of the extracellular matrix, which is a process necessary for a successful trophoblastinvasion. MMP3 has been produced in early pregnancy by decidual- and trophoblastcells. MMP3 activates MMP9 and MMP2 and these proteins have been associated with endothelial dysfunction. Annexin A5 has been

produced by syncytiotrophoblast- and endothelialcells and they play an important role in apoptosis. For this study, we used blood samples of a case-control study coordinated by the Leids Medical Centre. In our study, the rare T allele of MMP9 was associated with a lower risk for pre-eclampsia. No associations were found between MMP3 and annexin A 5 polymorphism, and pre-eclampsia.

In c**hapter 4** we provide a general discussion in which the studies observed in this thesis are described in a broader context and implications for future research are discussed. Awareness of risk factors for hypertensive disorders in pregnancy, intrauterine growth restriction and preterm birth gives the opportunity to improve pregnancy outcome.
Prediction models with maternal risk factors (table 1), biomarkers of abnormal placentation (table 2) and markers of an impaired vascular adaption in pregnancy (Doppler velocimetry artery uterine) will help us to select women with a higher risk for placental related complications. Future research will focus on a prenatal test in early pregnancy. Blood will be screened for fetal (and placental) markers associated with placental related complications of pregnancy.

SAMENVATTING VAN DIT PROEFSCHRIFT

Hypertensieve aandoeningen, intra-uteriene groeivertraging en spontane vroeggeboorte zijn zwangerschapscomplicaties waarvan de ontstaanswijze grotendeels onbekend is. Deze zwangerschapscomplicaties zijn belangrijke oorzaken van zowel maternale als perinatale sterfte en ziekte. Eerder onderzoek heeft aangetoond dat de ontwikkeling van de placenta een (belangrijke) rol kan spelen in de ontstaanswijze van zowel hypertensieve aandoeningen in de zwangerschap als intra-uteriene groeivertraging, maar ook van een spontane vroeggeboorte. De belangrijkste doelstelling van dit proefschrift is meer inzicht te geven in de ontstaanswijze van deze placentagerelateerde zwangerschapsaandoeningen. Een algemene inleiding en doelstellingen zijn beschreven in hoofdstuk 1. We beschrijven in hoofdstuk 2 de analyse van de associaties van risicofactoren (maternale, paternale, zwangerschap gerelateerde en familiaire factoren, en maternale en paternale comorbiditeit) en placentagerelateerde zwangerschapsaandoeningen. In hoofdstuk 3 beschrijven we de associaties van biomarkers (componenten van bloedstolling en extracellulaire matrixafbraak, en angiogene factoren) die gemeten zijn in bloedmonsters afgenomen in een vroeg stadium van de zwangerschap met placentagerelateerde zwangerschapscomplicaties.

De studies beschreven in hoofdstuk 2 zijn uitgevoerd binnen de Generation R Studie. Generation R is een groot, Rotterdams cohort studie met als doel meer inzicht te verkrijgen in omgeving -, sociale - en voeding determinanten van de groei, de ontwikkeling en de gezondheid in een multi-etnisch cohort vanaf de foetale periode tot aan de jongvolwassene leeftijd. Uiteindelijk zullen de resultaten van Generation R bijdragen aan de ontwikkeling van strategieën ter optimalisering van de gezondheid en zorg van zowel zwangere vrouwen als hun kinderen.

In **hoofdstuk 2.1** hebben we ons gericht op maternale en paternale risicofactoren voor hypertensieve aandoeningen in de zwangerschap, die in een vroeg stadium van de zwangerschap bekend zijn. Het is bekend dat vrouwen die geen hypertensie hebben ontwikkeld in de eerste zwangerschap een laag risico hebben om een zwangerschapshypertensie of pre-eclampsie te ontwikkelen. We hebben daarom gekeken naar overeenkomsten en verschillen in risicofactoren voor zwangerschapshypertensie en pre-eclampsie voor zowel nulli- als multi-para (tabel 1). Een verhoogde body mass index

(> 25) is geassocieerd met het ontstaan van zwangerschapshypertensie en pre-eclampsie bij zowel nulli- als multi-para. Een voorgeschiedenis met hypertensieve aandoeningen in de zwangerschap is geassocieerd met een verhoogd risico voor zowel zwangerschapshypertensie als pre-eclampsie bij multipara. Deze vrouwen hadden een tien maal verhoogd risico op de ontwikkeling van pre-eclampsie. Als er een hypertensieve aandoening ontstaat in de eerste zwangerschap heeft de vrouw daarmee een verhoogd risico op hypertensie in de volgende zwangerschappen (zowel zwangerschapshypertensie als pre-eclampsie). Als de eerste zwangerschap daarentegen niet gecompliceerd wordt door een hypertensieve aandoening is het risico op hypertensieve aandoeningen in de volgende zwangerschappen klein (< 1%). De eerste zwangerschap lijkt een stress-test voor de komende zwangerschappen. Een laag geboortegewicht van de moeder zelf is geassocieerd met pre-eclampsie bij nullipara. Hoofdpijn in het eerste trimester van de zwangerschap is geassocieerd met zwangerschapshypertensie en pre-eclampsie bij multipara. In tabel 1 zijn de risicofactoren voor zwangerschapshypertensie en pre-eclampsie voor nullipara en multipara weergegeven. Er is dus een verschil in risicofactoren tussen vrouwen die zwangerschapshypertensie of preeclampsie ontwikkelen en nullipara en mulipara met als uitzondering een verhoogde BMI die voor alle groepen een risico factor is.

Tabel 1. Risicofactoren voor zwangerschapshypertensie en pre-eclampsie

Ziektebeeld	Zwangerschapshypertensie		Pre-eclampsie	
Risicofactoren	**nulli**	**multi**	**nulli**	**multi**
Leeftijd > 40jaar	0	0	+	0
Verhoogde body mass index	+	+	+	+
Europese ethniciteit	+	0	0	-
Surinaamse ethniciteit	0	0	+	0
Hypertensieve aandoeningen in de voorgeschiedenis		+		+
Laag maternal geboortegewicht	0	0	+	0
Tweelingzwangerschap	0	0	+	0
Preexistente hypertensie			0	+
Hoofdpijn	0	+	0	+
Paternale gewrichtsklachten	0	0	0	+

O geen associatie, + positieve associatie (verhoogd risico), - negatieve associatie (beschermend)

In **hoofdstuk 2.2** hebben we de associaties beschreven tussen opleidingsniveau van de zwangere vrouw (als maat van de socio-economische status) en het ontstaan van pre-eclampsie. Naarmate vrouwen een lager opleidingsniveau hadden, nam het risico op pre-eclampsie toe. We vonden dat zwangere vrouwen met het laagste opleidingsniveau een vijfmaal verhoogd risico hadden op pre-eclampsie vergeleken met de vrouwen met het hoogste opleidingsniveau. We hebben geen factoren gevonden zoals financiële moeilijkheden, roken, werk gerelateerde problemen en body mass index die deze associatie zouden kunnen verklaren; de associatie tussen vrouwen met een lager opleidingsniveau en verhoogd risico op preeclampsie blijft hiermee grotendeels onverklaard.

In **hoofdstuk 2.3** hebben we beschreven dat vrouwen met een relatief laag opleidingsniveau een 30 tot 50% hoger risico op zwangerschapshypertensie hebben dan vrouwen met een laag opleidingsniveau. Obesitas en een relatief hoge bloeddruk bij de lager opgeleid vrouwen bij inclusie waren geassocieerd met een hoger risico op zwangerschapshypertensie. Deze bevindingen suggereren dat zwangerschapshypertensie bij vrouwen met een laag opleidingsniveau mogelijk gerelateerd is aan een aanleg tot een chronische hypertensie dat door de fysiologische stress van de zwangerschap tijdelijk zichtbaar wordt .

Het voorspellen van hypertensieve aandoeningen bij intake is moeilijk met name bij laag-risico nullipara. Er bestaat behoefte in de dagelijkse klinische praktijk aan predictiemodellen voor een laag-risico populatie om bij intake vrouwen te selecteren met een hoger risico op hypertensieve aandoeningen die zich later in de zwangerschap ontwikkelen. Recent is een predictiemodel beschreven voor het ontstaan van hypertensie (≥ 140 en/ of 90mmHg) vastgesteld voor de 36^{e} week van de zwangerschap. Dit model maakt gebruik van klinische variabelen die routinematig verzameld worden tijdens de intake van de zwangere vrouw voor de 16^{e} week van de zwangerschap. Hiervoor zijn nullipara afkomstig uit Tilburg en Amsterdam onderzocht. Het predictiemodel is voortgekomen uit een regressiemodel en getransformeerd tot een scorekaart bruikbaar voor de dagelijkse praktijk. Met behulp van het maternale gewicht, de diastolische en systolische bloeddruk (gemeten voor de 16^{e} week van de zwangerschap) kan een score berekend worden. Vrouwen met een lage score hebben een laag risico op zwangerschapshypertensie (<1%) en vrouwen met een hoge score hebben een hoger risico op zwangerschapshypertensie (>16%).

In **hoofdstuk 2.4** hebben we de validiteit van dit predictiemodel onderzocht in de Generation R populatie. De incidentie van hypertensie (≥ 140 en/ of 90mmHg) was 5.9%. Van de vrouwen met een lage score (13.4% van de populatie) ontwikkelde geen enkele vrouw een hypertensie. Bij de vrouwen met een hoge score (12.8% van de populatie) was het risico op hypertensie 15.9%. Deze validatie studie bevestigt het voorspellend vermogen van dit predictie model; Vrouwen met een laag risico en vrouwen met een hoog risico op hypertensie vastgesteld voor de 36e week van de zwangerschap kunnen accuraat worden onderscheiden. Toepassing van dit model zou kunnen leiden tot minder zwangerschapscontroles van vrouwen met een laag risico en intensievere controle van vrouwen met een hoog risico.

In epidemiologische studies worden vaak zelf ingevulde vragenlijsten gebruikt om risicofactoren te bestuderen. In h**oofdstuk 2.5** hebben we de validatie van zelf gerapporteerde pre-eclampsie in vragenlijsten gepresenteerd. We onderzochten 4330 vrouwen waarvan 2% van de vrouwen een pre-eclampsie had ontwikkeld volgens de ISSHP definitie. Van de 152 vrouwen die zelf rapporteerden dat ze een pre-eclampsie hadden gehad, bleek 50% niet een pre-eclampsie te hebben gehad. Van de 4178 vrouwen die aangaven geen pre-eclampsie te hebben gehad, bleek 0.3% een pre-eclampsie te

hebben gehad. De sensitiviteit en specificiteit van zelf gerapporteerde pre-eclampsie was respectievelijk 0.87 en 0.98. Een hogere opleiding en pariteit waren geassocieerd met een betere zelf gerapporteerde diagnose van pre-eclampsie. Een juiste diagnose is voor zowel de dokter als de patiënt belangrijk. Deze diagnose is essentieel voor preconceptie zorg, de behandeling van een volgende zwangerschap en mogelijk preventie van cardiovasculaire aandoeningen op oudere leeftijd. In onze studie was de validatie van de zelf gerapporteerde pre-eclampsie matig.

In hoofdstuk 3 van dit proefschrift hebben we hemostase (componenten van bloedstolling), fibrinolyse (extracellulaire matrixafbraak) en angiogenese, en het ontstaan van placentagerelateerde complicaties in de zwangerschap bestudeerd. In tabel 2 zijn de biomarkers en de associatie met pre-eclampsie, intra-uteriene groeivertraging of spontane vroeggeboorte weergegeven.

Voor een goede hemostase is er een complexe balans tussen inactiverende en activerende proteïnen noodzakelijk, die resulteert in een evenwicht tussen coagulatie en lysis. In een normale zwangerschap is er een relatieve hypercoagulatie. Een sterkere neiging tot hypercoagulatie wordt gezien bij bepaalde zwangerschapscomplicaties.

Hoofdstuk 3.1 beschrijft de concentraties van de activerende (tissue-type plasminogeen activator (tPA) en urokinase-type plasminogeen activator (uPA)) en inactiverende (plasminogeenactivator-inhibitor type-1 (PAI-1) en plasmingeenactivator-inhibitor type-2 (PAI-2)) componenten van het plasminogen activator-inhibitor systeem om zo meer inzicht te krijgen in dit systeem gedurende de gehele zwangerschap tot 6 weken postpartum. Voor deze studie hebben we gebruik gemaakt van bloedmonsters van een subgroep van 41 vrouwen, van een multicenterstudie die werd gecoördineerd door het Universitair Medisch Centrum Nijmegen. Inzicht in de longitudinale veranderingen in concentraties van de verschillende proteïnes van dit systeem kunnen ons helpen om zwangerschapscomplicaties, zoals pre-eclampsie beter te begrijpen. De concentraties van tPA en uPA daalden in de eerste 10 weken van de zwangerschap, waarna een stijging volgde in het derde trimester, om vervolgens weer tot preconceptionele waarde te dalen postpartum. De concentratie van PAI-2 nam toe gedurende de zwangerschap tot de geboorte en daalde tot een niet aantoonbare waarde ongeveer 6 weken postpartum. Een verminderde productie van PAI-2 zou mogelijk kunnen komen door een afwijkende

placentatie. Er lijkt een relatieve hypercoagulatie in een normale zwangerschap zoals eerder beschreven: in het 3e trimester door afname van activerende (tissue-type plasminogeen activator (tPA) en urokinase-type plasminogeen activator (uPA)) en toename van inactiverende plasmingeenactivator-inhibitor type-2 (PAI-2)) componenten van het plasminogen activator-inhibitor systeem.

In **hoofdstuk 3.2** beschrijven we de PAI-2 concentratie bepaald bij een zwangerschapsduur onder de 18 weken in relatie tot de ontwikkeling van placentagerelateerde afwijkingen, zoals vroeggeboorte, intra-uteriene groeivertraging en pre-eclampsie. Dit onderzoek werd uitgevoerd binnen de Generation R studie. Een lagere concentratie PAI-2 was geassocieerd met een lager geboortegewicht en een lager placentagewicht. Omdat PAI-2 wordt geproduceerd door trofoblastcellen, suggereert dit dat bij een abnormale ontwikkeling van de placenta, het trophoblastvolume kleiner is. Een lagere PAI-2 concentratie is ook geassocieerd met een grotere kans op spontane vroeggeboorte en intra-uteriene groeivertraging, maar niet met een verhoogd risico op pre-eclampsie.

Vele studies hebben de laatste tijd zich gericht op de angiogenese en de ontwikkeling van pre-eclampsie. Soluble fms-like tyrosine kinase-1 (sFlt-1) en placenta growth factor (PlGF) zijn factoren die al in een vroeg stadium van de zwangerschap geassocieerd worden met een abnormale placenta ontwikkeling. Een hoge concentratie sFlt-1 is door anderen geassocieerd met pre-eclampsie, zelfs aantoonbaar 5 weken voor de klinische manifestatie van de ziekte. En een lage concentratie van PlGF wordt geassocieerd met zowel pre-eclampsie als intra-uteriene groeivertraging. Deze resultaten suggereren een malplacentatie vroeg in de zwangerschap.

Hoofdstuk 3.2 beschrijft naast PAI-2 ook de associaties van sFlt-1 en PlGF concentraties bepaald bij een zwangerschapsduur onder de 18 weken en tussen de 18 en 25 weken met de uteroplacentaire vasculaire weerstand, placenta en geboortegewicht, en spontane vroeggeboorte, intra-uteriene groeivertraging en pre-eclampsie. Een hoge sFlt-1 concentratie (zowel bepaald bij een zwangerschapsduur onder de 18 weken als tussen de 18 en 25 weken) was geassocieerd met een lagere uteroplacentaire weerstand in het tweede trimester en een hoger geboortegewicht. Een hoge sFlt-1 concentratie was in onze studie niet geassocieerd met een spontane vroeggeboorte, intra-uteriene groeivertraging of pre-eclampsie. sFlt-1 lijkt geen factor waarmee al vroeg in de zwangerschap

placentagerelateerde complicaties kunnen worden voorspeld. Een lagere concentratie sFlt-1 in het eerste trimester lijkt meer een uiting van weinig angiogene activiteit als gevolg van een abnormale placentatie. Door de abnormale ontwikkeling van de placenta kan foetomaternale hypoxie ontstaan, waardoor de angiogene activiteit toeneemt in een verder gevorderd stadium van de zwangerschap. Een lage PlGF concentratie (zowel bepaald bij een zwangerschapsduur onder de 18 weken als tussen de 18 en 25 weken) was geassocieerd met een hogere uteroplacentaire vasculaire weerstand en een lager geboortegewicht. Een lage PlGF concentratie bepaald bij een zwangerschapsduur onder de 18 weken was geassocieerd met spontane vroeggeboorte, intra-uteriene groeivertraging en pre-eclampsie. PlGF is dus wel een potentiele marker die al vroeg in de zwangerschap gebruikt kan worden om placenta gerelateerde complicaties te voorspellen.

Tabel 2 Biomarkers voor pre-eclampsie, intra-uteriene groeivertraging en vroeggeboorte

Ziektebeelden	**Pre-eclampsie**		**Intra-uteriene groeivertraging**		**Spontane vroeggeboorte**	
Biomarkers	**<18 weken**	**18-25 weken**	**<18 weken**	**18-25 weken**	**<18 weken**	**18-25 weken**
PAI-2	0		+-		0	
sFlt-1	0	0	0	0	0	0
PlGF	+	+	+	+	0	+
sFlt-1/PlGF ratio	+	0	0	0	+	0

O geen associatie, + positieve associatie (verhoogd risico), - negatieve associatie (beschermend)

In **hoofdstuk 3.3** hebben we de functionele polymorfismen beschreven van MMP3 (-1562 C/T), MMP9 (-1612 5A/6A) en anexin-A5 (-1 C/T) in relatie tot het risico op pre-eclampsie. Dit zijn 3 functionele polymorfismen, die een invloed kunnen hebben op de hoeveelheid proteïne die wordt geproduceerd. Matrix metalloproteinases (MMP's) spelen een belangrijke rol in de trofoblastinvasie vroeg in de zwangerschap door hun invloed op de afbraak van de extracellulaire matrix, een proces noodzakelijk voor een succesvolle trofoblastinvasie. MMP3 wordt in het eerste trimester geproduceerd door decidua- en trofoblastcellen. MMP3 activeert MMP9 en MMP2 en wordt geassocieerd met

endotheeldysfunctie. Annexin A5 wordt geproduceerd door syncytiotrofoblast- en endotheelcellen en speelt een belangrijke rol bij apoptosis. Voor dit onderzoek hebben we gebruik gemaakt van bloedmonsters van een case-control studie gecoördineerd door het Leids Medisch Centrum. In onze studie bleek dat het zeldzamere T allel van MMP9 geassocieerd was met een lager risico op pre-eclampsie. Er konden geen associaties worden gevonden tussen MMP3 en annexin A5 polymorfisme enerzijds en pre-eclampsie anderzijds.

In **hoofdstuk 4** is een algemene discussie opgenomen over de bevindingen die zijn gedaan in dit proefschrift. Dit hoofdstuk bevat ook aanbevelingen voor toekomstig onderzoek.

Bewustwording van de risicofactoren voor hypertensieve aandoeningen in de zwangerschap, intra-uteriene groeivertraging en vroeggeboorte biedt de mogelijkheid om de zwangerschapsuitkomsten te verbeteren.

Met behulp van predictiemodellen, waarin maternale risicofactoren (zie tabel 1), biomarkers van een abnormale placentatie (zie tabel 2) en markers van een gestoorde vasculaire aanpassing in de zwangerschap (doppler velocimetrie arterie uterina) zal in de verschillende fases van de zwangerschap een risicoselectie kunnen plaatsvinden. Toekomstig onderzoek zal zich richten op een prenatale test vroeg in de zwangerschap waarin bloed van de zwangere gescreend wordt op foetale (en placenta) markers die geassocieerd zijn met placentagerelateerde zwangerschapscomplicaties.

CHAPTER 6

About the author

PhD portfolio

Dankwoord

ABOUT THE AUTHOR

Marianne Coolman was born on December 12, 1970 in Drachten, Smallingerland, The Netherlands. She attended the gymnasium at the secondary school "Het Drachtster Lyceum" in Drachten, where she graduated in 1989. Following this, she studied Medical Biology at the University of Amsterdam for two years. During these 2 years she decided to become a medical doctor. She studied Medicine at the University of Amsterdam (1991-1998). After obtaining her medical degree in 1998 she worked as an Obstetrics and Gynecology resident at the Flevoziekenhuis (1998-2001), Spaarneziekenhuis (2001) and Erasmus Medical Centre (2002-2003). She followed her specialty-training at the Erasmus Medical Centre and Reinier de Graaf Gasthuis, department of Obstetrics and Gynecology from 2003-2011. Scientific research remained of great interest, so she decided to start a PhD in 2003 at the Erasmus Medical Centre, department Obstetrics and Gynecology, devision prenatal medicine and obstetrics (under the supervision of Prof.dr.E.A.P. Steegers and Prof.dr.C.J.M. de Groot). Her research was partly embedded in the Generation R Study (Prof.dr.A Hofman and Prof.dr.V.W.V. Jaddoe). She combined this function with working as a medical doctor. Since 2012 Marianne is working as a medical doctor at Franciscus Gasthuis & Vlietland, department Gynecology and Obstetrics.

PhD Portfolio

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PhD training

Research skills

- Cursus werken met SPSS 2003
- Cursus wetenschappelijk schrijven 2004

Seminars, workshops and symposia

- Attending seminars at the department Obstetrics and Gynaecology 2003 – 2012
- Attending Generation R research meetings/seminars 2003 - 2006

International conferences & presentations

- Refereeravond Gynaecologie/Anesthesie, oral: Antihypertensiva 2003
- SGI, Houston, USA, poster 2004
  Concentrations of plasminogen activators and their inhibitors in blood, preconceptinally, during and after pregnancy.
- ISSHP, Wenen, Oostenrijk, poster: Early pregnancy risk factors for pre-eclampsia: a first analysis of the Generation R cohort. 2004
- Gynaecongres, Rotterdam, oral: Zoeken naar de oorzaak van pre-eclampsie, beginnen bij het begin 2004
- Nedwep, Rotterdam, oral: Zoeken naar de oorzaak van pre-eclampsie. 2004
- Gynaecongres, Noordwijk, poster: Early pregnancy risk factors for pre-eclampsia: a first analysis of the Generation R cohort. 2005

- SGI, Toronto, Canada, poster: Periconceptional maternal and risk factors for pre-eclampsia; a population-based study, generation R. 2006
- Gynaecongres, Nijmegen, poster and oral: Periconceptional maternal and paternal risk factors for preeclampsia; a population-based study, generation R. 2006
- ISSHP, Lissabon, Portugal, oral: MDR-1 polymorphism (C3435T) as a risk factor for HELLP syndrome in women with preeclampsia. 2006
- Refereeravond cluster Rotterdam gynaecologie, oral: risicofactoren voor pre-eclampsie in de vroege zwangerschap. 2008
- Onderzoeksmiddag RdGG : oral, risicofactoren voor pre-eclampsie in de vroege zwangerschap. 2008
- SGI, Miami, USA, poster: Angiogenic and Fibrinolytic Factors in Blood during the first Half of Pregnancy and Pregnancy Outcomes; The Generation R Study. 2011
- Werkgroep maternale ziekten, 2012
- oral: Angiogenic and Fibrinolytic Factors in Blood during first half of Pregnancy and Pregnancy Outcomes
- ISSHP, New Orleans, USA. 2014

Teaching activities

- Supervising several medical students

Other activities

- Speciality training Obstetrics and Gynaecology 2003 – 2011
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