Mona Høysæter Fenstad

Genetic Susceptibility to Preeclampsia

Studies on the Nord-Trøndelag
Health Study (HUNT) Cohort,
an Australian/New Zealand
Family Cohort and Decidua Basalis Tissue

Thesis for the degree of Philosophiae Doctor

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Norwegian University of Science and Technology
Faculty of Medicine
Department of Cancer Research and Molecular Medicine

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DET MEDISINSKE FAKULTET

Genetisk disposisjon for utvikling av svangerskapsforgiftning

Studier fra Helseundersøkelsen i Nord-Trøndelag, en familiekohort fra Australia/New Zealand og decidua basalis vev

Mona Høysæter Fenstad

Svangerskapsforgiftning er en alvorlig komplikasjon ved graviditet, og på verdensbasis bidrar tilstanden til økt sykelighet og dødelighet for både mor og foster. Både arvelige og miljø-/livsstilsfaktorer kan påvirke risikoen for utvikling av svangerskapsforgiftning. Selv om det fortsatt er uklart hva som forårsaker sykdommen, har forståelsen økt de siste årene, og genetisk forskning har vært en viktig bidragsyter i dette. Når morkaken fester seg til livmorveggen, bryter morkakens celler ned muskellaget i livmorens forsynende blodårer, slik at morkaken etter hvert får god blodgjennomstrømning med tilgang på surstoff og næring til fosteret. Et uheldig samspill mellom fosteret og mors immunsystem ser ut til å være sentralt i sykdomsutviklingen ved svangerskapsforgiftning. Det kliniske bildet er preget av en overdrevet betennelsesreaksjon og sirkulatoriske forandringer. Dette sees også ved hjerte-kar lidelser, og svangerskapsforgiftning deler mange risikofaktorer med disse sykdommene. Kvinner som har hatt svangerskapsforgiftning har dessuten økt risiko for hjerte-kar lidelser senere i livet. Svangerskapsforgiftning viser en klar opphopning i familier, og ulike modeller for det genetiske bakteppet er blitt foreslått.

Etter at man kartla hele den menneskelige arvestoffsekvensen (2003) kunne man begynne å analysere markører som er spredt i hele arvestoffet for å finne områder som påvirker risikoen for komplekse sykdommer som kreft, hjerte-kar sykdom og svangerskapsforgiftning. Da man begynte dette arbeidet trodde man at man i fremtiden ville kunne forutse sykdom hos enkeltpersoner ved å lese arvestoffsekvensen deres. Nå, syv år senere, har den teknologiske utviklingen snart gjort det mulig å lese hele arvestoffsekvensen til en person relativt raskt og til en overkommelig pris. Den genetiske forskningen som er gjort i løpet av disse årene har imidlertid endret vårt syn både på hvor stabilt og upåvirkelig arvestoffet er, og på hvor allmenn variasjonen som kan gi sykdom er.

Med utgangspunkt i den andre Helseundersøkelsen i Nord-Trøndelag (HUNT2) og Norsk Fødselsregister, har vi identifisert en relativt stor populasjonskohort av kvinner som har hatt svangerskapsforgiftning og kvinner som har hatt normale svangerskap. Kohorten er godt kartlagt med epidemiologiske data og vi har tilgang til blodprøver med mulighet for analyse av biokjemiske markører og isolering av arvestoff. Dette har gjort det mulig for oss å evaluere genetiske funn gjort i andre populasjoner. Vi har også undersøkt det globale genuttrykket i en samling av prøver tatt fra decidua basalis, møtepunktet for morkake og livmorvegg/mors blodårer, hos kvinner med kompliserte og normale svangerskap.

De funnene som presenteres i artiklene inkludert i denne tesen må sees i sammenheng med annen forskning for å kunne bidra til en økt forståelse av det genetiske og biologiske grunnlaget for svangerskapsforgiftning. Resultatene støtter teorien om at en forstyrret immunbalanse har betydning. Vi har knyttet *TNFSF13B*, et gen som er med på å regulere immuncellers aktiveringsgrad og funksjon, til svangerskapsforgiftning i den australske familiekohorten. Tidligere har dette genet vært vist å disponere for spontanabort. Vi viser også at en av de biologiske prosessene som ser ut til å være mest forstyrret ved svangerskapsforgiftning, er tryptofan metabolismen, som har betydning for normal utvikling av immunceller. Både *STOX1* og notch signalveier er involvert i nydannelse av blodårer og har vært knyttet til både svangerskapsforgiftning og nevrodegenerative sykdommer. Det er derfor fremsatt en teori om at disse tilstandene kan ha et felles genetisk grunnlag, og våre observasjoner støtter betydningen av disse prosessene for utvikling av svangerskapsforgiftning. Variasjon i *COMT* genet har vært vist å ha betydning

både for utvikling av hjerte-kar sykdom og svangerskapsforgiftning, via regulering av cellens respons på lav oksygentilførsel. Vi bekrefter at dette genet kan bidra til risiko for svangerskapsforgiftning. Flere av forandringen som vi finner i genuttrykks studien bekrefter også den tette forbindelsen mellom oksygenering-reoksygenerings skader og svangerskapsforgiftning.

Oppsummert har vi i løpet av de årene dette prosjektet har pågått opplevd en revolusjon i hvordan vi ser på genetisk variasjon som grunnlag for sykdomsutvikling. Vi har også opplevd en økende forståelse for de biologiske mekanismene som ligger bak utvikling av svangerskapsforgiftning. De funnene som presenteres her bidrar til noe av denne økte forståelsen og åpner for flere nye spørsmål. Videre forskning på dette feltet er nødvendig.

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1. Acknowledgements

"No realist politics in a civilized society is imaginable unless it is based on love of one's neighbor, mutuality, helpfulness and trust. This is the rock upon which all human cooperation must be built"

Fridtjof Nansen "Nestekjærlighet" (1922)

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knowledge and lab skills, Dr. John Blangero and Dr. Harald Göring for trying to convey some of their thoughts on statistics and common complex disorders. Dr. Matthew Johnson should have a page of his own, but I can only say that he is a great role model to me in the way he includes newcomers in his lab, follow up on the bench and analysis work and never lose patience or determination. A man´s character should be judged by the way he treats those that are below him in the hierarchy, and Matt gets the highest marks. Apart from that, he is also a very likable fellow and a good mate. Special thanks also to Eric and Andrea, Jac and Jo, Claire; my deepest gratitude for the way you took care of us and helped us establish as a family when arriving to Texas, and to Geir and Åsa for embarking upon a series of weekend camping trips into the Texan "wilderness". You made us feel that although far from home, we had both family and friends, and showed us the beauty hidden beyond the concrete. To all of you at SFBR, thanks for the fabulous lunches, coffees at Starbucks, jokes and laughs.

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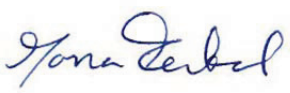
Trondheim, October 2010



2. Abbreviations

2-ME:	2-methoxyoestradiol
ACVR2A:	activin A receptor, type IIA
Aust/NZ:	Australian/New Zealand
BLAST:	Basic Local Alignment Search Tool
BP:	blood pressure
BRCA1:	breast cancer 1
BRCA2:	breast cancer 2
CADASIL:	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CDCV:	common disease – common variant
CDRV:	common disease – rare variant
CNV:	copy number variant
COMT:	catechol-o-methyl transferase
DBP:	diastolic blood pressure
DNA:	deoxyribonucleic acid
Eng:	endoglin
ER:	endoplasmic reticulum
ERAP:	endoplasmic reticulum aminopeptidase
FDR:	false discovery rate
FGR:	fetal growth restriction
GOPEC:	The Genetics of Preeclampsia Collaboration
HIF1α:	hypoxia inducible factor 1α
HLA:	human leukocyte antigen
HUNT:	the Nord Trøndelag Health Study
HUNT2:	the second Nord Trøndelag Health Study
IUGR:	intrauterine growth restriction

LD:	linkage disequilibrium
MAQC:	microarray quality control consortium
MBRN:	the Medical Birth Registry of Norway
MHC:	major histocompatibility complex
mRNA:	messenger ribonucleic acid
NK cells:	natural killer cells
OR:	odds ratio
PlGF:	placental growth factor
PIH:	pregnancy induced hypertension
qRT-PCR:	quantitative real-time polymerase chain reaction
QTDT:	quantitative transmission disequilibrium test
QTL:	quantitative trait locus
RNA:	ribonucleic acid
ROS:	reactive oxygen species
SBP:	systolic blood pressure
SD:	standard deviations
SEPS1:	selenoprotein S
sFlt:	soluble fms-like tyrosin kinase 1
SGA:	small for gestational age
SNPs:	single nucleotide polymorphisms
SOLAR:	Sequential Oligogenic Linkage Analysis Routines
STOX1:	storkhead box 1
STOX2:	storkhead box 2
TGF-ß:	transforming growth factor-β
Th17 cells:	T helper 17 cells
TNFSF13B:	tumor necrosis factor (ligand) superfamily member 13B
Treg cells:	regulatory T cells
VEGF:	vascular endothelial growth factor

3. List of papers

I M. H. Fenstad, M. P. Johnson, M. Løset, S. B. Mundal, L. T. Roten, I. P. Eide, L. Bjørge, R. K. Sande, Å.K. Johansson, T. D. Dyer, S. Forsmo, J. Blangero, E. K. Moses and R. Austgulen. ***STOX2*** **but not** ***STOX1*** **is differentially expressed in decidua from preeclamptic women: data from the Second Nord-Trøndelag Health Study.** Mol. Hum. Reprod., July 19, 2010

II M. Løset*, S.B. Mundal*, M.P. Johnson, M. H. Fenstad, K. A. Freed, I.A. Lian, I. P. Eide, L. Bjørge, J. Blangero, E. K. Moses, R. Austgulen. **A transcriptional profile of the decidua in preeclampsia**, AJOG, October 8, 2010

III M. H. Fenstad*, M. P. Johnson*, L.T. Roten, P. A. Aas, S. Forsmo, K. Klepper, C. E. East, L. J. Abraham, J. Blangero, S. P. Brennecke, R. Austgulen, E. K. Moses. **Genetic and Molecular Functional Characterization of Variants within** ***TNFSF13B*****, a Positional Candidate Preeclampsia Susceptibility Gene on 13q**, Plos ONE, September 29, 2010

IV L.T. Roten*, M.H. Fenstad*, S. Forsmo, M.P. Johnson, E.K. Moses, R. Austgulen, F. Skorpen. **A low** ***COMT*** **activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2),** Mol.Hum.Reprod., submitted October 2010

*These authors have contributed equally

4. Introduction

4.1 Preeclampsia

"A young, healthy pregnant woman suddenly had seizures and died" Celcus

Preeclampsia phenotype

Eclampsia (=lightening), pregnancy induced seizures and accompanying convulsions, was known to the ancient Greek, Egyptians and Chinese. The preceding syndrome of preeclampsia has been described since the mid 1800s [2]. However, there is still controversy about the classification and description of preeclampsia and eclampsia. The guidelines of different national working groups vary in some debated aspects, although there seems to be consensus about the general preeclampsia criteria of new onset of hypertension and proteinuria in pregnancy [3-6] The failure to provide uniform definitions, reflects that preeclampsia is a syndrome, where diagnosis is based on symptoms rather than pathophysiological manifestations [4]. For research purposes, we strive to use a stringent, standardized diagnosis. In clinical practice, however, clinical evaluation and individual assessment is encouraged, thereby increasing sensitivity and achieving the overall goal of reducing perinatal and maternal morbidity and mortality [4].

Definition

The [US] National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy [4] defines preeclampsia as gestational blood pressure elevation in a woman who was normotensive before 20 weeks of pregnancy, accompanied by proteinuria (Table 1). This definition is used by leading obstetrical societies, such as the American College of Obstetricians and Gynecologists [7], the Australasian Society for the Study of Hypertension in Pregnancy [3], and the Canadian Hypertension society [5], as well as the Norwegian Society of Gynecology and Obstetrics[6].

Table 1: Diagnostic criteria for preeclampsia

Hypertension	SBP ≥140 mmHg and/or DBP ≥90 mmHg
Proteinuria	≥0,3g/L in a 24 hour urine sample, or ≥1+ on a qualitative (dipstick) reading

To maintain consistency, the technique in which blood pressure is measured is important. Automated blood pressure measurements have been shown to affect the outcome in preeclamptic women [8]. It is recommended that standardized guidelines with a manual sphygmomanometer is used [9], and that two measurements of at least four to six hours but not more than one week apart should be performed [3,4,10]. Systolic blood pressure (SBP) is determined at Korotkoff phase V. Earlier diagnostic criteria for preeclampsia included an increase in blood pressure of 30mmHg systolic or 15mmHg diastolic. Since there is no evidence of increased adverse outcomes of this group, the criterion is no longer included, although special clinical consideration is warranted [4].

Proteinuria is most accurately measured in a 24 hour urine sample. When this is not feasible, a timed measure corrected for creatinine excretion is recommended. Excretion of ≥0.3 g protein/24 hours usually corresponds to ≥30mg/dL (≥1+) on a dipstick in a random urinary sample where there is no indication of urinary tract infection. It is possible to have all the features of severe preeclampsia without proteinuria [11], which shows the variable nature of this syndrome, and underscores the need for individual clinical assessment. Earlier classifications of preeclampsia used edema as an alternative criterion to proteinuria [12] but being highly unspecific, this criterion has later been abandoned [4].

An unambiguous preeclampsia diagnosis must demarcate from impinging conditions. Hypertension in pregnancy without proteinuria is termed “gestational hypertension”, and specified as “transistent hypertension of pregnancy” if blood pressure returns to normal by 12 weeks after delivery, and “chronic hypertension” if the elevation persists[4]. Preeclampsia superimposed on chronic hypertension represents a diagnostic challenge, and is associated with poor maternal and fetal outcome [4]. Thus pregnant women with

chronic hypertension should be closely followed, to detect new onset of proteinuria, or in case of preexisting proteinuria, signs of developing severe preeclampsia.

Preeclampsia and public health

Preeclampsia affects 3-5% of pregnancies [13] and is a leading cause of maternal and fetal mortality worldwide [14,15]. There has been a gradual incline in preeclampsia rates in Norway, but since 2001 this trend has been broken, and there has been a decline from 6,2% in primigravid women in 2001 to 4.9% in 2008 [16]. In the developed world, mortality and complication rates have dropped steadily [15]. In developing countries, preeclampsia has been reported to account for approximately 63.000 maternal deaths each year [14,17]. However, a recent publication shows an encouraging decline of global overall maternal mortality rates, and some of this decline is attributed to better obstetric care [18].

Infant mortality and morbidity is increased in preeclampsia both secondary to fetal growth restriction (FGR), increased incidence of placental abruption and iatrogenic preterm labor [19]. Notwithstanding major improvements in premature care in the developed world, the premature infant faces a wide range of acute (e.g. ventilation failure, infections) [20,21] and long term (e.g. cerebral palsy, mental retardation) [21-23] medical problems. Thus, preeclampsia is also a disease of the infant. The known risk factors can represent possible targets for primary prevention and guide further research into pathophysiological mechanisms and therapeutic targets for the disease. Some of the most important risk factors for preeclampsia are summarized in Table 2.

Table 2: Risk factors for preeclampsia (odds ratio = OR)

Partner-related	Risk *
Null parity/primipaternity [24]	4: 1
Limited sperm exposure (OR <4 months cohabitation) [25]	12: 1
Partner fathered a preeclamptic pregnancy in another woman [26]	2: 1
Sperm donation [27]	2: 1
Maternal	
Previous preeclampsia [26]	12: 1
Maternal age (OR per 5 year interval) [26]	3: 2
Interval between pregnancies (OR per 5 year interval) [26]	3: 2
Family history of preeclampsia [28]	3: 1
Constitutional	
Chronic hypertension [24]	4: 1
Renal disease [29]	20: 1
Obesity, insulin resistance, low maternal birth weight [30]	3: 1
Gestational diabetes, type-1 diabetes mellitus [31,32]	3: 1
Thrombophilias [33,34]	3: 1
Antiphospholipid antibodies [35 36]	2-20: 1
Exogenous	
Smoking (reduced risk) [24]	2: 3
Physically demanding, stressful work [37,38]	3: 1
Pregnancy associated	
Multiple pregnancy [24]	2: 1
Urinary tract infection [39]	3: 2
Trisomy 13, [40,41]	5: 1
Triploidy [42,43], Hydatiform mole [44]	1-10: 1

* Based on a review by Dekker and Sibai [45]. Examples of risk estimates from cited studies.

Notably, 98% of medical publications concern developed countries, representing 20% of the population in the world and only 12% of births annually [46]. This needs to be considered when interpreting these data. Some important characteristics of the population under study (developed world), is of special relevance to preeclampsia.

Social and reproductive patterns have changed over the last two generations. There are fewer children in each family and the use of barrier contraception and the maternal age at first birth have increased. Partner changes are more common [46]. Traditionally, preeclampsia was considered a disease of the first pregnancy. After a comprehensive review of epidemiological data comparing different reproductive cultures, partner

changes and data concerning miscarriages, abortions and donor insemination, primipaternity was introduced as a risk factor for preeclampsia rather than primigravidity [47]. Studies from the Medical Birth Registry of Norway (MBRN) contributed to this hypothesis [26].

Also, a growing proportion of the adult population in the developed world is classified as obese, and insulin resistance is becoming more common, presumably increasing the proportion of preeclamptic cases that are less dependent upon placental changes [13,45].

Severe preeclampsia

Preeclampsia sometimes presents with more severe manifestations; generalized organ failure (pulmonary oedema, oliguria, elevated liver enzymes, cerebral disturbances), thrombocytopenia, eclampsia (seizures, convulsion) and death. In affected women this is predominantly a gradual development, but might also occur in the course of days, even hours [4,48]. The diagnosis of severe preeclampsia is not well defined, but includes assessment of both maternal and fetal phenotypes (Table 3). Women with severe preeclampsia are monitored closely and are considered for delivery. The rationale for management by close observation and symptomatic treatment is reducing perinatal morbidity and mortality. Preeclampsia developing at term is considered for delivery irrespective of signs of severe preeclampsia.

Different maternal and fetal severe manifestations of preeclampsia show only moderate overlap [10,24,49-51]. Thus, severe preeclampsia presents as a rather heterogeneous condition, and this needs to be addressed when searching for genetic and biomolecular markers of severe disease. It has formerly been hypothesized that preterm and term preeclampsia represent separate pathogenic conditions [52]. However, both epidemiological and biological observations may rather support a continuous distribution of severe and less severe manifestations of the disease. Early onset preeclampsia (before 34 weeks) is complicated by FGR more often than late onset [53]. Although both early and late onset preeclampsia displays placental morphological changes, these are more extensive and pronounced in early onset preeclampsia [54]. Maternal risk factors and adverse outcomes are also more common in this group [50,55,56].

Table 3: Severe manifestations of preeclampsia indicating consideration for delivery

Maternal	Fetal
Blood pressure ≥160 mmHg systolic or ≥110 mmHg diastolic	Ultrasonographical estimate of fetal weight <10th percentile for gestational age
Proteinuria of ≥2g in 24 hours (2+ or 3+ on a qualitative (dipstick) measure	
Increase in serum creatinine >1,2 mg/dL	Oligohydramnios (amniotic fluid index ≤5cm) by ultrasonographic assessment of amniotic fluid volume
Platelet count < 100.000 cells/mm^3	
Microangiophatic hemolytic anemia (increase in lactic acid dehydrogenase (LDH))	Ultrasonographic assessment of fetal activity and/or fetal movement counts are unsatisfactory
Increased hepatic enzyme activities	
Persistent headache or other cerebral or visual disturbances	

Fetal growth restriction

FGR is associated with increased perinatal morbidity and mortality, as well as long term health consequences [7,57], and can be caused by maternal (infections, nutrition, smoking, medical conditions), fetal (multiple gestation, chromosomal aberrations), external (exposure to teratogens) or placental factors. Somewhat varying with the definition of FGR used and population studied, FGR is seen in 42-53% of early onset and 7-10% of late onset preeclamptic cases [50,58].

FGR is defined as a failure to reach the genetically determined growth potential. The term is used interchangeably with intrauterine growth restriction (IUGR) [57,59], although the American College of Obstetricians and Gynecologists include normal fetuses at the lower end of the growth spectrum in the IUGR definition [7]. Thus, the term IUGR refers to a prenatally estimated fetal weight that appears to be less than expected, whereas the term small for gestational age (SGA), refers to a measured infant weight of less than expected for gestational age. Both IUGR and SGA are most commonly defined as a measured or estimated birth weight below the 10^{th} percentile, although a more stringent use of 5^{th} or 3^{rd} percentile, or ≤2 standard deviations (SD) of expected weight will help identify infants at increased risk of adverse outcome [57,60]. Individually adjusted growth curves, serial ultrasonographic biometry and/or fundal height measurements and

assessment of umbilical artery wave form and amniotic fluid volume increases the specificity of the diagnosis and also helps identify patients at risk of adverse outcome[59,61].

Maternal long term health consequences

Women experiencing preeclampsia are at increased risk of later life renal, hypertensive and cardiovascular disease [62]. These conditions are also independent predictors of preeclampsia and share many of the constitutional factors seen in preeclamptic women. Thus it is not straightforward to distinguish whether preeclampsia and cardiovascular diseases are manifestations of the same underlying pathology, or if a preeclamptic pregnancy in itself constitutes a strain that might ultimately lead to cardiovascular disease. Pregnancy can be seen as a stress test for later life cardiovascular disease [63], where the increased metabolic and vascular demands of normal pregnancy provokes preeclampsia in some women and reveals a vulnerable constitution. However, it has been hypothesized that preeclampsia can be an independent risk factor for later cardiovascular disease even in healthy women with no predisposing vulnerability, possibly through persistent subclinical systemic vascular damage [64]. A large Canadian population and registry based study, show an additive effect of placental disease and other cardiovascular risk factors leading to premature cardiovascular disease [65]. This neither confirms, nor excludes causality, however, the authors propose metabolic syndrome as a possible intermediate phenotype between preeclampsia and cardiovascular diseases [65]. Data from the Nord Trøndelag Health Study (HUNT) support this [66]. Regardless of causality, a history of preeclampsia in a woman is consistent with both an increase in short term risk of morbidity (20% develop hypertension or microalbuminuria within 7 years [62]), serious cardiovascular events [65] and end-stage renal disease [67], and special attention in clinical practice is warranted. Adverse outcomes are more common in women experiencing early onset or recurrent preeclampsia and in women delivering SGA neonates [24,56,68].

Pathogenesis and etiology

Preeclampsia has been recognized as a disease of the placenta since the ancient accounts of the syndrome. The placental origin of preeclampsia is supported by evidence of occurrence in pregnancies outside the uterus [69], and in pregnancies without a fetus (molar pregnancies) [50]. Furthermore; increased placental load (multiple gestation, hydatiform mole) predispose to the condition. Preeclampsia only occurs during pregnancy, and delivery of the placenta relieves symptoms. The placental component of preeclampsia has been ascribed to reduced placental perfusion following impaired spiral artery remodeling shown morphologically [70]. However these morphological disturbances manifest in different ways (asymptomatic, FGR, preterm birth) [71]. Thus, placental changes alone cannot fully explain the preeclamptic syndrome. Epidemiological studies and animal model studies report independent maternal contributions to the syndrome [50,55,72,73]. The two-stage model for preeclampsia [50] (Figure 1) includes both placental and maternal factors in the etiology (stage 1) and proposes that a combination of these factors leads to proteinuria (due to renal glomerular endotheliosis) and hypertension (due to diffuse endothelial dysfunction) later in pregnancy (stage 2). Oxidative stress is proposed to be the link between the two stages[71,74]. The two stage model has been the prevailing understanding of the preeclamptic syndrome, guiding research in the area for the last one and a half decades.

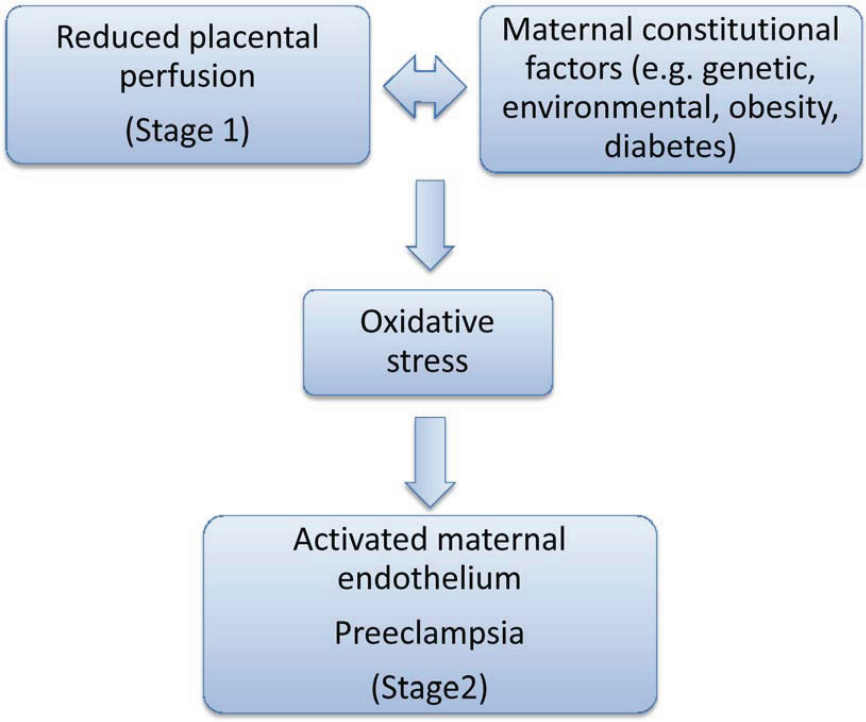


Figure 1: The two-stage model of preeclampsia (as by Roberts and Hubel [71]

Immune maladaptation

The adaption of a growing fetus to a maternal environment, and the maternal acceptance of this semiallograft, can be seen as a beautifully choreographed *pas de deux* [75]. Epidemiological data suggest that immunological priming might be important for a successful pregnancy [26,76-80]. Primipaternity and limited sperm exposure (Table 2) are risk factors for preeclampsia pointing in this direction. Biomolecular evidence of immune maladaptation is also emerging [81].

It is known that the pregnant woman's innate immune system is shifted towards a type 2/humoral response. This adaption is thought to contribute to acceptance of the fetal allograft [82]. Regulatory T cells (Treg cells) accumulate in the uterus and lymph nodes draining the uterus following increased estrogen levels at the time of ovulation. Seminal

fluid activates and expands the Treg cell pool [83]. These, and other observations are suggestive of immune priming prior to conception by exposure to seminal plasma [83,84]. In addition to paternal major histocompatibility complex (MHC) antigens, seminal plasma contains high concentrations of transforming growth factor β (TGF-β), which induces Treg cells and to a lesser extent T helper 17 (Th17) cells. Treg cells are important for the maternal immune tolerance of the fetal allograft [85] and Th17 cells are involved in host defense against bacteria, virus and fungi. The balance between these cells is important for the normal development of pregnancy, and is disturbed in complicated pregnancies [86].

Once pregnancy is established, there are two important materno-fetal immune interfaces; decidual cells interacting with invasive extravillous trophoblast and maternal blood interacting with the villous syncytiotrophoblast layer [82]. The syncytiotrophoblast does not seem to express MHC antigens at all, but the extravillous trophoblasts express human leukocyte antigen (HLA) C , E and G instead of the classical MHC antigens (HLA A, B and D) [82]. Non-cytotoxic uterine natural killer cells (uNK cells) are the main leukocytes in the decidua. Uterine NK cells stimulate trophoblast invasion and angiogenesis by secretion of cytokines. They express killer immunoglobulin (KIR) receptors, for which HLA-C is the primary ligand. Some combinations of genetic KIR and HLA-C variants have been shown to be unfavorable for pregnancy [87]. Toll-like receptors (TLRs) are expressed on uNK cells and are also implicated in pregnancy-associated complications [88]. They are a part of the innate immune system that helps discriminate between "self" and "non-self", and have been shown to recognize infectious agents and endogenous danger signals [89,90].

These proposed mechanisms of immune maladaptation for preeclampsia, imply preeclampsia as an intermediate phenotype between miscarriage and a normal pregnancy [81]. If immunological tolerance is not established, implantation and placentation fail, leading to spontaneous abortion of the pregnancy. If tolerance is perturbed, impaired placentation and later development of preeclampsia might be the outcome.

Impaired placentation

Three basic processes maintain a successful pregnancy; decidualization, placenta formation and embryogenesis. Decidualization of the endometrium starts in the secretory phase of the menstrual cycle in humans, regardless of the implantation of a blastocyst, and continues in pregnancy. A role for defective decidualization in the development of preeclampsia has been discussed [91-93].

During the formation of a placenta in the first half of a normal pregnancy, the spiral arteries of the uterus undergo changes in order to meet the nutritional needs of the growing fetus. Absence of these changes have been shown in pregnancies complicated by FGR and preeclampsia [70,94], but normal pregnancies and pregnancies with gestational or chronic hypertension may display similar disturbances [93]. Initial physiological spiral artery changes; disorganization of vascular smooth muscle, lumen dilation and basophilia are independent of trophoblast interaction, and are seen in ectopic pregnancies, as well as in non-implantation regions in a normal pregnant uterus [93,95,96]. These early changes are thought to be mediated through local artery renin-angiotensin systems [97], a mechanism possibly involved in preeclampsia pathogenesis [98-100]. Furthermore, it is widely acknowledged that the fetal trophoblast cells play an important part in normal and aberrant maternal spiral artery remodeling, and these mechanisms are a matter of continuous research and debate [48,53,93,101,102]. Physiological trophoblast-induced arterial changes include mural incorporation of trophoblasts and loss of smooth muscle cells, elasticity and vasomotor control [101]. These changes are less pronounced in preeclamptic placenta [70]. Shallow trophoblast invasion of the endometrium into the myometrium, and subsequent lack of changes to the more proximal spiral arteries in a later stage of gestation, are also key features of preeclampsia pathogenesis [93,103].

Angiogenic factors

Angiogenic factors are important for placental vascular development [62]. Uterine NK cells are involved in angiogenesis by production of a number of pro-angiogenic factors, like vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and angiopoietin 2 [48]. These factors are important for endothelial integrity. Soluble fms-like tyrosin kinase 1 (sFlt) is low in normal early pregnancy, but rising in the third trimester,

possibly reflecting an antiangiogenic shift in the placenta preceding the repair processes that are to take place after delivery of the baby and the placenta. This normal process is shown to be exaggerated in preeclampsia, where excess sFlt1 might lead to steep decreases in VEGF and PlGF levels, contributing to maternal endothelial dysfunction[104,105]. A higher level of TGF-β and its receptor endoglin (Eng) is another antiangiogenic factor shown to be increased in placenta from preeclamptic pregnancies, and hypothesized to contribute to maternal endothelial dysfunction [106]. Moreover, sFlt has been shown to decrease trophoblast invasiveness *in vitro* by binding of VEGF [107], suggesting that angiogenic factors also play a part in the balance of invasive/angiomodulating effects vs. non invasive behavior or cell death of trophoblasts[107].

Placental stress

Early placentation takes place in a hypoxic environment important for both embryonic development and trophoblast differentiation [108,109]. The transcription factor hypoxia inducible factor 1α (HIF1α) is highly expressed in early pregnancy and mediates the effects of low oxygen tension, including regulation of trophoblast to a proliferative, non invasive phenotype [108,109]. A continued high HIF1α level after the shift to a more oxygen rich environment at gestational week 10-12 might impair deep trophoblast invasion and lead to preeclampsia. Inadequate levels of the enzyme catechol-o-methyl transferase (COMT) and its metabolite 2-methoxyoestradiol (2-ME) might lead to inadequate inhibition of HIF1α and consequently up regulation of hypoxia induced genes and antiangiogenic factors (sFlt, Eng) in the placenta [110]. However, hypoxia due to poor placentation has been viewed as the driving mechanism of the systemic inflammatory activation in preeclampsia [111]. Poor placentation leads to both oxidative[111] and endoplasmic reticulum (ER) stress [112]. Intermittent hypoxia and re-oxygenation, creating reactive oxygen species (ROS) explains the clinical preeclampsia continuum better than chronic hypoxia [111]. ROS can influence cellular processes in a number of ways, including regulation of transcription factors (e.g. HIF1α), direct oxidative modifications of enzymes and interaction with proteins involved in cell survival and inflammation [113].

Inflammation

The inflammatory response involves intravascular activation of immune cells, clotting factors and complement, as well as endothelium. Normal pregnancy is a state of maternal systemic inflammation influencing all these systems. This is shown by the increase in circulating pro-inflammatory cytokines, activated leucocytes, platelets and clotting factors in maternal plasma during pregnancy [51]. Systemic inflammatory changes are enhanced in preeclampsia, and the role of inflammation in preeclampsia has recently been comprehensively reviewed [114]. Notably, there is a far more pronounced difference in the inflammatory state of non-pregnant vs. pregnant women than in normal pregnant vs. preeclamptic pregnant women [51]. An important clinical consequence is that it will be challenging to find good diagnostic and/or predictive markers distinguishing between the normal physiological changes of pregnancy and the enhanced response seen in preeclampsia.

Apoptotic debris resulting from the physiological renewal of the syncytiotrophoblast layer is one activator of the normal and perturbed inflammatory state of pregnancy. Both the quantity and quality of this debris has been shown to be different in preeclamptic women, in that an increase in peroxidized lipids, concordant with an increased oxidative state, as well as increased amount of cytokeratin, syncytial cellular fragments and soluble fetal deoxyribonucleic acid (DNA), concordant with an increased placental size, is observed [53]. In addition, syncytiotrophoblast secrete many bioactive factors (e.g. sFlt, activin A, corticotrophin releasing hormone and leptin) [114]. Hypoxia-reoxygenation and ER stress are potent activators of apoptotic changes in the syncytiotrophoblast [113].

Insulin resistance is a feature of normal third trimester pregnancies, thought to have a physiological role in sustaining a sufficient nutrition of the fetus [115]. Insulin resistance is also a feature of non-pregnant systemic inflammatory conditions. Several studies show an association between preeclampsia and insulin resistance [116-118], thus this is one interception between the normal physiological changes of pregnancy and the disease-causing exaggerated inflammatory state seen in overt preeclampsia.

Atherosclerosis is a focal large vessel disease, but an activated microvasculature endothelium might contribute substantially to the inflammatory milieu facilitating atherosclerosis [119]. Atherosclerotic placental infarctions are seen as alternative or complementary inductors of impaired placental blood flow leading to the preeclampsia syndrome [120,121]. However, the morphological studies describing these cannot unequivocally determine the timeline/cause-effect as well as the degree and specificity of these features [120]. The current view is that these changes might occur late in the disease process, as a possible end stage of preeclampsia (C.W. Redman, PremUp symposium, Paris May 2010).

Overt preeclampsia

Established preeclampsia may proceed to a vascular crisis, presenting as a state of hypoperfusion of many vital organ systems. This is thought to be secondary to an increased sensitivity to vasopressor agents and reduced expression of the vasorelaxant factor nitric oxide (NO) [122], leading to peripheral vasoconstriction in the clinical phase of preeclampsia [123]. Activation of platelets and the coagulation cascade further compromise perfusion [13]. The renin-angiotensin system, regulating plasma fluid volume, has also been shown to be implicated in preeclampsia pathogenesis [100,124].

The pathological processes of oxidative and ER stress, endothelial dysfunction, insulin resistance macrophage activation and atherosclerosis are shared between preeclampsia, maternal metabolic risk factors (hypertension, obesity, diabetes) and the increased later life cardiovascular morbidity and mortality [125-127].

The three-stage model of preeclampsia

Based on the increasing understanding of the complexity of preeclampsia pathogenesis, new presentations have been made based on the two stage model. Figure 2 summarizes some of these latest presentations of the preeclampsia syndrome.

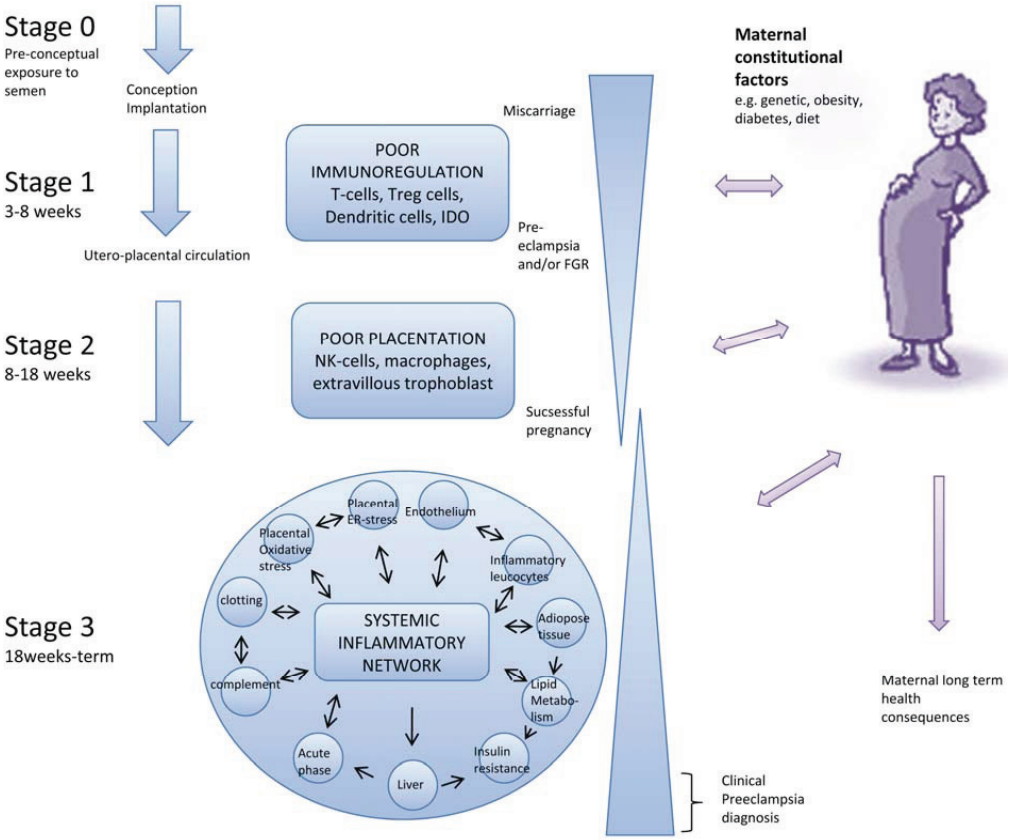


Figure 2: The three stage model of preeclampsia (modified from Roberts and Hubel and Redman and Sargent [81,128].

4.2 Genetics

"Today, we are learning the language in which God created life. (...) With this profound new knowledge, humankind is on the verge of gaining immense, new power to heal." Bill Clinton, 2000[1]

Population genetics

Population genetics had a rather narrow birth, due in large to personal conflicts between the first generation of prominent leaders in the field, as brilliantly described by William B. Provine in his dissertation [129]. After Darwin published his "*On the Origin of Species"* in 1859, his natural selection evolution theory gained many followers. However, there was a divide between those who believed evolution to have taken place by a gradual, continuous development driven by selection (later called the biometricians), and those who argued that evolution rather took place more rapidly in leaps of substantial changes, mutations (later called the Mendelians) [130]. Mendel was a contemporary of Darwin, and his work on the heritable characteristics of pea plants "*Versuche über Pflantzen-hybriden*" (1866) was published at the same time as Darwin's "*Provisional Hypothesis of Pangenesis"* (1868) describing inherited units. Mendel's work lay forgotten, however, until it was rediscovered by the "mendelians" in 1900 [131]. Mendel's work inspired the next generation of population geneticists to test their hypotheses by experiments. William Bateson introduced the term "genetics" in 1903, and in 1909 the distinction between genotype (inherited unit) and phenotype (measurable trait) was made by the Danish botanist Wilhelm Johannsen [132]. The following half century established the statistical and theoretical basis of most of the genetic tests that are run today, and the biometricians view and mendelian view merged to the field of population genetics [133]. Today, population genetics is divided in two branches: *Evolutionary genetics*, which deals with mathematical theories describing phylogenies and (theoretical) developmental processes from the past and *Genetic epidemiology*, describing inherited distribution, cause and consequence of disease in current populations.

[1]Bill Clinton: speaking at the joint press conference given by Bill Clinton and Tony Blair announcing the completion of the Human Genome project. The phrase is inspired by Galileo, who pictured the laws of mechanics and mathematics as "the language in which God created the universe".

From Mendelian to common, complex disorders

Several metabolic diseases follow the same patterns of inheritance as the color of Mendel's pea flowers (autosomal recessive or dominant). Sir Archibold Garrod introduced the concept of "inborn errors of metabolism" in the early 1900s [134]. In the following decades the idea of "one gene-(one protein)-one disease" was prevailing. Simple genetic diseases caused by one or a few mutations in single genes (Huntington's chorea, Fölling's phenylketonuria), were studied to great success [130], using linkage analyses in family pedigrees. The "one gene-one disease" concept evolved into "one wild-type healthy allele-one mutant disease allele" [134]. As early as 1928, Fisher postulated that mutant genes are inherently neither dominant nor recessive, but rather produce intermediate heterozygotes, and that numerous modifying genes influence their dominance [130]. However, it was only as we approached the new millennium that it became commonly appreciated that most diseases are complex, influenced by dozens or hundreds of genes, as well as environmental factors [134].

Table 4: Some important concepts in genetics

Deoxyribonucleic acid (DNA): a double helix molecule consisting of 4 bases; adenine (A), thymine (T), guanine (G), cytosine (C), forming the molecular basis of the genome.

Gene: a unit of DNA that codes for a protein (including both non-coding and coding elements). The ~30 000 genes of the human genome is packed into 23 chromosome pairs.

Ribonucleic acid (RNA): RNA, which is structurally similar to DNA, is transcribed from DNA by enzymes. Messenger RNA (mRNA) carries information from DNA to structural units in the cell where the sequence information is translated into the chain of amino acids forming a protein.

Locus: location sometimes used interchangeably with gene but more often to describe a particular site in the gene where a base is situated. Different forms of the gene (alleles) may occupy the locus.

Allele: the specific variant/base at a particular locus in the genome.

Genotype: the combination of alleles on corresponding loci in the two chromosomes. Usually, two bases are possible at a given locus, e.g. A and G. Two genotypes of equal information on both chromosomes (AA and GG) and a third with different information (AG or GA) are possible.

Phenotype: a measurable property of an individual (e.g. height, weight, hair color, blood pressure).

Penetrance: the fraction of phenotypic variance explained by a particular genotype.

Epistasis: interaction/dependence between different loci/genes.

Epigenetics: modifications (methylation, imprinting etc.) to DNA that influences expression of genes.

Complex trait: a trait that is influenced by multiple genes and environmental factors and the interaction between them.

Heritability: the proportion of phenotypic variance that is attributable to genetic effects (h^2).

Linkage analysis: a test for co-segregation of phenotype and genotype within families/pedigrees.

Linkage disequilibrium (LD): All alleles on the two strands of DNA do not separate randomly during meiosis. Non-random association of alleles on two or more loci is called LD [1]. At a locus, at least two different bases are possible. LD describes the extent to which an allele variant at one locus predicts the variant at another locus.

Association analysis: The genotype and the phenotype is said to be associated if the genotype-phenotype combination occurs more frequently than what would have been expected from their separate frequencies.

The Book of Life

Human genome project

The human genome project was initiated in 1990 by the U.S. National Institute of Health, aiming at determining the ~3 billion base pairs that constitute the human DNA and identify all human genes (www.genome.gov). The project was an international collaboration and took place at multiple centers around the world, making it an unprecedented explorative effort in the history of modern science. A parallel project was conducted by the commercial company Celera. The first draft of the human genome was published by the International Human Genome Sequencing Consortium [135] and the Celera group [136] in 2001. The full sequence was completed in 2003 [137], 50 years after Watson and Crick first published the double helix structure of DNA [138]. The consequences of these achievements are of course wide-ranging. The most immediate insights were that, surprisingly, the number of human genes appeared to be fewer (25.000) than first estimated (80.000 – 140.000). Over 1.4 million single nucleotide polymorphisms (SNPs) were initially identified. The preliminary conclusions of the sequencing project were discussed in a special edition of Nature [135]. The authors end their paper with a tribute to Watson and Crick: "Finally, it is has not escaped our notice that the more we learn about the human genome, the more there is to explore." (Reiterating the famous understatement made in the one-page paper describing the DNA helix structure: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.")

HapMap

The most common variation found in the human genome, are SNPs. At the completion of the human genome sequence, these SNPs were thought to represent 90% of human genetic variation, and 10 million SNPs with a frequency of 1% or more were predicted[139]. When linkage disequilibrium (LD) (Table 4) between a group of SNPs is high, most of the genetic information from these SNPs can be obtained from genotyping only one SNP instead of all. Such a group of SNPs is called a haplotype [140]. The international HapMap project, initiated in 2002, aimed to construct genome-wide maps

of LD patterns in multiple populations, thus identifying "Tag" SNPs predicting a larger proportion of SNP variance and facilitating more effective genotyping. In 2005, a haplotype map of the human genome was published [141]. New sequencing technologies were developed in line with the new discoveries. Tag SNPs from the HapMap project were included in commercially available SNP "Chips", giving genome-wide coverage of the most common variation in the human genome, and making it possible to type as many as 500.000-1 million SNPs in one experiment. The speed and magnitude of sequencing projects world-wide exploded [142]. At the release of NCBIs dbSNP Build 131 in March 2010, a total of 105.098.087 submitted SNPs and 23.652.081 reference SNPs were reported.

Common vs. rare allele hypotheses for complex diseases

After the completion of the Human Genome Project it has been possible to start unfurling the genetic framework as well as the biological mechanisms of complex, genetically heterogeneous diseases, like cardiac disease, cancer, and preeclampsia. In starting this work, the debate between the Biometricians and the Mendelians seems to have resurfaced in the scientific community. The common disease-common variant (CDCV) hypothesis (Reich and Lander 2001), somewhat simplified, states that a limited number of common (>1%) genetic variants, each with a low disease penetrance, primarily contribute to complex genetic disease susceptibility. These variants are hypothesized to be ubiquitous throughout human populations, as they predate the relatively recent considerable human population expansion and have a low calculated mutation rate [143]. The common disease-rare variant (CDRV) hypothesis challenges this, and states that a large number of uncommon variants (<1%), each with a higher disease penetrance will underlie the genetic susceptibility to complex diseases. Only the first hypothesis was initially economically and practically feasible to test [144].

Since the introduction of "Whole genome SNP Chip" technology, there have been quite a few discoveries of common genetic variation predisposing to disease [145]. Considering the total number of tests run, though, the findings have been surprisingly few. It is interesting to note, that some of the most studied disease genes, the breast cancer 1 (*BRCA1)* and breast cancer 2 (*BRCA2)* genes show multiple rare variants, some of them

with a higher OR for disease in the affected families than the more common variants [146]. Characterizing population specific variation and the possible functional role of rare population specific variants is therefore also of interest, and most geneticists agree that both the CDCV and CDRV hypotheses can co-exist.

Genomics

Examining differences in gene expression is an alternative way of elucidating the etiology of common complex disorders [147,148]. Measuring biological differences at the messenger ribonucleic acid (mRNA) level places the researcher one step closer to what is actually taking place in the cell. Microarray platforms provide the opportunity of measuring tens of thousands of expressed gene transcripts within a particular mRNA sample [149]. This reduces the cost, time and labor for measuring the expression of each gene and the need for biological material, which is often limited. When addressing the full dataset, a list of up or down regulated genes, does not easily translate into knowledge. Therefore, different ways of looking at patterns of gene expression are utilized [150]. In class comparison studies, a number of bioinformatics tools and statistical methods have been developed for analyzing the data more comprehensively in terms of known functions of genes and interactions between genes (http:// www.geneontology.org / GO.tools. browsers. shtml). Furthermore, combining the information about co-expression of genes in the dataset with prior knowledge about gene interactions is utilized [151,152]. Another utilization of gene expression profile data is to combine it with SNP data, to identify variants that affect gene expression (putative functional variants) [153]. This approach has revealed that SNPs influence gene expression in a cell-type specific manner [154].

Microarray expression data are normally made publically available, following the Microarray consortium of 2001 standards (minimum information about a microarray experiment, MIAME) [155]. The requirements include information about the experiment, the samples and all the detected, normalized transcript level values for each sample. The inclusion of these standards in most journal requirements for publication, has led to a wealth of information accumulating on gene expression, and new bioinformatics tools for interpretation of these data are continuously being developed.

Genetic variation – more than SNPs

The genetic variation leading to human disease is, as predicted, turning out to be more complex the more we explore the matter. Diseases that were originally thought to be caused by one or a few highly penetrant mutations in one gene, are turning out to be influenced considerably by additional genes [133]. Therefore, even if the distinction is often made when discussing methods, there is no clear divide between purely "mendelian", or monogenic, and "complex" disorders. SNPs have so far been the major tool of investigation for common complex disorders. However, the studies of less complex diseases show a considerable contribution to disease susceptibility from structural variation, like gene copy number variants (CNV), deletions and insertions [145,156]. There is an increasing understanding that interaction between genes and the environment is important [157]. Different chemical modifications to DNA have been shown to affect gene expression [156,157]. Imprinting of genes results in mono allelic gene expression, and a parent-of-origin effect of specific allelic variants. Several proteins can be expressed from one gene by alternative splicing of exons, and alternative splicing can also be a way of regulating gene expression [158]. Research on small ribonucleic acid (RNA) transcripts of 20-100 base pairs in length (microRNA) has shown widespread context specific regulatory functions of these microRNAs, and RNA-protein interactions are being explored further [159]. The non-coding "dark matter" of the genome is being studied, and an estimated 47% to 80% of total transcripts may map to locations separate from known exons (introns and intergenic areas) [160].

At the beginning of this century, the belief in individualized medicine based on genetic information was strong, and Roche Diagnostics issued microarray chips in 2007, claiming to predict drug metabolism, efficiency and adverse outcomes by the transcriptional profile of two CYP450 genes. The increasing understanding of the complexity of the human genome make researchers more cautious, some stating that individualized drug therapy is "*impossible now, or in the foreseeable future*" [134]. However, microarrays have proven to be useful clinical tools for example for classifying and diagnosing different tumor and leukemia types [161,162]. Also, increased understanding of pathophysiology may identify new targets for diagnosis and treatment of common complex diseases.

Preeclampsia genetics

Preeclampsia heritability is as high as 54% [163], and identification of genetic factors conferring susceptibility to the disease is one strategy for elucidating its pathophysiology. The history of preeclampsia genetics has been reviewed comprehensively by others [123,164,165]. In line with the general understanding of genetics, early family-based studies suggested different modes of inheritance for preeclampsia. A possibility of preeclampsia as a recessive single gene disease was predominant in the 1980s [166,167], later a dominant gene-reduced penetrance model was introduced [166,168]. The understanding of preeclampsia as a multifactorial disease was not established until the late 1990s [123]. This realization was followed by a large number of candidate gene studies with conflicting results [164]. Many of the studies were underpowered. Negative results were therefore uninformative and may have complicated meta analysis [169]. Of all the candidate gene studies performed, approximately 70% concerned a small number of genes involved in known preeclampsia disease mechanisms, such as the renin-angiotensin system, inherited thrombophilias, regulation of endothelial nitiric oxide synthase (eNOS, a vasorelaxant) and the inflammatory response cytokine TNF-α [164]. The Genetics of Pre-Eclampsia Collaboration (GOPEC) study was designed to identify genetic factors conferring susceptibility to preeclampsia in U.K. families, recruiting at the time of diagnosis, using strict diagnostic criteria [170]. A report published in 2005 including 398 maternal triads (an affected woman and her parents or one parent and one or more siblings) and 536 fetal triads (an affected woman, her partner and baby), in total 2.504 individuals, could not confirm disease association to any of the seven most studied preeclampsia candidate genes (encoding angiotensinogen, the angiotensin receptors, factor V Leiden variant, methylene tetrahydrofolate reductase, nitric oxide synthase, and tumor necrosis factor α) [170].

Genome-wide linkage studies in preeclampsia families were initiated to circumvent the problem of only looking at single candidate genes within biological systems affected in the disease. These studies use microsatellite markers (40-400) throughout the whole genome to identify chromosomal regions of interest. By examining the probability of co-segregating loci, several loci most likely to harbor maternal susceptibility genes were identified [171-176]. Although some susceptibility loci on chromosomes 2 and 4 are thought

to be shared [171,173,175,177], the poor overlap between studies, emphasized the genetic complexity of preeclampsia.

Susceptibility loci for preeclampsia may harbor 100-400 genes [164]. Thus, different approaches for prioritizing and fine-mapping these regions have been undertaken [177,178]. Several studies suggest the involvement of epigenetic mechanisms in preeclampsia [178,179]. The group reporting a linkage locus on chromosome 10q [174], hypothesized that preeclampsia might be associated with genetic imprinting, and re-analyzed their data under this model [178]. Maternally inherited, shared alleles under the 10q locus were confirmed. A Basic Local Alignment Search Tool (BLAST) search identified DNA sequence features characteristic of imprinted genes. Furthermore, using mRNA from first trimester placenta and first trimester hydatiform moles (only containing paternal nuclear DNA), they identified two clusters of genes that were transcribed only in placental tissue with maternally expressed genes. This information was combined to prioritize 17 candidate genes for genotyping, and storkhead box 1 (*STOX1*) was identified as a potential preeclampsia candidate gene [180].

The Australian group reporting linkage to chromosome 2q [176], hypothesized an underlying continuous distribution of susceptibility to preeclampsia, using a variance components-based linkage analysis method [181]. This analysis utilizes pedigree information to infer effect sizes and localization of possible quantitative trait loci (QTLs). Furthermore, transcription levels (mRNA) of genes residing within the linkage region on 2q were investigated in preeclamptic and normotensive decidua [177]. Finally, the computer program GeneSniffer (www.genesniffer.org) was used to prioritize candidate genes. This program retrieves information from the NCBI's Gene, OMIM and PubMed databases, and examines the text using a list of keywords provided by the researcher. The results of these QTL, gene expression and GeneSniffer analyses were combined to prioritize the Activin A receptor, type IIA (ACVR2A) gene for further investigation [177]. Known SNPs in this gene were genotyped, and association to *ACVR2A* was confirmed both in the Australian/New Zealand (Aust/NZ) and Norwegian (the second Nord Trøndelag Health Study (HUNT2)) cohorts [177,182].

The above examples illustrate the integrated approach now undertaken by most scientists in the field, and which this thesis also rests on, utilizing genome-wide screening, as well as biological knowledge, bioinformatic tools, RNA and protein analysis to generate study hypotheses.

Transcriptional profiling

Microarray-based transcriptional profiling, looking at transcripts across the whole genome, has been performed on placental [149,183,184] as well as decidual tissues [100,177,185,186] (paper II) from preeclamptic pregnancies. These studies have shed light on some biological processes known to be implicated in preeclampsia, such as immune regulation, angiogenesis and inflammation, and have also supported new hypotheses, such as the involvement of notch signaling pathways [183] and extracellular matrix proteins [186]. The results have, however, been inconsistent. This may simply reflect the mixed etiology and complex genetics of preeclampsia. Differences in study design; patient characteristics, sampling protocols, microarray procedures and aims make them hard to compare [149]. Diverging results may also be due to the relatively small number of samples analyzed. Furthermore, the vulnerability of gene expression to possible bias (discussed in **section 8**) has not been clearly enough recognized. The method has been in its infancy, and more stringent protocols and reporting standards are now being applied.

Animal models

Transgenic animal models are widely used to study effects of genes on disease development. There is no ideal animal model for human placentation, and we do not know any naturally occurring parallel to preeclampsia in other mammals. Non-human primates share some of the characteristics of human placentation, but lack deep trophoblast invasion, a mechanism central to preeclampsia development [187]. Rodents are evolutionary close to primates, and mouse-models for preeclampsia have been used [110,188,189]. Mice share many of the genes involved in human placentation [190], and preeclampsia-like symptoms can be induced by knocking out (removing) specific genes. Both maternal [191] and fetal [189] effect genes have been identified, as well as disease-causing combinations of maternal and fetal genotypes (renin-angiotensin) [192]. Maynard

et al. created a rat model producing sustained elevation of sFlt, developing preeclampsia like symptoms and renal lesions [104] and *COMT* was introduced as a preeclampsia susceptibility gene in 2008 by Kanasaki and co-workers, presenting work on COMT knockout mice [110]. COMT-/- mice developed a preeclampsia-like syndrome, with elevated blood pressure, albuminuria, glomerular changes, placental thrombosis and hypoxia, and preterm birth [110]. However, there are important limitations to using these rodent-models [187]. Trophoblast invasion in mice is shallow and the trophoblasts are less important for arterial remodeling. Immune mechanisms also differ, and syngenic or allogenic matings in cloned animals cannot truly evaluate the proposed allograft rejection hypothesis for preeclampsia [193].

Evolutionary perspective

With an incidence ranging from 1-10% preeclampsia represents a reproductive disadvantage to humans [46]. Another reproductive disadvantage is a low fertility success rate (25% compared to 90% in other mammals), with an average 7-8 months before conception (possibly facilitating immunological adaption to paternal antigens?) [46]. It has been speculated whether these two reproductive disadvantages are in equilibrium [46], supporting the immune maladaptation theory for preeclampsia (referring to the Red Queen hypothesis [194], describing evolution as an equilibrium between "pray" and "hunter"). Preeclampsia has also been conceived as a consequence of the struggle between maternal and paternal genes. A rise in blood pressure (seen as a physiological phenomenon towards late pregnancy) helps provide enough oxygen and nutrition for the fetus, at the cost of an increased risk for the mother [195].

The ENCODE consortium has estimated that 60% of mammalian DNA bases under strict evolutionary constraint are functional variants [196]. However, these functional elements differ greatly in their sequence variability within the human population [196]. Different regions of genomes evolve at different rates, and some regions might evolve more rapidly in particular lineages [197]. Immune genes are the most rapidly evolving genes in both humans and other mammals [197,198]. It has also been shown that transcription factors evolve more rapidly in humans than in any other primates [197].

An important implication of the evolutionary perspective on preeclampsia genetics is that the different underlying pathophysiological mechanisms of the disease stages probably are based on different kinds of genetic variation, as the two first stages might be more prone to evolutionary pressure mechanisms than the last [199]. Immune effect genetic variation may be more recent, and thus differ more between populations, and metabolic genetic variation conferring preeclampsia susceptibility more established and thus more similar between populations.

5. Aims

The overall aim of this work was to identify functional genetic variation influencing maternal preeclampsia susceptibility. The following queries were undertaken:

Paper I

Linkage studies in a Dutch family cohort have identified *STOX1* as a preeclampsia susceptibility gene under the chromosome 10q locus. Missense mutations in this gene were shared between affected sisters and were shown to co-segregate with the preeclampsia phenotype [180]. Overexpression of *STOX1A* in cultured trophoblast cells has been shown to affect invasiveness through regulation of the cell to cell adhesion complex protein αT-catenin (*CTNNA3*) [200]. In a separate study, global transcriptional alterations in trophoblast cells overexpressing *STOX1A* was compared to transcriptional alterations in term preeclamptic compared to non-preeclamptic placenta [201].

We aimed at evaluating the hypothesis that genetic variation in the *STOX1* gene residing under the 10q linkage region can be a causal factor for developing severe preeclampsia using our HUNT2 preeclampsia population cohort. Expression of the *STOX1* gene and related transcripts were investigated in our deciduas basalis material, including women with different pregnancy complications of presumed placental origin.

Paper II

Microarray-based transcriptional profiling can be a powerful strategy for identification of disease-related genes and pathways, and has been performed on decidual tissue previously [100,177,185,186]. The results have been somewhat inconsistent. Some of the divergence might be explained by differences in study design and relatively small numbers of samples analyzed.

We aimed to indentify biological processes that are perturbed in preeclampsia by a comprehensive investigation of gene-expression at the maternal-fetal interface (measuring ≥48,000 transcripts from all known genes) in our larger collection of decidua basalis samples from normal pregnancies and pregnancies complicated by preeclampsia.

Paper III

Three chromosomal regions of interest (2q22, 5q and 13q) have previously been reported from Aust/NZ families [173,176]. Literature searches and analyses in the software GeneSniffer identified 20-30 candidate genes under the 13q preeclampsia susceptibility locus.

We aimed to interrogate the chromosome 13q QTL for preeclampsia further by identifying potential functional and structural variants in the positional candidate gene tumor necrosis factor (ligand) superfamily member 13B (*TNFSF13B*) under the 13q linkage peak.

Paper IV

A recent study based on gene expression in placenta and studies on knock-out mice, has suggested that deficiency in COMT is associated with preeclampsia (Kanasaki *et al*., 2008). The low activity *COMT* rs4680 A/Met variant was subsequently shown to be associated with preeclampsia in a Korean population cohort of 164 preeclamptic and 182 normotensive patients (Lim *et al*., 2010).

We aimed to evaluate the hypothesis that genetic variation in the *COMT* gene conferring low activity may contribute to preeclampsia pathogenesis using our HUNT2 preeclampsia cohort.

6. Data sources

The second Nord Trøndelag Health Study

HUNT2 is a multipurpose health survey conducted from 1995-1997, focusing on the total population in the rural county of Nord-Trøndelag. All residents of Nord-Trøndelag above 19 years of age were invited to participate, and 75.5% of women (n=35.280) were included. The participants went through a clinical examination, extensive questionnaires and a large biobank was established. The collection of data and biological material is described in detail by Holmen *et al*. [202]. The county of Nord Trøndelag is considered to be representative of the rural Norwegian population, stable (with a net annual out migration of 0, 3%) and rather homogeneous (less than 3% non-Caucasians). Preeclamptic women and women with normal pregnancies were retrospectively selected using personal identification numbers to cross-link the HUNT2 database with the MBRN.

DNA samples were available for 1.139 women registered with preeclamptic pregnancies and 2.269 non-preeclamptic women [203]. Of the available cases, 1.003 women were registered with one (non-recurrent) and 136 women with more than one (recurrent) preeclamptic pregnancy. Mean follow up time from diagnosis in the MBRN to inclusion in the present study was 25±10 years.

Clinical characterization of the cohort showed expected differences in gestational age and birth weight between neonates in preeclamptic and non-preeclamptic pregnancies. The preeclamptic women had a higher risk of delivering preterm (<37 weeks [4]), and of delivering a FGR neonate. Furthermore, metabolic syndrome, evaluated by data from the HUNT2 study, was higher in the case groups as compared to controls (Table 5). We also observed clinical differences between the recurrent and non-recurrent preeclamptic groups (Table 5).

Table 5: Clinical characteristics of the HUNT2 preeclampsia cohort*

	Pre-eclampsia (recurrent[1], *n* = 136)	Pre-eclampsia (non-recurrent, *n* = 1.003)	Control (*n* = 2.269)
Maternal age at index pregnancy (years)	25 ± 5	27 ± 6*	25 ± 5
Gestational age (days)	271 ± 20*	275 ± 22*	282 ± 18
Birthweight (g)	3.040 ± 846*	3.238 ± 837*	3.483 ± 592
FGR[2]	26 (20)*	147 (15)*	87 (4)
Preterm birth[3]	29 (22)*	132 (14)*	114 (5)
Maternal age at inclusion in HUNT2	37 ± 9*	40 ± 11	40 ± 11
MetabolicSyndrome[4]	30 (22)*	163 (16)*	212 (9)

Data presented as mean ± SD or number (percentage). *P*-values are computed based on *t*-test statistics, each pre-eclamptic group is compared with the non-pre-eclamptic group. IDF, the International Diabetes Federation; HDL, high-density lipoprotein; CI, confidence interval.
[1]More than one pre-eclamptic pregnancy.
[2]≤2 SD of expected weight.
[3]Delivery before week 37.
[4]IDF-proxy definition; waist circumference ≥80 plus any two of (HDL cholesterol <1.29, treatment for hypertension or blood pressure ≥130/≥85 mm Hg, diabetes diagnosed after age of 30 or fasting plasma glucose ≥5.6 mmol/l).[43].
*$P < 0.001$.

*Table from Paper I

The Medical Birth Registry of Norway

The MBRN was founded in 1967. Obstetrical data of all deliveries in Norway after 16 weeks of gestation have been registered by doctors and midwives in standardized questionnaires completed shortly after delivery. More than 1.8 million births are included. Each person is registered with a personal identification number, which is common for all national registries in Norway. Preeclampsia was defined as hypertension (blood pressure of ≥140/ 90 mmHg) and proteinuria (≥0,3 g/d or ≥1+ according to a dipstick test), developing after 20 weeks of pregnancy [4]. Diagnostic codes ICD-8 (before 1998) and ICD-10 (after 1998) reported to the MBRN were used to represent these criteria. Cross-linking of the MBRN and HUNT2 data was performed at the MBRN, and data was made available to the researchers in an anonymized form. A total of 1.179 women having experienced preeclampsia were identified in the HUNT2 cohort. Controls (n=2.358) were selected randomly on the basis of the next two normal pregnancies in the HUNT2 cohort (non pair-wise matching).

Decidual samples

Decidual specimens were collected at St. Olavs Hospital, the University Hospital of Trondheim and Haukeland University Hospital from 2002 to 2006. Decidual tissue was collected at delivery by vacuum aspiration of the placental bed during cesarean section [204,205]. The vacuum suction method was chosen because it benefits from providing a representative and adequate amount of decidual material [204,205], as well as from avoiding the possible effects of labor on gene expression [205]. No complications to this method have been observed, by us or others [204,205]. Women with pregnancies complicated by preeclampsia and FGR, alone or in combination were included. Cesarean section in the control group was undergone for reasons considered irrelevant to the study hypotheses (breech presentation, cephalopelvic disproportion in an earlier pregnancy or maternal request). FGR was assessed by prenatal ultrasound measures [206] and birth weight confirmation (birth weight ≤2 SD, corresponding to the 2.5 percentile for gestational age). Preeclampsia was defined as persistent hypertension (blood pressure of ≥140/90 mmHg) plus proteinuria (≥0,3 g/l or ≥1+ according to a dipstick test), developing after 20 weeks of pregnancy [4]. Multiple pregnancies and pregnancies with chromosomal aberrations, fetal and placental structural abnormalities or suspected perinatal infections were not included. None of the included mothers were in labor prior to cesarean section. Only samples containing extravillous trophoblasts were included. The quality of the decidual material was assessed by immunohistochemistry, as described in [204]. Samples consisting mostly of blood or contaminated with placental tissue were excluded. In agreement with other reports [205], spiral arteries were identified in 89% of the collected samples tested [204].

Australian/New Zealand family cohort

The Aust/NZ familial cohort was recruited over a 15-year period through the Royal Women's Hospital and the Monash Medical Centre in Melbourne, Australia and the National Women's Hospital in Auckland, New Zealand. QTLs for preeclampsia on chromosomes 2q, 5q and 13q has been reported from an original set of 34 (26 Australian and eight New Zealand) families [207-210]. An additional 40 (Australian) preeclampsia families have subsequently been ascertained [211]. The extended cohort

includes 480 individuals from 74 families. Family members are coded as 1) affected, 2) unaffected or 3) unknown (male or non-fertile women). Preeclampsia was defined according to the criteria of the Australasian Society for the Study of Hypertension in Pregnancy [3,212]. Women were considered to have severe preeclampsia if they had either 1) an increase from baseline systolic blood pressure of ≥25 mmHg, and/or diastolic pressure of ≥15 mmHg; or 2) systolic pressure of ≥140 mmHg, and/or diastolic pressure of ≥90 mmHg on at least two occasions 6 h or more apart. Proteinuria (≥0,3 g/l in a 24 h specimen, or ≥2+ on a dipstick in a random urine collection) was also required for the diagnosis. Convulsions or unconsciousness in the prenatal period in addition to preeclampsia was classified as eclampsia. Hypertension without proteinuria was classified as mild preeclampsia. Only women experiencing preeclampsia in their first pregnancy (primiparous women) were included, and women with known predisposing medical conditions (e.g. renal disease, diabetes, twin pregnancies or fetal chromosomal abnormalities) were excluded. All family members were of Caucasian origin.

Bioinformatic tools and online resources

The publication of information within the field of molecular biology has increased vastly in volume and complexity over the last decade. Hence, a number of applications aimed at assisting researchers in finding, interpreting and integrating information are being developed [213]. Many of these bioinformatic "tools" are based on the same mathematical/statistical methods, and exploit the same information sources [133]. The primary source of information in the form of text in molecular biology and biomedicine is Medline, the United States National Library of Medicine (NLM)'s bibliographic database including more than 12.000.000 abstracts. The most widely used interface for this information is the PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). All the bioinformatic tools utilize text-mining technology based on automated computer-learning methods, depending upon proper annotation of text submitted to NLM and other repositories. Medline records are indexed with NLM's controlled vocabulary, the Medical Subject Headings (MeSH), and NLM also provides medical ontology, described by the Unified Medical Language System (UMLS) metathesaurus (http:// www.nlm.nih.gov/ research/ umls/ index.html). The Gene Ontology (GO) project

provides the most widely used classification system in molecular biology [213,214], aiming at providing consistent descriptions of gene products in different databases.

Table 6: Some important bioinformatic tools used in this thesis

BLAST	The Basic Local Alignment Search Tool (BLAST) compares nucleotide or protein sequences to sequences submitted to databases. The statistical significance of possible matches is calculated.
Entrez Genomes	Entrez is a retrieval system designed to search several linked databases. The Genome database provides integrated genetic and physical maps, with views for a variety of genomes, complete chromosomes, and sequences. Map Viewer is a tool developed specifically for eukaryotic genomes.
MIAMExpress	MIAMExpress is a web-based tool for submitting microarray data to the publicly available ArrayExpress database, complying with MIAME (Minimum Information About a Microarray Experiment) [155] requirements.
Match™/Transfac®	Match™ uses a library of mononucleotide weight matrices from TRANSFAC® database, and is designed for searching potential transcription factor binding sites in nucleotide sequences.
Ingenuity Pathway Analysis (IPA®)	Ingenuity® is a commercially available database of information regarding genes, drugs, biomarkers, chemicals, cellular and disease processes, signalling and metabolic pathways from the full text of the scientific literature. IPA® indicates pathways of genes that are over-represented in the list of differentially expressed genes from the users' own material.

7. Main results

Paper I

Decidual gene expression of the *STOX1* paralog storkhead box 2 (*STOX2*) was perturbed in preeclamptic women delivering FGR neonates. There was also a correlation between transcriptional alterations observed in preeclamptic decidua (relative to controls) and alterations previously reported by Rigourd *et al*. [201] in cultured trophoblast (JEG-3) cells overexpressing *STOX1A* (relative to mock-transfected JEG-3 cells). The strongest correlation to the previously reported dataset [201] was observed in preeclamptic pregnancies complicated by FGR.

We could not confirm the association of candidate SNPs in *STOX1* with preeclampsia. We found that women experiencing recurrent preeclampsia had a higher risk of complications and comorbidity (preterm birth, lower birth weight and development of metabolic syndrome) compared to those experiencing preeclampsia once. We observed a tendency towards higher frequency of the C genotypes for the previously reported *STOX1A*-Y153H variation in this group of women.

Paper II

Genomewide transcriptional profiling was performed on decidua basalis tissue from preeclamptic (n=37) and normal (n=58) pregnancies. Of the 26.504 transcripts detected, 455 were differentially expressed (P <0,05 , FDR P <0,1). Both novel and previously reported preeclampsia-associated genes were identified. Pathway analysis revealed that 'tryptophan metabolism', 'endoplasmic reticulum stress', 'linoleic acid metabolism', 'notch signaling', 'fatty acid metabolism', 'arachidonic acid metabolism' and 'NRF2-mediated oxidative stress response' were overrepresented pathways among differentially expressed genes.

Paper III

Ten sequence variants (nine SNPs and one single base insertion) were identified in the putative promoter region of *TNFSF13B* and seven SNPs were successfully genotyped in

the total Aust/NZ family cohort. Borderline association to preeclampsia (p=0,0153) was observed for three rare SNPs (rs16972194, rs16972197 and rs56124946) in strong LD with each other. Functional evaluation by electrophoretic mobility shift assays (EMSA) showed differential nuclear factor binding to the minor 'A' allele of the rs16972194 SNP, residing upstream of the translation start site. The observed genetic associations were not confirmed in our Norwegian case/control cohort.

Paper IV

We observed an increased OR of for carrying the wild-type allele (G/Val) of the *COMT* rs4680 SNP in women experiencing recurrent preeclampsia. Isolated, the A/Met variant at this position has been shown to confer low enzyme activity [215]. However, the G-allele of rs4680 is included in both a low activity and high activity haplotype [216]. An increased risk of carrying the low activity haplotype (OR 1,8 , p=0,018) was observed in the recurrent preeclampsia group of the HUNT2 preeclampsia cohort.

8. Some methodological considerations

"Six by nine. Forty two.

That's it. That's all there is.

I always thought something was fundamentally wrong with the universe"

Douglas Adams, 1980; "The Restaurant at the End of the Universe"

Understanding the etiology of preeclampsia has been called the "holy grail" in obstetrics. Appreciating the complexity of the pathogenesis, and the genetics involved, as outlined in previous chapters, it is apparent that a full understanding of the syndrome is still incomprehensible. In a spirit of discussion, the quest of unraveling the genetic output of complex diseases can be seen as somewhat analogous to the plot in the science fiction series "the Hitchhikers' guide to the galaxy" by Douglas Adams. In this series, a group of hyper-intelligent beings ask a supercomputer the Ultimate Question of Life the Universe and Everything. After 7.5 million years of computation, it turns out the answer is 42.

Study design

If your question does not make sense, neither will the answer. The point has probably been stressed in innumerable ways throughout the history of science, maybe most elegantly by Aristotle: "Prudens quæstio dimidium scientiæ" (To know what to ask is already to know half). Study design is therefore the single most important issue to address in (genetic) studies. An ideal approach would be to 1) set a stringent aim for your study 2) design the study in terms of the characteristics of the study population and the appropriate statistical methods to be used 3) ascertain samples and 4) perform experiments, evaluate the result by relatively simple statistical methods. More often, though, the situation is that we have some biological samples and we want to utilize them to answer an (ill defined?) question. Poorly designed studies however, are seldom recoverable, even by the most skilled statistician [133]. When dealing with the wealth of genetic and biological information provided by public databases and new technology, awareness of this is increasingly important. Which biological samples are available to us, will continue to restrict the ideal study outlined above. As a consequence it becomes

all the more important to put resources into generating good study questions, and including statistical expertise in the planning phase of studies. Bateson recognized the importance of restricting the aims of genetic studies already in 1900, addressing the Royal Horticultural Society, lecturing about the task at hand: *"Now this is pre-eminently a subject in which we must distinguish what we* can *do from what we* want *to do. We want to know the whole truth of the matter; we want to know the physical basis, the inward and essential nature, "the causes", as they are sometimes called, of heredity (...)"* [131].

Hypothesis testing

Determining whether variations between two sample distributions can be explained by chance is called hypothesis testing. A null-hypothesis is formed, usually describing the "neutral" state (e.g. "allele frequencies are not different in the case group vs. the control group"), as well as an alternative hypothesis (e.g. "allele frequencies are different in the case group vs. the control group").

Type 1 vs. Type 2 errors

The probability of rejecting the null-hypothesis when it in fact is true ("false positive") is called a type 1 error (α), and can also be called the significance, or the specificity of the test. The probability of accepting the null-hypothesis, when it in fact is not true ("false negative"), is called a type 2 error (β), and can also be called the sensitivity of the test (Neyman-Pearson theory) [169]. Type 2 error estimates cannot be made without knowing the actual distribution of the variables in the hypothesis (e.g. population genotype frequency and disease prevalence). The actual values of these variables are rarely known, but estimates from large population samples can usually be used for computations. It can be argued that, in complex diseases type 2 error (β) estimates cannot be made, as calculations would demand knowledge about the mode of disease inheritance [133,217].

Random errors vs. bias

Random errors are errors that are expected from the study design, that is to say, if a significance level of 1% is chosen, then one out of 100 conclusions are expected to be (falsely) positive by pure chance. Random errors are an accepted consequence of the usage of statistical tests in experimental biology. Systematical bias introduced in the data collection process or when performing experiments will hamper the interpretation of results in an unpredictable way, resulting in misleading conclusions. Investigators seek to avoid bias, although this is not always possible. Due to the unpredictable effect of bias on the study outcome, it is not easily controlled for, but should nonetheless be recognized as a factor possibly influencing the result.

Overfitting

Ideally, the number of measured cases in the study (n) should be considerably larger than that of measured features analyzed. When handling data from microarray platforms, the case is typically the opposite; a large number of measured features (e.g. transcripts or SNP genotypes) are tested in a much smaller number of samples. This is called "overfitting", and special care in handling the data is warranted, in order to avoid describing random errors and fluctuations in the data sets instead of biological relationships [218].

Multiple testing

With the advent of whole genome technologies, the simultaneous assessment of numerous markers or data points increases the likelihood of false positive findings. For example in microarray experiments, testing 48.000 transcripts for differential expression, applying an α-level of 0,05 yields 2.400 expected false positive findings. Different approaches are used for correcting for multiple testing [219-221]. All methods have in common that they are aiming at reducing the number of false positive results at the cost of accepting a larger proportion of false negative results [222]. Thus, the kind of biological variation that is observed is limited in that low level signals will be missed [147]. The justification for multiple testing has been questioned, since it can be seen as being based on the universal null hypothesis, stating that the neutral state is a lack of

association between any of the observed phenomena. This of course, contradicts all that we know about biology [222]. In line with this, a simple Bonferroni correction, dividing the α-level with the number of comparisons to get a new threshold of significance, is considered to be too conservative, as many of the outcomes (e.g. transcript levels) are expected to be connected. A less conservative approach, calculates a family wise false discovery rate (FDR) [219], by simulating the experiment ~10.000 times, using real data and random grouping of the samples. In summary, when performing hypothesis-generating or explorative studies, we need some means of rationally prioritizing candidate findings that we wish to look further into. Both p-values and FDR corrected p-values can be useful for this, but biological knowledge and information can be equally important.

Power

Power estimates should be made before a study is performed (*a priori*), in order to determine whether the method of choice is expected to be able to answer the study question. In other words, the statistical power of a study is the likelihood that a type 2 error (β) will not occur. Thus, power can be expressed as 1-β. Mathematically, the power (ψ) of a study can be determined as a function of the significance level (α), sample size (n) and effect size (ε). As it can be argued whether β and/or ε estimates are possible to make reliably in common complex diseases [133], the rationale for performing power-calculations is questionable. Furthermore, both study design, experimental design and analysis method influence the power of a genetic association study [133]. It is generally accepted that a study that does not provide a test-statistic significant at the 0,05 level, and has a power of less than 80% probability of detecting a true difference, must be considered inconclusive [169].

Power-calculations *ad modum* Lalouel and Rohrwasser [169] were performed in the HUNT2 preeclampsia cohort for a given single nucleotide polymorphism, including information about the known frequency of the minor allele for the given SNP (using information from publically available databases). Possible deviations from this frequency in the case population were set in a table. Effect sizes with according power (1-β) were calculated *a priori by* equation 1 [169].

Equation 1:

$$Z_{1-\beta} = \left(h\sqrt{\frac{n}{2}}\right) - Z_{1-\alpha}$$

Where $Z_{1-\beta}$ is the Z-score of 1-β (statistical power; ψ), h is the effect size index (OR = $p_R q_C / p_C q_R$) for a difference between case allele frequency (p_C)and reference allele frequency (p_R), the geometric mean *n*, is calculated from sample sizes n_C and n_R and $Z_{1-\alpha}$ is the Z-score for the significance (α) level. Figure 3 is an example of such a power-calculation, using a population minor allele frequency of 0,35 (relevant for the STOX1A-Y153H polymorphism).

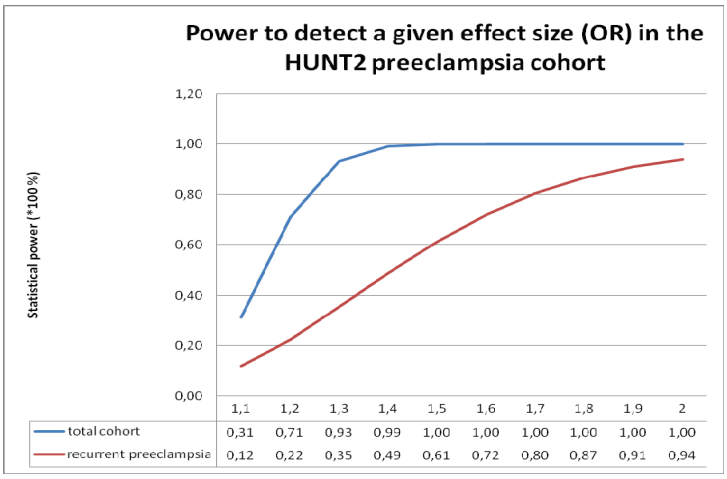


Figure 3: Powercalculations

Notably, the OR (hypothetical effect size) is calculated from (hypothetical) deviations from the control allele frequencies in cases. Thus, these calculations report the power to detect an *effect on the allele frequency* of the SNP *from the disease status*. What we want to deduce from this is the effect *from allele frequency on disease status*, which is assumed to be the same. Computations made in sequential oligogenic linkage analysis routines (SOLAR) software [223], regarding the general power of the total cohort, given a

genome-wide investigation, reports an 80% power to identify a SNP accounting for 2% of the total variation in the dichotomous preeclampsia phenotype [203].

Hypothesis testing vs. hypothesis generating

We always have to make assumptions about biology in order to narrow our question, and limit the number of hypothesis we are testing. Even "genome wide" studies performed with commercial SNP chips are based on a number of assumptions. In genetic studies, there is a rough divide between so-called "hypothesis testing" and "hypothesis generating" projects, although all projects are in reality testing a number of hypotheses and usually always generate new ones. The papers presented in this thesis are attempts at balancing the line between explorative and confirming approaches. The *STOX* genes, as well as the *TNFSF13B* gene are positional candidate genes. The positional candidate gene approach utilizes genomic regions identified by "whole genome" linkage studies in affected families, and combines this information with prior knowledge about biology, to form the study question. Also, the microarray transcriptomic data yields a list of differentially expressed genes. To translate this list into knowledge, we utilized available information about biological pathways (paper II). We also tested hypotheses regarding expression of given candidate gene transcripts (paper I).

Phenotype

Preeclampsia is a heterogeneous condition where the diagnosis is based on some of the main features of the disease. It has been argued that analyzing women in groups that are assumed to be more etiologically homogeneous will improve the identification of reliable prognostic clinical and biochemical markers [50]. Is this principle also applicable to genetic risk factors for preeclampsia? The ultimate (100% penetrant) complex phenotype, death, has been used as a model for testing different approaches to breaking down genetic susceptibility to complex phenotypes (Figure 4) [133], illustrating that new groups must be substantially less complex when we aim at gaining power by subgrouping [133]. One common strategy is to "enrich the tails", that is, to look at subjects in the extreme ends of a continuous distribution for a particular trait. The recurrent

preeclampsia group in our analyses may represent an extreme tail of the preeclampsia phenotype.

The concept of "endophenotypes" as "internal phenotypes discovered by a biochemical test or microscopic examination" was introduced by John and Lewis [224] and Gottersman and Shields [225]. Assessment of such endophenotypes has led to progress in the field of cardiovascular disease genetics [133,226]. Attempts have been made at setting requirements for endophenotypes, incorporating their heritability [132]. Generally, continuous phenotypes are regarded as the most informative. Any discretization of such continuous phenotypes (e.g. hypertension, low birth weight, diabetes II, proteinuria and obesity; all diagnoses that are based on cut off values of continuous phenomena) greatly reduces the power of a study. As the nature of the underlying gene action is also thought to be continuous, utilizing all the information in the data will increase chances of success [227]. Thus, although a stringent preeclampsia diagnosis is useful for research purposes; forcing threshold values on continuous distributions of hypertension and proteinuria may not only mask the phenotypic diversity seen in clinical practice [164], but also possibly the underlying mechanisms of genetic susceptibility.

Validity of the diagnosis

Both a poor specificity and sensitivity of the preeclampsia diagnosis will negatively influence the power to detect a true association. In the Norwegian HUNT2 cohort, preeclampsia was defined in accordance with the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy criteria [4], using diagnoses reported to MBRN (ICD-8 before 1998, ICD-10 after 1998). The preeclampsia diagnosis is based on cut-off values of hypertension and proteinuria, but is also a clinical diagnosis based on symptoms (e.g. epigastric pain, persistent headache or other cerebral or visual disturbances) [4]. Diagnostic criteria have changed over time, thus diagnoses reported by obstetricians and midwives to MBRN might be based on individual interpretations of the total clinical presentation rather than criteria set by MBRN. In 1995 the Norwegian Society of Gynecology and Obstetrics defined preeclampsia in a similar manner to the diagnostic criteria used in the Aust/NZ family cohort: blood pressure (BP)≥ 140/90 mmHg or an increase of diastolic blood pressure

(DBP) ≥15mmHg in pregnancy combined with proteinuria ≥0,3 g/L in a 24 hour urine sample (≥1+ on a dipstick reading) [228], while the criteria used today are more restrictive; BP≥140/90 mmHg combined with proteinuria ≥0,3 g/L in a 24 hour urine sample (≥1+ on a dipstick reading) on at least two occasions 4-6 hours apart [229]. The registration forms used in MBRN from 1967 until 1998 included the ICD-8 diagnoses preeclampsia, eclampsia and toxemia. In addition to this, the MBRN included extensions of the ICD-8 codes (e.g. hypertension developing during pregnancy, hypertension and edema, proteinuria without kidney disease, hypertension and proteinuria, threatening eclampsia). Combinations of hypertension and proteinuria developing in pregnancy reported by these codes have also been used to define preeclampsia in the HUNT2 cohort. After 1998, ICD-10 codes for preeclampsia have been used, adding information about severity and time of onset [230].

The prevalence and recurrence rates of preeclampsia in the MBRN are comparable to those reported in other Nordic countries [231]. We have initiated an independent validation of the preeclampsia diagnoses reported by the MBRN. The obstetric departments where the women of the HUNT2 preeclampsia cohort gave birth have compared the reported diagnoses to information in medical journals. Preliminary results show that the diagnosis was confirmed in 86% of the women, evaluated with MBRN criteria [232]. Lack of documented proteinuria was the most common cause of exclusion. A more thorough evaluation of these findings is needed in order to discuss the implications. For genotyping of the three *TNFSF13B* SNPs in the Norwegian cohort (paper III), only samples from women with a validated diagnosis were used. A clinical evaluation of the included samples showed that the main difference from the rest of the case-cohort was a shorter follow up time (newer diagnoses). This might imply that the older reports were less reliable, or that older (paper based) journals have been more difficult to acquire and validate. Also, severe signs of preeclampsia other than the strict preeclampsia criteria have not yet been evaluated. A similar evaluation of clinically determined preeclampsia diagnoses in a predominantly Hispanic preeclampsia cohort, showed that among patients without documented proteinuria, 75% had abnormal laboratory values suggestive of severe disease (elevated liver enzymes, uric acid, lactose dehydrogenase or decreased platelets), symptoms of preeclampsia (headache, epigastric pain, right

upper quadrant pain, visual disturbances), and/or a history of pregnancy induced hypertension (PIH) in a previous pregnancy (having been normotensive between pregnancies) [233].

In summary, presuming a continuum from normal pregnancy, via pregnancy complications to miscarriage, retrospectively ascertained samples based on registry data probably captures a broader proportion of this continuum than samples from women recruited at inclusion to the study in obstetrical departments. More homogeneous groups in the end tail of the distribution (early onset preeclampsia, recurrent preeclampsia) might add power to genetic association studies.

Sampling

When choosing which samples to collect, the study design is also largely decided. It is not always easy to determine which design is best suited to answer the research question, and there are always limitations to which samples it is feasible to collect. Thus, there is no straight forward answer to which is the "best" sampling strategy. Nonetheless it is important to be aware of the possibilities and limitations each design presents with.

Random vs. targeted

When ascertaining only individuals who are affected with a rare trait, or whose phenotype is in the extreme tail of a continuous distribution, a strong analysis of qualitative signal might be feasible. However, quantitative analysis would be difficult, if not impossible due to limited possibilities in adjusting for ascertainment bias [133]. Samples ascertained randomly without regard to the phenotype under study, would probably not include enough individuals presenting with a rare disease or extreme tail trait to allow for qualitative analysis, but would give the opportunity to study the (normal) quantitative variation that is more closely related to the underlying genetic variation. This approach would also provide the opportunity to assess pleiotropy, or different phenotypic effects of the same genetic variation [133]. Using the HUNT population and MBRN, we identified approximately 1.000 preeclamptic women and 2.000 non-preeclamptic women, making it possible to investigate genetic variants

associated to this trait. An alternative study design, including 3.000 random women from the HUNT population would be too small to study normal variation and probably only identify approximately 100 -50 preeclamptic women, too few to study the qualitative phenotype. However, six different projects are undertaken currently on HUNT material concerning the genetics of Weight/ Fitness/ Diabetes/ Cardiovascular disease/ Metabolic syndrome. Most biobanks now request that genotypes are reported back to them after projects are finished. In future, this might give us the opportunity to select larger random sample sets for the study of continuous variation underlying disease.

Family vs. Population cohort

Ascertaining families or non-familial population samples when studying common complex disorders is largely a question of practical feasibility. In family studies, linkage [234], describing the segregation of informative meiosis in the pedigree with a disease trait, is utilized. Association studies in population samples use LD [1], which is essentially the degree of relatedness in the last generation(s) of the largest theoretical pedigree possible. Thus, LD, or allele association predicts something about the historical, unobserved meiotic events in the pedigree. Arguably, the most powerful study, would utilize both these approaches simultaneously [133,227]. Generally, the more information we have about relatedness in the sample, and the larger the pedigree is, the greater power the study has. Thus, it is better to ascertain one large pedigree, than several smaller ones (e.g. sibling pairs or mother-father-child triplets) [133]. However, population samples are easier to ascertain in large numbers, thus increasing power. Also, for the study of normal genetic variation, random selections from the total population will give a more complete picture than family samples, when accepting that sample sizes need to be considerable [227]. Furthermore, some population studies benefit from not being based on volunteer recruitment, a possible bias in family studies [235].

Replication

Reproducibility or replication of biological findings is of essential importance in determining whether a given result is true, or a result of random error or bias. True biological replication would require that the phenotype is defined in the same manner as

in the original research, and that the study design is comparable. When designing a replication study, it should be noted that to contradict a study rejecting a null-hypothesis (H_0) with an error probability (α), a probability of rejecting the hypothesis H_1 of true difference in subsequent studies should be at least α [169]. That is to say, the power of a replication study should be at least one minus the lowest α-level at which the original finding is statistically significant. Very few replication studies fulfill such criteria. More commonly, replication of a finding by an independent research team is performed by looking at the study question from a different angle, possibly elaborating the result, but neither confirming nor contradicting it. This would also be true for our observations in paper I and IV.

Technical replication, using the same biological samples and the same method, can be used to control for the possibility that the results presented are technical artifacts (bias). This is different from replicating a finding using the same biological samples, on a different technological platform, as platforms probably vary in their ability to answer the research question. To illustrate this; when performing quantitative real-time polymerase chain reaction (qRT-PCR) to confirm some of the most extreme findings in the microarray gene expression study (paper II), we were testing for reproducibility across technical platforms. Three technical replicates were also made for each recorded data value in the qRT-PCR experiments, providing a standard error of the mean of the reported values.

Genotyping error

We have used genotyping on different technological platforms (SNPlex, TaqMan and Golden Gate Illumina) as one approach to control for genotyping error. Also, statistical analysis programs (e.g. SimWalk2 [236]) utilize information in the obtained data, like the concordance with Hardy-Weinberg equilibrium and the expected genotypes in a known pedigree structure, to predict genotyping errors. We observe a 99-100% concordance between the reported genotypes on different technological platforms, similar to what others have reported [170], making the genotype estimate a robust one. However, platforms providing low genotyping success-rates may generate false positive association results even though they conform to Hardy-Weinberg proportions. We saw

this in our preliminary genotyping of the 13q locus, where one of the SNPs genotyped showed a significant association to preeclampsia when the Aust/NZ and Norwegian cohorts were analyzed together. However, the SNP had a low genotyping success rate on the SNPlex platform (Norwegian cohort). Although the genotypes conformed to Hardy Weinberg proportions, discrepancies in the LD patterns made us cautious of the result. The SNP was genotyped again with the SNPs of the study presented in paper III, using TaqMan technology. The second genotyping confirmed the reported genotypes, but we found deviation from the observed allele frequencies in the (previously) non-genotyped part of the data set. This might reflect a different sensitivity for detecting a given genotype, and illustrates the need for high success rates when reporting association results with confidence. The additional SNP tested was in LD with the three SNPs reported to be associated to preeclampsia in paper III, and we concluded that the association signal is limited to the Aust/NZ family samples.

Expectations about effect sizes

Francis Collins of the US National Human Genome Research Institute held a speech in 1999 describing a hypothetical consultation in 2010 [144,237]. The patient was a 23 year old man with high cholesterol. Genetic screening was undertaken to assess susceptibility to cardiovascular disease as well as other future illness. By assessment of the apolipoprotein B (*APOB*) and cholesterol ester transfer (*CETB*) genes, the hypothetical patient was ascribed an increased relative risk of 2,5 for cardiovascular disease. Similarly, the assessment of lung cancer risk based on the information that he was a smoker and the N-acetyltransferase 2 (*NAT2*) genotype gave an increased relative risk (RR) of 6,0. These examples were taken from research done at that time [144]. Collin`s vision of a "genetically based, individualized preventive medicine" was based on the prevailing CDCV hypothesis, assuming that variants with minor allele frequency of 10% or more would explain the major genetic factors involved in human disease [133,238]. Researchers expected these factors to be defined within 5-10 years of the completion of the human genome sequence [239]. Within short time, however, this notion was quashed by evidence from larger studies, showing more moderate effect sizes (OR 0,96 for *NAT2*, 0,94 and 1,15/0,95 for *CETB* and *APOB*) [144]. Although it became widely recognized that the effect sizes seen in small studies were expected to be much lower in

follow up studies [240,241], there was still a belief that most complex diseases would also hold at least one genetic risk factor of greater effect size [227]. In 2005 researchers were encouraged by the identification of a common susceptibility variant for age-related macular degeneration (AMD) by genome wide association screening [242]. This variant showed an OR for disease between 2,4 and 7,4 in different studies and large attributable disease risks [243,244]. The AMD finding, however, has turned out to be the exception from the rule. Results from studies of common complex diseases like hypertension and type 2 diabetes are making researchers speculate that instead of 10, there are probably 10.000 alleles of small effects influencing common complex diseases [238]. Effect sizes observed are typically in the range of OR 1,05-1,50. Our observations are in accordance with this.

Genes may harbor common variation with modest effect on complex traits (e.g. lipid levels) as well as rare variants with large effects on "mendelian" disorders (e.g. dyslipidemia) [156]. It is therefore still a matter of discussion whether the dissection of "mendelian" high penetrance disorders, or the low penetrant genetic susceptibility genes of common complex disorders will teach us more of normal biological variation and improve public health (or the ultimate phenotypes of quality and quantity of life) [133,238,245] (Figure 4).

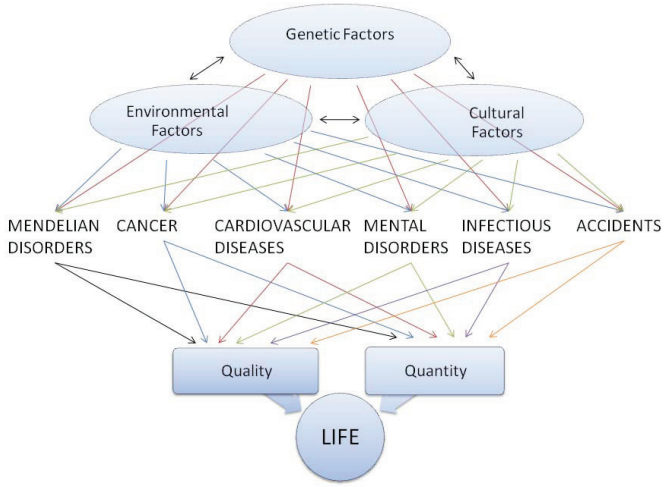


Figure 4: Life (adjusted from Terwillinger and Göring 2000 [133])

Transcriptomics

It is becoming apparent that there are many more pitfalls to using microarray technology for gene expression than using high throughput genotyping technology, as the genotype is relatively stable compared to the transcription of the genetic code. Slight differences in sampling technique, isolation of RNA, storage, treatment and kinetics of hybridization can be detrimental for the outcome of the study [150,246,247]. Therefore, care must be taken in ensuring consistency throughout sample collection and processing, and a number of quality assessment steps (e.g. principal components analysis and outlier detection) are warranted before interpreting the results. When stringent quality control criteria are applied, a high inter- and intra platform reproducibility is observed [246]. Generally, microarray platforms are more accurate and consistent when reporting ratios, or differences in expression between groups, than absolute measurements [248].

Cell lines vs. tissue samples

Using cell lines for gene expression studies enables the researcher to control and reduce the number of possible factors influencing gene expression (possible bias), thus increasing the power of the study. Cell cultures consist of only one cell type and are grown in a controlled environment. On the other hand, the environment the cells are kept in is artificial, and they have been manipulated to be able to grow outside their normal context, possibly making them less relevant for the question under study. Natural tissues, however, consist of a number of cell types, probably varying in expression patterns [154]. Also, a number of environmental factors may alter gene expression *in vivo* [249-251], obscuring possible differences in gene expression due to the trait under study. Notwithstanding this, results from cell-culture studies and natural tissue have been shown to correlate well [153]. Our decidual material consists of approximately 40% maternal leucocytes, 20% extravillous trophoblast and 30% decidual stromal cells [252]. Our results (paper I) are interpreted in relation to, and are consistent with, earlier observations in trophoblast cell lines, as well as placental tissue [201,253].

Gestational age – a probable bias

Decidual tissue is of interest in studying pregnancy-related complications since it represents the materno-fetal interface where important disease processes are thought to take place. However, representative samples can only be collected at delivery. Preeclamptic and FGR pregnancies are often terminated prematurely; normal controls cannot be obtained. Since gestational age is expected to influence gene expression [249], this is a probable bias in our material.

Some researchers have chosen to use premature deliveries with no signs of infection as controls [185]. Infection is a common cause of premature delivery [254], and is expected to affect gene expression [255,256]. A possible bias in these studies, is unrecognized infection or other obscure causes of prematurity [255]. A novel prospective study approach has also been introduced, using spare material from chorionic villous sampling voluntarily performed at gestational week 10-12 for cytogenetic diagnosis [257,258]. This is an interesting supplement to the literature, providing information from an early stage of disease development. However, the material is not representative for the entire decidua basalis and the women in the study groups are generally older than the average pregnant woman, as advanced maternal age is the primary indication for the procedure. Furthermore, relatively few preeclamptic cases are identified (four out of 160 consenting participants recruited over a five-year period). Other researchers have, as us, chosen to use normal pregnancies with delivery at term as controls [259]. Winn *et al*. performed a microarray study comparing gene expression in placental bed biopsies from healthy pregnant women over a range of gestational ages [249]. Comparing our set of differentially expressed transcripts to this data set (paper II), we identified some of the genes in our dataset that are highly likely to be differentially expressed due to gestational age related changes (four and a half LIM domains 1 (FHL1), SH3 and multiple ankyrin repeat domains 3 (SHANK3), notch 4 (NOTCH4), roundabout homolog 4 (ROBO4), notch-regulated ankyrin repeat protein (NRARP), G protein-coupled receptor 116 (GPR116), transmembrane protein 97 (TMEM97), kiaa1598 (KIAA1598), phospholipase A2, group VII (PLA2G7), ubiquitin associated and SH3 domain containing B (UBASH3B), interleukin 6 signal transducer (IL6ST), low density lipoprotein receptor (LDLR), signal recognition particle receptor, B subunit (SRPRB)

and kringle containing transmembrane protein 1 (KREMEN1)) (paper II). However, due to the limited sample size and spectrum of samples included in Winn`s dataset, and methodological differences between the two studies, Winn`s data set has only a limited value as a reference, and the influence of gestational age in other genes identified as differentially expressed in our material cannot be excluded.

Identification and standardization of expression values

Expression patterns have been shown to be consistent across different microarray technologies. However, differences in signal detection algorithms and data analysis influence the power to detect a transcript as differentially expressed [246]. We included transcripts that passed a tail test determining if there was a sufficient quantitative signal over that expected by chance in our analyses. In addition to detecting highly expressed transcripts, our approach allows for the detection of transcripts that are clearly present above baseline levels in most, if not all individuals [153]. We further used information from all recorded transcripts to standardize abundance values within individuals, thus minimizing the influence of overall signal levels (which might reflect RNA quality or quantity instead of biological differences). These normalized phenotypes are comparable between individuals and across transcripts [153].

Microarray vs. qRT-PCR

Microarrays have been shown to correlate well with qRT-PCR [150,185,257,260], and this is confirmed in our study (paper II). In contrast to intervention studies (or studies comparing different tumors or other tissues) the absolute and fold change values expected when looking at complex disease susceptibility genes are much lower [261]. Measuring these effects by a fold change cut-off value (for example set to two) has obvious limitations [150]. A high degree of variance and large fold change values in an abundant protein could be less important than a minimal fold change in a less variable regulatory protein. Therefore, reporting data based on deviation between means in case and control groups (as with the β-values generated by SOLAR) makes sense biologically. Furthermore, microarray platforms benefit from the possibility to integrate more information into the statistical analyses and normalization procedures when handling data [153]. This advantage could be especially important when looking at

complex disease susceptibility genes. As discussed by the microarray quality control consortium (MAQC), the standards that are published and the expected overlap between technological platforms are based on tissues with high detectable differences, not expected in intervention trials [246], and certainly not in case control studies concerning common complex disorders. When looking at tissue samples with a maximum fold change of three, both the power to detect a gene as differentially expressed and the overlap between platforms decrease [246]. For complex disease susceptibility genes that fulfill stringent criteria for disease association [262], a majority of studies reported fold change values below two [261]. These genes are probably the genes presenting with the highest effect sizes, the "low hanging fruits" of complex disease genetics [133,238]. In this situation, taking advantage of a full microarray data set, normalizing values based on the general sample transcription level for all transcripts before looking at a subset of genes or one candidate gene may give a more robust measure of gene expression than qRT-PCR, where internal standardization is based on housekeeping genes that may in themselves display both a high degree of variability and heritability [153,263].

Consequence or cause?

Preeclamptic women are in a state of increased inflammation, and a number of transcripts are expected to be differentially expressed in their placenta, some of them to a high degree. The detection of these transcripts will confirm which biological processes are perturbed in the condition, but will not necessarily reveal information about causative genes. The causative genes will probably be the ones that have regulatory functions [133], e.g. transcription factors, with low copy numbers, many of them not detected with the current technology [150,246,260], and where minimal changes can have potentially important biological effects. Notwithstanding this, it has been shown that hypothesizing detectable differences in gene expression between normal and pathological tissues for complex disorder susceptibility genes is justified [261]. Gene expression profiling can also be a powerful tool for identifying possible causative variants [153]. The high interconnectivity of focus genes with other genes within the biological networks described in paper II might imply a functional/biological importance of these genes. However, we prefer to focus on canonical pathways and

networks rather than single genes when looking to the total dataset for an increased understanding of the pathophysiology of preeclampsia.

9. Summary and discussion of papers

The complex pathophysiology of preeclampsia is undisputable. Figure 5 places the most important findings of papers I-IV in relation to the three stage model of preeclampsia.

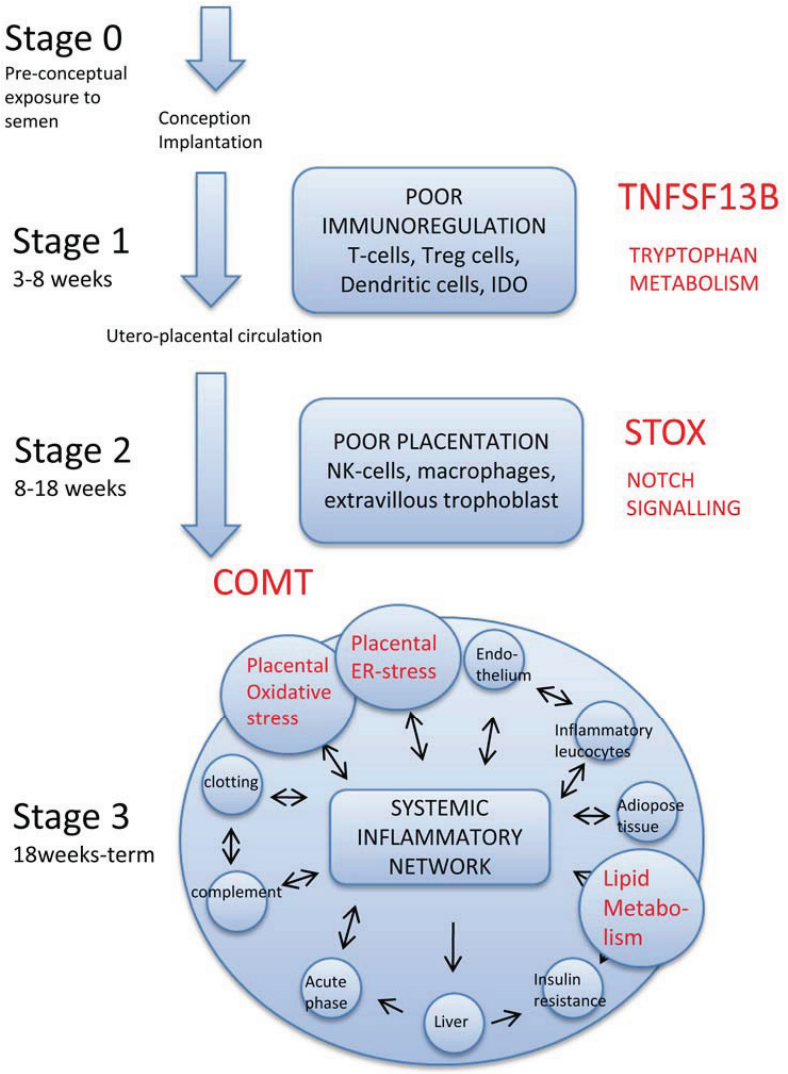


Figure 5: Findings in papers I-IV in relation to the three-stage model for preeclampsia.

Both the implication of the tryptophan metabolism pathway (paper II) and our *TNFSF13B* finding (paper III) may support the emerging biological evidence of an immune component to preeclampsia pathopysiology, and confirm the notion of preeclampsia as an intermediate phenotype between miscarriage and normal pregnancy, recently highlighted by Redman and Sargent [81].

Catabolites of tryptophan are believed to promote immunotolerance to foreign antigens by inhibiting proliferation of T- and NK cells [264]. Decreased tryptophan degradation by indoleamine 2,3 dioxygenase (IDO) increases T cell mediated rejection of allogenic fetuses in pregnant mice [193]. In mice treated with IDO inhibitor, the decidua of allogenic concepti turn out to be morphologically abnormal and show extensive hemorrhaging and mixed inflammatory cellular infiltrates (Mellor *et al*. 2001). Recently, tryptophan catabolism by IDO has been shown to alter the Th17/Treg balance in HIV infected patients [265]. This balance has also been proposed to be important for preeclampsia pathogenesis [86]. Furthermore, a rare coding variant in the IDO gene was recently identified in preeclamptic women [266]. Kudo *et al*. showed reduced mRNA expression of IDO in villous tissue from preeclamptic pregnancies, higher plasma concentration of tryptophan, but lower plasma levels of kynurenine, a tryptophan metabolite [267]. IDO expression was decreased in preeclamptic decidua in our study, but IDO was not significantly differentially expressed after FDR correction. The transcript encoding the enzyme kynureninase (KYNU) was upregulated. KYNU metabolizes L-kynurenine, which suppresses T cell proliferation and natural killer cells and influences immunotolerance to foreign antigens.

TNFSF13B is an important stimulator of immunoglobulin production [268,269] and is also a part of the innate immune system [270]. To the best of our knowledge, paper III is the first report of genetic variation in this gene related to adverse pregnancy outcome. Interference with the homeostatic regulation of *TNFSF13B* might disturb the finely tuned cytokine balance of pregnancy. However, verification of our finding in different preeclampsia cohorts and further functional evaluation is necessary in order to determine the biological importance of our finding.

Taken together, results presented in paper I and II might lend support to the implication of biological pathways involved in angiogenesis and preeclampsia pathogenesis that are shared between placenta and brain tissue.

Microarray-based transcriptional profiling has been applied both to placental and decidual tissues from preeclamptic pregnancies, using diverging study designs and small sample sizes [149]. A comprehensive placental global gene expression profile (33.000 transcripts; 21 cases and 21 controls) has been published [183], and we present a comparable profile from deciduas basalis (paper II, 48.000 transcripts, 37 cases and 58 controls). Interestingly, notch signaling was identified as a significant pathway in both these studies. Notch signaling is important for embryonic and placental vascular development [271-274]. Furthermore, Sitras *et al.* noted that the most common type of inherited stroke and vascular dementia in humans (CADASIL [275]) can be caused by mutations in the notch pathway, and that several identified notch-genes overlap and interact with genes involved in Alzheimer's disease pathways [183]. These observations support the hypothesis of shared disease mechanisms between preeclampsia and degenerative brain disorders recently put forward by van Dijk *et al.*, when showing a conserved pathway shared in placenta and brain, controlled by *STOX1* and up regulated in Alzheimer's disease [276].

STOX1 is hypothesized to mediate a balance of proliferation vs. invasion in the trophoblast cell column by activation/deactivation of the STOX1 nuclear localization signal [200]. The invasive trophoblast is an active modulator of maternal spiral arteries. The actions of *STOX1* are expected to be effective through interaction with other genes/proteins [277]. Recently, van Dijk *et al*. published data showing that STOX1 binds and transactivates the promoter of *CTNNA3* located close to *STOX1* on chromosome 10q22 [278]. Furthermore, overexpression of STOX1 in cultured choriocarcinoma cells (JEG-3 cells; a commonly used trophoblast cell model) gives a transcription profile similar to what is seen when comparing preeclamptic and normotensive placenta [201]. This supports the notion that the possible disease causing effects of *STOX1* expression is trophoblast derived, and that the regulation of this transcription factor is important for the homeostasis that ensures a successful interaction between fetal and maternal cells.

We observed differential expression of *STOX2* in preeclamptic pregnancies complicated by FGR. Little is known of the biological importance of *STOX2*, and its plausibility as a candidate gene for preeclampsia rests largely on research done on *STOX1*, the fact that it is its paralog (expected to be involved in some of the same biological processes) [279,280], and that it resides close to or under a replicated preeclampsia susceptibility locus [172,172,175,175,176,176,280] . Thus, future genetic and molecular work will be needed to evaluate the biological importance of our finding. However, available information in databases is increasing rapidly, and might also shed some light on the biological pathways in which the *STOX* genes are involved. The NCBI EST database (a collection of short single-read transcript sequences from GenBank) provides a new resource for evaluating gene expression, comparing expression patterns in different tissues of various transcripts. The transcript that had the most similar profile to *STOX2* was the delta/notch-like epidermal growth factor-related receptor (*DNER*) transcript. *DNER* is an epigenetically modulated gene encoding a noncanonical Notch ligand [281]. At present, the available database has too few measure points for us to draw any sound conclusions regarding *STOX2* and its interaction with other genes, but further elucidation of the interaction of genes in these pathways might in future make a more complete picture of findings presented here.

We observed a higher frequency of a low activity *COMT* haplotype in women experiencing recurrent preeclampsia (paper IV). The COMT enzyme converts estradiol to 2-ME, which inhibits HIF1α. It has been hypothesized that a premature increase in 2-ME disturbs hypoxia-driven trophoblast invasion and decidual vascular development and contributes to preeclampsia pathogenesis [282]. Thus, COMT is an upstream event of HIF1α and genetic variation in *COMT* influencing activity and/or protein levels might therefore be one causal factor in preeclampsia [110]. Also, decreased COMT-activity/decreased inhibition of HIF1α late in pregnancy could potentially cause vascular pathology and inflammatory activation [283]. The COMT enzyme is important for homocysteine metabolism, a known cardiovascular risk factor that has also been implicated in preeclampsia pathogenesis [284]. Furthermore, the COMT metabolite 2-ME acts like a pro-oxidant and has direct involvement in redox-regulated signaling [283], a possible shared disease mechanism between preeclampsia and cardiovascular diseases.

Increased generation of ROS has been shown in preeclamptic placenta [285], potentially increasing lipid peroxidation and consequently leukocyte activation, platelet adhesion and vasoconstriction [113]. Three of the pathways identified in paper II represented metabolism of lipids: linoleic acid metabolism, fatty acid metabolism, and arachidonic acid metabolism. Genes included in these networks are important in the generation of ROS (acyl-coenzyme A oxidase 1 (*ACOX1*)) and elimination of lipid peroxidation products (alcohol dehydrogenase 1a (*ADH1A*), aldehyde dehydrogenase 3 family member A2 (*ALDH3A2*)). A related canonical pathway significant in our transcription material, NRF2-mediated oxidative stress response, plays an essential role in the defense of oxidative stress by regulating the expression of antioxidant response elements. Furthermore, ER stress was the second most significant pathway in paper II. ER stress is a major source of ROS [113] and has previously been suggested as one of the main sources for the generation of placental oxidative stress and release of proinflammatory cytokines to the maternal circulation in preeclampsia and FGR [112]. Three genes (X-box binding protein 1 (*XBP1*), activating transcription factor 6 (*ATF6*) and PKR-like endoplasmic reticulum kinase (*PERK*)) representing the main signaling pathways of the unfolded protein response [112], a coordinated adaptive response to ER stress, were up-regulated in our material (paper II).

Previous findings from our Aust/NZ and Norwegian cohorts show that variation in the selenoprotein S (*SEPS1*) and endoplasmic reticulum aminopeptidase 2 (*ERAP2*) genes involved in ER stress are associated to preeclampsia [203,286]. Furthermore, *ACVR2A* has been identified as a common susceptibility gene. Activin A is an important regulator of reproductive function, endothelial functioning and vascular homeostasis, and has recently been shown to be involved in the generation of ROS [287]. The oxidative stress and inflammatory pathways (also involving *COMT*, *SEPS1*, *ACVR2A* and the *ERAP* genes) are dynamic, which means that cause and effect are not easily separated when describing gene expression or protein activity [114]. We propose that the observed association between genetic variation in these genes and preeclampsia reflects a constitution of increased vulnerability to hypoxia/ reoxygenation events, inflammation and ER stress.

Whole genome linkage data is reported from a handful of large preeclampsia family collections [171-176], the Aust/NZ families being one of these. Three chromosomal regions of interest (2q22, 5q and 13q) are reported from the Aust/NZ families, and *ACVR2A* and *ERAP2* have been identified as preeclampsia susceptibility genes under the chromosome 2 and 5 linkage peaks, respectively. The 13q preeclampsia susceptibility locus comprises of some 20-30 candidate genes. Preliminary SNP genotyping was done before the release of HapMap under this locus in the Norwegian and Aust/NZ cohorts, using SNPlex technology in the Norwegian cohort [288]. However, low genotyping success rates necessitated replication on a different technological platform. Furthermore, the SNPs selected for genotyping were somewhat arbitrary in light of the rapidly increasing available information in the field. When prioritizing the *TNFSF13B* gene for a more targeted genotyping and molecular analysis, results of preliminary SNP genotyping, literature searches, and the software GeneSniffer were employed. An explorative approach, identifying SNPs in the possible regulatory and coding regions of the gene was undertaken and the identified SNPs were genotyped in the total Aust/NZ cohort. We report borderline association to three rare SNPs in putative regulatory regions of *TNFSF13B* (paper III). We demonstrate differential nuclear factor binding to one of the SNPs, making this a possible functional variant. We did not replicate the finding in the Aust/NZ families by genotyping of the three associated SNPs in the HUNT2 cohort. The lack of replication in the HUNT2 cohort might imply a founder effect in the Aust/NZ cohort, and could support the CDRV hypothesis for preeclampsia. However, differences in study design might also influence our result. Women with a familial disposition generally display more severe manifestations of the disease [289], and the subjects of the Aust/NZ study were recruited at inclusion by obstetrician, possibly making the diagnosis more stringent. The available information about relatedness in the Aust/NZ pedigree sample set might also allow a more powerful statistical analysis. Considering both the measured genotype association test and the quantitative transmission disequilibrium test (QTDT) [290], the measured genotype test is asymptotically more powerful than the QTDT [291]. However, in the presence of certain types of latent stratification, the QTDT can be more powerful. Residual linkage, reflecting additional functional variants near the associated marker, can also lead to a

more powerful QTDT. Therefore, a more comprehensive investigation of the 13q locus is warranted, in order to identify other possible functional variants in this region.

The "one gene - one protein - one disease" hypothesis has been abandoned, but is still noticeable in the way we are modeling disease development. In 2007, Oudejans and van Dijk made a clear distinction between "placental" and "maternal" preeclampsia, ascribing the features of familial disposition, early onset and fetal growth restriction to the placental form of the disease, and maternal cardiovascular risk factors with late onset disease [277]. They proposed that this distinction would help identify genetic variants contributing to the diagnosis. They further hypothesized that the *STOX1* genetic association was specific for the "placental" form of preeclampsia [200]. However, the emerging evidence of the complexity of the genetic output of biological variation is changing how we describe disease development. In line with the current understanding of biology, the different clinical consequences of placental pathology are seen as manifestation of the same variation. Early onset preeclampsia is associated with a greater increase in cardiovascular disease risk than late onset [292]. Thus, maternal risk factors are hypothesized to be involved also in the placental (Stage 1) disease mechanisms [128]. Taken together with biological evidence of similar placental changes in a range of pregnancy disorders, as well as in normal pregnancies, clinical/epidemiological data in our HUNT preeclampsia cohort and transcriptional differences in the deciduas basalis material are consistent with preeclampsia as a continuous distribution of both placental and maternal risk factors mutually strengthening. This view is held by leading researchers in the field [81,111,128], and correlates well with observations made by others [10,24,49-51,293].

In the group of women experiencing recurrent preeclampsia, we observe a higher frequency of both "placental" and "maternal" risk factors. This group might therefore represent a "high penetrance" or "extreme tail" subgroup of preeclamptic women. The findings in paper IV (and possibly I) might imply that the recurrent preeclampsia group is more powerful than the total cohort for observing association to some of the genetic variation underlying preeclampsia liability. Alternatively, the low activity *COMT* haplotype, and possibly the functional *STOX1*A-Y153H variant (we only report a tendency for this variant) may be enriched in these women. However, in line with the

current understanding of preeclampsia liability it is less likely that these variants are specific for a certain group of preeclamptic women. Evidence is accumulating that the majority of genetic variation underlying common complex disorders are expected to have low effect sizes (in the order of 1.1-1.5) [238] and this is consistent with our observations. Epigenetic effects, like imprinting of *STOX1* (and possibly *STOX2*) effector genes, dietary status influence on the biological function of known SNPs within *COMT* [284], would lessen our power to detect an association, and might mask more substantial effect sizes.

In summary, far from being resolved, the understanding of preeclampsia pathogenesis and genetics has developed significantly during the last few years. Some of the observations in this dissertation are consistent with and may contribute to this increased understanding. Taken together with other observations, epidemiologic and genetic data in our cohorts support the concept of a continuous distribution of placental and maternal pathology. Also, it is evident that the more we learn about preeclampsia etiology, the more there is to explore.

10. Concluding remarks and future perspectives

We shall have to evolve
problem-solvers galore
-since each problem they solve
creates ten problems more.

Piet Hein

Conceptually, the last 5-10 years have represented a quantum-leap in the understanding of biological variation. Bell`s 1998 phrasing "it would be surprising if most of the major genetic factors involved in human disease were not defined over the next 5-10 years" [239] has the making of a classic, similar to the alleged 1943 statement from IBM head T. J. Watson: "I think there is a world market for maybe five computers". Deterministic views [294] underlying a lot of the hype surrounding the genetic revolution in medicine [237], has been disproved by biology. Just as the family linkage studies of the previous decade showed us that a small number of genetic variants with a large effect could not explain complex disorders, the last ten years of genome-wide association studies have demonstrated that they cannot be explained by a limited number of moderate effect common variants [156]. The genome, once perceived as a stable molecule, is turning out to be highly dynamic. This insight will guide genetic research in the coming decade. The study of regulatory genes as well as non-coding (regulatory) genetic variation will probably be an important part of this research.

The regulation of *STOX2* gene expression has not yet been explored, and this is a possible focus for elaborating findings presented in this thesis. Also the regulation and expression of *COMT* throughout pregnancy needs further investigation. The possibility of differential nuclear factor binding to one of the SNPs identified in *TNFSF13B* is a finding that necessitates verification by identification of the factor binding and by complementary *in vivo* approaches. Furthermore, our findings need validation in independent cohorts. The 13q locus should be subjected to a more comprehensive genetic investigation.

We believe our microarray expression results to provide a rich data source for further elucidation of preeclampsia pathogenesis. The pathways of the unfolded protein response (ER stress) have been explored by protein analyses [295]. Using new statistical approaches we have observed gene-gene interactions that might be disturbed in preeclamptic deciduas [296] and we have utilized a large randomly selected cohort of pedigrees to assess continuous phenotype in relation to our decidual material (Johansson *et al.* submitted to EJHG). These and other new approaches in handling large data sets will be developed and exploited further.

Several genome-wide SNP association studies for preeclampsia are presently being planned and performed (including the HUNT2 preeclampsia cohort and an independent Australian population cohort). The data obtained may help elucidate the importance of susceptibility loci identified in family linkage studies (e.g. on chromosome 10q harboring *STOX1*, 13q harboring *TNFSF13B* and 4q harboring *STOX2*), as well as identify new targets for investigation. We are not able to investigate possible parent-of-origin effects or fetal contributions to preeclampsia pathogenesis in our HUNT2 cohort. We have however organized four of the largest obstetrical departments in Norway in recruiting patients with familial disposition for the disease, identified in MBRN. New sequencing technology, allowing not only for SNP genotyping, but whole sequence genotyping (whole exome sequencing being the most financially feasible so far) will increase the amount of information we can get from these families. Combined efforts including the other large family cohorts might further expand the available genetic knowledge base. International collaboration will be crucial to succeeding in utilizing information extracted from both the large population cohorts and family cohorts in a comprehensive manner. As we move in to the next generation sequencing era, the need for posing appropriate research questions has increased, rather than diminished.

In a public health perspective, better nutrition and sanitary conditions in the developed world are still the main factors that will improve maternal health and neonatal survival. Notwithstanding this, better obstetric care is important, also in developing countries. In the developed world, better prognostic markers for preeclampsia could mean less resources used on surveillance of pregnant women in general and preeclamptic women in particular. Appreciating the complexity of the genetic and environmental

contributions to preeclampsia, markers on the genetic level sensitive and specific enough to justify a relaxation of surveillance procedures is not a realistic prospect. Prognostic test are being developed based on molecular studies. So far these tests have convincing sensitivity, but they all have a poor specificity [297]. However, combined with e.g. umbilical artery flow measurements they might be able to help guide clinical decision making, and identify women who should be followed more closely. Furthermore, the main concern both for the individual mother and the health care system is bearing the child to maturity. Genetic studies will continue to play an important part in clarifying preeclampsia pathogenesis, and exploring the pathophysiology of preeclampsia can potentially teach us better ways of managing preeclamptic patients. For the fetus, every extra day spent in the uterus is significant. Finally, an important philosophical point is illustrated by Clinton`s Galileo reference at the introduction to the genetics chapter: Learning the language in which we are "created" does hold a great value in itself. Not all pursuits need an immediate practical consequence to be justified.

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Paper I

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Paper II

GENETICS

A transcriptional profile of the decidua in preeclampsia

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OBJECTIVE: We sought to obtain insight into possible mechanisms underlying preeclampsia using genomewide transcriptional profiling in decidua basalis.

STUDY DESIGN: Genomewide transcriptional profiling was performed on decidua basalis tissue from preeclamptic (n = 37) and normal (n = 58) pregnancies. Differentially expressed genes were identified and merged into canonical pathways and networks.

RESULTS: Of the 26,504 expressed transcripts detected, 455 were differentially expressed ($P < .05$; false discovery rate, $P < .1$). Both novel (ARL5B, SLITRK4) and previously reported preeclampsia-associated (PLA2G7, HMOX1) genes were identified. Pathway analysis revealed that tryptophan metabolism, endoplasmic reticulum stress, linoleic acid metabolism, notch signaling, fatty acid metabolism, arachidonic acid metabolism, and NRF2-mediated oxidative stress response were overrepresented canonical pathways.

CONCLUSION: In the present study single genes, canonical pathways, and gene-gene networks that are likely to play an important role in the pathogenesis of preeclampsia have been identified. Future functional studies are needed to accomplish a greater understanding of the mechanisms involved.

Key words: decidua, genomewide gene expression, microarray, preeclampsia

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The etiology of preeclampsia is not fully understood, but a number of observations suggest that divergent abnormalities may be involved (immunological, inflammatory, vascular/ischemic).[1] In a normal pregnancy extravillous trophoblasts (of fetal origin) invade decidua basalis and modify the spiral arteries. In preeclampsia, this pregnancy-associated adaption of spiral arteries may fail, with a hypoperfused placenta as a result. Oxidative stress is suggested to play a central role in the pathogenesis of preeclampsia,[2] and may be generated in the decidua basalis.[3,4] Heritability of the disease has been estimated to be >50%,[5,6] with both maternal and fetal (paternal) contributions.[7]

Microarray-based transcriptional profiling can be a powerful strategy for identification of disease-related genes and pathways,[8] and this approach has been used for analysis of placental[9] as well as decidual[6,10,11] tissues from preeclamptic pregnancies. However, the data obtained have been inconsistent. In the case of the 3 decidual studies reported, the diverging results may be due to the relatively small number of samples analyzed (≤12 preeclamptic samples included).[6,10,11] In the current study, we have applied genomewide transcriptional profiling (measuring ≥48,000 transcripts from all known genes) on a large collection of decidual samples (from 37 preeclamptic and 58 normal pregnancies) to comprehensively investigate how gene expression at the maternal-fetal interface may be contributing to the pathogenesis of preeclampsia. We further aimed to identify the genetic canonical pathways and gene-gene interaction networks represented by the differently expressed genes using contemporary bioinformatic approaches.

Materials and Methods

Human subjects

Women with pregnancies complicated by preeclampsia (n = 43) and women with normal pregnancies (n = 59) were recruited at St. Olav's University Hospital (Trondheim, Norway) and Haukeland University Hospital (Bergen, Norway) from 2002 through 2006. Preeclampsia was defined as persistent hypertension (blood pressure of ≥140/90 mm Hg) plus

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proteinuria (≥0.3 g/L or ≥1+ by dipstick) developing >20 weeks of pregnancy.[12] Due to tissue sampling procedures, only pregnancies delivered by cesarean section were included. Women with preeclamptic pregnancies had cesarean section performed for medical indications, whereas women with normal pregnancies underwent cesarean section for reasons considered irrelevant to the aim of the study (eg, breech presentation, cephalopelvic disproportion in previous delivery, and fear of vaginal delivery). None of the included mothers were in labor prior to cesarean section. Exclusively healthy women with no history of preeclampsia were accepted in the normal pregnancy group. Multiple pregnancies, pregnancies with chromosomal aberrations, fetal and placental structural abnormalities, or suspected perinatal infections were excluded from both study groups. The study was approved by the Norwegian Regional Committee for Medical Research Ethics. Informed consent was obtained from all participants prior to collection of decidual samples.

Decidual tissue collection

Samples of decidua basalis tissue were obtained by vacuum suction of the placental bed, a procedure that allows the collection of tissue from the whole placental bed.[13] Collected samples were flushed with saline solution to remove excessive blood. The decidual tissue was immediately submerged in RNA-later (Ambion, Austin, TX).

Total RNA isolation

Total RNA was isolated using a TRIzol (Invitrogen, Carlsbad, CA) extraction protocol with chloroform interphase separation, isopropanol precipitation, and ethanol wash steps. Precipitated total RNA was resuspended in Rnase-free water and purified with an RNeasy Mini Kit using spin technology (Qiagen, Valencia, CA). Spectrophotometric determination of purified total RNA yield (μg) was performed using the NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE). Total RNA quality was measured using RNA 6000 Nano Series II Kit on a BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA). Ethical approval for total RNA processing and decidua expression analysis was obtained from the institutional review board at the University of Texas Health Science Center in San Antonio.

Synthesis, amplification, and purification of antisense RNA

Antisense RNA (aRNA) was synthesized, amplified, and purified using the Illumina TotalPrep RNA Amplification Kit according to manufacturer's instructions (Ambion, Austin, TX). Synthesis of aRNA was performed using a T7 Oligo(dT) primer, and the amplification underwent in vitro transcription with a T7 RNA polymerase to generate multiple copies of biotinylated aRNA from a double-stranded complementary DNA (cDNA) template. Purified aRNA yield was determined spectrophotometrically using the NanoDrop ND-1000.

Microarray data

Purified aRNA was hybridized to Illumina's HumanWG-6 v2 Expression BeadChip (Illumina Inc, San Diego, CA). Washing, blocking, and transcript signal detection (streptavidin-Cy3) was performed using Illumina's 6 × 2 BeadChip protocol. Samples were scanned on the Illumina BeadArray 500GX Reader using Illumina BeadScan image data acquisition software (version 2.3.0.13). Illumina's BeadStudio Gene Expression software module (version 3.2.7) was used to subtract background noise signals and generate an output file for statistical analysis.

Real-time quantitative polymerase chain reaction

We performed a verification of the microarray experiment with quantitative real-time (RT)-polymerase chain reaction (PCR) on 6 of the most differentially expressed transcripts using a 7900HT Fast RT-PCR instrument (Applied Biosystems, Foster City, CA). The 6 genes were prioritized for RT-PCR based on beta values, false discovery rate (FDR) *P* values, and manual literature searches. RT quantitative PCR was run with 93 samples. Two of the total collection of 95 samples were excluded due to shortage of biological material. Preoptimized TaqMan Gene Expression Assays (Applied Biosystems) were run, in triplicate, to measure messenger RNA expression levels relative to the reference genes, TATA box binding protein and glyceraldehyde-3-phosphate dehydrogenase. Reverse transcription and PCR amplification was performed in a 2-step procedure, following Applied Biosystems High-Capacity cDNA ReverseTranscription Kit Protocol and TaqMan Gene Expression Master Mix Protocol. Negative controls were run, in triplicate, without RT enzyme or no cDNA template.

Statistical analysis

Transcript data for each sample were preprocessed and analyzed using our Sequential Oligogenic Linkage Analysis Routines (SOLAR) statistical analysis software program,[14] as previously described.[15] To evaluate the magnitude of differential gene expression the displacement of each detected transcript's mean expression value was measured between the 2 groups. A standard regression analysis was performed on the preeclamptic group to test whether the mean transcription level differed from that of the normal pregnancy group.

The messenger RNA expression levels were calculated by the Comparative threshold cycle (CT) method, as described elsewhere.[16] For each target gene, the mean CT value for each sample was used for analysis, after exclusion of outliers. Outliers were determined as values >2SD from the mean. Delta CT (ΔCT) values were computed as the difference between the given mean value for a target gene and the mean of the CT values for the 2 reference genes.[17] Fold change values were calculated, based on the differences in ΔCT values between tissue from preeclamptic women and women with normal pregnancy ($2^{-\Delta\Delta CT}$).[16] A *t* test statistic (SPSS, version 16; SPSS, Inc, Chicago, IL) evaluated the difference between the ΔCT values of the preeclamptic pregnancies, compared with the normal pregnancy group. Analyzing for the 2 reference genes separately did not change the results.

Canonical pathway and network identification

Differentially expressed transcripts in the preeclamptic group ($P < .05$; FDR,[18]

$P < .1$) were imported into Ingenuity Pathways Analysis (IPA, v7.5; Ingenuity Systems, Redwood City, CA). Transcripts' gene identifiers were mapped to their corresponding gene object in the Ingenuity Pathways Knowledge Base. IPA was used to bioinformatically identify canonical (ie, cell signaling and metabolic) pathways and gene-gene interaction networks potentially involved in preeclampsia within our dataset. IPA gene-gene networks were constructed from the published literature, and they diagrammatically represent molecular relationships between gene-gene products.

Significant IPA pathways were further analyzed with Rotation Gene Set Enrichment Analysis (ROMER; Fred Hutchinson Cancer Research Center, Seattle, WA) pathway analysis, using the *limma* package, available via the Bioconductor Project (Fred Hutchinson Cancer Research Center).[19]

RESULTS

Human subjects

The clinical information of women/pregnancies enrolled is presented in Table 1. Only those samples of sufficient RNA quality for gene expression analysis have been included. In the preeclamptic pregnancies, both mean gestational age and birthweight were lower than in the normal pregnancies (Table 1). As expected, the mean blood pressure was higher among preeclamptic than normal pregnancies (Table 1).

Decidual genomewide transcriptional profiling

In total, 43 women with pregnancies complicated by preeclampsia and 59 women with normal pregnancies were included in the study. Six samples from preeclamptic pregnancies and 1 sample from a normal pregnancy were excluded from gene expression analyses due to low RNA quality. The 95 samples with good RNA quality were hybridized onto Illumina's HumanWG-6 v2 genomewide Expression BeadChip.

The nonnormalized decidua basalis transcriptional profile data (n = 48,095) may be found at ArrayExpress (European Molecular Biology Laboratory-European Bioinformatics Institute, Hinxton, UK) (accession code E-TABM-682). We detected 26,504 significantly expressed transcripts (55.1%), of which 455 were differentially expressed after FDR correction ($P < .05$; FDR, $P < .1$); 285 were down-regulated and 170 were up-regulated. The significant differentially expressed transcripts are presented in Table 2, together with the corresponding *P* values (raw and FDR adjusted) and preeclampsia-correlated expression. The RT quantitative PCR for the 6 genes (PLA2G7, ANGPTL2, MAN1A2, SLITRK4, FZD4, and ARL5B) tested showed a high grade of correlation with the microarray data (Table 3).

TABLE 1
Clinical characteristics of study groups

Variable	Preeclamptic pregnancies[a] (n = 37)	Normal pregnancies[a] (n = 58)
Gestational age, wk	32 ± 4[b]	39 ± 1
Systolic blood pressure, mm Hg	152 ± 16[b]	116 ± 10
Diastolic blood pressure, mm Hg	96 ± 10[b]	70 ± 9.0
Birthweight, g	1555 ± 769[b]	3619 ± 469
Body mass index, kg/m^2[c]	27.7 ± 6.2	25.3 ± 5.7

Values are means ± SD.

[a] In all, 43 women with pregnancies complicated by preeclampsia and 59 women with normal pregnancies were included in the study. Six samples from preeclamptic pregnancies and 1 sample from normal pregnancy were excluded from gene expression analysis due to low RNA quality; [b] $P < .001$ obtained with *t* test statistics with software (SPSS, version 16; SPSS, Inc, Chicago, IL); [c] Body mass index was measured at first antenatal care visit.

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Canonical pathways and network

The 455 differentially expressed transcripts were analyzed using IPA. The significant canonical pathways ($P < .01$) are shown in Table 4, along with the included genes and *P* values. They included tryptophan metabolism, endoplasmic reticulum (ER) stress, linoleic acid metabolism, notch signaling, fatty acid metabolism, arachidonic acid metabolism, and NRF2-mediated oxidative stress response. All the canonical pathways identified in IPA were also found to be significant ($P < .01$) using ROMER (Table 4), with the exception of the NRF2-mediated oxidative stress response canonical pathway (IPA, $P = .009$; ROMER, $P = .067$).

Using network analysis in IPA, 59 of the preeclampsia-associated genes could be connected into a single network of gene-gene product interactions (Figure). The genes in this network were among others involved in the function of ER, oxidative stress, notch signaling, and cell migration. The network included a cluster of 15 up-regulated genes (ATP2A2, TRAM1, FKBP2, HMOX1, SPCS2, ATF6, DNAJC3, EIF2AK3, PIGA, SEC23B, SEC24D, DNAJB9, SRPRB, DNAJB11, and X-box binding protein 1 [XBP1]) associated with ER stress and oxidative stress (Figure). All these genes were in a direct relationship to XBP1. Epidermal growth factor receptor (EGFR) was another focus molecule with a direct relationship to 7 other genes (PLCG1, NGF, MET, LRIG1, SLN, ATP2A2, and SHC2) in the network.

COMMENT

In this study, 455 differentially expressed transcripts were found when decidua basalis tissue from preeclamptic and normal pregnancies was compared. Some transcripts were novel findings (ie, ARL5B and SLITRK4), whereas others, such as PLA2G7[20] and HMOX1,[21,22] have been reported to be associated with preeclampsia previously. Pathway analysis identified 7 significant canonical pathways.

In our patient cohort, a lower gestational age was found in the preeclamptic group (average, 32 weeks; range, 28–36) compared with the normal pregnancy group (39 weeks; range, 38–40). This is not unexpected due to the need for early delivery in patients with severe preeclampsia. Since gene expression in uteroplacental tissues

TABLE 2
Differentially expressed transcripts[a]

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782259	NM_173078.2	SLITRK4	SLIT and NTRK-like family, member 4	X	−1.0363	4.6×10^{-8}	.0012
ILMN_1680465	NM_178815.3	ARL5B	ADP-ribosylation factor-like 5B	10	0.9122	4.5×10^{-7}	.0039
ILMN_1743367	NM_012193.2	FZD4	Frizzled homolog 4 (Drosophila)	11	−0.9122	4.1×10^{-7}	.0054
ILMN_1726210	NM_178172.2	LOC338328	High density lipoprotein–binding protein	8	−0.8672	3.7×10^{-6}	.0088
ILMN_1709222	NM_005692.3	ABCF2	ATP-binding cassette, subfamily F (GCN20), member 2, nuclear gene encoding mitochondrial protein, transcript variant 2	7	−0.8634	3.5×10^{-6}	.0093
ILMN_1772612	NM_012098.2	ANGPTL2	Angiopoietin-like 2	9	−0.8884	4.4×10^{-6}	.0097
ILMN_1659792	NM_014213.2	HOXD9	Homeobox D9	2	−0.8541	3.5×10^{-6}	.0102
ILMN_1813295	NM_018640.3	LMO3	LIM domain only 3 (rhombotin-like 2), transcript variant 1	12	−0.8992	3.3×10^{-6}	.0110
ILMN_1669023	NM_020482.3	FHL5	Four and a half LIM domains 5	6	−0.8489	3.2×10^{-6}	.0123
ILMN_1658677	NM_178502.2	DTX3	Deltex 3 homolog (Drosophila)	12	−0.9038	2.9×10^{-6}	.0130
ILMN_1812461	NM_003881.2	WISP2	WNT1 inducible signaling pathway protein 2	20	−0.8717	6.6×10^{-6}	.0134
ILMN_1776157	NM_080415.1	SEPT4	Septin 4, transcript variant 2	17	−0.8882	2.6×10^{-6}	.0140
ILMN_1794370	NM_001031702.2	SEMA5B	Sema domain, 7 thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, transcript variant 1	3	−0.8695	8.0×10^{-6}	.0141
ILMN_1719069	NM_213596.1	FOXN4	Forkhead box N4	12	−0.8803	7.8×10^{-6}	.0147
ILMN_1733667	NM_021931.2	DHX35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	20	−0.8537	9.0×10^{-6}	.0149
ILMN_1734276	NM_199169.1	TMEPAI	Transmembrane, prostate androgen-induced RNA, transcript variant 2	20	−0.8360	1.6×10^{-5}	.0153
ILMN_1701195	NM_005084.2	PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	6	0.8305	1.6×10^{-5}	.0155
ILMN_1687821	NM_033201.1	C16orf45	Chromosome 16 open reading frame 45	16	−0.8218	1.4×10^{-5}	.0156
ILMN_1736911	NM_003275.2	TMOD1	Tropomodulin 1	9	−0.8178	1.5×10^{-5}	.0157
ILMN_1744487	NM_015645.2	C1QTNF5	C1q and tumor necrosis factor-related protein 5	11	−0.8113	1.7×10^{-5}	.0157
ILMN_1767556	NM_007021.2	C10orf10	Chromosome 10 open reading frame 10	10	−0.7966	1.3×10^{-5}	.0158
ILMN_1668249	NM_022773.2	TMEM112	Transmembrane protein 112	16	−0.8079	1.6×10^{-5}	.0158
ILMN_1788462	NM_001033059.1	AMD1	Adenosylmethionine decarboxylase 1, transcript variant 2	6	0.8027	1.4×10^{-5}	.0160
ILMN_1665945	NM_022735.3	ACBD3	acyl-Coenzyme A binding domain containing 3	1	0.8297	1.3×10^{-5}	.0164

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TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1657803	NM_001014975.1	CFH	Complement factor H, transcript variant 2	1	−0.8780	2.5×10^{-6}	.0164
ILMN_1880012	NM_003966.2	SEMA5A	Sema domain, 7 thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	5	0.8208	2.7×10^{-5}	.0168
ILMN_1763036	NM_001286.2	CLCN6	Chloride channel 6, transcript variant CIC-6a	1	−0.8027	2.4×10^{-5}	.0170
ILMN_1710962	NM_014573.2	TMEM97	Transmembrane protein 97	17	0.8236	2.6×10^{-5}	.0171
ILMN_1801927	NM_001004311.2	FIGLA	Folliculogenesis-specific basic helix-loop-helix	2	−0.8616	1.1×10^{-5}	.0171
ILMN_1673773	NM_198516.1	GALNTL4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4	11	−0.7998	2.3×10^{-5}	.0172
ILMN_1711516	NM_001690.2	ATP6V1A	ATPase, H+ transporting, lysosomal 70 kDa, V1 subunit A	3	0.8101	1.2×10^{-5}	.0172
ILMN_1715555	NM_001352.2	DBP	D site of albumin promoter (albumin D-box) binding protein	19	−0.7916	1.3×10^{-5}	.0172
ILMN_1779632	NM_001001723.1	TMEM1	Transmembrane protein 1, transcript variant 2	21	0.8054	2.6×10^{-5}	.0172
ILMN_1685703	NM_003500.2	ACOX2	acyl-Coenzyme A oxidase 2, branched chain	3	−0.8253	2.2×10^{-5}	.0173
ILMN_1711157	NM_004557.3	NOTCH4	Notch homolog 4 (Drosophila)	6	−0.7709	2.5×10^{-5}	.0174
ILMN_1740160	NM_182811.1	PLCG1	Phospholipase C, gamma 1, transcript variant 2	20	−0.8077	2.1×10^{-5}	.0176
ILMN_1834017	N25708	Hs.573236	yx79f04s1 Soares melanocyte 2NbHM cDNA clone IMAGE: 267967 3 sequence		0.8058	2.3×10^{-5}	.0176
ILMN_1798076	NM_006898.4	HOXD3	Homeobox D3	2	−0.8238	2.3×10^{-5}	.0176
ILMN_1705985	NM_020473.2	PIGA	Phosphatidylinositol glycan anchor biosynthesis, class A (paroxysmal nocturnal hemoglobinuria), transcript variant 3	X	0.7983	2.6×10^{-5}	.0177
ILMN_1772302	NM_006441.1	MTHFS	5,10-Methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	15	0.7802	2.9×10^{-5}	.0178
ILMN_1781791	NM_000950.1	PRRG1	Proline-rich Gla (G-carboxyglutamic acid) 1	X	0.7681	3.2×10^{-5}	.0179
ILMN_1748812	NM_152913.1	TMEM130	Transmembrane protein 130	7	−0.7814	3.0×10^{-5}	.0179
ILMN_1680774	XM_001132373.1	LOC730994	Similar to NACHT, leucine-rich repeat and PYD (pyrin domain) containing 1, transcript variant 1	17	−0.8034	2.0×10^{-5}	.0179
ILMN_1755120	NM_006699.3	MAN1A2	Mannosidase, alpha, class 1A, member 2	1	0.8519	1.3×10^{-5}	.0180

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TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1788166	NM_003318.3	TTK	TTK protein kinase	6	0.8169	2.1×10^{-5}	.0181
ILMN_1685608	NM_002523.1	NPTX2	Neuronal pentraxin II	7	−0.7865	3.1×10^{-5}	.0181
ILMN_1678842	NM_003247.2	THBS2	Thrombospondin 2	6	−0.8054	3.2×10^{-5}	.0182
ILMN_1813430	NM_182985.3	TRIM69	Tripartite motif-containing 69, transcript variant a	15	−0.8120	3.5×10^{-5}	.0192
ILMN_1675936	NM_016438.2	HIGD1B	HIG1 domain family, member 1B	17	−0.8060	3.9×10^{-5}	.0202
ILMN_1877909	BX105647	Hs.125533	BX105647 Soares_NFL_T_GBC_S1 cDNA clone IMAGp998F143713 sequence		−0.7992	3.7×10^{-5}	.0202
ILMN_1803279	NM_016040.3	TMED5	Transmembrane emp24 protein transport domain containing 5	1	0.7904	3.8×10^{-5}	.0202
ILMN_1700202	NM_022918.2	TMEM135	Transmembrane protein 135	11	0.7615	4.1×10^{-5}	.0206
ILMN_1727589	NM_004605.2	SULT2B1	Sulfotransferase family, cytosolic, 2B, member 1, transcript variant 1	19	0.7826	4.2×10^{-5}	.0209
ILMN_1811873	NM_002889.2	RARRES2	Retinoic acid receptor responder (tazarotene induced) 2	7	−0.7690	4.4×10^{-5}	.0214
ILMN_1703955	NM_148177.1	FBXO32	F-box protein 32, transcript variant 2	8	−0.8049	4.8×10^{-5}	.0225
ILMN_1731358	NM_018181.4	ZNF532	Zinc finger protein 532	18	−0.7974	4.7×10^{-5}	.0226
ILMN_1682937	NM_001038633.2	RSPO1	R-spondin homolog (Xenopus laevis)	1	−0.7973	5.0×10^{-5}	.0230
ILMN_1695947	NM_174934.2	SCN4B	Sodium channel, voltage-gated, type IV, beta	11	−0.7948	5.8×10^{-5}	.0234
ILMN_1707342	NM_015541.2	LRIG1	Leucine-rich repeats and immunoglobulin-like domains 1	3	−0.7679	5.8×10^{-5}	.0235
ILMN_1781626	NM_001734.2	C1S	Complement component 1, s subcomponent, transcript variant 1	12	−0.7833	5.7×10^{-5}	.0236
ILMN_1676215	NM_001364.2	DLG2	Discs, large homolog 2, chapsyn-110 (Drosophila)	11	−0.7928	5.6×10^{-5}	.0238
ILMN_1880210	BC038188	Hs.179213	Homo sapiens, clone IMAGE: 3451765		0.7666	5.7×10^{-5}	.0239
ILMN_1767225	NM_006092.1	NOD1	Nucleotide-binding oligomerization domain containing 1	7	−0.7808	5.3×10^{-5}	.0239
ILMN_1793410	NM_021021.2	SNTB1	Syntrophin, beta 1 (dystrophin-associated protein A1, 59 kDa, basic component 1)	8	0.7636	5.5×10^{-5}	.0239
ILMN_1752837	NM_018184.2	ARL8B	ADP-ribosylation factor-like 8B	3	0.7644	5.3×10^{-5}	.0241
ILMN_1791949	NM_032507.2	PGBD1	PiggyBac transposable element-derived 1	6	−0.7478	5.5×10^{-5}	.0243
ILMN_1859863	BM458075	Hs.555181	AGENCOURT_6411402 NIH_MGC_71 cDNA clone IMAGE:5530423 5 sequence		0.7667	6.4×10^{-5}	.0248

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782788	NM_003651.3	CSDA	Cold shock domain protein A	12	−0.7756	6.3×10^{-5}	.0251
ILMN_1727740	NM_006372.3	SYNCRIP	Synaptotagmin binding, cytoplasmic RNA interacting protein	6	0.6949	6.7×10^{-5}	.0253
ILMN_1677396	NM_019080.1	NDFIP2	Nedd4 family interacting protein 2	13	0.7591	6.6×10^{-5}	.0253
ILMN_1744191	NM_003042.2	SLC6A1	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1	3	−0.7914	6.9×10^{-5}	.0253
ILMN_1656129	NM_020342.1	SLC39A10	Solute carrier family 39 (zinc transporter), member 10	2	0.7306	6.8×10^{-5}	.0253
ILMN_1809639	NM_178505.5	TMEM26	Transmembrane protein 26	10	0.7732	7.9×10^{-5}	.0287
ILMN_1786326	NM_024076.1	KCTD15	Potassium channel tetramerization domain containing 15	19	−0.7853	8.2×10^{-5}	.0291
ILMN_1651343	NM_001004439.1	ITGA11	Integrin, alpha 11	15	−0.7812	8.2×10^{-5}	.0292
ILMN_1739887	NM_031491.2	RBP5	Retinol-binding protein 5, cellular	12	−0.7607	8.7×10^{-5}	.0304
ILMN_1716247	NM_203371.1	FIBIN	Fin bud initiation factor	11	−0.7760	8.9×10^{-5}	.0307
ILMN_1752668	NM_015345.2	DAAM2	Disheveled-associated activator of morphogenesis 2	6	−0.7617	1.0×10^{-4}	.0309
ILMN_1789243	NM_018668.3	VPS33B	Vacuolar protein sorting 33 homolog B (yeast)	15	−0.7368	1.0×10^{-4}	.0312
ILMN_1763852	NM_001093.3	ACACB	acetyl-Coenzyme A carboxylase beta	12	−0.7651	9.6×10^{-5}	.0314
ILMN_1731561	NM_022370.2	ROBO3	Roundabout, axon guidance receptor, homolog 3 (Drosophila)	11	−0.7335	1.0×10^{-4}	.0314
ILMN_1672635	NM_182947.2	GEFT	RhoA/RAC/CDC42 exchange factor, transcript variant 1	12	−0.7711	9.3×10^{-5}	.0315
ILMN_1691181	NM_030755.4	TXNDC1	Thioredoxin domain containing 1	14	0.7498	1.1×10^{-4}	.0315
ILMN_1742034	NM_000261.1	MYOC	Myocilin, trabecular meshwork-inducible glucocorticoid response	1	−0.7416	1.0×10^{-4}	.0315
ILMN_1761968	NM_033256.1	PPP1R14A	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	19	−0.7785	9.5×10^{-5}	.0315
ILMN_1703142	NM_001005416.1	MARCH2	Membrane-associated ring finger (C3HC4) 2, transcript variant 3	19	−0.7337	1.0×10^{-4}	.0316
ILMN_1752225	NR_002330.1	ST7OT1	ST7 overlapping transcript 1 (antisense noncoding RNA)	7	−0.7606	9.8×10^{-5}	.0318
ILMN_1667692	NM_000961.3	PTGIS	Prostaglandin I2 (prostacyclin) synthase	20	−0.7787	9.5×10^{-5}	.0318
ILMN_1691457	NM_004900.3	APOBEC3B	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	22	0.7343	1.0×10^{-4}	.0319

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1728979	NM_207310.1	CCDC74B	Coiled-coil domain containing 74B	2	−0.7428	1.2×10^{-4}	.0320
ILMN_1688346	NM_176814.3	ZNF800	Zinc finger protein 800	7	0.7259	1.2×10^{-4}	.0323
ILMN_1682428	NM_144584.1	C1orf59	Chromosome 1 open reading frame 59	1	0.7635	1.2×10^{-4}	.0323
ILMN_1755173	NM_020904.1	PLEKHA4	Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4	19	−0.7470	1.1×10^{-4}	.0324
ILMN_1782954	NM_005339.3	UBE2K	Ubiquitin-conjugating enzyme E2-25K	4	0.7190	1.2×10^{-4}	.0324
ILMN_1735996	NM_016931.2	NOX4	NADPH oxidase 4	11	−0.7504	1.3×10^{-4}	.0325
ILMN_1680110	NM_006829.2	C10orf116	Chromosome 10 open reading frame 116	10	−0.7497	1.2×10^{-4}	.0325
ILMN_1755832	NM_000435.2	NOTCH3	Notch homolog 3 (Drosophila)	19	−0.7589	1.1×10^{-4}	.0325
ILMN_1800463	NM_017859.2	UCKL1	Uridine-cytidine kinase 1-like 1	20	−0.7338	1.2×10^{-4}	.0326
ILMN_1674337	NM_004470.2	FKBP2	FK506 binding protein 2, 13 kDa, transcript variant 1	11	0.7401	1.2×10^{-4}	.0327
ILMN_1807171	NM_000929.2	PLA2G5	Phospholipase A2, group V	1	−0.7349	1.3×10^{-4}	.0327
ILMN_1724671	NM_207577.1	MAP6	Microtubule-associated protein 6, transcript variant 2	11	−0.7623	1.2×10^{-4}	.0328
ILMN_1655117	NM_025132.3	WDR19	WD repeat domain 19	4	−0.7425	1.3×10^{-4}	.0328
ILMN_1706511	NM_003216.2	TEF	Thyrotrophic embryonic factor	22	−0.7288	1.1×10^{-4}	.0328
ILMN_1677018	NM_002141.4	HOXA4	Homeobox A4	7	−0.7424	1.3×10^{-4}	.0333
ILMN_1785646	NM_153321.1	PMP22	Peripheral myelin protein 22, transcript variant 2	17	−0.7487	1.3×10^{-4}	.0334
ILMN_1709661	NM_145276.1	ZNF563	Zinc finger protein 563	19	−0.7481	1.4×10^{-4}	.0334
ILMN_1736863	NM_018295.2	TMEM140	Transmembrane protein 140	7	−0.7336	1.3×10^{-4}	.0337
ILMN_1807379	NM_023034.1	WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1, transcript variant long	8	0.7237	1.4×10^{-4}	.0338
ILMN_1740842	NM_005407.1	SALL2	Sal-like 2 (Drosophila)	14	−0.7458	1.4×10^{-4}	.0340
ILMN_1734229	NM_032802.3	SPPL2A	Signal peptide peptidase-like 2A	15	0.7168	1.4×10^{-4}	.0343
ILMN_1696003	NM_006496.1	GNAI3	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	1	0.7101	1.5×10^{-4}	.0343
ILMN_1793770	NM_058246.3	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6, transcript variant 1	7	−0.7448	1.4×10^{-4}	.0343
ILMN_1797861	NM_002184.2	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor), transcript variant 1	5	0.7406	1.6×10^{-4}	.0353
ILMN_1720865	NM_145798.2	OSBPL7	Oxysterol binding protein-like 7, transcript variant 1	17	−0.7298	1.6×10^{-4}	.0355
ILMN_1713978	NM_006923.2	SDF2	Stromal cell-derived factor 2	17	0.7243	1.6×10^{-4}	.0356

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1682231	NM_001003682.2	TTMB	TTMB protein	1	−0.7581	1.6×10^{-4}	.0356
ILMN_1684554	NM_001856.3	COL16A1	Collagen, type XVI, alpha 1	1	−0.7316	1.5×10^{-4}	.0356
ILMN_1778595	NM_003063.2	SLN	Sarcolipin	11	−0.7375	1.6×10^{-4}	.0356
ILMN_1811790	NM_004118.3	FKHL18	Forkhead-like 18 (Drosophila)	20	−0.7197	1.6×10^{-4}	.0357
ILMN_1712461	NM_004352.1	CBLN1	Cerebellin 1 precursor	16	−0.7413	1.5×10^{-4}	.0358
ILMN_1815874	NM_018946.2	NANS	N-acetylneuraminic acid synthase (sialic acid synthase)	9	0.7205	1.7×10^{-4}	.0359
ILMN_1720819	XM_934796.2	LOC653566	Similar to signal peptidase complex subunit 2 (microsomal signal peptidase 25-kDa subunit) (SPase 25-kDa subunit), transcript variant 3	1	0.6515	1.7×10^{-4}	.0359
ILMN_1669898	NM_201446.1	EGFL7	EGF-like-domain, multiple 7, transcript variant 2	9	−0.6935	1.5×10^{-4}	.0359
ILMN_1740441	NM_000398.4	CYB5R3	Cytochrome b5 reductase 3, transcript variant M	22	−0.7263	1.7×10^{-4}	.0360
ILMN_1700274	NM_031442.2	TMEM47	Transmembrane protein 47	X	−0.7303	1.6×10^{-4}	.0360
ILMN_1720889	NM_001017369.1	SC4MOL	Sterol-C4-methyl oxidase-like, transcript variant 2	4	0.6822	1.7×10^{-4}	.0367
ILMN_1793543	NM_144697.2	C1orf51	Chromosome 1 open reading frame 51	1	−0.7115	1.8×10^{-4}	.0376
ILMN_1734288	NM_152511.3	DUSP18	Dual specificity phosphatase 18	22	−0.7243	1.9×10^{-4}	.0383
ILMN_1678998	NM_014665.1	LRRC14	Leucine-rich repeat containing 14	8	−0.7119	1.9×10^{-4}	.0383
ILMN_1791508	NM_024302.3	MMP28	Matrix metallopeptidase 28, transcript variant 1	17	−0.7246	1.9×10^{-4}	.0385
ILMN_1688295	NM_016423.1	ZNF219	Zinc finger protein 219	14	−0.7437	1.9×10^{-4}	.0388
ILMN_1770293	NM_001730.3	KLF5	Kruppel-like factor 5 (intestinal)	13	0.7122	1.9×10^{-4}	.0388
ILMN_1886424	BG621061	Hs.559870	602616941F1 NIH_MGC_79 cDNA clone IMAGE:4730410 5 sequence		−0.7236	1.9×10^{-4}	.0388
ILMN_1697006	XM_930748.2	LOC642361	Hypothetical protein LOC642361	10	−0.6926	2.2×10^{-4}	.0400
ILMN_1673543	NM_018290.2	PGM2	Phosphoglucomutase 2	4	0.6845	2.0×10^{-4}	.0401
ILMN_1742230	NM_182648.1	BAZ1A	Bromodomain adjacent to zinc finger domain, 1A, transcript variant 2	14	0.7376	2.1×10^{-4}	.0401
ILMN_1659843	NM_006260.2	DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	13	0.7094	2.1×10^{-4}	.0401
ILMN_1696585	NM_017671.4	C20orf42	Chromosome 20 open reading frame 42	20	0.7335	2.1×10^{-4}	.0402
ILMN_1763641	NM_025040.2	ZNF614	Zinc finger protein 614	19	0.7013	2.1×10^{-4}	.0402
ILMN_1726678	NM_014147.1	HSPC047	HSPC047 protein	7	−0.7153	2.0×10^{-4}	.0402
ILMN_1779034	NM_018161.4	NADSYN1	NAD synthetase 1	11	−0.6854	2.1×10^{-4}	.0402
ILMN_1705253	NM_130393.2	PTPRD	Protein tyrosine phosphatase, receptor type, D, transcript variant 4	9	0.7342	2.1×10^{-4}	.0403

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1837017	CB269825	Hs.543359	1008732 Human Fat Cell 5-Stretch Plus cDNA Library cDNA 5' sequence		−0.7281	2.1×10^{-4}	.0405
ILMN_1829490	BX106357	Hs.445732	BX106357 Soares_NFL_T_GBC_S1 cDNA clone IMAGp998B055155 sequence		0.6957	2.2×10^{-4}	.0409
ILMN_1714691	NM_002148.3	HOXD10	Homeobox D10	2	−0.7265	2.3×10^{-4}	.0413
ILMN_1803213	NM_015419.2	MXRA5	Matrix-remodeling-associated 5	X	−0.7061	2.3×10^{-4}	.0416
ILMN_1732158	NM_001460.2	FMO2	Flavin containing monooxygenase 2 (nonfunctional)	1	−0.6950	2.4×10^{-4}	.0424
ILMN_1681938	NM_022568.2	ALDH8A1	SIdehyde dehydrogenase 8 family, member A1, transcript variant 1	6	0.6875	2.4×10^{-4}	.0424
ILMN_1753243	NM_016306.4	DNAJB11	DnaJ (Hsp40) homolog, subfamily B, member 11	3	0.7183	2.5×10^{-4}	.0431
ILMN_1793846	NM_014670.2	BZW1	Basic leucine zipper and W2 domains 1	2	0.7033	2.7×10^{-4}	.0431
ILMN_1852159	BF753039	Hs.557431	RC3-BN0425-011200-022-c08 BN0425 cDNA sequence		−0.7234	2.4×10^{-4}	.0432
ILMN_1805992	NM_018330.4	KIAA1598	KIAA1598	10	0.7077	2.4×10^{-4}	.0433
ILMN_1740512	XM_936687.1	MGC39900	Hypothetical protein MGC39900	X	−0.7227	2.6×10^{-4}	.0433
ILMN_1708916	NM_032512.2	PDZD4	PDZ domain containing 4	X	−0.7075	2.7×10^{-4}	.0434
ILMN_1773563	NM_015927.3	TGFB1I1	Transforming growth factor beta 1-induced transcript 1, transcript variant 2	16	−0.7335	2.6×10^{-4}	.0435
ILMN_1674184	NM_153022.2	C12orf59	Chromosome 12 open reading frame 59	12	0.7122	2.6×10^{-4}	.0436
ILMN_1657483	NM_032985.4	SEC23B	Sec23 homolog B (S cerevisiae), transcript variant 2	20	0.6717	2.7×10^{-4}	.0436
ILMN_1772540	NM_015251.2	ASCIZ	ATM/ATR-Substrate Chk2-Interacting Zn2+-finger protein	16	0.6872	2.6×10^{-4}	.0438
ILMN_1756862	NM_145641.1	APOL3	Apolipoprotein L, 3, transcript variant beta/a	22	−0.7021	2.8×10^{-4}	.0438
ILMN_1685413	NM_024079.4	ALG8	Asparagine-linked glycosylation 8 homolog (S cerevisiae, alpha-1,3-glucosyltransferase), transcript variant 1	11	0.6986	2.8×10^{-4}	.0439
ILMN_1686645	NM_021645.4	UTP14C	UTP14, U3 small nucleolar ribonucleoprotein, homolog C (yeast)	13	0.6746	2.8×10^{-4}	.0440
ILMN_1813746	NM_003389.2	CORO2A	Coronin, actin-binding protein, 2A, transcript variant 1	9	0.7135	2.6×10^{-4}	.0440
ILMN_1765557	NM_015441.1	OLFML2B	Olfactomedin-like 2B	1	−0.6714	2.7×10^{-4}	.0441
ILMN_1740586	NM_000300.2	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1	−0.7049	2.6×10^{-4}	.0443

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1758750	NR_003501.1	EARS2	Glutamyl-tRNA synthetase 2, mitochondrial (putative), transcript variant 2, transcribed RNA	16	0.7199	2.6×10^{-4}	.0444
ILMN_1703178	NM_003469.3	SCG2	Secretogranin II (chromogranin C)	2	−0.7239	2.6×10^{-4}	.0444
ILMN_1710522	NM_175635.1	RUNX1T1	Runt-related transcription factor 1; translocated to, 1 (cyclin D-related), transcript variant 3	8	−0.6886	3.1×10^{-4}	.0444
ILMN_1730048	NM_024067.2	C7orf26	Chromosome 7 open reading frame 26	7	−0.6943	2.9×10^{-4}	.0444
ILMN_1722855	NM_003377.3	VEGFB	Vascular endothelial growth factor B	11	−0.7039	3.0×10^{-4}	.0445
ILMN_1752915	NM_004124.2	GMFB	Glia maturation factor, beta	14	0.6872	2.6×10^{-4}	.0445
ILMN_1702124	NM_153371.3	LNX2	Ligand of numb-protein X 2	13	0.7044	3.0×10^{-4}	.0445
ILMN_1695299	NM_014476.1	PDLIM3	PDZ and LIM domain 3	4	−0.7140	3.1×10^{-4}	.0445
ILMN_1666364	NM_144576.3	COQ10A	Coenzyme Q10 homolog A (S cerevisiae), transcript variant 1	12	−0.6949	2.6×10^{-4}	.0445
ILMN_1756942	NM_001017371.3	SP3	Sp3 transcription factor, transcript variant 2	2	0.6849	3.1×10^{-4}	.0445
ILMN_1750386	NM_006172.2	NPPA	Natriuretic peptide precursor A	1	−0.6947	3.1×10^{-4}	.0445
ILMN_1685433	NM_020351.2	COL8A1	Collagen, type VIII, alpha 1, transcript variant 2	3	−0.6900	2.9×10^{-4}	.0445
ILMN_1665095	NM_015537.3	NELF	Nasal embryonic LHRH factor	9	−0.7203	2.9×10^{-4}	.0445
ILMN_1695316	NM_022154.5	SLC39A8	Solute carrier family 39 (zinc transporter), member 8	4	0.6843	2.9×10^{-4}	.0446
ILMN_1749338	NM_173505.2	ANKRD29	Ankyrin repeat domain 29	18	−0.6916	3.0×10^{-4}	.0446
ILMN_1692340	NM_207404.2	ZNF662	Zinc finger protein 662	3	−0.7117	2.9×10^{-4}	.0447
ILMN_1730612	NM_001048223.1	DBNDD2	Dysbindin (dystrobrevin binding protein 1) domain containing 2, transcript variant 3	20	−0.7208	3.1×10^{-4}	.0447
ILMN_1778523	NM_001206.2	KLF9	Kruppel-like factor 9	9	−0.6988	3.0×10^{-4}	.0447
ILMN_1813175	NM_014921.3	LPHN1	Latrophilin 1, transcript variant 2	19	−0.6905	3.0×10^{-4}	.0447
ILMN_1800103	XM_001128785.1	LOC731196	Similar to proprotein convertase subtilisin/kexin type 7 precursor (proprotein convertase PC7) (subtilisin/kexin-like protease PC7) (prohormone convertase PC7) (PC8) (hPC8) (lymphoma proprotein convertase)	11	0.6977	2.9×10^{-4}	.0447
ILMN_1801583	NM_017680.3	ASPN	Asporin	9	−0.7233	2.9×10^{-4}	.0447
ILMN_1740024	NM_005467.2	NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2	11	−0.6949	3.0×10^{-4}	.0449
ILMN_1683133	NM_014079.2	KLF15	Kruppel-like factor 15	3	−0.6842	3.2×10^{-4}	.0451
ILMN_1801441	NM_144629.1	RFTN2	Raftlin family member 2	2	−0.6948	3.2×10^{-4}	.0452

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1719097	NM_013326.3	C18orf8	Chromosome 18 open reading frame 8	18	−0.7098	3.2×10^{-4}	.0452
ILMN_1723689	NM_003624.1	RANBP3	RAN binding protein 3, transcript variant RANBP3-a	19	−0.6899	3.2×10^{-4}	.0452
ILMN_1790052	NM_004659.1	MMP23A	Matrix metallopeptidase 23A	1	−0.7011	3.3×10^{-4}	.0456
ILMN_1679262	NM_001387.2	DPYSL3	Dihydropyrimidinase-like 3	5	−0.7208	3.3×10^{-4}	.0458
ILMN_1683487	NM_003444.1	ZNF154	Zinc finger protein 154 (pHZ-92)	19	−0.6905	3.3×10^{-4}	.0460
ILMN_1710284	NM_005524.2	HES1	Hairy and enhancer of split 1, (Drosophila)	3	−0.7019	3.4×10^{-4}	.0462
ILMN_1728710	NM_001031665.1	ZNF816A	Zinc finger protein 816A	19	0.6975	3.5×10^{-4}	.0462
ILMN_1685156	NM_020983.2	ADCY6	Adenylate cyclase 6, transcript variant 2	12	−0.6890	3.5×10^{-4}	.0464
ILMN_1721087	NM_012435.1	SHC2	SHC (Src homology 2 domain containing) transforming protein 2	19	−0.6788	3.5×10^{-4}	.0465
ILMN_1700811	NM_019116.2	UBFD1	Ubiquitin family domain containing 1	16	0.6888	3.5×10^{-4}	.0466
ILMN_1661066	XM_927710.1	LOC644596	Hypothetical protein LOC644596	X	−0.6663	3.5×10^{-4}	.0466
ILMN_1733769	NM_001033047.1	NPNT	Nephronectin	4	−0.7029	3.5×10^{-4}	.0466
ILMN_1784948	NM_144569.4	SPOCD1	SPOC domain containing 1	1	−0.7223	3.6×10^{-4}	.0467
ILMN_1727574	NM_178835.3	LOC152485	Hypothetical protein LOC152485		−0.6904	3.5×10^{-4}	.0467
ILMN_1724984	NM_004836.4	EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 3	2	0.6981	3.7×10^{-4}	.0467
ILMN_1660305	NM_177966.4	2'-PDE	2'-Phosphodiesterase	3	0.7031	3.5×10^{-4}	.0468
ILMN_1782057	NM_020452.2	ATP8B2	ATPase, class I, type 8B, member 2, transcript variant 1	1	−0.7041	3.6×10^{-4}	.0468
ILMN_1751072	NM_021203.2	SRPRB	Signal recognition particle receptor, B subunit	3	0.6672	3.7×10^{-4}	.0468
ILMN_1740609	NM_032964.2	CCL15	Chemokine (C-C motif) ligand 15, transcript variant 1	17	−0.6697	3.7×10^{-4}	.0468
ILMN_1669982	NM_001080433.1	CCDC85A	Coiled-coil domain containing 85A	2	−0.6858	3.6×10^{-4}	.0468
ILMN_1807515	NM_015235.2	CSTF2T	Cleavage stimulation factor, 3' pre-RNA, subunit 2, 64 kDa, tau variant	10	0.6892	3.5×10^{-4}	.0469
ILMN_1657361	NM_175709.2	CBX7	Chromobox homolog 7	22	−0.6904	3.8×10^{-4}	.0469
ILMN_1801043	NM_198252.2	GSN	Gelsolin (amyloidosis, Finnish type), transcript variant 2	9	−0.7028	3.7×10^{-4}	.0469
ILMN_1738116	NM_181724.1	TMEM119	Transmembrane protein 119	12	−0.6425	3.7×10^{-4}	.0470
ILMN_1760890	NM_206926.1	SEPN1	Selenoprotein N, 1, transcript variant 2	1	−0.6762	3.8×10^{-4}	.0473
ILMN_1728785	NM_015234.4	GPR116	G protein-coupled receptor 116, transcript variant 1	6	−0.6903	3.8×10^{-4}	.0475

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TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1744647	NM_018448.2	CAND1	Cullin-associated and neddylation-dissociated 1	12	0.6883	3.9×10^{-4}	.0479
ILMN_1757440	XM_001130258.1	FAM69B	Family with sequence similarity 69, member B	9	−0.6706	3.9×10^{-4}	.0480
ILMN_1783805	NM_013364.4	PNMA3	Paraneoplastic antigen MA3	X	−0.7005	3.9×10^{-4}	.0482
ILMN_1809098	NM_019599.2	TAS2R1	Taste receptor, type 2, member 1	5	0.7013	4.1×10^{-4}	.0490
ILMN_1719759	NM_002160.2	TNC	Tenascin C (hexabrachion)	9	−0.7107	4.1×10^{-4}	.0491
ILMN_1811313	NM_003062.1	SLIT3	Slit homolog 3 (Drosophila)	5	−0.6810	4.0×10^{-4}	.0491
ILMN_1700432	NM_002221.2	ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	1	−0.6983	4.1×10^{-4}	.0495
ILMN_1809488	NM_014752.1	SPCS2	Signal peptidase complex subunit 2 homolog (S cerevisiae)	11	0.6204	4.2×10^{-4}	.0498
ILMN_1795338	NM_013313.3	YPEL1	Yippee-like 1 (Drosophila)	22	−0.6528	4.3×10^{-4}	.0505
ILMN_1736242	NM_015432.2	PLEKHG4	Pleckstrin homology domain containing, family G (with RhoGef domain) member 4	16	−0.6879	4.3×10^{-4}	.0506
ILMN_1696568	NM_014382.2	ATP2C1	ATPase, Ca++ transporting, type 2C, member 1, transcript variant 1	3	0.6572	4.3×10^{-4}	.0507
ILMN_1766925	NM_001257.3	CDH13	Cadherin 13, H-cadherin (heart)	16	−0.7020	4.3×10^{-4}	.0509
ILMN_1698252	NM_152633.2	FANCB	Fanconi anemia, complementation group B, transcript variant 2	X	0.6928	4.5×10^{-4}	.0526
ILMN_1781149	NM_006774.4	INMT	Indolethylamine N-methyltransferase	7	−0.6688	4.6×10^{-4}	.0530
ILMN_1665437	NM_000773.3	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	10	−0.6839	4.6×10^{-4}	.0531
ILMN_1773395	NM_002905.2	RDH5	Retinol dehydrogenase 5 (11-cis/9-cis)	12	−0.6860	4.6×10^{-4}	.0533
ILMN_1665483	NM_014878.4	KIAA0020	KIAA0020	9	0.6892	4.6×10^{-4}	.0534
ILMN_1666545	NM_001097635.1	GCNT1	Glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-acetylglucosaminyltransferase), transcript variant 4	9	0.6953	4.6×10^{-4}	.0535
ILMN_1743864	NM_001453.2	FOXC1	Forkhead box C1	6	−0.6694	4.8×10^{-4}	.0542
ILMN_1709486	NM_006307.3	SRPX	Sushi-repeat-containing protein, X-linked	X	−0.6834	4.8×10^{-4}	.0543
ILMN_1676088	NM_198080.2	MSRB3	Methionine sulfoxide reductase B3, transcript variant 1	12	−0.6889	4.8×10^{-4}	.0543
ILMN_1771238	NM_000390.2	CHM	Choroideremia (Rab escort protein 1), transcript variant 1	X	0.6744	4.9×10^{-4}	.0544
ILMN_1656807	NM_000988.3	RPL27	Ribosomal protein L27	17	−0.6987	4.9×10^{-4}	.0545
ILMN_1711826	NM_020344.1	SLC24A2	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	9	−0.6804	5.0×10^{-4}	.0545

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1660730	NM_032803.4	SLC7A3	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 3, transcript variant 1	X	−0.6816	4.9×10^{-4}	.0546
ILMN_1849218	BX451947	Hs.559564	BX451947 FETAL BRAIN cDNA clone CS0DF008YL16 5-PRIME sequence		−0.6910	4.9×10^{-4}	.0547
ILMN_1726752	NM_175071.1	APTX	Aprataxin, transcript variant 5	9	0.6732	4.9×10^{-4}	.0548
ILMN_1739640	NM_003737.2	DCHS1	Dachsous 1 (Drosophila)	11	−0.6808	5.0×10^{-4}	.0550
ILMN_1686968	NM_152493.2	FLJ25476	FLJ25476 protein	1	−0.6856	5.1×10^{-4}	.0557
ILMN_1718044	NM_018127.5	ELAC2	elaC Homolog 2 (E coli)	17	−0.6695	5.3×10^{-4}	.0562
ILMN_1799836	NM_006735.3	HOXA2	Homeobox A2	7	−0.7036	5.3×10^{-4}	.0563
ILMN_1658847	XM_939432.1	MGC61598	Similar to ankyrin-repeat protein Nrarp	9	−0.6363	5.3×10^{-4}	.0565
ILMN_1764619	NM_207443.1	FLJ45244	FLJ45244 protein	14	−0.6691	5.3×10^{-4}	.0567
ILMN_1739521	NM_014932.2	NLGN1	Neuroligin 1	3	0.6893	5.4×10^{-4}	.0568
ILMN_1710675	NM_005080.2	XBP1	X-box binding protein 1, transcript variant 1	22	0.6814	5.3×10^{-4}	.0568
ILMN_1772810	XM_946142.2	SHANK3	SH3 and multiple ankyrin repeat domains 3, transcript variant 4	22	−0.6733	5.4×10^{-4}	.0570
ILMN_1693481	NM_021949.2	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3, transcript variant 1	X	0.6669	5.3×10^{-4}	.0570
ILMN_1671106	NM_002060.2	GJA4	Gap junction protein, alpha 4, 37 kDa	1	−0.6706	5.3×10^{-4}	.0572
ILMN_1773757	NM_138718.1	SLC26A8	Solute carrier family 26, member 8, transcript variant 2	6	0.6936	5.5×10^{-4}	.0573
ILMN_1680652	NM_003944.2	SELENBP1	Selenium binding protein 1	1	−0.6566	5.6×10^{-4}	.0585
ILMN_1813528	NM_133459.1	CCBE1	Collagen and calcium binding EGF domains 1	18	−0.6806	5.7×10^{-4}	.0587
ILMN_1715175	NM_000245.2	MET	Met protooncogene (hepatocyte growth factor receptor)	7	0.6834	5.7×10^{-4}	.0587
ILMN_1688160	NM_182552.3	WDR27	WD repeat domain 27	6	−0.6906	5.7×10^{-4}	.0587
ILMN_1805842	NM_001449.3	FHL1	Four and a half LIM domains 1	X	−0.6833	5.6×10^{-4}	.0587
ILMN_1806301	NM_002077.2	GOLGA1	Golgi autoantigen, golgin subfamily a, 1	9	−0.6603	5.8×10^{-4}	.0595
ILMN_1734653	NM_032532.2	FNDC1	Fibronectin type III domain containing 1	6	−0.6810	5.9×10^{-4}	.0596
ILMN_1706935	NM_022742.3	CCDC136	Coiled-coil domain containing 136	7	−0.6766	5.9×10^{-4}	.0597
ILMN_1727091	NM_138326.2	ACMSD	Aminocarboxymuconate semialdehyde decarboxylase	2	0.6688	5.9×10^{-4}	.0597
ILMN_1740385	NM_014956.4	CEP164	Centrosomal protein 164 kDa	11	−0.6244	5.9×10^{-4}	.0598
ILMN_1746517	NM_003937.2	KYNU	Kynureninase (L-kynurenine hydrolase), transcript variant 1	2	0.6445	6.0×10^{-4}	.0598

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1801246	NM_003641.3	IFITM1	Interferon-induced transmembrane protein 1 (9-27)	11	−0.6716	6.0×10^{-4}	.0599
ILMN_1756784	NM_014286.2	FREQ	Frequenin homolog (Drosophila)	9	−0.6824	6.1×10^{-4}	.0599
ILMN_1652389	NM_001031733.2	CALML4	Calmodulin-like 4, transcript variant 2	15	−0.6783	6.1×10^{-4}	.0600
ILMN_1794038	NM_030797.2	FAM49A	Family with sequence similarity 49, member A	2	0.6333	6.1×10^{-4}	.0601
ILMN_1758731	NM_000775.2	CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2	1	0.6767	6.1×10^{-4}	.0602
ILMN_1707380	NM_002725.3	PRELP	Proline/arginine-rich end leucine-rich repeat protein, transcript variant 1	1	−0.6844	6.1×10^{-4}	.0603
ILMN_1801226	NM_020812.1	DOCK6	Dedicator of cytokinesis 6	19	−0.6576	6.1×10^{-4}	.0605
ILMN_1766386	XR_017805.1	LOC401433	Hypothetical gene supported by AK127717, misc RNA	7	−0.6178	6.2×10^{-4}	.0607
ILMN_1763657	NM_025212.1	CXXC4	CXXC finger 4	4	−0.6560	6.3×10^{-4}	.0608
ILMN_1777221	NM_058182.2	C21orf51	Chromosome 21 open reading frame 51	21	−0.6266	6.3×10^{-4}	.0612
ILMN_1712199	NM_024738.1	C12orf49	Chromosome 12 open reading frame 49	12	0.6531	6.4×10^{-4}	.0619
ILMN_1741801	NM_003503.2	CDC7	Cell division cycle 7 homolog (S cerevisiae)	1	0.6725	6.6×10^{-4}	.0631
ILMN_1891067	AK127526	Hs.553187	cDNA FLJ45619 fis, clone BRTHA3027318		0.6413	6.6×10^{-4}	.0632
ILMN_1663843	NM_004161.3	RAB1A	RAB1A, member RAS oncogene family	2	0.6529	6.7×10^{-4}	.0632
ILMN_1792571	NM_173728.2	ARHGEF15	Rho guanine nucleotide exchange factor (GEF) 15	17	−0.6508	6.7×10^{-4}	.0632
ILMN_1790315	NM_001039706.1	FLJ21062	Hypothetical protein FLJ21062	7	−0.6657	6.6×10^{-4}	.0634
ILMN_1733756	NM_080645.2	COL12A1	Collagen, type XII, alpha 1, transcript variant short	6	−0.6799	6.8×10^{-4}	.0638
ILMN_1812701	NM_001099783.1	C4orf33	Chromosome 4 open reading frame 33, transcript variant 2	4	0.6666	6.8×10^{-4}	.0640
ILMN_1782257	NM_022734.2	METT11D1	Methyltransferase 11 domain containing 1, transcript variant 2	14	−0.6653	6.9×10^{-4}	.0643
ILMN_1691112	NM_176787.4	PIGN	Phosphatidylinositol glycan anchor biosynthesis, class N, transcript variant 1	18	0.6741	6.9×10^{-4}	.0646
ILMN_1756086	NM_023015.3	INTS3	Integrator complex subunit 3	1	−0.6306	6.9×10^{-4}	.0648
ILMN_1710303	NM_031421.2	TTC25	Tetratricopeptide repeat domain 25	17	−0.6482	7.0×10^{-4}	.0651
ILMN_1785765	NM_004800.1	TM9SF2	Transmembrane 9 superfamily member 2	13	0.6617	7.1×10^{-4}	.0656
ILMN_1684321	NM_030579.2	CYB5B	Cytochrome b5 type B (outer mitochondrial membrane)	16	0.6858	7.1×10^{-4}	.0658

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1722244	NM_001018011.1	ZBTB16	Zinc finger and BTB domain containing 16, transcript variant 2	11	−0.6734	7.2×10^{-4}	.0660
ILMN_1787906	NM_014629.2	ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	8	−0.6634	7.3×10^{-4}	.0668
ILMN_1736974	NM_006943.2	SOX12	SRY (sex determining region Y)-box 12	20	−0.6435	7.4×10^{-4}	.0668
ILMN_1808590	NM_000856.3	GUCY1A3	Guanylate cyclase 1, soluble, alpha 3	4	−0.6252	7.3×10^{-4}	.0669
ILMN_1751559	NM_024600.2	C16orf30	Chromosome 16 open reading frame 30	16	−0.6442	7.4×10^{-4}	.0671
ILMN_1774427	NM_020898.1	CALCOCO1	Calcium binding and coiled-coil domain 1	12	−0.6804	7.7×10^{-4}	.0682
ILMN_1657502	NM_001098515.1	MRGPRF	MAS-related GPR, member F, transcript variant 1	11	−0.6781	7.6×10^{-4}	.0682
ILMN_1652128	NM_018368.2	LMBRD1	LMBR1 domain containing 1	6	0.6306	7.8×10^{-4}	.0683
ILMN_1808417	NM_015102.2	NPHP4	Nephronophthisis 4	1	−0.6615	7.7×10^{-4}	.0684
ILMN_1657194	NM_018430.2	TSNAXIP1	Translin-associated factor X interacting protein 1	16	−0.6513	7.7×10^{-4}	.0684
ILMN_1680948	NM_012134.2	LMOD1	Leiomodin 1 (smooth muscle)	1	−0.6757	7.7×10^{-4}	.0684
ILMN_1703471	NM_007348.2	ATF6	Activating transcription factor 6	1	0.6569	7.6×10^{-4}	.0684
ILMN_1728742	NM_032385.3	C5orf4	Chromosome 5 open reading frame 4, transcript variant 2	5	−0.6564	7.8×10^{-4}	.0686
ILMN_1702861	NM_172244.2	SGCD	Sarcoglycan, delta (35 kDa dystrophin-associated glycoprotein), transcript variant 2	5	−0.6460	7.6×10^{-4}	.0686
ILMN_1868150	BX537697	Hs.98581	mRNA; cDNA DKFZp686D0853 (from clone DKFZp686D0853)		−0.6645	7.9×10^{-4}	.0686
ILMN_1694325	NM_002501.2	NFIX	Nuclear factor I/X (CCAAT-binding transcription factor)	19	−0.6525	7.8×10^{-4}	.0687
ILMN_1748432	XM_375646.3	ZNF525	Zinc finger protein 525	19	0.6710	8.0×10^{-4}	.0691
ILMN_1743357	NM_003399.5	XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	X	−0.6388	8.0×10^{-4}	.0694
ILMN_1782125	NM_024422.2	DSC2	Desmocollin 2, transcript variant Dsc2a	18	0.6142	8.0×10^{-4}	.0695
ILMN_1687967	NM_001007156.1	NTRK3	Neurotrophic tyrosine kinase, receptor, type 3, transcript variant 3	15	−0.6630	8.1×10^{-4}	.0699
ILMN_1685286	NM_017607.2	PPP1R12C	Protein phosphatase 1, regulatory (inhibitor) subunit 12C	19	−0.6754	8.3×10^{-4}	.0710
ILMN_1756937	NM_005668.3	ST8SIA4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4, transcript variant 1	5	0.6470	8.3×10^{-4}	.0711

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1794534	NM_021827.3	CCDC81	Coiled-coil domain containing 81	11	−0.6588	8.4×10^{-4}	.0712
ILMN_1793615	NM_001014811.1	ME3	Malic enzyme 3, NADP(+)-dependent, mitochondrial, nuclear gene encoding mitochondrial protein, transcript variant 2	11	−0.6720	8.5×10^{-4}	.0716
ILMN_1885397	BM311228	Hs.503590	ig62e09y1 HR85 islet cDNA 5 sequence		−0.6606	8.5×10^{-4}	.0717
ILMN_1759375	NM_001083330.1	ZNF133	Zinc finger protein 133, transcript variant 2	20	−0.6532	8.5×10^{-4}	.0719
ILMN_1796851	XM_001131060.1	FOXL2	Forkhead box L2	3	−0.6417	8.6×10^{-4}	.0720
ILMN_1703105	NM_139178.2	ALKBH3	alkB, Alkylation repair homolog 3 (E coli)	11	−0.6275	8.5×10^{-4}	.0720
ILMN_1678710	NM_032439.1	PHYHIPL	Phytanoyl-CoA 2-hydroxylase interacting protein-like	10	0.6064	8.8×10^{-4}	.0733
ILMN_1758398	NM_000858.4	GUK1	Guanylate kinase 1	1	−0.6158	8.9×10^{-4}	.0735
ILMN_1796734	NM_003118.2	SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	5	−0.6468	8.8×10^{-4}	.0737
ILMN_1653856	NM_032873.3	STS-1	Cbl-interacting protein Sts-1	11	0.6288	8.9×10^{-4}	.0739
ILMN_1795251	NM_004684.3	SPARCL1	SPARC-like 1 (mast9, hevin)	4	−0.6301	9.0×10^{-4}	.0746
ILMN_1717206	NM_175060.1	CLEC14A	C-type lectin domain family 14, member A	14	−0.6376	9.2×10^{-4}	.0754
ILMN_1739496	NM_006902.3	PRRX1	Paired related homeobox 1, transcript variant pmx-1a	1	−0.6477	9.2×10^{-4}	.0754
ILMN_1718552	NM_006419.1	CXCL13	Chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	4	−0.6037	9.2×10^{-4}	.0755
ILMN_1797191	NM_014656.1	KIAA0040	KIAA0040	1	0.6227	9.2×10^{-4}	.0757
ILMN_1737705	NM_015054.1	KIAA0701	KIAA0701 protein, transcript variant 1	12	0.6459	9.5×10^{-4}	.0770
ILMN_1682781	NM_003598.1	TEAD2	TEA domain family member 2	19	−0.6440	9.5×10^{-4}	.0771
ILMN_1673352	NM_006435.2	IFITM2	Interferon-induced transmembrane protein 2 (1-8D)	11	−0.6409	9.6×10^{-4}	.0772
ILMN_1750158	NM_007292.4	ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl, transcript variant 2	17	0.6554	9.7×10^{-4}	.0772
ILMN_1657156	NM_207306.2	KIAA0495	KIAA0495	1	−0.6716	9.7×10^{-4}	.0773
ILMN_1787576	NM_004070.3	CLCNKA	Chloride channel Ka, transcript variant 1	1	−0.6609	9.6×10^{-4}	.0773
ILMN_1665449	NM_019055.4	ROBO4	Roundabout homolog 4, magic roundabout (Drosophila)	11	−0.6256	9.7×10^{-4}	.0773
ILMN_1796018	NM_004554.3	NFATC4	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4	14	−0.6726	9.6×10^{-4}	.0774
ILMN_1765118	NM_003627.4	SLC43A1	Solute carrier family 43, member 1	11	−0.6174	9.9×10^{-4}	.0784

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1785424	NM_006720.3	ABLIM1	Actin-binding LIM protein 1, transcript variant 4	10	−0.6004	9.9×10^{-4}	.0784
ILMN_1701204	NM_005429.2	VEGFC	Vascular endothelial growth factor C	4	−0.6393	9.9×10^{-4}	.0784
ILMN_1769186	NM_001755.2	CBFB	Core-binding factor, beta subunit, transcript variant 2	16	0.6454	1.0×10^{-3}	.0785
ILMN_1651958	NM_000900.2	MGP	Matrix Gla protein	12	−0.6404	1.0×10^{-3}	.0786
ILMN_1770803	NM_004330.1	BNIP2	BCL2/adenovirus E1B 19 kDa interacting protein 2	15	0.6074	1.0×10^{-3}	.0786
ILMN_1720452	NM_001031855.1	LONRF3	LON peptidase N-terminal domain and ring finger 3, transcript variant 1	X	0.6477	1.0×10^{-3}	.0786
ILMN_1780349	NM_003292.2	TPR	Translocated promoter region (to activated MET oncogene)	1	−0.6328	1.0×10^{-3}	.0792
ILMN_1818018	DA321576	Hs.576997	DA321576 BRHIP3 cDNA clone BRHIP3014850 5 sequence		0.6451	1.0×10^{-3}	.0793
ILMN_1724424	NM_145239.1	PRRT2	Proline-rich transmembrane protein 2	16	−0.6496	1.0×10^{-3}	.0794
ILMN_1760849	NM_018092.3	NETO2	Neuropilin (NRP) and tolloid (TLL)-like 2	16	0.6201	1.0×10^{-3}	.0794
ILMN_1773742	NM_012328.1	DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	7	0.6452	1.0×10^{-3}	.0794
ILMN_1792529	NM_004783.2	TAOK2	TAO kinase 2, transcript variant 1	16	−0.6358	1.0×10^{-3}	.0796
ILMN_1740772	NM_133172.2	APBB3	Amyloid beta (A4) precursor protein-binding, family B, member 3, transcript variant 3	5	−0.6393	1.1×10^{-3}	.0796
ILMN_1737604	NM_018291.2	FLJ10986	Hypothetical protein FLJ10986	1	0.6581	1.0×10^{-3}	.0796
ILMN_1742272	NM_000537.2	REN	Renin	1	−0.6262	1.1×10^{-3}	.0798
ILMN_1806403	NM_016563.2	RASL12	RAS-like, family 12	15	−0.6338	1.1×10^{-3}	.0798
ILMN_1715647	NM_020335.1	VANGL2	Vang-like 2 (van gogh, Drosophila)	1	−0.6544	1.1×10^{-3}	.0800
ILMN_1655913	NM_005013.2	NUCB2	Nucleobindin 2	11	0.6408	1.1×10^{-3}	.0801
ILMN_1736080	NM_012432.2	SETDB1	SET domain, bifurcated 1	1	−0.6417	1.1×10^{-3}	.0805
ILMN_1663033	NM_138385.2	TMEM129	Transmembrane protein 129	4	−0.6319	1.1×10^{-3}	.0809
ILMN_1697585	NM_022496.3	ACTR6	ARP6 actin-related protein 6 homolog (yeast)	12	0.6055	1.1×10^{-3}	.0817
ILMN_1711124	NM_144724.1	MARVELD2	MARVEL domain containing 2, transcript variant 2	5	0.6463	1.1×10^{-3}	.0820
ILMN_1711919	NM_017988.4	SCYL2	SCY1-like 2 (S cerevisiae)	12	0.5937	1.1×10^{-3}	.0821
ILMN_1669142	NM_057175.3	NARG1	NMDA receptor regulated 1	4	0.6007	1.1×10^{-3}	.0822
ILMN_1678862	NM_173540.2	FUT11	Fucosyltransferase 11 (alpha [1,3] fucosyltransferase)	10	0.6192	1.1×10^{-3}	.0822
ILMN_1768393	NM_006938.2	SNRPD1	Small nuclear ribonucleoprotein D1 polypeptide 16 kDa	18	0.5974	1.1×10^{-3}	.0822

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782938	NM_018593.3	SLC16A10	Solute carrier family 16, member 10 (aromatic amino acid transporter)	6	0.5965	1.1×10^{-3}	.0822
ILMN_1899428	AW173494	Hs.483540	xj07f12x1 NCI_CGAP_Ut2 cDNA clone IMAGE:2656559 3 sequence		−0.6320	1.1×10^{-3}	.0825
ILMN_1748845	NM_002506.2	NGFB	Nerve growth factor, beta polypeptide	1	−0.6390	1.1×10^{-3}	.0832
ILMN_1767722	NM_203437.2	AFTPH	Aftiphilin, transcript variant 1	2	0.6445	1.2×10^{-3}	.0837
ILMN_1791545	NM_015515.3	KRT23	Keratin 23 (histone deacetylase inducible)	17	0.6391	1.2×10^{-3}	.0838
ILMN_1747183	NM_001099650.1	GLT8D3	Glycosyltransferase 8 domain containing 3, transcript variant 2	12	0.6353	1.2×10^{-3}	.0839
ILMN_1815666	NM_170665.2	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2, transcript variant 1	12	0.6340	1.2×10^{-3}	.0840
ILMN_1761425	NM_182487.2	OLFML2A	Olfactomedin-like 2A	9	−0.6347	1.2×10^{-3}	.0843
ILMN_1794825	NM_000382.2	ALDH3A2	Aldehyde dehydrogenase 3 family, member A2, transcript variant 2	17	−0.6101	1.2×10^{-3}	.0845
ILMN_1767459	NM_018082.4	POLR3B	Polymerase (RNA) III (DNA directed) polypeptide B	12	0.6220	1.2×10^{-3}	.0851
ILMN_1717905	NM_015726.2	WDR42A	WD repeat domain 42A	1	−0.6202	1.2×10^{-3}	.0853
ILMN_1682404	NM_006515.1	SETMAR	SET domain and mariner transposase fusion gene	3	−0.6306	1.2×10^{-3}	.0861
ILMN_1725338	NM_194284.2	CLDN23	Claudin 23	8	0.6369	1.2×10^{-3}	.0863
ILMN_1765371	NM_018032.3	LUC7L	LUC7-like (S cerevisiae), transcript variant 1	16	−0.6428	1.2×10^{-3}	.0863
ILMN_1756118	NM_014634.2	PPM1F	Protein phosphatase 1F (PP2C domain containing)	22	−0.5914	1.2×10^{-3}	.0864
ILMN_1793621	NM_001002262.1	ZFYVE27	Zinc finger, FYVE domain containing 27, transcript variant 3	10	0.6345	1.2×10^{-3}	.0865
ILMN_1654945	NM_153759.2	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha, transcript variant 2	2	0.6434	1.2×10^{-3}	.0866
ILMN_1754364	NM_001868.1	CPA1	Carboxypeptidase A1 (pancreatic)	7	0.6196	1.2×10^{-3}	.0866
ILMN_1663640	NM_000240.2	MAOA	Monoamine oxidase A, nuclear gene encoding mitochondrial protein	X	0.6353	1.3×10^{-3}	.0874
ILMN_1728581	NM_016210.2	C3orf18	Chromosome 3 open reading frame 18	3	−0.6159	1.3×10^{-3}	.0876
ILMN_1736834	NM_005414.2	SKIL	SKI-like oncogene	3	0.6206	1.3×10^{-3}	.0883
ILMN_1800731	NM_018328.3	MBD5	Methyl-CpG binding domain protein 5	2	−0.6235	1.3×10^{-3}	.0884

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1805098	NM_000924.2	PDE1B	Phosphodiesterase 1B, calmodulin-dependent	12	−0.6434	1.3×10^{-3}	.0884
ILMN_1769764	NM_001039935.1	ANKRD55	Ankyrin repeat domain 55, transcript variant 2	5	0.6090	1.3×10^{-3}	.0885
ILMN_1814015	NM_004063.2	CDH17	Cadherin 17, LI cadherin (liver-intestine)	8	−0.6315	1.3×10^{-3}	.0885
ILMN_1802669	NM_021132.1	PPP3CB	Protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform	10	−0.6361	1.3×10^{-3}	.0888
ILMN_1800512	NM_002133.1	HMOX1	Heme oxygenase (decycling) 1	22	0.5922	1.3×10^{-3}	.0888
ILMN_1772731	NM_005326.4	HAGH	Hydroxyacylglutathione hydrolase, transcript variant 1	16	−0.6280	1.3×10^{-3}	.0890
ILMN_1756573	NM_020142.3	NDUFA4L2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2	12	−0.6186	1.3×10^{-3}	.0891
ILMN_1686464	NM_180991.4	SLCO4C1	Solute carrier organic anion transporter family, member 4C1	5	0.6381	1.3×10^{-3}	.0903
ILMN_1769083	NM_000847.3	GSTA3	Glutathione S-transferase A3	6	0.6117	1.3×10^{-3}	.0904
ILMN_1687410	NM_022776.3	OSBPL11	Oxysterol binding protein-like 11	3	0.6035	1.4×10^{-3}	.0918
ILMN_1651611	NM_000527.2	LDLR	Low-density lipoprotein receptor (familial hypercholesterolemia)	19	0.6122	1.4×10^{-3}	.0937
ILMN_1665123	NM_178177.2	NMNAT3	Nicotinamide nucleotide adenylyltransferase 3	3	−0.6152	1.4×10^{-3}	.0938
ILMN_1651370	NM_001014443.2	USP21	Ubiquitin-specific peptidase 21, transcript variant 3	1	−0.6228	1.4×10^{-3}	.0939
ILMN_1774110	NM_004067.2	CHN2	Chimerin (chimaerin) 2, transcript variant 2	7	0.6275	1.4×10^{-3}	.0950
ILMN_1730662	NM_001008745.1	LOC401431	Hypothetical gene LOC401431	7	−0.6048	1.4×10^{-3}	.0950
ILMN_1753554	NM_022763.2	FNDC3B	Fibronectin type III domain containing 3B	3	0.6167	1.4×10^{-3}	.0950
ILMN_1734254	NM_014106.2	ZNF770	Zinc finger protein 770	15	0.5801	1.4×10^{-3}	.0951
ILMN_1801889	NM_015011.1	MYO16	Myosin XVI	13	−0.6401	1.4×10^{-3}	.0951
ILMN_1703074	NM_001304.3	CPD	Carboxypeptidase D	17	0.6178	1.4×10^{-3}	.0952
ILMN_1885728	XM_001130020.1	KIAA1147	KIAA1147	7	0.6203	1.5×10^{-3}	.0952
ILMN_1652594	NM_024855.3	ACTR5	ARP5 actin-related protein 5 homolog (yeast)	20	−0.6197	1.4×10^{-3}	.0953
ILMN_1672287	NM_018657.3	MYNN	Myoneurin	3	0.6237	1.5×10^{-3}	.0954
ILMN_1680113	NM_004758.1	BZRAP1	Benzodiazepine receptor (peripheral)-associated protein 1	17	−0.6340	1.5×10^{-3}	.0966
ILMN_1660282	NM_022135.2	POPDC2	Popeye domain containing 2	3	−0.6316	1.5×10^{-3}	.0967
ILMN_1683441	NM_015261.2	NCAPD3	Non-SMC condensin II complex, subunit D3	11	−0.6169	1.5×10^{-3}	.0968
ILMN_1761486	NM_024808.2	C13orf34	Chromosome 13 open reading frame 34	13	0.6187	1.5×10^{-3}	.0970

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1894569	BX093121	Hs.571048	BX093121 Soares_placenta_ 8to9weeks_2NbHP8to9W cDNA clone IMAGp998K13561; IMAGE:257796 sequence		0.6106	1.5×10^{-3}	.0970
ILMN_1751086	NM_015459.3	DKFZP564J0863	DKFZP564J0863 protein	11	0.5780	1.5×10^{-3}	.0970
ILMN_1656386	NM_014822.1	SEC24D	SEC24-related gene family, member D (S cerevisiae)	4	0.6073	1.5×10^{-3}	.0971
ILMN_1760271	NM_194314.2	ZBTB41	Zinc finger and BTB domain containing 41	1	0.6038	1.5×10^{-3}	.0972
ILMN_1702683	NM_004733.2	SLC33A1	Solute carrier family 33 (acetyl-CoA transporter), member 1	3	0.6196	1.5×10^{-3}	.0972
ILMN_1878019	AL512695	Hs.278285	mRNA; cDNA DKFZp547G133 (from clone DKFZp547G133)		0.6348	1.5×10^{-3}	.0973
ILMN_1806487	NM_001002034.2	FAM109B	Family with sequence similarity 109, member B	22	−0.5735	1.5×10^{-3}	.0973
ILMN_1779748	NM_004192.2	ASMTL	Acetylserotonin O-methyltransferase-like	X,Y	−0.6146	1.5×10^{-3}	.0974
ILMN_1770084	NM_006283.1	TACC1	Transforming, acidic coiled-coil containing protein 1	8	−0.6145	1.6×10^{-3}	.0974
ILMN_1707534	NM_017544.2	NKRF	NF-kappaB repressing factor	X	0.6044	1.6×10^{-3}	.0974
ILMN_1678086	NM_138770.1	CCDC74A	Coiled-coil domain containing 74A	2	−0.6070	1.6×10^{-3}	.0975
ILMN_1669064	NM_001080493.2	HSZFP36	ZFP-36 for a zinc finger protein	19	0.6087	1.5×10^{-3}	.0975
ILMN_1810093	NM_005725.3	TSPAN2	Tetraspanin 2	1	−0.5941	1.5×10^{-3}	.0976
ILMN_1673522	NM_017947.1	MOCOS	Molybdenum cofactor sulfurase	18	0.6122	1.6×10^{-3}	.0976
ILMN_1764309	NM_000667.2	ADH1A	Alcohol dehydrogenase 1A (class I), alpha polypeptide	4	−0.6229	1.6×10^{-3}	.0977
ILMN_1795325	NM_001615.3	ACTG2	Actin, gamma 2, smooth muscle, enteric	2	−0.6151	1.6×10^{-3}	.0977
ILMN_1773814	NM_205853.2	MUSTN1	Musculoskeletal, embryonic nuclear protein 1	3	−0.6206	1.5×10^{-3}	.0977
ILMN_1703576	NM_012334.2	MYO10	Myosin X	5	0.6086	1.6×10^{-3}	.0977
ILMN_1780937	NM_025128.3	MUS81	MUS81 endonuclease homolog (S cerevisiae)	11	−0.6341	1.6×10^{-3}	.0977
ILMN_1757162	XM_945736.2	LOC654085	Similar to Glycine cleavage system H protein, mitochondrial precursor, transcript variant 2	19	0.6181	1.5×10^{-3}	.0977
ILMN_1832155	AK094744	Hs.167721	cDNA FLJ37425 fis, clone BRAWH2001530		−0.6007	1.6×10^{-3}	.0977
ILMN_1782688	NM_024838.4	THNSL1	Threonine synthase-like 1 (S cerevisiae)	10	0.6374	1.6×10^{-3}	.0977
ILMN_1757298	NM_018167.3	BTBD7	BTB (POZ) domain containing 7, transcript variant 2	14	0.6056	1.6×10^{-3}	.0978
ILMN_1798975	NM_005228.3	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral [v-erb-b] oncogene homolog, avian), transcript variant 1	7	0.6186	1.6×10^{-3}	.0978

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2010. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1775974	NM_019012.2	PLEKHA5	Pleckstrin homology domain containing, family A member 5	12	0.5995	1.6×10^{-3}	.0979
ILMN_1872404	AK055652	Hs.478682	cDNA FLJ31090 fis, clone IMR321000102		−0.6351	1.6×10^{-3}	.0979
ILMN_1808999	NM_153213.3	ARHGEF19	Rho guanine nucleotide exchange factor (GEF) 19	1	−0.6104	1.6×10^{-3}	.0979
ILMN_1733703	NM_018006.4	TRMU	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase, nuclear gene encoding mitochondrial protein, transcript variant 1	22	−0.6098	1.6×10^{-3}	.0980
ILMN_1795574	XM_928045.1	LOC644968	Hypothetical protein LOC644968	4	0.5916	1.6×10^{-3}	.0980
ILMN_1700994	NM_001039571.1	KREMEN1	Kringle containing transmembrane protein 1, transcript variant 4	22	0.6255	1.6×10^{-3}	.0980
ILMN_1737146	NM_014294.4	TRAM1	Translocation-associated membrane protein 1	8	0.6212	1.6×10^{-3}	.0980
ILMN_1809889	NM_173510.1	CCDC117	Coiled-coil domain containing 117	22	0.6109	1.6×10^{-3}	.0981
ILMN_1735909	NM_001033678.2	TRPT1	tRNA phosphotransferase 1, transcript variant 1	11	−0.6155	1.6×10^{-3}	.0982
ILMN_1670472	NM_014613.2	UBXD8	UBX domain containing 8	5	0.6387	1.7×10^{-3}	.0986
ILMN_1700633	NM_022060.2	ABHD4	Abhydrolase domain containing 4	14	−0.5964	1.7×10^{-3}	.0988
ILMN_1914072	BQ718005	Hs.562762	AGENCOURT_8100698 Lupski_sympathetic_trunk cDNA clone IMAGE:6190431 5 sequence		0.6098	1.7×10^{-3}	.0989
ILMN_1651642	NM_152742.1	GPC2	Glypican 2	7	−0.6187	1.7×10^{-3}	.0990
ILMN_1671046	NM_001541.2	HSPB2	Heat shock 27-kDa protein 2	11	−0.6162	1.7×10^{-3}	.0990
ILMN_1662578	NM_020156.1	C1GALT1	Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1	7	0.5897	1.7×10^{-3}	.0990
ILMN_1693514	NM_001014795.1	ILK	Integrin-linked kinase, transcript variant 3	11	−0.6264	1.7×10^{-3}	.0992
ILMN_1800447	NM_001031835.1	PHKB	Phosphorylase kinase, beta, transcript variant 2	16	0.5895	1.7×10^{-3}	.0992
ILMN_1701933	NM_007308.1	SNCA	Synuclein, alpha (non-A4 component of amyloid precursor), transcript variant NACP112	4	−0.5903	1.7×10^{-3}	.0993
ILMN_1779547	NM_006665.2	HPSE	Heparanase	4	0.6297	1.7×10^{-3}	.0995
ILMN_1883624	DA589983	Hs.582952	DA589983 HLUNG2 cDNA clone HLUNG2011800 5 sequence		0.5802	1.7×10^{-3}	.0997
ILMN_1774717	NM_020182.3	TMEPAI	Transmembrane, prostate androgen-induced RNA, transcript variant 1	20	−0.5933	1.7×10^{-3}	.0998

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TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1789463	NM_021902.2	FXYD1	FXYD domain containing ion transport regulator 1 (phospholemman), transcript variant b	19	−0.6164	1.7×10^{-3}	.0999
ILMN_1651900	NM_002233.2	KCNA4	Potassium voltage-gated channel, shaker-related subfamily, member 4	11	0.6164	1.7×10^{-3}	.0999

FDR, false discovery rate; *mRNA*, messenger RNA.

[a] Gene expression in decidual tissue from preeclamptic and normal pregnancies has been compared; [b] Values are given in beta, measure of distance between group means, expressed in SD units–positive beta implies up-regulation and negative beta implies down-regulation in preeclamptic group compared with normal pregnant group; [c] $P < .05$, obtained with SOLAR; [d] FDR $P < .10$.

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may be influenced by gestational age,[23,24] it cannot be excluded that some of the differences observed between the preeclamptic and normal pregnancy groups are, in fact, gestational age related. Winn et al[23] compared global gene expression in basal plate (decidual) biopsies from normal pregnancies at mid-gestation (14-24 weeks) and at term (37-40 weeks) and found that 418 genes (of 39,000 transcripts examined) changed expression throughout gestation. This provides a useful dataset for comparison with the data obtained in this current study, albeit different profiling platforms were used. Winn et al[23] used the Affymetrix HG-U133 A&B chip for transcriptional profiling, whereas we used the Illumina HumanWG-6 v2 Expression BeadChip. By this, the number of possible comparisons was restricted to the 16,799 genes shared in both systems. Of the 455 transcripts found to be differentially expressed in this current study, 368 genes demonstrate no gestational age-influenced changes, according to the data of Winn et al.[23] It is therefore tempting to speculate that the differential expression of these 368 genes may be related to disease mechanisms at play in preeclampsia. Seventeen of our differentially expressed genes (TEMEM97, KIAA1598, SULT2B1, EGFR, FHL1, PLA2G7, SHANK3, NOTCH4, UBASH3B, ROBO4, NRARP, GPR116, IL6ST, LDLR, ANGPTL2, SRPRB, and KREMEN1) are reported to change expression with gestational age.[23] For 2 of these genes (SULT2B1 and EGFR), expression increases toward term.[23] Thus, isolated gestational age-related influences in the preeclampsia group would suggest a lower expression of SULT2B1 and EGFR, but both were up-regulated in our dataset. Similarly, the ANGPTL2 gene is down-regulated toward term,[23] but in contrast to what might be expected from gestational age–related changes, expression was lower in the preeclampsia group than in the normal pregnancy group. Based on this, we conclude that the differential expression of these 3 genes may also be ascribed to disease-related mechanisms. However, with regard to the remaining 14 genes in our dataset previously shown to exhibit gestational age-dependent changes in expression, conclusions are hampered by the fact that gestational age may have contributed to the differences observed between preeclamptic and normal pregnancies. To illustrate: expression of FHL1, SHANK3, NOTCH4, ROBO4, NRARP, and GPR116 increases toward term[23] and was down-regulated in the preeclampsia group, whereas TMEM97, KIAA1598, PLA2G7, UBASH3B, IL6ST, LDLR, SRPRB, and

TABLE 3
Results for selected genes from microarray and real-time quantitative polymerase chain reaction expression

		Microarray		RT-qPCR	
Gene symbol	Up/down	Beta value	*P* value[a]	Fold change	*P* value[b]
SLITRK4	↓	−1.04	4.59×10^{-8}	−1.98	< .0001 1.73 x 10-5
FZD4	↓	−0.91	4.05×10^{-7}	−1.35	.001 7.71 x 10-4
ANGPTL2	↓	−0.89	4.39×10^{-6}	−1.74	< .0001 4.79 x 10-5
PLA2G7	↑	0.83	1.58×10^{-5}	1.26	.068 6.79 x 10-2
MAN1A	↑	0.85	1.29×10^{-5}	1.30	.025 2.49 x 10-2
ARL5B	↑	0.91	4.46×10^{-7}	1.22	.017 1.66 x 10-2

RT-qPCR, real-time quantitative polymerase chain reaction.

[a] $P < .05$, obtained with SOLAR; [b] $P < .10$, obtained with *t* test statistics with software (SPSS, version 16; SPSS, Inc, Chicago, IL).

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TABLE 4
Canonical pathway analysis

Canonical pathway[a]	Genes	*P* value[b] Ingenuity Pathway Analysis	*P* value[c] Rotation Gene Set Enrichment Analysis
Tryptophan metabolism	ACMSD, ALDH3A2, ASMTL, CYP2E1, CYP2J2, INMT, KYNU, MAOA	5.51×10^{-4}	2.0×10^{-4}
Endoplasmic reticulum stress pathway	ATF6, DNAJC3, EIF2AK3, XBP1	5.81×10^{-4}	5.3×10^{-3}
Linoleic acid metabolism	CYP2E1, CYP2J2, PLA2G5, PLA2G2A, WISP2	3.91×10^{-3}	1.5×10^{-3}
Notch signaling	DTX3, HES1, NOTCH3, NOTCH4	6.72×10^{-3}	7.9×10^{-3}
Fatty acid metabolism	ACOX1, ACOX2, ADH1A, ALDH3A2, CYP2E1, CYP2J2	7.90×10^{-3}	10.0×10^{-5}
Arachidonic acid metabolism	CYP2E1, CYP2J2, PLA2G5, PLA2G2A, PTGIS, WISP2	8.66×10^{-3}	10.0×10^{-5}
NRF2-mediated oxidative stress response	ACTG2, DNAJB6, DNAJB9, DNAJB11, DNAJC3, EIF2AK3, GSTA3, HMOX1, UBE2K	9.99×10^{-3}	6.7×10^{-2}

[a] Ingenuity Pathway Analysis (www.ingenuity.com; Ingenuity Systems, Redwood City, CA) was used to bioinformatically identify canonical (ie, cell signaling and metabolic) pathways potentially involved in preeclampsia within our dataset; [b] *P* value obtained with Fisher's exact test; [c] *P* value obtained with use of *limma* package.

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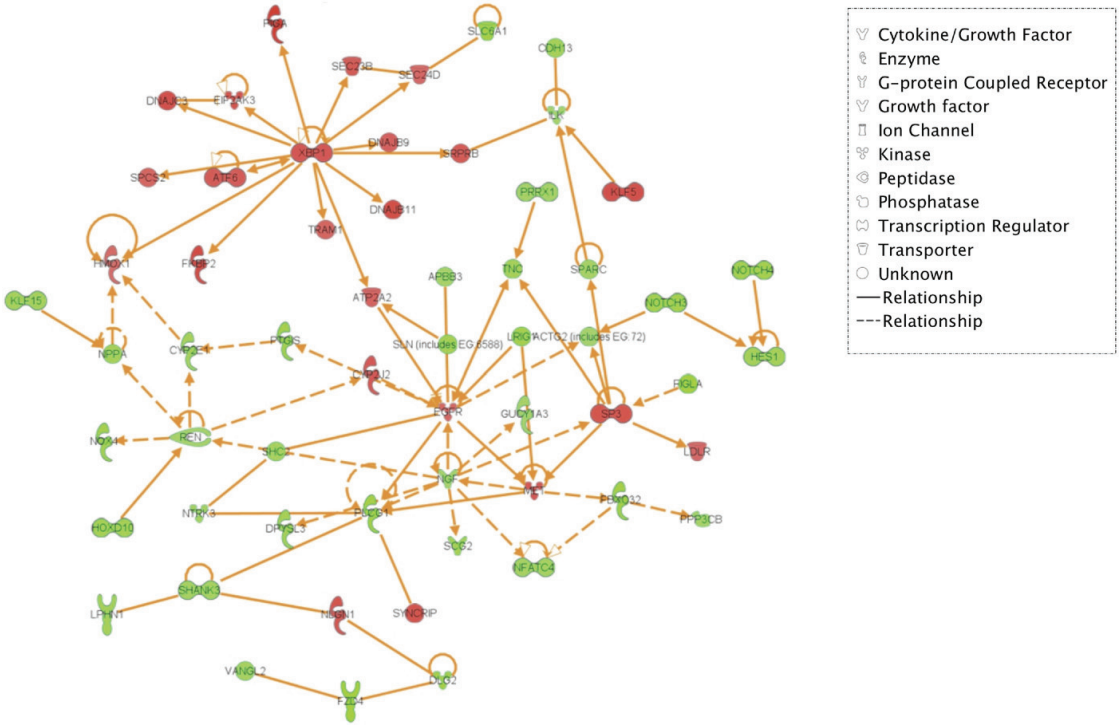
KREMEN1 expression decreases toward term[23] and was up-regulated in the preeclampsia group.

In genomewide transcriptional profiling, analysis of *groups* of genes is a strategy to increase power and reduce the dimensionality of the underlying statistical problem following multiple testing.[25] Further, it may be advantageous to put focus on canonical pathways and networks instead of single genes when the aim is to obtain insight in the pathophysiology of complex diseases, such as preeclampsia. The high interconnectivity of focus genes with other correlated genes within a biological network may imply functional and biological importance of these genes.[26,27] To be able to assess this in a comprehensive manner, we increased the FDR cutoff to 0.1 and consequently the number of genes included in the analysis. Using this approach, 7 significant canonical pathways were found to be represented by the differentially expressed genes identified in this current study (Table 4).

The most significant canonical pathway detected was tryptophan metabolism. The metabolism of tryptophan, through the kynurenine pathway, has previously been suggested to be involved in preeclampsia pathogenesis,[28,29] and, in accordance with this, the activity of the first enzyme of the kynurenine pathway, indoleamine 2,3 dioxygenase, has been reported to be reduced in placenta from preeclamptic pregnancies.[28] We found no disease-associated changes in indoleamine 2,3 dioxygenase expression, but the transcript encoding the enzyme kynureninase (KYNU) was up-regulated. KYNU metabolizes L-kynurenine, which suppresses T-cell proliferation and natural killer cells and influences immunotolerance to foreign antigens.[30] This implies that a consequence of KYNU up-regulation may be an increased inflammatory response (due to lack of L-kynurenine). An additional 7 genes were assigned to this canonical pathway (Table 4).

The second most significant canonical pathway identified was the ER stress pathway. Three genes (EIF2AK3, ATF6, and XBP1) included in the unfolded protein response, a coordinated adaptive response to ER stress, were up-regulated. ER stress has previously been suggested as one of the main sources for generation of placental oxidative stress.[31] Yung et al[32] have reported similar associations of the unfolded protein response signaling pathways to preeclampsia in placental tissue, but these findings are reported for the first time in decidual tissue. There is a close connection between oxidative stress and ER stress,[31,33] also indicated by the many direct relationships of the ER and oxidative stress–related genes in the generated network (Figure). The canonical pathway NRF2-mediated oxidative stress response was also among the significant pathways identified (Table 4). The nuclear factor NFR2 plays an essential role in the defense of oxidative stress by regulating the expression of antioxidant response elements.[34] In case of excessive oxidative stress, activation by reactive oxygen species, nitrogen oxide, and proinflammatory cytokines results in translocation of NRF2 to the nucleus. NRF2 binds to antioxidant response element sequences, leading to transcriptional activation of antioxidant genes (eg, glutathione and HMOX1). NRF2-mediated oxidative stress response included 9 genes, of which 3 genes have pre-

FIGURE
Network of preeclampsia-correlated genes



Ingenuity Pathway Analysis (www.ingenuity.com; Ingenuity Systems, Redwood City, CA) generated gene-gene product interaction network of preeclampsia-correlated genes. Genes or gene products are represented as nodes, and biological relationship between 2 nodes is represented as edge (*line*). All edges are supported by at least 1 published reference. *Solid edges* represent direct relationship, and *dashed edges* represent indirect relationship. Node color represents correlation of expression level with preeclampsia, and color intensity indicates degree of correlation (*red* is positive and *green* negative). Shape of each node represents functional class of gene product, as shown in *key*.

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viously been associated with preeclampsia (EIF2AK3,[32] GSTA3,[10] and HMOX1[21,22]). Several enzymes metabolize reactive oxygen species to exportable compounds, and in this study the transcripts encoding the antioxidant enzymes GSTA3, HMOX1, and UBE2K were up-regulated.

Three of the remaining significant canonical pathways generated by IPA represented metabolism of fatty acids: linoleic acid metabolism, fatty acid metabolism, and arachidonic acid metabolism. The genes included in these pathways were partly overlapping, as shown in Table 4. Decidual arterioles of preeclamptic women show atherosclerotic-like lesions,[35] suggesting an underlying atherogenic process of low-density lipoprotein lipid peroxidation.[36] Lipid peroxidation contributes to the development of preeclampsia,[37] and decidua basalis tissue from preeclamptic women has an increased content of lipid peroxides.[4] The first enzyme of the fatty acid β-oxidation pathway, acyl-coenzyme A oxidase (ACOX)1/palmitoyl-coA oxidase, donates electrons directly to molecular oxygen, thereby producing hydrogen peroxides. ACOX1 was found to be up-regulated, whereas ACOX2/branched chain ACOX, which is involved in the degradation of long branched fatty acids and bile acid intermediates in peroxisomes, was found to be down-regulated. Two genes involved in elimination of lipid peroxidation products were also down-regulated in the material: alcohol dehydrogenase 1a, which metabolizes a wide variety of substrates including lipid peroxidation products, and aldehyd-edehydrogenase 3 family member A2 isozymes, thought to play a major role in the detoxification of aldehydes generated by alcohol metabolism and lipid peroxidation. Increased generation or decreased elimination of lipid peroxidation products may be among the factors activating the maternal endothelium[38] and triggering systemic inflammation in preeclampsia.

Finally, the pathway analysis suggested a role of notch signaling, with inclusion of 4 down-regulated genes: DTX3, HES1, NOTCH 3, and NOTCH 4. Notch signaling is known to be involved in cell differentiation, proliferation, apopto-

sis,[39] and blood vessel formation,[40] processes neatly regulated in the placenta to maintain a normal pregnancy. Notch receptors are expressed on extravillous trophoblasts and are hypothesized to be involved in the differentiation and proliferation of both extravillous trophoblasts and endothelial cells.[41] Placental villi from preeclamptic pregnancies show down-regulation of notch pathway members.[42] Notch signaling in placenta has been suggested to play a role in the development of preeclampsia,[42,43] and the altered expression of DTX and HES1 in tissue from preeclamptic pregnancies compared with normal pregnancies is presented for the first time.

In summary, we have provided a comprehensive transcriptional profile of the decidua in preeclampsia. Our network analysis has demonstrated extensive connectivity between the differently expressed genes. Alteration of the expression level of 1 gene may influence the transcription of others included in the network. Due to this, it is difficult to pinpoint the genes having primary roles in perpetuating preeclampsia from our dataset. Some of our findings confirm and elaborate the current knowledge on the pathophysiology of preeclampsia, while others are novel. Further studies are warranted to replicate findings and confirm involvement of specific genes that have been identified. ■

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Paper III

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PLoS one

Genetic and Molecular Functional Characterization of Variants within *TNFSF13B*, a Positional Candidate Preeclampsia Susceptibility Gene on 13q

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Abstract

Background: Preeclampsia is a serious pregnancy complication, demonstrating a complex pattern of inheritance. The elucidation of genetic liability to preeclampsia remains a major challenge in obstetric medicine. We have adopted a positional cloning approach to identify maternal genetic components, with linkages previously demonstrated to chromosomes 2q, 5q and 13q in an Australian/New Zealand familial cohort. The current study aimed to identify potential functional and structural variants in the positional candidate gene *TNFSF13B* under the 13q linkage peak and assess their association status with maternal preeclampsia genetic susceptibility.

Methodology/Principal Findings: The proximal promoter and coding regions of the positional candidate gene *TNFSF13B* residing within the 13q linkage region was sequenced using 48 proband or founder individuals from Australian/New Zealand families. Ten sequence variants (nine SNPs and one single base insertion) were identified and seven SNPs were successfully genotyped in the total Australian/New Zealand family cohort (74 families/480 individuals). Borderline association to preeclampsia ($p = 0.0153$) was observed for three rare SNPs (rs16972194, rs16972197 and rs56124946) in strong linkage disequilibrium with each other. Functional evaluation by electrophoretic mobility shift assays showed differential nuclear factor binding to the minor allele of the rs16972194 SNP, residing upstream of the translation start site, making this a putative functional variant. The observed genetic associations were not replicated in a Norwegian case/control cohort (The Nord-Trøndelag Health Study (HUNT2), 851 preeclamptic and 1,440 non-preeclamptic women).

Conclusion/Significance: TNFSF13B has previously been suggested to contribute to the normal immunological adaption crucial for a successful pregnancy. Our observations support *TNFSF13B* as a potential novel preeclampsia susceptibility gene. We discuss a possible role for *TNFSF13B* in preeclampsia pathogenesis, and propose the rs16972194 variant as a candidate for further functional evaluation.

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Introduction

Preeclampsia is a major cause of fetal and maternal morbidity and mortality in pregnancy, with an incidence ranging from 2–5% [1]. A complete understanding of the etiology and pathogenesis of the preeclampsia syndrome remains elusive. The clinical manifestations of hypertension and proteinuria usually emerge after 20 weeks of pregnancy, and are caused by inflammatory changes and endothelial dysfunction [2,3]. Impaired placentation in the earlier stages of pregnancy is an underlying pathological feature [4]. However, immunological changes occurring before placentation [5] and even before implantation [6] are also implied in the pathogenesis. Therefore a three stage model for preeclampsia is proposed [7,8] in which immunological dysfunction (stage 1) is followed by impaired placentation (stage 2), leading to an enhanced inflammatory state and overt preeclampsia (stage 3).

Maternal-fetal immune maladaption is an intriguing aspect of preeclampsia pathogenesis, for which there is both epidemiological and biological evidence [9–14]. Importantly, the theory implies a mechanism which by partial failure will lead to poor placentation, but by more severe failure will cause spontaneous abortion. Indeed, observations of immunological pathogenic factors place preeclampsia as an intermediate phenotype between miscarriage and successful pregnancy [8].

Like in the majority of other common complex disorders, the mode of preeclampsia inheritance is unclear [15–17]. By examining the probability of co-segregating loci within familial cohorts, several loci most likely to harbor maternal susceptibility genes have been identified [18–24]. Genome-wide linkage studies in our Australian/New Zealand (Aust/NZ) familial cohort initially identified a maternal preeclampsia susceptibility locus to chromosome 2q [23,25]. Re-analysis of the Aust/NZ data set, assuming an underlying inherent quantitative liability for preeclampsia, resolved and strengthened the chromosome 2 linkage signal to 2q22 [24]. Two additional novel maternal preeclampsia susceptibility quantitative trait loci (QTLs) on chromosomes 5q and 13q were revealed [20,24]. An extended Aust/NZ familial cohort and an independent retrospectively ascertained Norwegian case/control cohort (the HUNT2 cohort) have been utilized to identify maternal preeclampsia susceptibility genes at these QTLs. Association to the activin A receptor, type IIA (*ACVR2A*) [26,27] and the endoplasmic reticulum aminopeptidase 2 (*ERAP2*) [28] genes at the 2q22 and 5q QTLs, respectively, has been reported. Priorization of candidate susceptibility genes at the 13q QTL, was performed using the database text-mining program GeneSniffer (www.genesniffer.org) [20,24,28], literature searches and interrogating publically available SNP loci in the Aust/NZ and Norwegian cohorts (NCBI SNP database, dbSNP build 125, Sep 2005) [29]. This preliminary assessment identified the tumor necrosis factor (ligand) superfamily 13B (*TNFSF13B*) as our most promising candidate gene [29].

TNFSF13B, also known as BAFF, BLYS, TALL-1, zTNF4, THANK, CD257, TNFSF20 and DTL, is a member of the TNF superfamily. This protein is active both as a membrane-bound and soluble ligand. Originally discovered as an important stimulator of B-cell proliferation and immunoglobulin production [30,31], TNFSF13B has later been shown to hold various roles in the innate immune system [32]. Both malignant [33–35] and autoimmune [36,37] B-cell diseases have been linked to this protein. Furthermore, TNFSF13B has been implicated in normal placental development [38,39], with reduced expression in recurrent spontaneous miscarriage patients [40].

The current study aimed to identify potential functional and structural variants in *TNFSF13B* by re-sequencing the proximal promoter area and coding regions of the gene in preeclamptic individuals from our Aust/NZ families. Identified variants were tested for association with maternal preeclampsia genetic susceptibility in the extended Aust/NZ families. Associated variants were further assessed by formal molecular genetics analyses followed by attempts to independently replicate genetic association findings in a large Norwegian case/control cohort.

Materials and Methods

Ethics

Australia. Ethical approval for the recruitment of Aust/NZ preeclampsia family members was granted by the Royal Women's Hospital Research and Ethics Committees, Melbourne, Australia. Written informed consent was obtained from study participants prior to them being phlebotomized. Ethical approval for the molecular genetic investigation across the 13q QTL in The 74 Family Cohort was obtained from The University of Texas Health Science Center at San Antonio, Institutional Review Board. Data were analyzed anonymously.

Norway. Prior approval to link the information in the HUNT and MBRN databases, to use the Norwegian case/control cohort for genetic studies, and to export samples was obtained by the Regional Committee for Medical Research Ethics, Norway and approved by the National Data Inspectorate and The Directorate of Health and Social Welfare. Ethical approval for genotyping and statistical analysis of the Norwegian case/control cohort was also obtained from The University of Texas Health Science Center at San Antonio, Institutional Review Board. Data were analyzed anonymously.

Aust/NZ Study Population

The Aust/NZ familial cohort consists of the original set of 34 (26 Australian and eight New Zealand) families that we have previously used to localize the 2q, 5q and 13q preeclampsia susceptibility QTLs and an additional 40 (Australian) preeclampsia families that we have subsequently ascertained and recently described [26]. The entire familial sample is herein called "The 74 Family Cohort". All family members are of Caucasian origin. Preeclampsia diagnosis in the Aust/NZ study population was performed by qualified clinicians, using criteria set by the Australasian Society for the Study of Hypertension in Pregnancy (new onset proteinuria, $\geq$0.3 g/d and either an increase from baseline blood pressure of 15/25 mmHg or absolute values $\geq$140/90 mmHg on at least two occasions 6 h or more apart) [41,42] as described in detail elsewhere [23,26]. Women who met the preeclamptic criteria of new onset of hypertension and proteinuria in pregnancy, and experienced convulsions or unconsciousness in the prenatal period were classified as having had eclampsia. Women with pre-existing hypertension or other medical conditions known to predispose for preeclampsia (e.g. renal disease, diabetes, twin pregnancies or fetal chromosomal abnormalities) were excluded. Family members were coded as 1) affected, 2) unaffected or 3) unknown (e.g. male, non-fertile women).

Norwegian Study Population

All women in the Norwegian cohort were identified from Nord-Trøndelag County in Norway as part of a large multipurpose health survey conducted during 1995–1997 (the Nord-Trøndelag Health Study, HUNT2) [43]. Preeclamptic women and women who had non-preeclamptic pregnancies were retrospectively identified in the HUNT cohort by linking the HUNT database to the database at the Medical Birth Registry of Norway (MBRN) as previously described [44,45]. Preeclampsia was defined in accordance with the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (new onset hypertension, blood pressure $\geq$140/90 mmHg, and proteinuria, $\geq$0.3 g/d in pregnancy) [46] using diagnosis codes ICD-8 (before 1998) and ICD-10 (after 1998) as previously described [44,45]. Preeclamptic women with multiple pregnancies were excluded. Two controls per case were identified at random among parous women in the HUNT2 cohort with no registered preeclamptic pregnancy in the MBRN. Information stored in hospital records was retrospectively examined by an independent obstetrician for validation of the diagnosis reported to MBRN.

Positional Candidate Gene Sequencing

Two kb of the proximal promoter (upstream of the translation start site) and all six exons (translated or untranslated) of the 13q preeclampsia QTL candidate gene, *TNFSF13B* (NM_006573.3)

were sequenced in 48 preeclamptic women. These women are a selection of founders or probands chosen from the most informative pedigrees in The 74 Family Cohort. This sequencing sample set will give a greater than 99% probability of detecting any polymorphism that has a frequency of 0.05 or greater.

Primer Design for *TNFSF13B* Gene Sequencing

Sequence information for use as a reference template was obtained from the UCSC Genome Browser (Human, Mar. 2006 [NCBI/hg 18]). Sequencing primers were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Primers were designed to be between 20 and 27 bp in length with an annealing temperature between 55°C and 63°C and within 1°C of each other (Table 1).

TNFSF13B Gene Sequencing

Extraction of genomic DNA from peripheral blood samples has been previously described [23]. PCR was performed with 20 ng genomic DNA in a 5 µl reaction containing 0.25 U HotStarTaq DNA Polymerase (QIAGEN), 1× QIAGEN PCR buffer, 0.2 mM dNTP, and 0.2 mM of each forward and reverse primer (Table 1). A GeneAmp 9700 thermal cycler (Applied Biosystems) was used for PCR amplification. After an initial denaturation step at 95°C for 15 min, 40 cycles of 94°C for 30 s, a primer pair specific annealing temperature (Table 1) for 30 s, and 72°C for 30 s were run followed by a final extension step of 72°C for 10 min. PCR products were purified using ExoSAP-IT (Amersham Biosciences) according to the manufactures instructions before they were used as a template for sequencing. Sequencing reactions were performed independently for both sense and anti-sense DNA strands using 1 µl purified PCR product in a 5 µl reaction, containing 0.25 µl AB BigDye Terminators v3.1 (Applied Biosystems), 1× AB BigDye Terminator v3.1 buffer (Applied Biosystems) and 1.6 mM of either forward or reverse primer. Sequence reaction amplification was performed on a GeneAmp 9700 thermal cycler using standard cycling conditions, 96°C for 1 min followed by 25 cycles of 96°C for 10 s, 50°C for 10 s and then 60°C for 4 min. The Applied Biosystems BigDye XTerminator purification kit was used according to manufacturer's instructions to purify all sequenced products. Purified sequence reactions were electrophoretically separated on an Applied Biosystems 3730xl DNA Analyzer. Sequence variant identification was performed using Applied Biosystems' SeqScape software v2.6.

SNP Genotyping in the Aust/NZ Study Population

All *TNFSF13B* SNPs identified in our sequencing experiments were incorporated into a custom Illumina SNP pool and genotyped back in The 74 Family Cohort. Briefly, SNP designs were uploaded to Illumina's Assay Design Tool to design a custom GoldenGate SNP pool with VeraCode technology (Illumina Inc., CA). The design of two allele specific oligos and one locus specific oligo in conjunction with a universal set of amplification primers followed by hybridization to complementary VeraCode bead types makes the GoldenGate assay with VeraCode technology highly robust and specific in a small to medium multiplex reaction. Each VeraCode microtitre bead plate was imaged on the Illumina BeadXpress Reader System using Illumina VeraScan image data acquisition software (version 1.1.9.2). SNP genotype clustering and individual sample genotype calls were interrogated using the Illumina GenomeStudio software, Genotyping Module (version 1.1.9). As an added measure we confirmed genotype calls made by GenomeStudio against the sequence data obtained from our sequencing sub-set of The 74 Family Cohort (n = 48).

Table 1. Primers used for *TNFSF13B* PCR amplification and sequencing.

Name	Primer Sequence	Fragment size (bp)	Annealing temperature (°C)
Promoter 1 F	AGACGTTACAAGCACAGTTGTAGAA	652	60
Promoter 1 R	CCGAGCAGTGTACACATTGAA		60
Promoter 2 F	CATAGGAATGATCTAATGGACTTTAG	631	57
Promoter 2 R	CATTCTAGTCCTGCCTTATCCT		57
Promoter 3 F	TTCTCCACTTTGCACTATATCATTTC	585	58
Promoter 3 R	AACATGCATAAACTTTTTCCTTCTG		58
Promoter 4 F	TAGTATCATATTGAGCGGGGACTTA	728	58
Promoter 4 R	CTTTCTGCATCTCTACCCCTACTG		58
Exon 1 F	TAAGGGGTTTTAAATCTACTTGAGCAT	664	60
Exon 1 R	TGCAAACTCACTTTCAGTCCC		60
Exon 2 F	TCACGGTGGTGTCTTTCTACC	661	62
Exon 2 R	GCATTATCTACCTGAGGAAACACATA		62
Exon 3 F	AATGTCATGCAATCAATGTAAAAAGT	639	57
Exon 3 R	TCTAAGTGGAAAAAGTACTGGGGATA		57
Exon 4/5 F	GAGGTAGCTTAACAACTAAATGGAGG	559	60
Exon 4/5 R	TTGAGGAATGTCTTTCTGTCTATTTG		60
Exon 6 F	AGATAATTGCAATGGTTTAGAAGTCC	430	58
Exon 6 R	TAGTTTCAGCAAACCAAAACAAATAG		58
Exon 6 seq F	TTTATTTAAGATTCTTTTCTTTTCTGTTG	261	
Exon 6 seq R	TTGGTATTTTCAGTTAGATTCTTTCTT		

F; Forward primer, R; Reverse primer. For exon 6 an extra set of sequencing primers internal to the PCR amplicon of 430 bp was used.
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Bioinformatic Evaluation of SNPs

To predict possible functional relevance of the detected *TNFSF13B* variants, we used different publicly available bioinformatic tools for identifying transcription factor binding sites in DNA sequences (http://www.gene-regulation.com), as well as Transfac® Professional and MotifScanner. The programs use different approaches to utilize the library of mononucleotide weight matrices in the TRANSFAC® [47] and Jaspar [48] databases.

Electrophoretic Mobility Shift Assays (EMSA)

HeLa and T47D total nuclear protein extract was prepared and stored as described [49]. Total protein was determined using the Bio-Rad Protein Assay Reagent. The DNA oligonucleotides (0.025 µmol) (Sigma-Aldrich) used to assay the three *TNFSF13B* variants are presented in Table 2. All oligonucleotides were 5′ end-labeled using T4 polynucleotide kinase (PNK) (New England Biolabs) and [γ33P] ATP (3000 Ci/mmol) (PerkinElmer) and annealed to their complementary unlabeled oligonucleotides as previously described [49]. The samples were purified according to manufacturers' instructions through G25 Microspin™ columns (GE Healthcare). The EMSA reactions were carried out in binding buffer (4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris–HCl (pH 7.5), 0.25 mg/ml poly(dI-dC)) in a final volume of 10 µl. Nuclear extract (7 µg) was incubated with double-stranded competitor oligonucleotides for 30 min at room temperature, followed by the addition of 50 fmol of P33 labeled oligonucleotide and then incubated for another 30 min. Samples were mixed with 1 µl of 10× loading buffer (250 mM Tris–HCl (pH 7.5), 0.1% bromophenol blue, 40% glycerol) and run on a 4% polyacrylamide gel (37.5:1 acrylamide:bisacrylamide, 2.5% glycerol, 0.5× TBE) at 300 V. The gels were fixed in 50% ethanol and 10% acetic acid for 1 h followed by Phosphor Imager analysis (Bas-1800II) (Fujifilm) of the dried gel.

Replicated SNP Genotyping in the Norwegian Study Population

DNA for genotyping was extracted from peripheral blood samples stored in the HUNT biobank as described elsewhere [27,45]. Replicated SNP genotyping was performed at Southwest Foundation for Biomedical Research, Texas, using TaqMan genotyping assays (Applied Biosystems) on an Applied Biosystems' 7900HT Fast Real-Time PCR System. For each TaqMan SNP assay 50 ng of genomic DNA was used in a 5 µl reaction volume with 2.5 µl TaqMan Genotyping master mix, 0.125 µl TaqMan assay mix (40×) and 1.375 µl water. Four no template (water) controls were incorporated into each 384-well plate. SNP genotype clustering and individual sample genotype calls were interrogated using Applied Biosystems' Sequence Detection Systems software v2.2.2.

Statistical Methods

Genotype Error Checking. Genotypes pertaining to the Aust/NZ study population not conforming to Mendelian inheritance laws were identified and assessed using SimWalk2 [50]. Mendelian discrepancies and spurious recombinations were removed by blanking those genotypes identified in SimWalk2 as having a high probability of being in error. Norwegian genotypes in this current study were compared to Norwegian genotypes in our preliminary study using SNPlex technology which prioritized the *TNFSF13B* gene [29].

SNP Allele Frequency Estimation. We used the statistical genetics analysis program SOLAR [51] to estimate SNP allele frequencies by using maximum likelihood techniques that account for pedigree structure. Tests for deviations from Hardy-Weinberg equilibrium (HWE) were also performed in SOLAR.

SNP Linkage Disequilibrium Estimates. Estimates of pairwise linkage disequilibria parameters were used in a basic correlation method to assess all disequilibria jointly in SOLAR. In this approach, SNP genotypes are scored as -1, 0 and 1 (for the AA, AB and BB genotypes, respectively) and the correlations among these data vectors are calculated to give an unbiased estimate of the squared LD correlation, rho (ρ).

SNP Association Analysis. Power calculations and SNP association analyses were performed in SOLAR [51]. SNP association analyses were conducted using SOLAR's QTLD procedure [52]. This procedure performs a test for population stratification and two commonly used association tests: the quantitative transmission disequilibrium test (QTDT) [53], and the measured genotype test [54]. The QTDT procedure is not limited to the scoring of allele transmission from parents to offspring but extends further to assess the entire pedigree structure. The scoring of allele transmission can be performed for quantitative or

Table 2. Oligonucleotides used for Electrophoretic mobility shift assays.

SNP	Allele	F/R*	Sequence
rs16972197	G	F	5′-GCTTTCCCTTGACTGTGCCAATCC-3′
	G	R	5′-GGATTGGCACAGTCAAGGGAAAGC-3′
	C	F	5′-GCTTTCCCTTCACTGTGCCAATCC-3′
	C	R	5′-GGATTGGCACAGTGAAGGGAAAGC-3′
rs16972194	G	F	5′-AAACTTCTTACTTAAGACTGTGTGGAAATGTAGAGT-3′
	G	R	5′-ACTCTACATTTCCACACAGTCTTAAGTAAGAAGTTT-3′
	A	F	5′-AAACTTCTTACTTAAGACTGTATGGAAATGTAGAGT-3′
	A	R	5′-ACTCTACATTTCCATACAGTCTTAAGTAAGAAGTTT-3′
rs56124946	C	F	5′-GCTGCCTCTCCCTCGCCTCAGCTGTCTTT-3′
	C	R	5′-AAAGACAGCTGAGGCGAGGGAGAGGCAGC-3′
	G	F	5′-GCTGCCTCTCCCTGGCCTCAGCTGTCTTT-3′
	G	R	5′-AAAGACAGCTGAGGCCAGGGAGAGGCAGC-3′

*orientation of oligo: Forward (F)/Reverse (R) strand.
doi:10.1371/journal.pone.0012993.t002

Table 3. *TNFSF13B* variants tested in the Aust/NZ and Norwegian study populations.

SNP	Chr. Post. (bp)*	Function	Aust/NZ study population				Norwegian study population		
			Allele (frequency)		MG_p	$QTDT_p$	Allele (frequency)		MG_p
SNP_A-1967C	107718278	pp	Failed genotyping assay				Not tested		
rs16972194	107718962	pp	G (0.993)	A (0.007)	0.380	0.015	G (0.997)	A (0.003)	0.421
rs9514828	107719374	pp	G (0.566)	A (0.434)	0.406	0.915	Not tested		
rs36206504	107719569	pp	A (0.965)	C (0.035)	0.714	0.162	Not tested		
rs36206505	107719584	pp	A (0.931)	G (0.069)	0.125	0.618	Not tested		
rs16972197	107719892	pp	G (0.993)	C (0.007)	0.380	0.015	G (0.997)	C (0.003)	0.357
rs56124946	107720644	Intron 1	C (0.993)	G (0.007)	0.380	0.015	C (0.991)	G (0.009)	0.737
SNP_A17071G	107737282	Intron 3	Failed assay design				Not tested		
rs33926705	107757082^107757083	Intron 5	Not tested				Not tested		
rs61972017	107757114	Intron 5	A (0.988)	C (0.012)	1.000	0.197	Not tested		

Novel SNPs are denoted SNP_[UCSC reference template allele][bp position from TSS][alternative allele]. Alleles reported are orientated on the TOP strand (ftp://ftp.ncbi.nih.gov/snp/database/Illumina_top_bot_strand.note.txt). * ref_assembly, human genome build 36.3, Abbreviations: TSS; translation start site, Chr.; chromosome, Post.; position, bp; base pair, MG_p; measured genotype test p-value, $QTDT_p$; quantitative transmission disequilibrium test p-value, pp; proximal promoter.
doi:10.1371/journal.pone.0012993.t003

qualitative traits and it has been modified in SOLAR to work with discrete traits using a threshold model [55]. The measured genotype test uses a standard threshold model assuming an underlying normal distribution of liability. The threshold model and its assumptions are near identical to those used in standard logistic regression but benefits from the ease of interpretation with regard to genetic effects. The measured genotype test of association can assess the extent of genotypic mean differences (or the liability or risk scale) between case and control singletons assuming a model of additive gene action [54]. Due to the non-familial structure of the Norwegian study population, we can only present the measured genotype test statistic for this cohort.

Multiple Hypothesis Testing. To accommodate for multiple hypothesis testing, we used the approach of Moskvina and Schmidt [56] to determine the effective number of independent SNPs (i.e. tests) based on the pair-wise genotypic correlations. This algorithmic approach has been implemented into SOLAR and it evaluates the strength of correlation amongst the observed genotypes at each SNP locus within a gene.

Results

Statistical Power analyses

We performed formal power calculations to assess the power to detect an association (between a SNP and the dichotomous preeclampsia phenotype – where affected are scored as 1 and unaffected as 0) of a given relative size in the population. In the Aust/NZ families, with a SNP-specific heritability of 0.01 to 0.05, we predicted 80% power to identify functional effects that account for as little as 3.5% of the total phenotypic variation with a nominal alpha (significance) of 0.05. In the Norwegian case/control cohort, we estimated an 80% likelihood of identifying a SNP accounting for at least 2% of the total (dichotomous) phenotypic variation.

TNFSF13B Gene Sequencing

The proximal promoter (2 kb upstream of the translation start site), the 5′UTR, 3′UTR and all coding regions were sequenced. In total, we identified nine SNPs (two novel, seven known) and one known, single base insertion in the proximal promoter or intronic sequence flanking the exons (Table 3 and Figure 1).

TNFSF13B Genotyping and Association Analysis in the Aust/NZ Families

The 74 Family Cohort (n = 480) included 140 affected women (20 with eclampsia, 120 with preeclampsia) and 146 unaffected women (normotensive and non-proteinuric). At the time of custom SNP pool design the single base insertion variant (rs33926705)

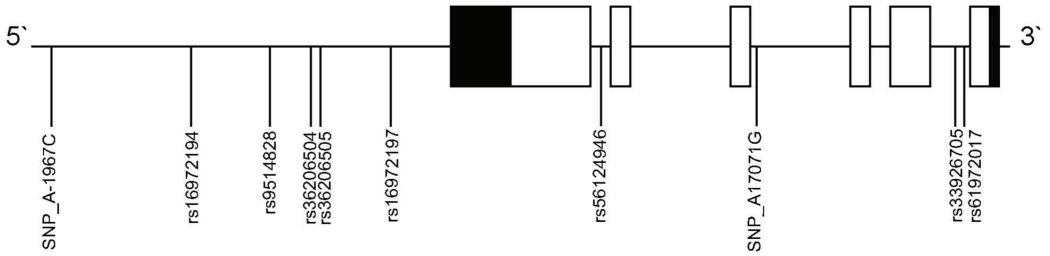

Figure 1. Schematic representation of the *TNFSF13B* gene and variants detected in a sub-set of founding or proband preeclamptic women from the Aust/NZ study population. Solid blocks; untranslated exons, open blocks; translated exons.
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could not be included into the assay. Additionally, one novel SNP (SNP_A17071G) failed assay design due to it residing within a duplicate or repetitive region and the other novel SNP (SNP_A-1967C) could not be successfully genotyped. The seven successfully typed SNPs in the Aust/NZ study population exhibited a high sample genotype success rate (≥98.5%) and all SNPs conformed to Hardy-Weinberg expectations ($p>0.05$).

We observed association to preeclampsia ($p = 0.0153$) for three rare SNPs (rs16972194, rs16972197 and rs56124946) (Table 3). Based on the extent of linkage disequilibrium (LD) between these SNPs (Figure 2) we were effectively testing five independent SNPs in our association analyses. To correct for multiple testing, these SNP correlations return an adjusted p-value threshold of 0.0102. Therefore, we present a borderline association for three *TNFSF13B* SNPs (Figure 2) with preeclampsia susceptibility in the Aust/NZ families with the QTDT statistic (Table 3).

Bioinformatic Evaluation

Bioinformatic analysis of rs16972194, rs16972197 and rs56124946 using MATCH^{TM} 1.0 [57] revealed that the rare rs16972194 (A) allele created a promoter sequence with high core similarity (core match; 0.948, matrix match; 0.932) to the binding motif of transcription factor Oct-1. Oct-1 is a member of the POU domain transcription factor family [58], and the DNA recognition sequence is the octamer motif 5′-ATGCAAT-3′, which is shared between several Oct/POU transcription factor family members [59]. A more stringent bioinformatics analysis, examining whether any other known transcription factor(s) could bind preferentially to the minor allele, but not the major allele of rs16972194, was also

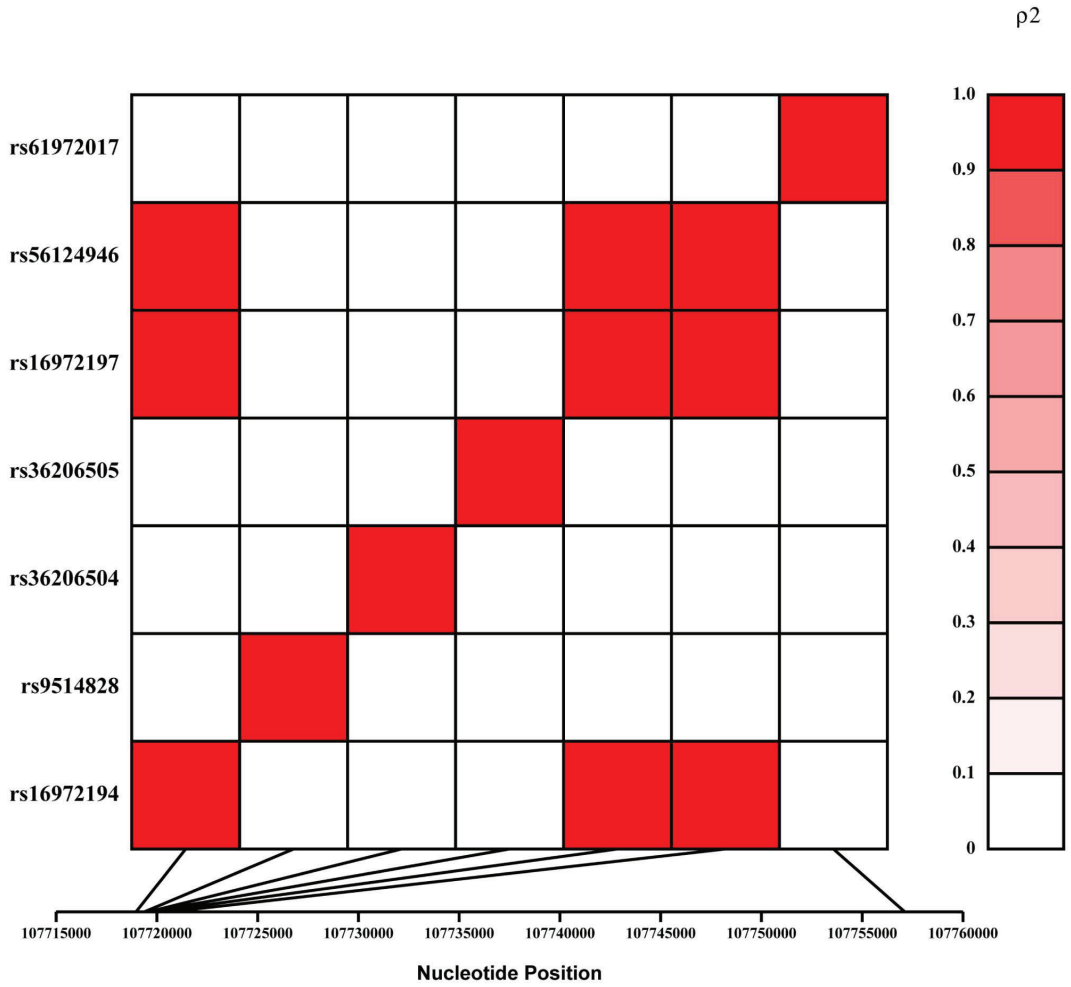

Figure 2. Linkage disequilibrium (LD) pattern for the successfully genotyped *TNFSF13B* SNPs in the Aust/NZ study population. LD is measured by the squared value of the pair wise correlation (rho) amongst intra-genic genotypes and the strength of correlation is depicted in the colored bar to the right of the LD plot. The intensity of red color increases with the strength of SNP allele correlation from white (0) indicating no correlation (i.e. no LD) to red (1.0) indicating a perfect correlation (i.e. complete LD).
doi:10.1371/journal.pone.0012993.g002

performed. A total of 1,351 binding models for transcription factors were collected from the TRANSFAC (version 2009.2) and JASPAR CORE databases [48,60]. The Oct- motif was confirmed, and additional shorter core sequences exhibiting a preference to the rs16972194 minor (A) allele were identified. Of these, the FOXC1 and YY1 transcription factor-motifs were the most relevant.

Electrophoretic Mobility Shift Assays (EMSA)

We subsequently carried out electrophoretic mobility shift assays (EMSA), using nuclear extracts from HeLa and T47D cells. Radioactively labeled double stranded DNA oligonucleotide probes representing the major and minor allele of each of the three rare and associated SNPs were run with both nuclear extracts to visualize binding of nuclear protein (Figure 3). All probes demonstrated non-specific electrophoretic mobility shifts (Figure 3). Unlabelled double stranded oligos for the wild type and mutant alleles, as well as an unspecific competitor, were added in separate reactions. The unspecific shifts were inhibited by these competitors. Interestingly, the rs16972194 SNP demonstrated a specific shift for the minor, but not the major, allele probe (Figure 3). The minor allele unlabelled probe suppressed the shift whereas the major allele unlabelled probe and the unspecific competitor did not (Figure 3). This strongly suggests the creation of a nuclear factor binding site by this variant. Antibodies for transcription factors Oct1, Oct2, Oct3/4, Oct6, YY1 and FOXC1 were run in separate reactions, but no supershift was observed under the current running conditions (result not shown).

Replicated *TNFSF13B* SNP Genotyping and Association Analysis in the Norwegian Singletons

DNA samples were available for 851 confirmed cases of women with preeclampsia and 1,440 women with a history of non-preeclamptic pregnancies (controls). Of the available cases, 737 women were registered with one and 114 women with more than one preeclamptic pregnancy. As expected, gestational age (273 d vs. 282 d, $p<0.001$) and birth weight (3156 g vs. 3457 g, $p<0.001$) differed between the neonates in preeclamptic and non-preeclamptic pregnancies. Maternal age at first pregnancy was higher in the case group (23.6 yrs vs. 22.8 yrs, $p<0.001$), but the groups did not differ with respect to parity (2.56 vs. 2.55, $p>0.05$). After adjusting for maternal age, the differences in clinical phenotype between case and control groups remained significant ($p<0.001$). All three rare SNPs associated to preeclampsia were successfully genotyped in the Norwegian study population. A high genotyping success rate ($\geq$97.1%) was observed and all three SNPs were in Hardy-Weinberg equilibrium ($p>0.05$). Independent genotyping of these SNPs did not replicate the results attained in the Aust/NZ families (Table 3).

Discussion

The elucidation of genetic risk factors contributing to preeclampsia susceptibility has become a priority of obstetric research. It is well known that both maternal and paternal factors influence the preeclampsia phenotype [9,11,13,14]. To identify maternal genetic contributions to preeclampsia our positional cloning approach identified a susceptibility QTL on chromosome 13q [20], and the *TNFSF13B* gene was prioritized as the most promising candidate under this QTL [29]. In the current study, a targeted molecular genetic evaluation of TNFSF13B was undertaken. We report borderline association to a putative functional SNP within the proximal promoter region of *TNFSF13B* with preeclampsia susceptibility in affected Aust/NZ families. The finding is not replicated in a Norwegian case/control population cohort.

The early changes of pregnancy include a shift of the Th1/Th2 cytokine balance towards Th2 predominance [61]. Inflammatory/infectious processes may alter this balance towards a Th1 profile less favorable for pregnancy [61]. TNFSF13B is regulated by inflammatory response cytokines [62–64] and stimulates macrophages to secrete proinflammatory cytokines, enhancing the cascade [32]. Interference with the homeostatic regulation of *TNFSF13B* could therefore potentially disturb the finely tuned cytokine balance of pregnancy. Decidual stromal cells (DSCs) have been shown to express *TNFSF13B* mRNA and protein [40] and DSCs are involved in a number of different functions that are important for the immunological cross-talk between mother and fetus [65]. Our finding may therefore reflect an abnormal immunological function of DSCs at the maternal-fetal interface.

The interaction between decidual natural killer (NK) cells and the allogenic extravillous trophoblast (EVT) cells is suggested to contribute to the depth of EVT cell invasion during implantation and placentation [66,67]. NK-cells are the predominant leucocytes found in decidua [67] and NK- cell activity is elevated by TNFSF13B in mice [68,69]. In humans, TNFSF13B has been shown to relay immunological response to toll-like receptor (TLR) 3 and 4 binding [70,71]. TLRs are expressed on placental NK cells. They help discriminate between "self" and "non-self", and have been shown to recognize infectious agents as well as endogenous danger signals [61,72]. These biological functions are implicated in preeclampsia pathogenesis [5,10,73], and TLRs have been assigned a role in pregnancy-associated complications such as intrauterine growth restriction, pre-term delivery and preeclampsia [74]. It is therefore tempting to speculate, that disturbed TLR signaling might be one mechanism by which aberrant *TNFSF13B* regulation could confer susceptibility to preeclampsia.

We observe differential nuclear binding to the minor allele of the *TNFSF13B* promoter area rs16972194 SNP, thus suggesting it as a putative functional, albeit rare, variant. A recent report showed that SNPs contribute substantially to genetic variation leading to aberrant transcription factor binding, and that this might be an important evolutionary mechanism [75]. Transcriptional regulation is proving to be highly complex, as illustrated by the FANTOM consortiums attempt to describe the transcriptional landscape of the mouse genome [76]. In the human genome, over 2,500 proteins with DNA binding motifs are predicted, and it is estimated that about 8% of human proteins are transcription factors [77]. Of these, only about 10% are well characterized and included in available databases for motif searches [48,60]. HeLa cells are widely used as a model system for biomedical research on both normal and disease molecular processes [78]. A wide variety of nuclear factors are expressed in this cell type, including transcription factors only expressed in embryonic stem-cells and not in differentiated tissues [79]. Our EMSA results show differential binding of a nuclear factor to the sequence in question. The finding was replicated using T47 cells. However, further investigation of the protein band representing nuclear factor binding to the rs16972194 minor allele and in vivo confirmation of the result is warranted. The role of the identified putative functional variant in other TNFSF13B related diseases should also be subject of further investigation.

The Aust/NZ sequencing sample set ensures a high probability of detecting common frequency variants within the population. However, our choice of affected women who are either pedigree founders or probands for re-sequencing will also increase the likelihood of identifying rare functional variants that are enriched

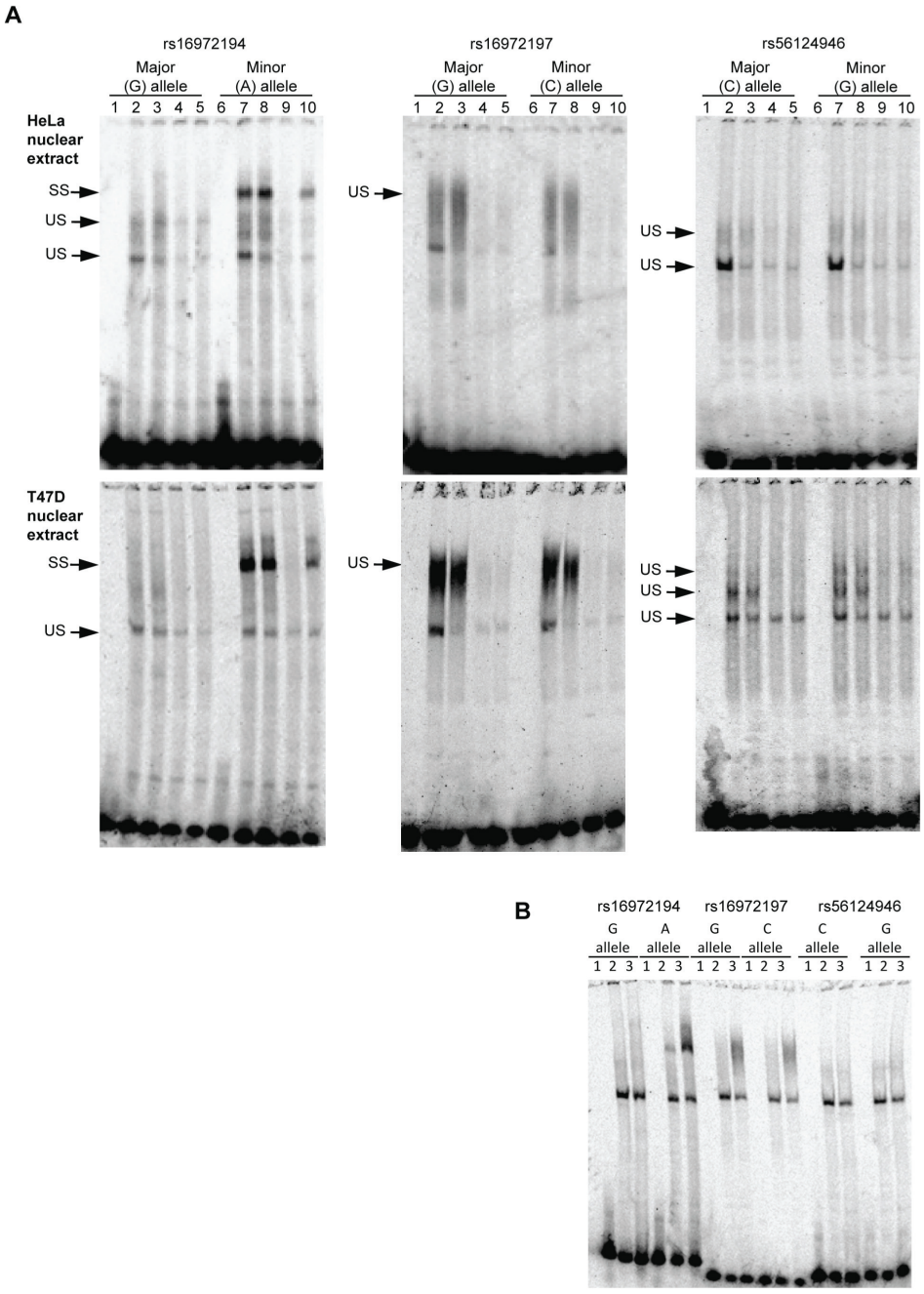

Figure 3. Electrophoretic mobility shift assays for the *TNFSF13B* SNPs associated with preeclampsia in the Aust/NZ families. Panel A: Lanes 1 and 6; No nuclear extract, Lanes 2 and 7; nuclear extract only, Lanes 3 and 8, Nuclear extract with unspecific competitor, Lanes 4 and 10; Nuclear extract with specific competitor (unlabelled double stranded oligo for the major allele), Lanes 5 and 9; Nuclear extract with specific competitor (unlabelled double stranded oligo for the minor allele). Panel B: Major shifts without competitor. Lane 1; no nuclear extract, Lane 2; HeLa nuclear extract, Lane 3; T47D nuclear extract. SS; specific shift, US; unspecific shift.
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in these preeclamptic women. Over the last decade, a large number of genome wide association studies have been undertaken for numerous common complex diseases, assuming that common disease is caused by common variation (the common disease-common variant (CDCV) hypothesis) [80]. This approach has provided new insight [81], but a notable knowledge "gap" of 90–95% of the genetic liability to these diseases is left unaccounted for [80]. As shown for extensively studied disease genes, such as *BRCA1* and *BRCA2*, rare variants might be population specific, but yield a higher individual risk of disease than common variants (the common disease rare-variant (CDRV) hypothesis) [82,83]. Therefore, most geneticists appreciate that the CDCV and CDRV hypotheses both have their place in the understanding of heterogeneous genetic disorders. The rare *TNFSF13B* variants exhibiting borderline association with preeclampsia susceptibility in the Aust/NZ families were not replicated in the Norwegian population sample. Confirming the biological importance of rare predisposing variants between populations is a challenge [80], and further genetic and molecular investigation in other populations is required.

The Norwegian population cohort has a larger sample size than the Aust/NZ family cohort. However, the power of a study is also influenced by the stringency of the diagnosis and the pedigree information included in the statistical analyses. In both the Aust/NZ and Norwegian study populations, the preeclampsia diagnosis was based on the development of new onset hypertension and proteinuria during pregnancy. However, preeclampsia is a complex disease, and preeclamptic cases selected from a population sample represent a more heterogeneous group, than a collection of family samples. The MBRN did not include absolute values of blood pressure and proteinuria, and severity of preeclampsia was not reported to the registry before 1998. Thus, we are not able to include this information in our analyses. However, women with a familial disposition generally display more severe manifestations of the disease [84], and this may have influenced our results. The available information about relatedness in the Aust/NZ pedigree sample set allows a wider range of potential test statistics to be considered. Hence, we applied both the measured genotype association test and the QTDT which controls for any potential latent stratification in the data. In the absence of hidden stratification and residual linkage effects, the measured genotype test is asymptotically more powerful than the QTDT [85]. However, in the presence of certain types of latent stratification, the QTDT can be more powerful. Similarly, residual linkage (reflective of additional functional variants near the associated marker) can also lead to a more powerful QTDT. Such additional potential genetic signals have no influence in the analysis of unrelated individuals. Thus, even though the sample size of the Norwegian cohort is larger its composition of solely unrelated females may have rendered it somewhat less powerful for detecting the observed effect if such complexities are involved.

In conclusion, we observe borderline association between three rare *TNFSF13B* SNPs, one of which exhibits putative functional characteristics, and maternal preeclampsia genetic susceptibility in our Aust/NZ families. Our observation supports *TNFSF13B* as a potential preeclampsia susceptibility gene in a region of known genetic linkage, and adds evidence to its importance for a successful human pregnancy. Furthermore, showing differential nuclear factor binding to the minor allele of rs16972194, we propose this variant as a candidate for additional functional evaluation.

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Author Contributions

Conceived and designed the experiments: MHF MPJ LTR PAA LJA JB EKM. Performed the experiments: MHF MPJ PAA KK. Analyzed the data: MHF MPJ SF JB. Contributed reagents/materials/analysis tools: LTR PAA KK CEE JB SPB RA EKM. Wrote the paper: MHF MPJ LTR.

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Paper IV

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A low *COMT* activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2)

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A low *COMT* activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2)

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ABSTRACT

The aetiology of preeclampsia is complex, with susceptibility being attributable to multiple environmental factors and a large genetic component. Although many candidate genes for preeclampsia have been suggested and studied, the specific causative genes still remain to be identified. Catechol-*O*-methyltransferase (COMT) is an enzyme involved in catecholamine and estrogen degradation and has recently been ascribed a role in development of preeclampsia. In the present study we have examined the *COMT* gene, by genotyping the functional *Val108/158Met* polymorphism (rs4680) and an additional SNP, rs6269, predicting *COMT* activity haplotypes in a large Norwegian case/control cohort (n_{cases}=1,135, $n_{controls}$=2,262). A low *COMT* activity haplotype is associated with recurrent preeclampsia in our cohort. This may support the role of redox-regulated signaling and oxidative stress in preeclampsia pathogenesis. The *COMT* gene might be a genetic risk factor shared between preeclampsia and cardiovascular diseases.

KEY WORDS

Preeclampsia/ catechol-*O*-methyltransferase/ *COMT*/ *Val108/158Met*/ haplotypes

INTRODUCTION

The pregnancy-associated complication preeclampsia is a leading cause of maternal and fetal morbidity and mortality. Approximately 3% of all pregnant women in populations of European descent are affected by preeclampsia (Saftlas *et al.*, 1990). In severe cases of preeclampsia the only effective treatment is delivery, irrespective of gestational age. The classical clinical manifestations of preeclampsia are elevated blood pressure and proteinuria. The etiology is complex and like in other common complex disorders both genetic and environmental factors influence the risk of developing the disease. Genetic factors are suggested to be responsible for more than 50% of the liability to preeclampsia (Moses *et al.*, 2006, Salonen Ros *et al.*, 2000), and several candidate genes have been studied. However, the results are inconsistent and specific causative genes involved in preeclampsia still remain to be identified (Broughton Pipkin, 1999, Chappell and Morgan, 2006, Consortium, 2005, Mutze *et al.*, 2008, Nejatizadeh *et al.*, 2008, Roberts and Cooper, 2001).

A recent study put forward that deficiency in catechol-*O*-methyltransferase (COMT) is associated with preeclampsia (Kanasaki *et al.*, 2008). COMT is a key enzyme in the degradation of both catecholamines and estrogens (Creveling, 2003). High- and low-activity variants of COMT, due to single base changes, have been discovered (Diatchenko *et al.*, 2005). One polymorphism with functional implications is a non-synonymous *G* to *A* base change (rs4680; NM_000754.2), the *COMT Val108/158Met* polymorphism. The Met(*A*)-allele of this polymorphism is associated with a three- to four-fold decrease in COMT enzyme activity (Lotta *et al.*, 1995), and several clinical conditions such as pain perception (Diatchenko *et al.*, 2005, Zubieta *et al.*, 2003), psychiatric disorders (Azzam and Mathews, 2003, Prasad *et al.*, 2008, Woo *et al.*, 2002), hypertension (Annerbrink *et al.*, 2008, Hagen *et al.*, 2007, Happonen *et al.*, 2006) and heart disease (Eriksson *et al.*, 2004, Hagen *et al.*, 2007, Voutilainen *et al.*, 2007) have been reported to be associated with this single base change.

Inspired by Kanasaki *et al.*'s hypothesis that COMT deficiency is associated with preeclampsia we examined the potential role of the functional *COMT Val108/158Met* polymorphism in a large Norwegian case/control cohort. Furthermore, an additional SNP (rs6269) was genotyped to account for the three major haplotypes observed in the central region of *COMT* in populations of European decent (Diatchenko *et al.*, 2005, Gabriel *et al.*, 2002).

MATERIALS AND METHODS

The HUNT population

All women subjected to genotyping were retrospectively identified from the second Nord-Trøndelag Health Study (HUNT2) (Holmen *et al.*, 2003). Preeclampsia was defined as onset of persistent hypertension (exceeding 140/90 mmHg), in combination with proteinuria (exceeding 300 mg/l per day) after 20 weeks gestation. Women with preeclamptic (cases) and non-preeclamptic (controls) singleton pregnancies in the HUNT2 cohort were identified by linking the HUNT database to the Medical Birth

Registry of Norway (MBRN) (Moses *et al.*, 2008). The inhabitants of Nord-Trøndelag county are well suited for genetic studies due to ethnic homogeneity (<3% non-Caucasians) (Holmen *et al.*, 2003, Holmen *et al.*, 2004). The HUNT2 preeclampsia cohort is described in detail elsewhere (Fenstad *et al.*, 2010, Moses *et al.*, 2008).

Clinical characterisation of the HUNT2 preeclampsia cohort
Preterm delivery was defined as delivery before 37 weeks (Gifford *et al.*, 2000). Small for gestational age (SGA) was defined as f an infant with a birth weight ≤2 standard deviations (SD) below the expected weight for gestational age and sex, corresponding to the 2.5 percentile (Marsal *et al.*, 1996). For assessment of metabolic syndrome, an International Diabetes Federation (IDF) proxy definition (waist circumference ≥80 cm plus any two of HDL cholesterol <1.29, treatment for hypertension or blood pressure ≥130/≥85 mm Hg, diabetes diagnosed after age of 30) (Hildrum *et al.*, 2007) was used, as fasting blood glucose was not available for all the individuals in the study cohort. Using the IDF proxy definition in a cross sectional analysis of 10,206 HUNT2 participants, Hildrum *et al.* showed that there was no differences in the prevalence of metabolic syndrome between fasting and non-fasting groups (Hildrum *et al.*, 2007).

SNP genotyping
DNA for genotyping was extracted from blood samples stored in the HUNT biobank, as described elsewhere (Moses *et al.*, 2008). Applied Biosystems' TaqMan genotyping assays (Applied Biosystems, Foster City, U.S.A) were selected to genotype the rs4680 (*Val108/158Met*) and rs6269 SNPs using 5 ng of genomic DNA from each of the case and control samples. The assays were performed on an Applied Biosystems 7900HT Fast Real-Time PCR System at HUNT biobank and sample genotypes were interrogated using the integrated 7900HT system data analysis software.

Haplotype analysis
Haplotypes were predicted from genotype information from each individual using the computer program Phase (http://stephenslab.uchicago.edu/home.html) (Stephens *et al.*, 2001, Stephens and Donnelly, 2003). Only individuals with both SNPs successfully genotyped were included in the haplotype analysis (n=3,036; $n_{controls}$=2,029, n_{cases}=1,007; $n_{non\text{-}recurrent}$=888, $n_{recurrent}$=119). The frequency of the haplotypes was also calculated based on this number of individuals.

Only a few common *COMT* haplotypes are observed in populations of European decent (Diatchenko *et al.*, 2005, Gabriel *et al.*, 2002), and three major *COMT* haplotypes accounting for approximately 96% of all detected haplotypes in the coding region determine the COMT activity in humans (Diatchenko *et al.*, 2005). Figure 1 show these three haplotypes which are demonstrated to constitute of four central SNPs (rs6269, rs4633, rs4818, rs4680) (Diatchenko *et al.*, 2005). These three haplotypes are associated with very different COMT enzyme activities (Nackley *et al.*, 2006), and have also been demonstrated to be associated with variation in sensitivity to experimental pain. They were therefore designated as Low Pain Sensitivity (LPS), Average Pain Sensitivity

(APS) and High Pain Sensitivity (HPS) (Diatchenko *et al.*, 2005). There is an inverse correlation between pain sensitivity and COMT activity, meaning that the LPS haplotype represents the high *COMT* activity haplotype, whereas the HPS represents the low *COMT* activity haplotype, and the APS represents the intermediate *COMT* activity haplotype.

In this study, the genotyped SNPs (rs4680 and rs6269) were selected based on the observation that only two of the central four SNPs were needed to tag the variation in a Norwegian sample set (Halleland *et al.*, 2009). It was observed that there is strong pair wise linkage disequilibrium (LD) with almost perfect correlation ($r^2 > 0.98$) between rs6269-rs4818 and rs4633-rs4680, and that the rs6269 SNP tags the high *COMT* activity haplotype (Halleland *et al.*, 2009).

Statistical Analysis

Clinical characterisation

The software package SPSS 16.0 for Windows was used to compute descriptive statistics means and standard deviations. P-values were computed based on t-test statistics. Cases registered with one preeclamptic pregnancy (non-recurrent) and cases with more than one preeclamptic pregnancy (recurrent) were analyzed separately. Each preeclamptic group was compared to the non-preeclamptic group. Multivariate logistic regression was used to model preeclampsia as the (dichotomous) dependent variable against maternal age. A threshold of $\alpha=0.05$ was set for statistical significance of all computed analyses.

SNP and haplotype association analysis

Concordance with Hardy-Weinberg proportions was tested using a χ^2 goodness-of-fit statistic. The SNP association analyses for the *Val108/158Met* (rs4680) and rs6269 SNPs and haplotype association analyses for the four possible haplotypes (Figure 1) were carried out in PASW Statistics version 17 using a Pearson's χ^2 statistic. The SNPs and haplotypes were analyzed separately for the subgroups of preeclamptic women (non-recurrent, recurrent) against non-preeclamptic control women. An additive (*A* allele frequency vs. *G* allele frequency) genetic model was used for the SNP association analysis. For the haplotype association analyses we tested whether carrying one of the four possible haplotypes was associated with disease state. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. A threshold of $\alpha=0.05$ was set for statistical significance of all computed analyses.

Ethics

The study was approved by the Regional Committee for Medical Research Ethics, the National Data Inspectorate and The Directorate of Health and Social Welfare in Norway.

RESULTS

Statistical power analysis

Using a relevant range of minor allele frequencies (30-50%) (NCBI SNP database), a priori power calculations *ad modum* Lalouel and Rhorwasser (Lalouel and Rohrwasser, 2002) for the genotyped SNPs demonstrated 80% power to detect an effect size (OR) difference of 1.25 for the non-recurrent group (n=1003) and 1.65-1.75 for the subgroup of women with recurrent preeclampsia (n=136).

Clinical characterisation

The original HUNT2 preeclampsia cohort (1,139 cases and 2,269 controls) was used when performing the clinical characterisation (Fenstad *et al.*, 2010, Moses *et al.*, 2008). Mean follow up time from index pregnancy recorded in MBRN to inclusion in the present study was 25±10 years. Gestational age and birth weight differed between the neonates in preeclamptic and non-preeclamptic pregnancies, the preeclamptic women had a higher risk of preterm delivery, and of delivering a SGA neonate (Table I, $p<0.001$). Metabolic syndrome was evaluated by data from the HUNT2 study and was higher in the case groups as compared to controls (Table I, $p<0.001$). After adjusting for maternal age, the differences in clinical phenotype between case and control groups remained significant (Table I, $p<0.001$).

Table I: Clinical characteristics of the HUNT2 preeclampsia case/control cohort.

	Preeclampsia (non-recurrent, n=1,003)	Preeclampsia (recurrent[1], n=136)	Control (n=2,269)
Maternal age at index pregnancy (years)	27±6*	25±5	25±5
Gestational age (days)	275±22*	271±20*	282±18
Birth weight (g)	3.238±837*	3.040±846*	3.483±592
SGA[2]	147 (15)*	26 (20)*	87 (4)
Preterm birth[3]	132 (14)*	29 (22)*	114 (5)
Maternal age at inclusion in HUNT2	40±11	37±9*	40±11
Metabolic syndrome[4]	163 (16)*	30 (22)*	212 (9)

Data presented as mean ± standard deviation or number (percentage). P-values are computed based on T-test statistics, each preeclamptic group is compared to the non-preeclamptic group.

[1]More than one preeclamptic pregnancy.

[2]≤2SD of expected weight.

[3]Delivery before week 37.

[4]IDF-proxy definition; waist circumference ≥80 cm plus any two of (HDL cholesterol <1.29, treatment for hypertension or blood pressure ≥130/≥85 mm Hg, diabetes diagnosed after age of 30 or fasting plasma glucose ≥5.6 mmol/L)[43]

IDF; the International Diabetes Federation, HDL; high-density lipoprotein, CI; confidence interval

**p<0.001*

We also observed clinical differences between the group of women with recurrent and non-recurrent preeclampsia (Table I). The women with recurrent preeclampsia delivered earlier (p=0.018) and had a higher prevalence of preterm birth (22%) compared to the women with non-recurrent preeclampsia (16%) (p<0.01). The neonates from the recurrent preeclamptic pregnancies had a lower birth weight (adjusted for gestational age, p=0.055), but the seemingly different prevalence of SGA (20% vs. 15%) was not statistically significant (p=0.2). The p-values were adjusted for maternal age. The group of women with recurrent preeclampsia also had higher prevalence of metabolic syndrome at inclusion in the HUNT2 study compared to the women with non-recurrent preeclampsia when adjusting for age at inclusion (p=0.019).

COMT genotyping and association analysis

DNA samples were available for 1,135 women registered with preeclamptic pregnancies and 2,262 controls. We observed a high genotyping success rate for the rs4680 and rs6269 SNPs in both cases (94%) and controls (95%), and both SNPs conformed to Hardy-Weinberg proportions (p>0.05). No association between the two studied *COMT* SNPs and non-recurrent preeclampsia was observed in our Norwegian cohort (Table II). However, a significant overrepresentation of the wild type allele (*Val* (*G*)), not the low activity allele (*Met* (*A*)), of the *Val108/158Met* polymorphism (rs4680) was observed in the group of women with recurrent preeclampsia (p=0.047, OR=0.77, CI 0.6-1.0) (Table II). No association was observed between rs6269 and recurrent preeclampsia (Table II).

Table II: Distribution of *COMT* genotypes and alleles in the HUNT2 preeclampsia case/control cohort.

SNP	Genotype (NN) / Allele (N)	Preeclampsia non-recurrent n (proportion of total)	Preeclampsia recurrent n (proportion of total)	Control n (proportion of total)	OR	CI
rs4680 (*Val108/158Met*)	GG	174 (0.18)	36 (0.28)	412 (0.19)		
	AG	461 (0.48)	60 (0.46)	1,097 (0.50)		
	AA	335 (0.35)	35 (0.27)	678 (0.31)		
	A (*Met*)	1,131 (0.58)	130 (0.50)	2,453 (0.56)	1.1 [a] 0.8 [b] *	1.0-1.2 [a] 0.6-1.0 [b]
	G (*Val*)	809 (0.42)	132 (0.50)	1,921 (0.44)		
rs6269	AA	361 (0.39)	47 (0.39)	771 (0.37)		
	GA	412 (0.45)	52 (0.43)	1,035 (0.49)		
	GG	143 (0.16)	23 (0.19)	289 (0.14)		
	A	1,134 (0.62)	146 (0.60)	2,577 (0.62)	1.0 [a] 0.9 [b]	0.9-1.1 [a] 0.7-1.2 [b]
	G	698 (0.38)	98 (0.40)	1,613 (0.39)		

[a] *preeclampsia non-recurrent vs. control*

[b] *preeclampsia recurrent vs. control*

** Significantly different from the value for the control group when compared with the frequency of the G allele using Pearson's χ^2 analysis in a 2 x 2 contingency table (χ^2=4.185, p=0.047)*

OR; odds ratio, CI; 95% confidence interval

The three common *COMT* haplotypes, as well as the less frequent *G-A* (rs6269-rs4680) haplotype were detected in our Norwegian cohort (Table III and Figure 1). The frequencies of the three common haplotypes in our cohort are consistent with frequencies observed in other studies (Figure 1) (Diatchenko *et al.*, 2005, Halleland *et al.*, 2009, Rakvag *et al.*, 2008). We found that carrying the low *COMT* activity haplotype was significantly associated with recurrent preeclampsia (p=0.018, OR=1.8, CI 1.1-2.8) (Table III). The non-recurrent preeclampsia group did not show association with any of the haplotypes.

Table III: *COMT* haplotypes in the HUNT2 preeclampsia case/control cohort.

Haplotype	rs6269 – rs4680 (N-N)	Preeclampsia non-recurrent (proportion)	Preeclampsia recurrent (proportion)	Control (proportion)	OR	CI
1 (high activity)	G – G	516 (0.58)	72 (0.61)	1,237 (0.61)	0.9 [a] 1.0 [b]	0.8-1.0 [a] 0.7-1.4 [b]
2 (intermediate activity)	A – A	710 (0.80)	87 (0.73)	1,627 (0.80)	1.0 [a] 0.7 [b]	0.8-1.2 [a] 0.4-1.0 [b]
3 (low activity)	A – G	110 (0.12)	25 (0.21)	263 (0.13)	1.0 [a] 1.8 [b] *	0.8-1.2 [a] 1.1-2.8 [b]
4 (unknown activity)	G – A	30 (0.03)	2 (0.02)	58 (0.03)	1.2 [a] 0.6 [b]	0.8-1.9 [a] 0.1-2.4 [b]

Proportions represent the proportion of individuals being a carrier of the haplotype tested (number of individuals carrying haplotype X divided on total number of individuals in the studied subgroup)

[a] *preeclampsia non-recurrent vs. control*

[b] *preeclampsia recurrent vs. control*

** Significantly different from the value for the control group when compared with the frequency of the other haplotypes combined using Pearson's χ^2 analysis in a 2 x 2 contingency table (χ^2= 0.57, p=0.018)*

OR; odds ratio, CI; 95% confidence interval

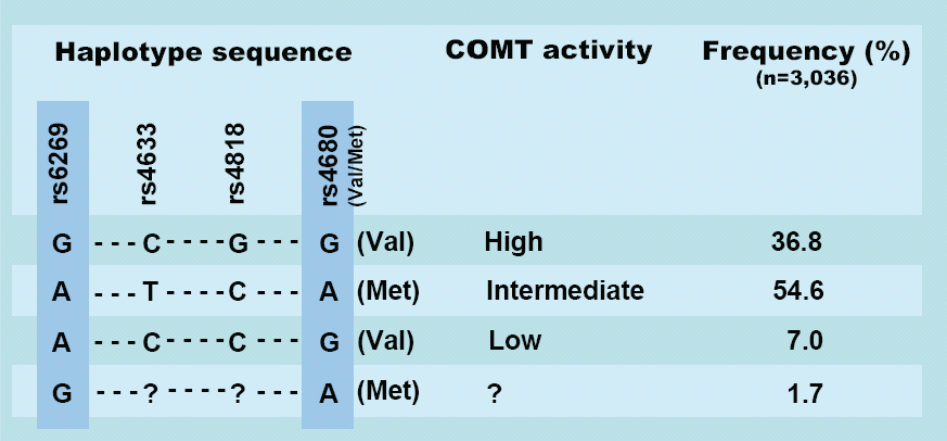

Figure 1: Haplotypes in the central region of the *COMT* gene.
Figure modified from (Andersen and Skorpen, 2009). A total of four central SNPs in the *COMT* gene have been demonstrated to combine into three common haplotypes (Diatchenko *et al.*, 2005) which have been associated with variation in COMT enzyme activity (Nackley *et al.*, 2006). The two SNPs marked with a pale blue rectangle, rs6269 and rs4680, in combination differentiate between the three common activity haplotypes (Halleland *et al.*, 2009) and were the ones genotyped in the present study. Frequencies for the haplotypes detected are consistent with previous findings.

DISCUSSION

Growing evidence supports the role of COMT in human pregnancy. The COMT enzyme is reported to be active in both placenta (Barnea *et al.*, 1988) and decidua (Casey and MacDonald, 1983), and expression in human fetal membranes has recently been reported (Harirah *et al.*, 2009). Decreased placental COMT activity was first reported to be associated with hypertension in pregnancy (Barnea *et al.*, 1988). More recently, reduced placental COMT protein expression has been observed in women with severe preeclampsia (Kanasaki *et al.*, 2008). On the basis of the latter observation, together with observations from studying *COMT* knockout mice, *COMT* was introduced as a preeclampsia susceptibility gene (Kanasaki *et al.*, 2008). The *Comt-/-* mice developed a preeclampsia-like syndrome, with elevated blood pressure, albuminuria, glomerular changes, placental thrombosis and hypoxia and preterm birth. However, administration of 2-methoxyestradiol (2-ME), a natural estrogen metabolite produced by COMT, to pregnant *Comt-/-* mice ameliorated the preeclampsia-like symptoms (Kanasaki *et al.*, 2008). It was suggested that genetic variation within the *COMT* gene could be an explanation for disruption of COMT and 2-ME in preeclamptic women (Kanasaki *et al.*, 2008).

SNPs in the *COMT* gene have been shown to significantly affect enzyme activity (Diatchenko *et al.*, 2005, Lotta *et al.*, 1995, Nackley *et al.*, 2009). It was therefore reasonable to hypothesize that SNPs in this gene are associated with preeclampsia pathogenesis. Recently, the *Val108/158Met* polymorphism was shown to be associated

with preeclampsia in a Korean population cohort of 164 preeclamptic and 182 normotensive patients (Lim *et al.*, 2010). They found that preeclamptic women tended to be homozygous for the low activity allele (*Met* (*A*)) and that this genotype increased risk significantly in severe preeclampsia and preeclampsia with SGA neonates. The present study confirms association between the *Val108/158Met* polymorphism and recurrent preeclampsia. However, in contrast with the Korean study we found that the wild type allele (*Val* (*G*)) was more frequent. It has become clear that the *Val108/158Met* polymorphism alone is not likely to account for the variation of COMT enzyme activity. Four central SNPs (rs6269, rs4633, rs4818, rs4680) in the *COMT* gene combine to form three common haplotypes (Diatchenko *et al.*, 2005), and these are associated with varying levels of COMT enzyme activity (Nackley *et al.*, 2006) (Figure 1). The fact that the wild type *Val108/158* (*G*) allele is present in both the high and low *COMT* activity haplotypes (Diatchenko *et al.*, 2005) demonstrates the importance of studying haplotypes rather than single SNPs. We therefore performed haplotype analysis to see if any of the three common haplotypes (Figure 1) were associated with preeclampsia. We found that the low *COMT* activity haplotype was significantly associated with recurrent preeclampsia (p=0.018), with an OR of 1.8 (CI 1.1-2.8) for carrying this haplotype. Consistent with other studies, our group of women with recurrent preeclampsia showed the highest risk of preterm labour, low fetal birth weight and the highest risk of later life cardiovascular disease (assessed as metabolic syndrome) (Magnussen *et al.*, 2009, Odegard *et al.*, 2000, Sibai *et al.*, 1991).Therefore, our findings support the hypothesis that lower maternal COMT enzyme activity predisposes to severe preeclampsia.

Angiogenesis, the formation of new blood vessels, is a central process in development of both preeclampsia and cardiovascular diseases. Alterations in angiogenesis during early pregnancy contribute to incomplete remodelling of uterine spiral arteries and abnormal placental vascular development (Roberts and Cooper, 2001). Decreased COMT activity and subsequent reduced levels of estrogen metabolites, such as 2-ME, may impair vascular health in several ways. (Barchiesi *et al.*, 2006, Dubey *et al.*, 2007, Dubey and Jackson, 2009, LaVallee *et al.*, 2003). It has been demonstrated that 2-ME has antiangiogenic effects (Fotsis *et al.*, 1994), suppressing hypoxia inducible factor 1α (HIF-1α) which is essential in angiogenesis. This transcription factor is responsible for induction of genes that facilitate the adaption and survival of cells during low-oxygen levels (Semenza, 1998, Wang *et al.*, 1995), including soluble fms-like tyrosine kinase (sFlt-1). 2-ME has recently been suggested to be an important co-stimulator together with low oxygen levels for induction of the invasiveness of trophoblasts (Lee *et al.*, 2010). Thus, it has been suggested that 2-ME plays a role in maintaining placental homeostasis (Kanasaki and Kalluri, 2009). A premature increase in 2-ME has been hypothesized to disturb hypoxia-driven trophoblast invasion and vascular remodelling and therefore contribute to preeclampsia pathogenesis (Lee *et al.*, 2010). In late pregnancy decreased COMT activity, thus lower levels of 2-ME and decreased inhibition of HIF-1α could potentially cause vascular pathology and inflammatory activation (Banerjee *et al.*, 2009).

Acting as a pro-oxidant, 2-ME has direct involvement in redox-regulated signaling (Banerjee *et al.*, 2009), a possible shared disease mechanism between preeclampsia and cardiovascular diseases. Furthermore, the COMT enzyme is also important for homocysteine metabolism, a known cardiovascular risk factor (Shenoy *et al.*, 2010). A combination of high serum homocysteine levels and the low activity *Val108/158* allele conferred increased risk (hazard risk ratio of 2.94) of acute coronary events in middle-aged men from eastern Finland (Voutilainen *et al.*, 2007). A similar mechanism has been suggested for preeclampsia (Shenoy *et al.*, 2010). Such epigenetic effects might explain the diverging results found in both genetic studies concerning *COMT* and epidemiologic studies concerning vitamin B and homocysteine levels (Annerbrink *et al.*, 2008, Ciaccio and Bellia, 2010, Eriksson *et al.*, 2004, Guven *et al.*, 2009, Hagen *et al.*, 2007, Happonen *et al.*, 2006, Hintsanen *et al.*, 2008, Lim *et al.*, 2010, Mignini *et al.*, 2005, Nackley *et al.*, 2009, Ntaios *et al.*, 2009, Ray and Laskin, 1999, Sanchez *et al.*, 2001, Voutilainen *et al.*, 2007). In summary, the *COMT* gene may be a candidate gene for the genetic liability possibly shared between preeclampsia and cardiovascular disease. Altered COMT enzyme activity and 2-ME production is likely to be of great importance in development of both preeclampsia and cardiovascular diseases (Dubey and Jackson, 2009).

In the present study we have examined only two SNPs, representing haplotypes in the central region of COMT. However, we do acknowledge that the multiple SNPs within the *COMT* gene give rise to a multitude of possible haplotype combinations, where minor differences between haplotypes may have profound effects on the COMT activity. To further investigate the role of genetic variation affecting COMT activity one should extend the haplotype analysis to include the entire *COMT* gene. Only then can the 'true' effect of the high, intermediate and low activity haplotypes examined in the present study be controlled for. This can be done by looking at how these haplotypes are combined with the different haplotypes of flanking haploblocks. However, this will require very large study samples in order to account for the multitude of possible diplotype combinations (Andersen and Skorpen, 2009). In addition, seven different mRNA splice variants exist for the *COMT* gene which potentially exacerbates the complexity of COMT in biological mechanisms (Tunbridge *et al.*, 2007). In future studies it would also be of great interest to look at the fetal contribution since placental COMT is likely to be of importance.

In conclusion, the available evidence makes *COMT* a likely and interesting candidate gene for preeclampsia development. The present study confirms that a low *COMT* activity haplotype contributes to the genetic liability of recurrent preeclampsia in our Norwegian HUNT2 cohort. Nonetheless, further genetic and functional studies are needed to validate our finding and clarify the role of the COMT enzyme in preeclampsia pathogenesis. Studies examining the expression and activity of the COMT enzyme throughout pregnancy are warranted.

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73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

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74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

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82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

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92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

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104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.

108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

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110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANCER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

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124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs.

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132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.
133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.

138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

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141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

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158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
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160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.

168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

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178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM

198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

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201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

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216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES

223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

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235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

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248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS

251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

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