Original Article

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The antihypertensive *MTHFR* gene polymorphism rs17367504-G is a possible novel protective locus for preeclampsia

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Objective: Preeclampsia is a complex heterogeneous disease commonly defined by new-onset hypertension and proteinuria in pregnancy. Women experiencing preeclampsia have increased risk for cardiovascular diseases (CVD) later in life. Preeclampsia and CVD share risk factors and pathophysiologic mechanisms, including dysregulated inflammation and raised blood pressure. Despite commonalities, little is known about the contribution of shared genes (pleiotropy) to these diseases. This study aimed to investigate whether genetic risk factors for hypertension or inflammation are pleiotropic by also being associated with preeclampsia.

Methods: We genotyped 122 single nucleotide polymorphisms (SNPs) in women with preeclampsia ($n = 1006$) and nonpreeclamptic controls ($n = 816$) from the Norwegian HUNT Study. SNPs were chosen on the basis of previously reported associations with either nongestational hypertension or inflammation in genome-wide association studies. The SNPs were tested for association with preeclampsia in a multiple logistic regression model.

Results: The minor (*G*) allele of the intronic SNP rs17367504 in the gene methylenetetrahydrofolate reductase (*MTHFR*) was associated with a protective effect on preeclampsia (odds ratio 0.65, 95% confidence interval 0.53–0.80) in the Norwegian cohort. This association did not replicate in an Australian preeclampsia case–control cohort ($P = 0.68$, odds ratio 1.05, 95% confidence interval 0.83–1.32, minor allele frequency = 0.15).

Conclusion: *MTHFR* is important for regulating transmethylation processes and is involved in regulation of folate metabolism. The *G* allele of rs17367504 has previously been shown to protect against nongestational hypertension. Our study suggests a novel association between this allele and reduced risk for preeclampsia. This is the first study associating the minor (G) allele of a SNP within the *MTHFR* gene with a protective effect on preeclampsia, and in doing so identifying a possible pleiotropic protective effect on preeclampsia and hypertension.

Keywords: blood pressure regulation, hypertension, methylenetetrahydrofolate reductase, pleiotropy, preeclampsia, single nucleotide polymorphism

Abbreviations: AGER, advanced glycosylation end product-specific receptor; ATP2B1, ATPase Ca^{2+} transporting, plasma membrane 1; BP, blood pressure; CI 95%, 95% confidence interval; CVD, cardiovascular diseases; ENCODE, Encyclopedia of DNA Elements; eQLTs, expression quantitative trait loci; GTEx, Genotype-Tissue Expression Project; GWAS, genome-wide association studies; HUNT, the HUNT Study; LD, linkage disequilibrium; MAF, minor allele frequency; MBRN, Medical Birth Registry of Norway; MTHFR, methylenetetrahydrofolate reductase; NHGRI Catalog, National Human Genome Research Institute Catalog of Published Genome-Wide Association Studies; NLRP, NOD-like receptor containing pyrin domain; NOD, nucleotide-binding oligomerization domain; OR, odds ratio; PAX5, paired box 5; PheGenI, phenotype–genotype integrator; PLCD3, phospholipase C delta 3; RGS, regulator of G-protein signaling; SNP, single nucleotide polymorphism; TLR, Toll-like receptor; VEGF, vascular endothelial growth factor

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INTRODUCTION

Preeclampsia is a disease of pregnancy with potentially severe and fatal outcomes for mother and fetus. The condition occurs in 2–8% of pregnancies and is commonly defined as *de novo* hypertension and proteinuria after gestational week 20 [1]. Women who experience preeclampsia have a two to eight-fold increased risk of subsequent development of cardiovascular diseases (CVD) [2], a major cause of death in women worldwide [3].

Preeclampsia and CVD share lifestyle-related, pathophysiological, and genetic risk factors [4–10]. For the joint pathophysiologic mechanisms, genetic and molecular functional studies have demonstrated endothelial dysfunction, inflammation, and vascular remodeling to be crucial to the development of chronic hypertension [9,10]. Changes in these biological processes are also thought to play a role in preeclampsia [11]. Aberrant blood pressure (BP) regulation can lead to sustained hypertension and CVD in susceptible individuals [12]. Hypertension developing during pregnancy (gestational hypertension) is the only consistent part of the preeclampsia diagnosis included in all definitions of the disease, whereas other diagnostic criteria of the disorder, including proteinuria, have varied between populations and over time [1,13–15]. Both gestational and non-gestational hypertension are usually defined by BP at least 140/90 mmHg, but it is not known whether these two conditions share a common cause. Preeclampsia as well as CVD involves release of angiogenic factors, oxidative stress, and adaptive metabolic changes like hyperlipidemia and increased insulin resistance, resulting in sustained inflammation and endothelial dysfunction [10,16]. The physiologic low-grade systemic inflammation of pregnancy is dysregulated and enhanced in preeclampsia at the maternal–fetal interface in the oxidatively stressed placenta and in the maternal vascular system [16,17]. In CVD periods of reduced tissue perfusion and ischemia with subsequent tissue reperfusion increase the level of cellular oxidative stress and thereby establish a sustained inflammatory response locally in tissues and generalized in the circulatory system [9,10].

The genetic contributions to preeclampsia and CVD have been established separately in large cohorts based on national registries and longitudinal health surveys as well as through family studies [4–7,18,19]. The maternal heritability estimates for preeclampsia are in the range 35–60% [6,19], whereas the heritability of BP regulation is estimated to 16–63% [4–7,19]. Genome-wide association studies (GWAS) on either preeclampsia or BP regulation have identified genetic variants associated with each of these conditions. The first GWAS published on preeclampsia identified two loci near the *Inhibin beta B* gene that could not be replicated in two other cohorts, whereas the second GWAS had a smaller numbers of cases and did not find any genome-wide significant associations [20,21]. Large GWAS have identified several genetic loci associated with BP regulation [22–30] and other inflammatory diseases such as inflammatory bowel disease, diabetes mellitus type 2, and lung function [31–33].

The concept of pleiotropy is receiving increasing attention in studies on complex diseases. Pleiotropy concerns how a single genetic variant directly regulates the expression of two or more phenotypes, either by inducing the same biological effect on two phenotypes or influencing opposite phenotypic effects through inducing both a protective and a disease promoting phenotype [34,35]. GWAS have resulted in identification of single nucleotide polymorphisms (SNPs) in genetic regions significantly associated with several seemingly unrelated conditions, indicating presence of pleiotropic influences on the development of complex human conditions. Despite an increasing awareness of a pathophysiologic relationship between preeclampsia and chronic hypertension, both risk factors for CVD, few studies have investigated possible pleiotropic genetic effects on these conditions or focused on their shared pathophysiological mechanisms of disease development [36–39].

In this study, our aim was to test the association between 122 SNPs previously associated with the phenotypes BP regulation and inflammation with preeclampsia. These phenotypes were chosen on the basis of their prominent involvement in both CVD and preeclampsia.

METHODS

Ethics statement

The Norwegian Regional Committees for Medical and Health Research Ethics (REK 2012/1876) and the HUNT Research Centre (HUNT 13/4894) have approved the project. Informed consent was obtained from all participants when enrolled in the HUNT surveys.

The Australian study was approved by the Royal Women's Hospital Research and Ethics Committees, Melbourne, Australia, and the Institutional Review Board of the University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA. All study participants gave their written informed consent when enrolled in the study.

Study population

In this report, the HUNT Study (HUNT), a longitudinal health study containing a biorepository, was linked to the Medical Birth Registry of Norway (MBRN) to identify female HUNT participants who had been diagnosed with preeclampsia and a control group with unaffected pregnancies.

The Medical Birth Registry of Norway

The MBRN is a national databank based on unique identification numbers and compulsory notification that contains information collected since 1967 on all pregnancies in Norway of at least 16 weeks gestation. The registered data detail maternal health before and during pregnancy, complications during pregnancy and delivery, and pregnancy outcomes [40].

The HUNT Study

The HUNT Study is a collaboration between the HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology), Nord-Trøndelag County Council, the Central Norway Health Authority, and the Norwegian Institute of Public Health. The HUNT Study

consists of three separate multipurpose health surveys (HUNT1–3) in the adult population of Nord-Trøndelag County in Norway and has been described in detail elsewhere [41,42]. The ethnicity of participants is not requested in the surveys, but the population in Nord-Trøndelag is stable and ethnically homogenous with more than 97% of Northern European origin. The main purpose of the HUNT Study is to investigate etiological factors of human disease. The three parts of the study took place in 1984–1986 (HUNT1), 1995–1997 (HUNT2), and 2006–2008 (HUNT3), and the available data on participants consist of health information based on questionnaires and physical examinations, as well as biological samples from individuals taking part in HUNT2 and HUNT3. Approximately 41,500 women participated and donated biological samples in at least one part of the study. Of these, over 20,000 women took part in both HUNT2 and HUNT3, and about 15,300 women participated in all three surveys (http://www.ntnu.no/hunt/oppmote, accessed 11 May 2015).

Data linkage

By means of the unique national identification numbers, data from the MBRN have been linked with the HUNT Databank to identify a preeclampsia case–control cohort of female participants in HUNT2 and HUNT3. These women gave birth between 1967 and 2009 and have pregnancies registered in the MBRN either with preeclampsia (cases) or as having had only pregnancies unaffected by preeclampsia, gestational hypertension, and chronic hypertension (controls).

The Australian preeclampsia case–control cohort

The replication cohort from Australia has previously been described [20]. Briefly, 1092 women (545 preeclampsia cases and 547 controls) of European ancestry were recruited at the Royal Women's Hospital, Melbourne, Australia. Of the 1092 individuals included 1018 women (471 preeclampsia cases and 547 controls) were ascertained from a larger case–control cohort assembled over a 5-year period (2007–2011), and an additional 74 unrelated preeclampsia cases were from a family-based cohort recruited over a 15-year period (1984–1999) [43].

Clinical definition of phenotypes

Data on gestational health and outcomes from the MBRN and well defined clinical and laboratory information from the HUNT Databank were used to identify phenotypes and covariates related to preeclampsia. Time of participation in HUNT was not related in time to the pregnancies and births of the included women.

Data from the MBRN contained information on all pregnancies and births of each woman, including maternal health prior to and during the pregnancies. For the women who had participated in more than one HUNT survey, all available relevant information was utilized, including data on diabetes, asthma, inflammatory diseases, use of medication, risk factors of CVD, established CVD and family history of CVD, as well as BP measurements and results from biochemical blood analysis.

In the MBRN preeclampsia is registered according to the set criteria of the repository: hypertension (BP $\geq$ 140/90 mmHg) with proteinuria ($\geq$+1 protein on a urine dipstick). We have previously found the diagnostic validity of these criteria to be 88.3% when examined against medical records of women in HUNT who gave birth at two hospitals (Levanger Hospital and Namsos Hospital) [15].

Cases and controls lacking phenotypic information on pregnancies and births in the MBRN were excluded. Individuals originally selected as controls were omitted if they had any pregnancies registered in the MBRN as stillbirths or complicated by placental abruption, or if they, prior to pregnancy, had an established kidney disorder, maternal heart disease, or a chronic inflammatory disorder such as rheumatoid arthritis. Numbers for excluded cases and controls can be found in SDC2 Table 2, http://links.lww.com/HJH/A681 in Supplemental digital content.

In the Australian cohort preeclampsia was defined as de novo hypertension with proteinuria after 20 weeks of gestation. Hypertension was defined as BP at least 140/90 mmHg or, an increase in SBP and/or DBP of at least 25 and/or 15 mmHg, respectively. Proteinuria was defined as either at least 0.3 g/l in a 24-h urine specimen, at least a '2+' dipstick reading from a random urine sample, or a spot protein : creatinine ratio at least 0.03 g/mmol. The preeclampsia diagnosis was determined by clinicians examining information in the medical hospital records of the participant. Exclusion criteria were preexisting hypertension or other medical conditions known to predispose for preeclampsia such as renal disease and diabetes mellitus. Further, women pregnant with twins or fetuses with chromosomal abnormalities were excluded.

Genotyping and quality control

Single nucleotide polymorphism selection

The SNP selection was based on results from GWAS performed in populations of European descent to match our study cohort. The studies were registered in one of two databases: the National Human Genome Research Institute Catalog of Published Genome-Wide Association Studies (NHGRI Catalog), containing published GWAS data [44], and the NCBI resource phenotype–genotype integrator (PheGenI) [45]. PheGenI contains genetic results assembled from several sources and by several methods, including published and unpublished raw data from GWAS. In the NHGRI Catalog (http://www.genome.gov/gwastudies/, accessed 25 February 2013), we utilized search filters 'blood pressure' and 'hypertension' as well as P value 10^{-6} or less, at which point 87 SNPs mapped to 65 unique genic regions were reported at this P value.

We next applied the following inflammatory genes one by one as filters in the PheGenI (http://www.ncbi.nlm.nih.gov/gap/phegeni, accessed 25 March 2013) including only results with P value 10^{-6} or less: Toll-like receptor (*TLR*) *1*, *TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR9*, nucleotide-binding oligomerization domain (*NOD*) *1*, *NOD2*, advanced glycosylation end product-specific receptor (*AGER*), NOD-like receptor containing pyrin domain (*NLRP*)*3*, *NLRP12*, vascular endothelial growth factor A (*VEGF*)-A, *VEGF-B*,

regulator of G-protein signaling 5 (*RGS5*), and NLR family CARD domain-containing protein 4 (*NLRC4*). For 10 of these genes, GWA results below the set P value were identified: *TLR1*, *TLR4*, *TLR5*, *NOD1*, *NOD2*, *AGER*, *NLRP3*, *NLRP12*, *VEGF-A*, and *RGS5*, resulting in $n = 163$ inflammatory SNPs for replication.

Of the in total 250 identified SNPs, 61 were found in duplicate and 74 were in linkage disequilibrium with another SNP in the dataset (SNAP [46] $r^2 \geq 0.8$, HapMap release 21, Northern Europeans from Utah population). The SNP with the highest *rs*-number in each linkage disequilibrium pair was excluded, leaving 115 SNPs for genotyping (SDC1 Table 1, http://links.lww.com/HJH/A681).

Genotyping

DNA was extracted by the HUNT Biobank from whole blood or blood clots sampled at inclusion in the HUNT2 and HUNT3 and stored at the HUNT Biobank, Levanger, Norway [41,42]. The genotyping was performed on the Sequenom MassARRAY system (Sequenom iPLEXassay, Sequenom Inc., San Diego, California, USA) by the Australian Genome Research Facility Ltd., St Lucia, Queensland, Australia. Of the 115 SNPs, 12 failed designability, 11 of which were replaced by all suitable surrogates ($r^2 = 1$). Finally a total of 122 SNPs were successfully genotyped, effectively covering 114 of the 115 SNPs originally planned genotyped.

Quality control

Standard quality control methods were applied as described in Supplementary material SDC2 Table 2, http://links.lww.com/HJH/A681 and SDC3 Table 3, http://links.lww.com/HJH/A681. Of 2187 samples genotyped, we excluded 125 individuals with very low quality DNA who failed all genotyping assays. From the remaining samples only individuals with average call rates more than 95% were included in the genetic analysis (SDC2 Table 2, http://links.lww.com/HJH/A681). SNPs failing genotyping and SNPs with call rates less than 98% were excluded from further analysis (SDC3 Table 3, http://links.lww.com/HJH/A681). Of the 122 SNPs successfully genotyped in the cohort, 119 SNPs passed the quality control and were analyzed in the 1822 individuals who passed quality control ($n = 1006$ cases and $n = 816$ controls).

Statistical analyses

All analyses in the Norwegian cohort were performed using the software packages PLINK version 1.07 [47] and R version 2.15.2 [48]. Quality control checks were performed with the R software. For phenotyping and identification of covariates the various birth-related outcomes were treated separately for each pregnancy, whereas data regarding the total obstetric history of a woman (including ever having had a pregnancy resulting in a stillbirth) were treated as binary variables. Longitudinal measures like BMI and BP were averaged. Participants were classified as smokers if they acknowledged to ever have smoked.

Variables associated with increased risk for developing preeclampsia were examined against the main phenotype preeclampsia in a generalized linear model (SDC5 Table 5, http://links.lww.com/HJH/A681). Adjustment for smoking as a risk factor was made based on whether participants acknowledged having ever smoked (ever vs never smokers). Only those variables significantly associated with the phenotype at P values 0.01 or less were incorporated as covariates in the final model: maternal age, total number of births per woman, average BMI at HUNT participation of the woman, twin pregnancies, year of giving birth, and if a woman had ever smoked (SDC5 Table 5, http://links.lww.com/HJH/A681).

The associations between phenotypes and SNPs were analyzed under an additive genetic model and tested in a multiple logistic regression model with the PLINK software [47]. Statistical tests were two-sided, and associations with a Bonferroni-corrected P value less than 0.05 for 119 independent tests (SNPs) ($P < 0.00042$) were considered statistically significant.

We had 90% power to detect SNPs with an effect size equivalent to odds ratio (OR) at least 1.6 with minor allele frequency (MAF) 0.05, or OR at least 1.4 with MAF 0.10 under an additive model assuming a type 1 error rate of 0.05 (adjusted for 119 SNP tests) and a two-sided test (QUANTO power calculator [49], http://biostats.usc.edu/Quanto.html).

The Australian preeclampsia genome-wide association study

The preeclampsia GWAS in Australian samples has been described in detail elsewhere [20,43]. Briefly, DNA was extracted from the 1092 Australian case–control blood samples using Qiagen's Blood & Cell Culture DNA Midi Kit (Qiagen Pty Ltd, Doncaster, Victoria, Australia) and samples were genotyped on Illumina's Human OmniExpress-12 BeadChip (Illumina Inc., San Diego, California, USA). Quality control performance measures for individual SNPs (genotype success rate, Hardy Weinberg equilibrium, and minor allele copy number) and samples (genotype call rate, random genotype error, and cryptic relatedness) were conducted in PLINK [47]. Samples were excluded when average call rates fell below 95%, where X chromosome homozygosity rates were at least 0.2 (PLINK [47]), or if estimates in PLINK indicated possible distant relations between women in the cohort. The potential confounding effect of population structure in the Australian preeclampsia GWAS cohort was negligible (genomic inflation factor = 1.002). Point-wise, asymptotic association tests were performed in PLINK assuming an additive model of gene action. The GWAS analyses included only SNPs passing genotyping and demonstrating call rates above 98%.

RESULTS

Descriptive characteristics of the selected study population from HUNT ($n = 1822$) are shown in Tables 1 and 2, containing continuous and binary variables, respectively. In the genetic association analysis, 1006 cases with preeclampsia and 816 controls with unaffected pregnancies were included (SDC2 Table 2, http://links.lww.com/HJH/A681). Significantly higher values were identified for preeclampsia cases compared with controls at time of participation in HUNT for BMI ($P < 0.001$), weight

TABLE 1. Continuous descriptive variables for the HUNT cohort included in the genotype-phenotype analyses

Descriptive variable	HUNT cohort ($n = 1822$)		Cases ($n = 1006$)		Controls ($n = 816$)		Cases vs controls
	Mean (±SD)	Missing (*n*)	Mean (±SD)	Missing (*n*)	Mean (±SD)	Missing (*n*)	*P* value
Birth year of participant	1956 (±11.1)	0	1957 (±10.5)	0	1955 (±11.7)	0	<0.01[a]
BMI (kg/m^2)[c]	26.5 (±4.7)	1	27.3 (±4.9)	0	25.6 (±4.3)	0	<0.001[b]
Height (m^2)[c]	165.4 (±5.7)	0	165.3 (±5.7)	0	165.5 (±5.8)	0	0.55[a]
Weight (kg)[c]	72.6 (±13.5)	1	74.5 (±14.1)	1	70.1 (±12.3)	0	<0.001[b]
SBP (mmHg)[c]	128.1 (±15.9)	0	131.0 (±15.7)	1	124.4 (±15.5)	0	<0.001[b]
DBP (mmHg)[c]	77.1 (±9.8)	0	79.0 (±9.6)	0	74.8 (±9.7)	0	<0.001[b]
Age in years of mother at birth of first child	23.3 (±4.1)	123	23.6 (±4.3)	71	22.8 (±3.9)	52	<0.001[a]
Age in years of mother at birth of last child	29.8 (±5.0)	390	30.1 (±4.7)	220	29.4 (±5.3)	170	<0.05[a]
Waist circumference (cm)	86.05 (±12.7)	50	87.67 (±12.9)	26	84.05 (±12.1)	24	<0.001[b]
Year of giving birth[c]	1984 (±10.57)	0	1985 (±10.24)	0	1984 (±11.0)	0	<0.01[a]
Total number of births[d]	2.79 (median 3.0) (±1.00)	0	2.85 (median 3.0) (±0.96)	0	2.71 (median 3.0) (±1.05)	0	<0.001[a]
Age in years of mother at time of giving birth[c]	26.9 (±5.36)	0	27.0 (±5.36)	0	26.7 (±5.36)	0	<0.05[b]

Data collected at time of participation in HUNT irrespective of when the woman gave birth. *HUNT*, the HUNT Study; *n*, numbers.
[a]Welch two sample *t* test.
[b]Mann–Whitney *U* test.
[c]Based on calculated averages for each individual according to all measurements given for the variable.
[d]According to the latest information registered for the woman.

($P < 0.001$), SBP and DBP ($P < 0.001$), diabetes of undefined type ($P < 0.001$), and waist circumference ($P < 0.001$) (Table 1). In addition, cases had higher parity ($P < 0.001$) and were younger at time of participation in HUNT when compared with controls (Table 1). Finally, a significantly higher percentage of the case women reported chronic hypertension ($P < 0.001$), a diagnosis of diabetes mellitus ($P < 0.001$), as well as a family history of hypertension ($P < 0.01$) in the HUNT surveys. A higher proportion of controls reported to ever have smoked compared with the women who had experienced preeclampsia ($P < 0.05$) (Table 2).

Of the 122 SNPs genotyped in the cohort, one SNP, rs17367504, was significantly associated with preeclampsia with the *G* (minor) allele demonstrating a protective effect on disease development after correction for multiple testing [$P = 3.52 \times 10^{-5}$, OR 0.65, 95% confidence interval (CI) 0.53–0.80, allele frequency 13%]. The SNP rs17367504 is located in intron 10 of the methylenetetrahydrofolate reductase (*MTHFR*) gene on chromosome 1.

The top 10 association results for the preeclampsia phenotype are given in Table 3, whereas a complete list of association results for this trait can be found in Supporting information (SDC4 Table 4, http://links.lww.com/HJH/A681). Three further SNPs demonstrated nominally significant associations with preeclampsia: the intergenic SNP rs17249754 located just downstream of the ATPase, Ca^{2+} transporting, plasma membrane 1 (*ATP2B1*) gene ($P = 0.02$, OR 0.80, CI 95% 0.66–0.97), the intronic SNP rs16933812 in the paired box 5 (*PAX5*) gene ($P = 0.04$, OR 0.85, CI 95% 0.74–0.99), and the intronic SNP rs12946454, located in the phospholipase C delta 3 gene (*PLCD3*) ($P = 0.046$, OR 0.85, CI 95% 0.73–0.80).

The top SNP (rs17367504) in our HUNT cohort was successfully genotyped in the Australian preeclampsia GWAS cohort but unlike in the HUNT cohort, the G-allele was not associated with preeclampsia ($P = 0.68$, OR 1.05, 95% CI 0.83–1.32, MAF = 0.15). A possible risk for carriers of the G-allele in the Australian cohort was not supported by the CI.

A holistic examination of the associations with preeclampsia of the 122 SNPs previously demonstrating association with BP regulation or inflammation does not indicate that using this selection strategy introduces an overall enrichment for associations with preeclampsia. In fact, we saw fewer significant associations than we would have expected due to chance (SDC6 Fig. 1, http://links.lww.com/HJH/A681).

TABLE 2. Binary descriptive variables for the HUNT cohort included in the genotype-phenotype analyses

Descriptive variable	HUNT cohort ($n = 1822$)	Cases ($n = 1006$)	Controls ($n = 816$)	Cases vs controls[a]
	n (%)	*n* (%)	*n* (%)	*P* value
Chronic hypertension	754 (41.4)	502 (49.9)	252 (30.9)	<0.001
Inflammatory disease[b]	221 (12.1)	119 (11.8)	102 (12.5)	0.70
Family history of hypertension	821 (45.1)	483 (48)	338 (41.4)	<0.01
Diabetes (unspecified)	483 (26.5)	335 (33.3)	148 (18.1)	<0.001
Smoking in pregnancy ever	278 (15.3)	158 (15.7)	120 (14.7)	0.62
Smoking ever	674 (37.0)	346 (34.4)	328 (40.2)	<0.05

n, numbers.
[a]Two-sample test for equality of proportions with continuity correction.
[b]Includes rheumatoid arthritis, ankylosing spondylitis, psoriasis, psoriasis arthritis, and hyperthyroidism.

TABLE 3. Top associations with preeclampsia after multiple logistic regression analysis

Rank	Chromosome	Gene	Base pair	SNP	Effect allele	MAF	*P* value	OR (CI 95%)
1	1	*MTHFR*	11862778	rs17367504[a]	G	0.130	3.52e − 05	0.65 (0.53–0.80)
2	12	*ATP2B1*	90060586	rs17249754[a]	A	0.146	0.022	0.80 (0.66–0.97)
3	9	*PAX5*	36969205	rs16933812[a]	G	0.338	0.040	0.85 (0.74–0.99)
4	17	*PLCD3*	43208121	rs12946454[a]	T	0.255	0.046	0.85 (0.73–0.80)
5	11	*AX747213/ADAMTS8/ BC144419/BC144418*	130273230	rs11222084[a]	T	0.349	0.055	0.87 (0.75–1.00)
6	8	*NOV*	120353267	rs2469997[a]	G	0.168	0.062	1.19 (0.99–1.43)
7	6	*VEGFA/C6orf223*	43951656	rs910609[b]	T	0.212	0.074	0.86 (0.72–1.02)
8	3	*SLC4A7*	27537909	rs13082711[a]	C	0.212	0.082	0.86 (0.72–1.02)
9	9	*TLR4/DBC1*	121278291	rs4132476[b]	T	0.097	0.083	0.81 (0.64–1.03)
10	8	*ZFAT*	135567046	rs1372662[a]	C	0.348	0.084	1.13 (0.98–1.30)

CI 95%, 95% confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Previously associated with blood pressure regulation.
[b]Located to an inflammation gene.

DISCUSSION

Despite an increased focus on preeclampsia as a risk factor for CVD development, few studies have investigated whether preeclampsia and CVD-related risk factors share a genetic background. Here we have performed a candidate SNP study in a preeclampsia-based cohort with candidates selected at loci previously associated with BP regulation and inflammation in GWAS. We identified one intronic SNP, rs17367504, in the *MTHFR* gene in which the G allele is significantly associated with a protective effect on preeclampsia development in the HUNT cohort, although not in the Australian cohort. The rs17367504 SNP is located within the DNase1 hypersensitivity cluster in 125 cell types from the Encyclopedia of DNA Elements and in a binding site for 16 transcription factors according to the UCSC Genome Browser (http://genome.ucsc.edu/, accessed 5 July 2015) [50]. Despite this strong evidence for a regulatory role for this SNP, no significant expression quantitative trait loci were found for rs17367504 in the Genotype-Tissue Expression Project portal (http://gtexportal.org, accessed 5 July 2015) [51]. The G-allele of this SNP has repeatedly been associated with lower SBP as well as DBP in different ethnic populations [25,26], but this is the first time this SNP has been associated with preeclampsia.

The pleiotropic nature of the *MTHFR* gene is indicated by more than 2900 publications on the term *MTHFR* gene. Changes in the *MTHFR* gene have been indicated in conditions such as hyperhomocysteinemia (a risk factor for CVD), stroke, neural tube defects, cancer, and obstetric complications [38,52–54]. The *MTHFR* gene produces the enzyme MTHFR, which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the active form of folate. Connections between the folate cycle and lipid metabolism have been demonstrated repeatedly including that excess folate leads to reduced MTHFR activity and increased lipogenesis [55], and that folate deficiency is associated with elevated homocysteine due to excess methionine scavenging by glycine *N*-methyltransferase, a key regulator of intracellular cholesterol trafficking shown associated with increased risk for CVD [56–58]. The similarities in preeclampsia and CVD pathophysiology support a role for MTHFR in preeclampsia, but so far no studies have investigated the functional implications of changes in rs17367504 in preeclampsia or CVD.

The novel association suggested by our findings in the HUNT cohort, between the BP-associated SNP rs17367504 and preeclampsia, adds to the increasing evidence that the *MTHFR* gene influences the development of preeclampsia and may point to a central role of the *MTHFR* gene in the pathophysiology of both preeclampsia and chronic hypertension. Genetic studies of *MTHFR* in relation to development of preeclampsia, hypertensive gestational diseases, or CVD have mainly investigated genetic associations with the separate disorders, not focusing on possible pleiotropic effects on both conditions. The preeclampsia studies conducted to date have largely focused on a missense *MTHFR* SNP, rs1801133, and a regulatory *MTHFR* SNP, rs1801131 [38,59] and identified an association between the minor alleles and an *increased* risk of preeclampsia. The *MTHFR* SNP associated with preeclampsia in our HUNT cohort, rs17367504, is not in linkage disequilibrium with either of the two SNPs (rs1801133 and rs1801131, $r^2 < 0.5$). Further, a recent study has identified the *MTHFR* gene promoter to be hypermethylated in placental tissue and maternal blood of preeclamptic women [60].

Despite the increasing evidence from candidate gene research and other genetic studies, no SNPs in the *MTHFR* gene did reach genome-wide significance in the two GWAS on preeclampsia reported to date [20,21]. In our study, the top association from the HUNT Study was examined for replication in the Australian GWAS cohort [20], resulting in a nonsignificant finding not supporting the link between preeclampsia and rs17367504 and rising the question about a possible false positive finding. The lack of replication of the association could also be due to the relatively small sample size, which, coupled with the diagnostic and genetic complexity of preeclampsia, leaves these studies underpowered to detect SNPs with modest effect sizes. The design of GWAS chips means that genotyped SNPs are likely to tag causative variants rather than be functional themselves, and different patterns of linkage disequilibrium between populations means these associations may weaken or disappear altogether. Indeed, both published GWAS on preeclampsia were unable to replicate their findings in independent cohorts [20,21]. To validate the finding identified in the HUNT Study, further replication

and functional studies on the SNP rs17367504 are required in new preeclampsia cohorts.

The minor alleles of the three SNPs that were nominally associated with a protective role against preeclampsia development in our study have previously been associated with BP regulation [25,26,28]. These SNPs are located in or close to the *ATP2B1*, *PAX5*, and *PLCD3* genes. Given the nominal *P* values for association achieved by these variants in this study, both further replication and molecular functional studies are warranted to identify a possible role as disease modifiers in preeclampsia and CVD.

The main strength of this study is the deep phenotyping including longitudinal variables that was performed on all cases and controls. This might be one of the reasons why we, in a cohort of this size, are able to identify a significant association between preeclampsia and a genetic locus with an acknowledged link to BP regulation. A weakness of the present study is the lack of replication of the link between preeclampsia and the SNP rs17367504 in an independent preeclampsia cohort. Although nonsignificant, the G allele in the Australian cohort did not demonstrate a trend toward protection against preeclampsia, as identified in the HUNT cohort. The result could have been strengthened further had the result replicated in an independent preeclampsia cohort. Further, the pleiotrophic effect of the SNP would have been strengthened had the study aim included replication of the associations with BP regulation identified through GWAS, but as the cohort was selected on the basis of the preeclampsia diagnosis, not hypertension, the study contained inadequate power for such a replication. A relatively small number of women had developed chronic hypertension at time of participation in HUNT, probably because the preeclampsia cohort selected from HUNT contained relatively young women (mean age 40.3 years in HUNT2 and 52.1 years in HUNT3).

In summary, we have comprehensively assessed SNPs previously reported to be associated with the phenotypes BP regulation and inflammation for association with preeclampsia. Women who carry the G (minor) allele of the intronic *MTHFR* SNP rs17367504 in the Norwegian HUNT Study are significantly less prone to develop preeclampsia than other women. Ours is the first study linking this BP associated *MTHFR* SNP to preeclampsia, thereby identifying a possible pleiotropic protective effect of the minor allele of this variant on preeclampsia and hypertension. Further genetic and functional studies in other cohorts are required for validation of the association. Discovery of common genetic associations between preeclampsia and hypertension will help identify shared etiological pathways. Increased comprehension of pathophysiologic mechanisms may lead to improved screening for preeclampsia and for subsequent risk of CVD.

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Conflicts of interest

There are no conflicts of interest.

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