A Family Based Study of Carbon Monoxide and Nitric Oxide Signalling Genes and Preeclampsia

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Abstract

Background: Preeclampsia is thought to originate during placentation, with incomplete remodelling and perfusion of the spiral arteries leading to reduced placental vascular capacity. Nitric oxide (NO) and carbon monoxide (CO) are powerful vasodilators that play a role in the placental vascular system. Although family clustering of preeclampsia has been observed, the existing genetic literature is limited by a failure to consider both mother and child.

Methods: We conducted a nested case–control study within the Norwegian Mother and Child Birth Cohort of 1545 case-pairs and 995 control-pairs from 2540 validated dyads (2011 complete pairs, 529 missing mother or child genotype). We selected 1518 single-nucleotide polymorphisms (SNPs) with minor allele frequency >5% in NO and CO signalling pathways. We used log-linear Poisson regression models and likelihood ratio tests to assess maternal and child effects.

Results: One SNP met criteria for a false discovery rate Q-value <0.05. The child variant, rs12547243 in adenylate cyclase 8 (*ADCY8*), was associated with an increased risk (relative risk [RR] 1.42, 95% confidence interval [CI] 1.20, 1.69 for *AG* vs. *GG*, RR 2.14, 95% CI 1.47, 3.11 for *AA* vs. *GG*, Q = 0.03). The maternal variant, rs30593 in *PDE1C* was associated with a decreased risk for the subtype of preeclampsia accompanied by early delivery (RR 0.45, 95% CI 0.27, 0.75 for *TC* vs. *CC*; Q = 0.02). None of the associations were replicated after correction for multiple testing.

Conclusions: This study uses a novel approach to disentangle maternal and child genotypic effects of NO and CO signalling genes on preeclampsia.

Keywords: *preeclampsia, genetic epidemiology, family based design, mother–child dyad, case–control, Norwegian Mother and Child Cohort Study, MoBa.*

Preeclampsia is a serious pregnancy complication, affecting approximately 2%–7% of pregnant women, characterized by new-onset gestational hypertension and proteinuria after 20 weeks' gestation.[1] The only

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definitive treatment is delivery, and it is associated with serious maternal and foetal morbidity and mortality.[1]

Although incompletely understood, preeclampsia is hypothesized to originate during placentation.[1] Thus, both maternal and foetal components may contribute to the condition. During normal placentation, the foetal cytotrophoblast invades the maternal decidua,

penetrating maternal spiral arteries and increasing vascular dilation. It is hypothesized that incomplete remodelling and perfusion of the spiral arteries leads to placental ischaemia and hypoxia,[2,3] increasing maternal endothelial dysfunction and the subsequent clinical symptoms of preeclampsia.[3]

Nitric oxide (NO) and carbon monoxide (CO) are powerful vasodilators[4,5] that may improve vascular capacity during spiral artery remodelling, thereby promoting healthy placental development and reducing risk of preeclampsia. NO and CO are produced endogenously as well as inhaled from exogenous sources.[6,7] Measured NO production and serum metabolites of NO are lower among women with preeclampsia than during normal pregnancy[7,8] and women with preeclampsia have decreased amounts of CO concentrations in their exhaled breath compared to those with healthy pregnancies.[9,10] NO and CO are important in the placental vascular system[5,11] and it has been suggested that NO and CO are required for proper trophoblast differentiation and invasion.[5,11] CO is directly produced by foetal trophoblast cells,[5] and chorionic and umbilical endothelial cells release NO.[11] Because NO and CO are associated with smooth muscle relaxation and blood pressure regulation, they have been posited as potential explanations for the well-known inverse relationship of maternal smoking with preeclampsia.[3,5,7]

Numerous studies have found a familial predisposition for preeclampsia[12] and heritability is high (approximately 50%),[13] however, few consistent genetic associations have emerged. Prior genetic studies of preeclampsia have almost exclusively focused on maternal DNA, which ignores the potential contributions of child genetics in disease aetiology. Isolated variants in CO or NO-related genes (*NOS3, NOS2, HMOX1, HIF1A*) have been examined in relation to preeclampsia, or cardiovascular disease (*CAV1, ESR1, GUCY1A3, GUCY1B3, PRKCA*), but these studies have generally been small and results have been mixed.[8,14,15] A meta-analysis of the most widely investigated SNP in *NOS3* (rs1799983) showed a slightly increased risk of preeclampsia (OR 1.19, 95% CI 1.00, 1.42).[14] In addition, in knockout mice and human placental expression studies, NOS3 expression is lower in preeclamptic placentas[11] and HMOX1 deficiency is associated with insufficient spiral artery remodelling.[16]

The objective of this study was to determine if maternal or child single-nucleotide polymorphisms

(SNPs) in NO and CO signalling pathways were associated with preeclampsia using a mother–child dyad design, nested within the Norwegian Mother and Child cohort (MoBa). We examined SNPs within three canonical pathways important for both CO and NO activity. Exploring both maternal and child genotype and identifying variants that may play a role in both endogenous and exogenous NO and CO may help establish potential therapeutic targets for this serious and life-threatening condition.

Methods

Study population

This study is a nested case–control study within the Norwegian Mother and Child Cohort Study (MoBa), conducted by the Norwegian Institute of Public Health (MoBa data Version 8).[17] MoBa is a large prospective birth cohort of pregnant women and their offspring, recruited throughout Norway from 1999 to 2008 ($N = 112\,908$ pregnancies). All pregnant women living in Norway who gave birth at a hospital or maternity unit with more than 100 births annually and who could speak Norwegian were eligible; MoBa investigators applied no other exclusion criteria. Pregnant women were recruited by mail prior to their routine ultrasound appointment at 17 to 20 weeks' of gestation. Of all women invited to participate, 41% enrolled in the study.[17] Participants completed two prenatal questionnaires about their health and environment.[17] Maternal blood was collected at the first ultrasound appointment and cord blood was collected at birth.

Outcome assessment

Birth outcome information was obtained from the Medical Birth Registry of Norway.[18] Preeclampsia case/control status was verified using antenatal records and hospital discharge codes, as described by Klungsøyr and colleagues[19] and in the Supplementary Methods. Preeclampsia was defined using American College of Obstetrics and Gynecologists (ACOG) criteria.[20] All observations registered as preeclampsia cases ($n = 4081$) and a random sample ($n = 2000$) of pregnancies registered as being unaffected by preeclampsia were selected from MoBa to be verified by antenatal records. Of the 3500 registered preeclampsia cases and 1840 registered to be

unaffected by preeclampsia for which records were received, 2936 pregnancies registered with preeclampsia cases in the MBRN were verified to have been affected by preeclampsia, and 1745 pregnancies without preeclampsia registered in the MBRN were found to be negative for preeclampsia. For this analysis, we included from among these validated records, women with a singleton pregnancy who conceived spontaneously, were verified cases or controls, returned both early and late pregnancy questionnaires, had blood stored in the MoBa biobank, and had no history of chronic hypertension. After exclusion criteria, there were 1564 cases (of which 1118 had both mother and child DNA, and 446 had only maternal DNA) and 999 controls (of which 968 had mother and child DNA, and 31 had only maternal DNA) that were genotyped, for total of 4649 samples across all cases and controls, mothers and children (Figure S1). Criteria for preeclampsia subtypes are presented in Table 1, and Supplementary Methods. The same set of control samples was used for each subtype analysis.

Gene and single-nucleotide polymorphisms selection

For this study, the three established Ingenuity (www. ingenuity.com, QIAGEN, Redwood City, CA, USA) canonical pathways involved in CO and NO signalling and synthesis were selected, which included: (i) endothelial nitric oxide synthase (eNOS) signalling pathway, which accomplishes the synthesis of NO from L-arginine, (ii) haeme degradation, which accomplishes the breakdown of haemoglobin into CO and bilirubin, and (iii) hypoxia-inducible factor 1-alpha (HIF1A), which regulates oxygen homeostasis and response to hypoxia. Sixty-six genes (Table S1) in these pathways were selected for analysis, using a 10 kb upstream and downstream margin around the transcription start and end sites for each gene. We utilized TagZilla (http://tagzilla.nci.nih.gov) to identify haplotype tagging SNPs with an R^2 criteria of 80%, using a 10 kb upstream and downstream margin around the start and end transcription site, to capture important promoter and enhancer sites, resulting in a of 1518 SNPs.

DNA genotyping and quality control

Single-nucleotide polymorphisms were genotyped by the UNC Mammalian Genotyping Core using the HumanCoreExome+ array from Illumina (Illumina, Inc., San Diego, CA, USA). Samples and SNPs were assessed for quality control using PLINK 1.07 (http://pngu.mgh.harvard.edu/purcell/plink). SNPs were excluded for missingness >5%, deviation from Hardy–Weinberg Equilibrium ($P < 1 \times 10^{-3}$), and minor allele frequency <5%. Samples were assessed for call rate, sex discrepancies, relatedness, and inbreeding. The full quality control process is described in Supplementary Methods and Figures S1 and S2.

Table 1. Preeclampsia and preeclampsia subtype criteria

Phenotype	Criteria	Case pregnancies
Preeclampsia[a]	• New onset systolic blood pressure ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg AND • Proteinuria ≥0.3 g/24-h or ≥1+ on urine dipstick	1545
Preeclampsia subtypes		
Severe preeclampsia[a]	General requirements of preeclampsia plus • Systolic blood pressure of ≥160 mm Hg or diastolic blood pressure of ≥110 mm Hg OR • Proteinuria ≥5 g/24-h or ≥3+ on urine dipstick	308
Early onset preeclampsia	General requirements of preeclampsia plus • Diagnosis prior to 34 completed weeks of gestation	277
Preeclampsia with early delivery	General requirements of preeclampsia plus • Delivery prior to 34 completed weeks of gestation	132
Preeclampsia with small-for-gestational-age	General requirements of preeclampsia plus • Infant born <10th percentile weight for gestational age[b]	349

[a]Cases with a validated diagnosis of eclampsia in the Medical Birth Registry of Norway were included in the preeclampsia and severe preeclampsia phenotypes.
[b]Population percentiles derived from Norwegian distribution, eSnurra Norway.

The top three principal components of genetic variation were plotted for the MoBa data together with the 1000 Genomes reference populations to assess evidence of admixture (Figure S3). The final analysis sample (n = 4551 total samples) included dyads with both mother and child genotype data as well as incomplete dyads with only mother or child genotype data [n = 2621 preeclampsia case samples (1076 mother/child pairs, 459 mother only, 10 child only), n = 1930 control samples (935 mother/child pairs, 46 mother only, 14 child only)].

Statistical analysis

To simultaneously account for maternal and child genotype, we used the case-mother control-mother log-linear modelling approach proposed by Shi et al.[21] This method uses Poisson regression to model expected counts of each possible genetic mating type combination under the assumption of Mendelian inheritance. This method allows one to account for the correlation between maternal and child genotype and improves power compared to a logistic model.[21,22] Two maternal and two child genetic risk parameters were included in the model to saturate for codominant genetic main effects, as follows:

$$\ln[E(N_{mcd})] = \theta_{mc} + \delta d + \alpha_1 dI_{m=1} + \alpha_2 dI_{m=2}$$
$$+ \beta_1 dI_{c=1} + \beta_2 dI_{c=2}$$

where $E(N_{mcd})$ is the expected value of the counts of families with each of maternal genotypes, child genotypes, and case or control status; $d = 1$ for a case and $d = 0$ for a control; and $I_{(m=j)}$ and $I_{(c=i)}$ are indicators for whether a mother or child has j (=one or two) copies of the variant allele. The θ_{mc} parameters allow flexibility of the control-mother distribution and ensure that the parental genotype distribution is only constrained by the family relationships.

To explore the potential for maternal–foetal interactions, we expanded our model to include an indicator for when the mother has more copies of the variant allele than the child, described in the Supplementary Methods.

LEM software[23] was used to fit these models. The expectation maximization algorithm was used to incorporate dyads with missing genotypes. Likelihood ratio tests comparing reduced models with the saturated model were performed to determine

P-values for both maternal and child genetic effects, each adjusted for the other genotype. A 4 degree-of-freedom likelihood ratio test was used to determine joint P-values for simultaneous tests of maternal/child genetic effects. Point estimates and 95% confidence intervals for relative risks were calculated for each SNP for both maternal and child genotype.

To account for multiple comparisons, we calculated the false discovery rate (FDR), which is the expected proportion of type 1 errors (false positives) among all positive tests.[24] We used an FDR of <0.05 (reported as Q-values) as our threshold for considering a finding noteworthy.

Replication methods

All SNPs with Q-values ≤ 0.2 for both preeclampsia overall and preeclampsia sub-phenotypes were sent to the InterPregGen consortium for attempted replication analysis.[25] Within InterPregGen, cases came from the UK Genetics of Pre-eclampsia (GOPEC) consortium. The same standard definition of preeclampsia defined cases. Population controls came from the Wellcome Trust Case-Control Consortium. Maternal samples (1875 cases, 5088 controls) and child samples (1004 cases, 5286 controls) were analysed separately for SNP associations with preeclampsia using logistic regression, assuming a logit-additive model. A subset included early preeclampsia information, so were also analysed as a proxy for the subtypes of preeclampsia with additional complications (505 maternal cases, 5051 maternal controls, 276 child cases, 5297 child controls). Complete methods for recruitment, genotyping, and quality control of the replication sample are described in Supplementary Methods.

Results

The final analysis sample consisted of 4551 individual samples for 2011 complete mother–child dyads, 505 samples with only maternal genotype data (459 cases), and 24 children with only child genotype data (10 cases; n = 2540 pregnancies; Table 2). Mean maternal age was 29.6 years (SD 4.7) and most had a university degree. As expected, a greater proportion of women with preeclamptic pregnancies were nulliparous and of high body mass index (overweight or obese) compared to those without preeclampsia. Babies born to women with preeclampsia were more often preterm

Table 2. Demographic characteristics of pregnancies in the final study sample (n = 2540 pregnancies, 4551 samples)

	Preeclampsia cases (N = 1545)		Controls (N = 995)	
	No.	%	No.	%
Maternal age [mean(SD), years]	29.3 (4.9)		30.1 (4.4)	
Maternal education				
<High school	132	8.5	73	7.3
High school graduate	439	28.4	264	26.5
University degree	831	53.8	574	57.7
Missing	143	9.3	84	8.4
Body mass index (kg/m^2)				
Underweight (<18.5)	25	1.6	32	3.2
Normal weight (18.5–24.9)	712	46.1	629	63.2
Overweight (25.0–29.9)	444	28.7	186	18.7
Obese (30.0+)	264	17.1	95	9.6
Missing	100	6.5	53	5.3
Maternal smoking				
Smoking in weeks 11–20	120	7.8	95	9.6
Missing	83	5.4	44	4.4
Smoking in third trimester	71	4.6	64	6.4
Missing	159	10.3	86	8.6
Nulliparous	1012	65.5	407	40.9
Preterm (< 37 weeks)	332	21.5	33	3.3
Small for gestational age (SGA) (<10th percentile)[a]	349	22.6	77	7.7
Preeclampsia subtypes				
Severe	308	19.9		
Onset <34 weeks	277	17.9		
Delivery <34 weeks	132	8.5		
Accompanied by SGA[a]	349	22.6		

[a]Population percentiles derived from Norwegian distribution, eSnurra Norway.

and small-for-gestational-age (SGA). Severe preeclampsia (including eclampsia) was present in 20% of women with preeclampsia.

Results of tests for maternal genotypic associations controlling for child genotype are summarized in Figure S3a and for child genotypic associations controlling for maternal genotype are summarized in Figure S3b. In the joint 4-degree of freedom test, we found one SNP to be significant ($Q \leq 0.05$), however, this SNP was only individually significant in the child and not in the mother. We found a child association of increasing risk in the variant allele of this SNP (rs12547243, MAF = 0.29), a synonymous substitution in a coding region of *ADCY8* on chromosome 8. The estimated relative risk (RR) was 1.41, and 95%

confidence interval (CI) 1.19, 1.67 for 1 copy of the minor allele and RR 2.12, 95% CI 1.46, 3.07 for 2 copies (Q = 0.04) (Table 3). Although no maternal genotypic associations met our FDR threshold, there were a number of suggestive maternal genotypic associations for SNPs in *ESR1*, *PDE1C*, *PIK3C2G*, and *GUCY1A3*. Generally, the *ESR1* and *PDE1C* SNPs were associated with a reduced risk of preeclampsia and the *PIK3C2G* and *GUCY1A3* SNPs were associated with an increased risk of preeclampsia, however, few showed a dose–response pattern and risk ratios were mostly null for the homozygous genotype. Table 3 shows both mother and child effect estimates, and the joint test results, for all top SNPs with FDR Q-values ≤0.20. Excluding population outliers along axes of ancestral variation did not substantially alter our findings (See Figure S3, Table S2). All results for potential maternal–foetal interactions were null (Table S4).

Because preeclampsia is a heterogeneous condition for which underlying aetiologies may differ, we repeated the analysis within preeclampsia subtypes. Results for subtype associations with $Q \leq 0.2$ are presented in Table 4. Within subtypes, we found associations for one SNP within *PDE1C*, a maternal association of rs30593 (MAF = 0.35) for preeclampsia accompanied by early delivery (RR 0.45, 95% CI 0.27, 0.75 for 1 copy; RR 1.44, 95% CI 0.63, 3.30 for 2 copies; Q = 0.02). A similar child pattern was present for rs30562, another SNP in *PDE1C*, but was just above our significance threshold (Q = 0.06).

We provided the SNPs with $Q \leq 0.2$ overall and within subtypes to the InterPregGen Consortium for analysis. Because we found different lead SNPs among the general preeclampsia phenotype and preeclampsia subtypes, in the replication sample we assessed these SNPs for both associations with overall preeclampsia (Table S3a) and early preeclampsia (Table S3b), the only sub-phenotype for which we had replication data. None of the SNPs analysed were associated in the replication dataset after correction for multiple testing. Our lead SNP for preeclampsia with early delivery, a maternal association of rs30593 in *PDE1C*, was nominally associated (uncorrected P = 0.05) in the replication cohort, but in the child population. As with this SNP in the MoBa study, we saw a similar reduced risk of preeclampsia (RR 0.90, 95% CI 0.82, 1.00) in the replication study. Complete replication results are reported in Table S3.

Table 3. Summary of SNPs with FDR $Q \leq 0.2$ for tests of maternal genetic effects, adjusting for child genotype, and child genetic effects, adjusting for maternal genotype for pre-eclampsia overall

Marker[a]	Chr	Position	MAF Mother	MAF Child	Gene	Genotype	Mother RR (95% CI)	Mother P value	Mother FDR Q value	Child RR (95% CI)	Child P value	Child FDR Q value	Joint test P value	Joint test FDR Q value
rs7435347	4	156654735	0.17	0.17	GUCY1A3	AA	1.00 (Reference)	6.17×10^{-4}	0.09	1.00 (Reference)	0.09	0.76	3.36×10^{-3}	0.30
						GA	1.47 (1.20, 1.80)			0.83 (0.69, 0.99)				
						GG	1.00 (0.63, 1.58)			0.67 (0.40, 1.14)				
rs1569788	6	152328616	0.30	0.30	ESR1	TT	1.00 (Reference)	3.02×10^{-4}	0.09	1.00 (Reference)	0.61	0.88	2.66×10^{-3}	0.30
						CT	0.70 (0.58, 0.84)			1.07 (0.89, 1.28)				
						CC	0.93 (0.67, 1.30)			1.18 (0.83, 1.68)				
rs3020366	6	152368758	0.37	0.36	ESR1	TT	1.00 (Reference)	5.45×10^{-4}	0.09	1.00 (Reference)	0.40	0.88	4.49×10^{-3}	0.30
						CT	0.70 (0.58, 0.84)			1.11 (0.92, 1.32)				
						CC	0.86 (0.64, 1.15)			1.23 (0.90, 1.67)				
rs6462324	7	32120897	0.40	0.39	PDE1C	CC	1.00 (Reference)	2.05×10^{-4}	0.09	1.00 (Reference)	0.32	0.88	1.23×10^{-3}	0.25
						AC	0.69 (0.57, 0.84)			1.04 (0.87, 1.25)				
						AA	0.93 (0.69, 1.23)			1.24 (0.92, 1.69)				
rs6470860	8	131905190	0.41	0.42	ADCY8	AA	1.00 (Reference)	0.96	0.98	1.00 (Reference)	3.12×10^{-4}	0.15	9.88×10^{-5}	0.07
						GA	1.03 (0.85, 1.24)			1.32 (1.10, 1.58)				
						GG	1.02 (0.77, 1.35)			1.85 (1.36, 2.51)				
rs12547243	8	131921956	0.29	0.31	ADCY8	GG	1.00 (Reference)	0.09	0.89	1.00 (Reference)	2.69×10^{-5}	0.04	6.27×10^{-7}	8.78×10^{-4}
						AG	1.14 (0.94, 1.37)			1.41 (1.19, 1.67)				
						AA	0.82 (0.58, 1.17)			2.12 (1.46, 3.07)				
rs7459573	8	131928401	0.34	0.36	ADCY8	AA	1.00 (Reference)	0.92	0.98	1.00 (Reference)	1.61×10^{-4}	0.11	3.14×10^{-4}	0.11
						GA	0.97 (0.81, 1.17)			1.43 (1.20, 1.70)				
						GG	0.94 (0.69, 1.28)			1.73 (1.25, 2.38)				
rs17475920	12	18478126	0.12	0.13	PIK3C2G	AA	1.00 (Reference)	9.39×10^{-4}	0.10	1.00 (Reference)	0.13	0.80	7.12×10^{-3}	0.34
						TA	1.52 (1.21, 1.90)			0.85 (0.69, 1.04)				
						TT	1.65 (0.80, 3.40)			0.54 (0.27, 1.07)				
rs9634063	12	18593252	0.15	0.15	PIK3C2G	CC	1.00 (Reference)	2.70×10^{-4}	0.09	1.00 (Reference)	0.04	0.76	1.95×10^{-3}	0.30
						TC	1.54 (1.25, 1.91)			0.80 (0.66, 0.97)				
						TT	1.39 (0.75, 2.60)			0.56 (0.30, 1.06)				

[a]Single-nucleotide polymorphisms that also met $Q \leq 0.2$ and were in high linkage disequilibrium ($R^2 > 0.8$ using 1000 Genomes Pilot 1 CEU data): rs3796578 ($R^2 = 1.0$ with rs7435347); rs722208 ($R^2 = 1.0$ with rs1569788); rs3020365 ($R^2 = 0.93$ with rs3020366); rs11044095, rs11044129, rs7311726, rs1447408 ($R^2 \geq 0.92$ with rs9634063).

Table 4. Summary of SNPs with FDR $Q \leq 0.2$ for tests of maternal genetic effects, adjusting for child genotype, and child genetic effects, adjusting for maternal genotype for preeclampsia subtypes

Marker*	Chr	Position	MAF Mother	MAF Child	Gene	Genotype	Mother			Child			Joint test	
							RR (95% CI)	P value	FDR Q value	RR (95% CI)	P value	FDR Q value	P value	FDR Q value
Severe preeclampsia														
No SNPs with $Q \leq 0.2$														
Early onset preeclampsia (diagnosis <34 weeks)														
No SNPs with $Q \leq 0.2$														
Preeclampsia with delivery <34 weeks														
rs30593	7	32105096	0.35	0.35	PDE1C	CC	1.00 (Reference)	1.69×10^{-5}	0.02	1.00 (Reference)	0.40	0.75	5.38×10^{-5}	0.07
						TC	0.45 (0.27, 0.75)			0.63 (0.31, 1.30)				
						TT	1.44 (0.63, 3.30)			0.89 (0.27, 2.89)				
rs12785615	11	106869624	0.14	0.14	GUCY1A2	GG	1.00 (Reference)	6.32×10^{-4}	0.20	1.00 (Reference)	0.66	0.75	2.80×10^{-3}	0.62
						CG	0.63 (0.35, 1.15)			0.85 (0.35, 2.08)				
						CC	4.31 (1.42, 13.10)			—				
rs1455590	11	106869973	0.23	0.23	GUCY1A2	GG	1.00 (Reference)	3.50×10^{-4}	0.15	1.00 (Reference)	0.44	0.75	6.10×10^{-4}	0.34
						AG	0.63 (0.38, 1.03)			1.43 (0.73, 2.80)				
						AA	2.50 (1.16, 5.37)			0.65 (0.07, 5.70)				
rs11636443	15	52319696	0.44	0.44	MAPK6	GG	1.00 (Reference)	3.17×10^{-4}	0.15	1.00 (Reference)	0.21	0.75	1.89×10^{-3}	0.62
						AG	0.68 (0.42, 1.08)			0.55 (0.27, 1.11)				
						AA	1.98 (0.97, 4.02)			0.47 (0.17, 1.32)				
Preeclampsia with small for gestational age														
rs30562	7	32065264	0.35	0.35	PDE1C	CC	1.00 (Reference)	0.04	0.96	1.00 (Reference)	4.42×10^{-5}	0.06	2.76×10^{-5}	0.04
						TC	1.40 (1.04, 1.88)			0.50 (0.36, 0.71)				
						TT	1.04 (0.62, 1.73)			1.02 (0.62, 1.70)				

Comment

Principal findings

In this large, family based case–control study of genetic variants in CO and NO pathways and preeclampsia, there were no replicated associations between CO and NO variants and risk of preeclampsia. However, this study demonstrates the utility of using a dyad approach and provides information about potential genetic associations of interest within both the mother and the child. In the child, rs12547243 in *ADCY8*, a SNP with no previously reported associations, was associated with preeclampsia in Norwegian women. The *AG* and *AA* genotypes were associated with increased risk of preeclampsia as compared with the *GG* genotype. There were no maternal genotypic associations with preeclampsia overall that met the FDR threshold ($Q < 0.05$). Among preeclampsia subtypes, there was a decreased risk of preeclampsia accompanied by early delivery associated with the *TC* maternal genotype as compared to *CC* genotype of rs30593 in *PDE1C*. However, none of these associations were replicated in the InterPregGen study. Of all the lead SNPs that were sent for replication testing, only rs30593 in *PDE1C* was nominally associated with preeclampsia in the replication sample ($P = 0.05$), however, this association was found in the child (rather than the mother as in the MoBa cohort) and was not significant after adjustment for multiple testing.

Interpretation

The potential role of nitric oxide and carbon monoxide in the development of preeclampsia is an ongoing area of research. In this study, none of the SNPs in the most widely studied candidate genes for preeclampsia in the CO and NO pathways, *NOS2* or *NOS3*, were associated with preeclampsia, however, prior associations were found in small and ethnically diverse populations.[8,15] Although not replicated, there were some interesting associations that have been supported by animal studies of preeclampsia[26] or are more broadly associated with cardiovascular outcomes in GWA studies[27–29] and warrant further investigation. Clinical trials are currently underway for the treatment of preeclampsia with pharmaceuticals whose mechanism is via nitric oxide signalling, including hydroxymethylglutaryl-CoA reductase inhibitors ('statins')

and phosphodiesterase inhibitors.[30] Several SNPs of interest in our analysis ($Q < 0.1$) are located in the genes *ADCY8, GUCY1A2, GUCY1A3,* and *PDE1C*, which are all part of the canonical pathway for cellular effects of sildenafil ('Viagra'), a phosphodiesterase inhibitor that operates through nitric oxide signalling to increase vasodilation.[31] Mouse studies have demonstrated resolution of the preeclampsia phenotype with the administration of sildenafil[26] and it is now being investigated for treatment of preeclampsia and foetal growth restriction.[30]

Although there were a few associations of interest in the MoBa sample, these were not replicated in the InterPregGen analysis. It is plausible that some of the associations failed to replicate due to different analytic methods and a somewhat different outcome assessment in the primary analysis and replication datasets. While both populations used the same definition of preeclampsia, the MoBa analysis used only validated cases and non-cases, whereas the InterPregGen Consortium used validated cases but population controls that may or may not be pregnant women. Overall allele frequencies for SNPs in the replication analysis were similar between the MoBa sample and the InterPregGen sample, but differed by case–control status in both mothers and children for SNPs associated with preeclampsia in the MoBa sample (Table S5). In addition, in the MoBa analysis, both mother and child genotypes were included and modelled codominantly with a genetic mating type parameter to account for mother-child family structure; by contrast, the replication analysis independently modelled mother and child genotype logit-additively. To investigate how a similar type of analysis might alter these results, maternal and child samples from the MoBa sample were analysed using standard logistic regression analysis modelled additively in PLINK. Associations with rs12547243 in the child were stronger, but other associations were attenuated (data not shown).

The largely null findings of this study demonstrate the challenges of the genetic study of preeclampsia. Despite a clear *a priori* hypothesis, large sample size, well-validated outcome, and population with little admixture used to study a condition for which there is strong evidence of heritability, associations were not replicated in another population. Although both maternal and foetal genes should be considered because they may differentially affect preeclampsia independently or through their interaction, the

aetiology of preeclampsia likely includes both poly-genecity as well as phenotypic heterogeneity, underscoring the need for very large sample sizes.

Strengths of the study

This study is one of the largest genetic studies of preeclampsia to date, with 1076 case pairs (2152 samples) and 935 control pairs (1870 samples), with access to both maternal and child DNA. Given the suspected pathophysiology underlying preeclampsia, it is biologically plausible that both maternal and child genotypes contribute to this pregnancy complication. This dyad analysis accounted for family structure, and has been implemented for other child phenotypes in which both maternal and child genotype may play a role,[32] but has not been explored for pregnancy complications. Although a few other studies of preeclampsia have examined child genotype,[33,34] to our knowledge, none have simultaneously modelled both mother and child. Mother and child genotype may independently contribute to the development of preeclampsia,[13] but it is also possible that maternal and child genetic factors may act synergistically or antagonistically through their interaction. The dyad design allowed for preliminary investigation of maternal–foetal interactions.

Candidate gene studies have inconsistently replicated, possibly in part due to the fact that few studies adequately considered variability across the gene. This study aimed to improve upon prior candidate gene studies by providing a more comprehensive coverage of genetic variation across the genes, while continuing to apply a hypothesis-driven approach to conserve statistical power.

A significant strength of our study was the verification of the clinical endpoint by antenatal medical records and hospital diagnostic codes through medical record validation.[19] This also enabled classification of cases into subtypes that may have differing underlying aetiologies.

Limitations of the data

Although the analytic method allowed control for paired genotypes, which is likely the biggest confounder, a limitation of this study design is the inability to control for external confounders, such as using principal components to adjust for population admixture. Family based case-parent trio designs are protected from population stratification bias because the families essentially serve as their own controls, but use of dyads in which mating-type frequencies may differ between cases and controls retains possible confounding by ancestry; thus, the case-mother control-mother design is not as robust to population stratification as a trio design would be. To address this limitation, population stratification was assessed in the quality control process to determine if ancestral homogeneity was a plausible assumption within this Norwegian cohort. Survey information about ethnicity is limited to first-language spoken by parents and grandparents. Comparing the MoBa population with the 1000 Genomes reference populations, a handful of MoBa participants were identified who clustered with Chinese, Amerindian, and Nigerian ancestral populations (Figure S3). Excluding such observations in a sensitivity analysis, however, did not change results, indicating that population stratification bias is not a serious issue (Supplementary Methods). However, the results of this study are most generalizable to populations of European descent. This study may be affected by selection bias if similar genetic factors that influence preeclampsia also influence study participation. When MoBa study participants were compared with the population of all births in Norway during the same period, prevalence of preeclampsia was similar, but women in MoBa were less likely to be young, live alone, smoke, and have more than two births.[35] When associations between covariates and birth outcomes in the two populations were compared, however, there were few differences (e.g. the ratio of adjusted odds between smoking and low birthweight and between parity and preeclampsia were approximately 1.0).[35] To address the possibility that prior birth outcome may affect participation in the study, a sensitivity analysis was conducted restricting the analysis to primiparous women and results were unchanged (data not shown). In addition, despite being the largest study, power is still limited. A dyad design improves statistical power, but is slightly less powerful than other family based designs, such as trios. Although there were some interesting trends and suggested associations, larger studies are needed to study these associations in greater detail.

Conclusions

In conclusion, this study uses a novel design to disentangle maternal and child genotypic effects of NO and CO signalling genes on preeclampsia. The

results of this study highlight the challenges of discovering replicated SNPs across multiple populations, even for highly heritable perinatal conditions, like preeclampsia, and demonstrate the unique challenges of conditions that may be influenced by both mother and child. Future research of genetics and preeclampsia should continue to incorporate maternal and child genetic components and expand to explore maternal–child genotypic interactions as well as interactions with exogenous sources of CO and NO.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Quality control and sample selection process.

Figure S2. Quality control quantile–quantile plots for maternal and child genotypic effects among genome-wide data of 263 494 variants to assess genomic inflation. Test results (observed -$\log_{10}$p values) are plotted against the expected -$\log_{10}$p values for each of 263 494 SNPs in the sample.

(a) Q-Q plot of maternal genotypic effects among maternal samples. Genomic inflation factor, lambda = 1.01. (b) Q-Q plot of child genotypic effects among child samples. Genomic inflation factor, lambda = 1.03.

Figure S3. Top three axes of genetic variation based on common SNPs for our Norwegian Mother and Child Cohort sample (MOBA) compared to 1000 Genomes reference populations. Plots are show for axes 1 and 2, axes 1 and 3, and axes 2 and 3. Reference populations are: CEU: Utah Residents with Northern and Western Ancestry; CHB: Han Chinese in Beijing, China; PUR: Puerto Ricans from Puerto Rico; MXL: Mexican Ancestry from Los Angeles, USA; CLM: Colombians from Medellin, Colombia; YRI: Yoruban in Ibadan, Nigeria.

Figure S4. Quantile–quantile plots for maternal and child genotypic effects. Test results (observed -$\log_{10}$p values) are plotted against the expected -$\log_{10}$p values for each of 1518 SNPs across 66 loci in the sample.

Figure S4. (a) Q-Q plot of maternal genotypic effects, adjusting for child genotype. Genomic inflation factor, lambda = 1.18. (b) Q-Q plot of child genotypic effects, adjusting for maternal genotype. Genomic inflation factor, lambda = 1.09.

Figure S5. Case and control status of the study sample plotted along the first two axes of variation for genetic ancestry.

Table S1. List of genes and canonical pathways.

Table S2. (a) Sensitivity analysis results. Summary of SNPs with FDR $Q \leq 0.2$ in the full cohort analysis excluding samples with first principal component >0.04 (n = 1994 mother-child dyads). (b) Sensitivity analysis results. Summary of SNPs FDR $Q \leq 0.2$ in the full cohort analysis excluding samples with first principal component >0.01 (n = 1964 mother-child dyads).

Table S3. (a) Replication results for preeclampsia overall in the UK GWAS cohort for SNPs with FDR $Q \leq 0.2$ in the MoBa analysis. (b) Replication results for early preeclampsia in the UK GWAS cohort for SNPs with FDR $Q \leq 0.2$ in the MoBa analysis.

Table S4. Summary of SNPs with $P < 0.01$ for tests for maternal–child genotype interactions for preeclampsia overall, indicated by number of maternal copies of the variant allele being greater than number of child copies of the variant allele.

Table S5. Minor allele frequencies for cases and controls in the MoBa sample and InterPregGen replication sample for all SNPs included in the replication analysis (SNPs with FDR $Q \leq 0.2$).

Table S6. Reference alleles for mothers and children.