Severe preeclampsia is a common cause of maternal and perinatal morbidity worldwide. The disease clusters in families; however, individual genetic studies have produced inconsistent results. We conducted a review to examine relationships between maternal genotype and severe preeclampsia. We searched the MEDLINE and Embase databases for prospective and retrospective cohort and case-control studies reporting associations between genes and severe preeclampsia. Four reviewers independently undertook study selection, quality assessment, and data extraction. We performed random-effects meta-analyses by genotype and predefined functional gene group (thrombophilic, vasoactive, metabolic, immune, and cell signalling). Fifty-seven studies evaluated 50 genotypes in 5,049 cases and 16,989 controls. Meta-analysis showed a higher risk of severe preeclampsia with coagulation factor V gene (proaccelerin, labile factor) \((F5)\) polymorphism rs6025 (odds ratio = 1.90, 95% confidence interval: 1.42, 2.54; 23 studies, \(I^2 = 29\%\)), coagulation factor II (thrombin) gene \((F2)\) mutation G20210A (rs1799963) (odds ratio = 2.01, 95% confidence interval: 1.14, 3.55, 9 studies, \(I^2 = 0\%\)), leptin receptor gene \((LEPR)\) polymorphism rs1137100 (odds ratio = 1.75, 95% confidence interval: 1.15, 2.65; 2 studies, \(I^2 = 0\%\)), and the thrombophilic gene group (odds ratio = 1.87, 95% confidence interval: 1.43, 2.45, \(I^2 = 27\%\)). There were no associations with other gene groups. There was moderate heterogeneity between studies and potential for bias from poor-quality genotyping and inconsistent definition of phenotype. Further studies with robust methods should investigate genetic factors that might potentially be used to stratify pregnancies according to risk of complications.
preeclampsia and perinatal death (6). Accurate quantification of risk could also aid obstetric decision making on the timing of delivery to prevent both maternal and neonatal complications. However, risk assessment cannot be achieved reliably by conventional clinical evaluation of symptoms, history, and investigations (7–10).

Thus, because preeclampsia clusters in families, and a positive family history is associated with a 3-fold higher risk of preeclampsia (11), the role of genetic factors in assessing the risk of preeclampsia and its complications is an active area of investigation. Genetic tests could potentially be combined with conventional prognostic indicators to construct better predictive models for more accurate stratification of women according to risk.

The mode of inheritance of predisposition to preeclampsia is complex and likely to be polygenic, with influence from environmental factors (12). Candidate genes from various biological pathways involving the immune system, control of vascular resistance, blood coagulation, as well as those involved in cell signalling pathways and metabolic processes, have been the subject of many genetic association studies because of their putative roles in causing preeclampsia and its complications. However, most of the existing genetic studies assess the risk of developing any form of preeclampsia and, thus, provide less information on the clinically relevant outcome, which is severe preeclampsia (13). In addition, their small sample sizes often render them powerless to detect weak associations. Similarly, whereas previous systematic reviews in this area have examined genetic associations with preeclampsia, attention to severe preeclampsia has been restricted mainly to subanalyses (14–16). Alternatively, some systematic reviews have combined adverse pregnancy outcomes that are not always associated with preeclampsia, such as venous thromboembolism or nonhypertensive growth restriction (17, 18). We therefore set out to evaluate the association between any maternal candidate genes and severe preeclampsia through a systematic review and meta-analysis.

METHODS

Definition of phenotype

We conducted the review in compliance with HuGENet (19) guidelines for genetic meta-analysis with a prospective protocol registered in PROSPERO (20). We defined “severe preeclampsia” as preeclampsia (hypertension (blood pressure of ≥140/90 mm Hg) and proteinuria (≥300 mg/24 hours or protein-creatinine ratio ≥30 mg/dL) (21)) and at least 1 of the following conditions: severe hypertensive (systolic blood pressure over 160 mm Hg and/or diastolic blood pressure over 110 mm Hg); eclampsia (convulsions that cannot be attributed to other causes); hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, (with platelet count level <100,000/dL and raised serum transaminases above the upper limits of normal) (22); or intrauterine growth restriction with an estimated fetal weight less than the tenth centile. This definition was based on the recent classification and general agreement from the International Society for the Study of Hypertension in Pregnancy (Groningen, the Netherlands), suggesting that difficulty controlling blood pressure, deterioration of clinical condition such as HELLP syndrome, impending eclampsia, worsening thrombocytopenia, and fetal growth restriction were indicators of severity and an indication to expedite delivery (22).

However, because there is a lack of consensus on criteria for the definition of severe preeclampsia, we took a pragmatic approach and also included any study in which there was a clear distinction between mild and severe preeclampsia.

Selection criteria

Studies that examined an association between severe preeclampsia and any genetic variation (e.g., single nucleotide polymorphisms, insertion/deletions, microsatellite markers, repeat sequences) were eligible. We included prospective and retrospective cohort and case-control studies.

Identification of studies and study selection

The MEDLINE and Embase databases were searched using the OVID platform from inception to August 2013 without language restrictions. We used HugeNavigator to supplement the search, and references from reviews were hand searched (23). The search strategy was developed with input from an experienced librarian (Web Appendix 1, available at http://aje.oxfordjournals.org/).

Studies were selected in a 2-stage process. In the first stage, we identified relevant citations from abstracts and titles. In the next stage, we reviewed full texts of selected abstracts for inclusion. Four independent reviewers undertook study selection. The citations were divided into 2 groups, and 2 reviewers were assigned per group for screening, study selection, and data extraction. Disagreements were resolved by discussion with another reviewer.

We included studies that provided genotype frequencies enabling the construction of 2 × 2 tables for each genotype and complication of preeclampsia. Studies were excluded if the genotype frequency for the homozygous minor homozygous and heterozygous groups combined was 0.

Methods to assess risk of bias in individual studies

We used the Newcastle Ottawa Scale and the Strengthening the Reporting of Genetic Association Studies (STREGA) recommendations for quality assessment (24–26). The 3 main parameters evaluated by the Newcastle Ottawa Scale are selection of study groups, comparability of groups, and outcome assessment. For cohort studies, we assessed selection bias by representativeness of the exposed cohort, selection of the nonexposed cohort, ascertainment of exposure, and whether the assessed outcome of interest was present at the start; comparability was assessed by the study analysis and design; and outcome bias was assessed by the duration and adequacy of follow-up for the outcome to occur. For case-control studies, selection was assessed by definition and representativeness of cases and controls; comparability of the groups was assessed by evaluating the study design and analysis; and outcome bias was assessed by ascertainment of exposure, method of ascertainment for cases and controls, and the nonresponse rate (26).

Risk of bias was regarded as low if a study obtained 4 stars for selection (stars were awarded according to criteria in the Newcastle Ottawa Scale manual (26)), 2 stars for comparability, and 3 stars for ascertainment of exposure. Risk of bias was considered to be medium in studies with 2 or 3 stars for selection, 1 star for comparability, and 2 stars for exposure. Any study scoring 1 or 0 stars for selection, comparability, or exposure was assigned a high risk of bias.

Genotyping methodology, success rates, population stratification, and deviation from Hardy-Weinberg equilibrium and Hardy-Weinberg equilibrium were taken into account, following the STREGA recommendations. Where data allowed, we calculated statistics for Hardy-Weinberg equilibrium and compared results to those given by the authors. Epidemiologic credibility was assessed for positively associated genetic variants using the Venice criteria, which evaluate sample size, replication, and protection from bias (28, 29).

Data extraction and analysis

Four investigators independently extracted data from the included studies. Heterozygous and homozygous genotypes of the minor (least frequent) allele were combined and compared with homozygous genotypes of the major allele. We compared women who were homozygous for the major allele to those with 1 or more copies of the variant to give a clinically relevant estimate of the genetic risk of complications for individual women. Where percentages of groups were provided, these were converted to actual numbers.

We calculated odds ratios with 95% confidence intervals. Random-effects meta-analyses were performed according to individual genes and by predefined gene function groups (i.e., thrombophilic, vasoactive, metabolic, immune-related, and cell signalling) representing physiological pathways associated with severe pre-eclampsia. Normal pregnancy and uncomplicated pre-eclampsia controls (including women with gestational hypertension or mild pre-eclampsia) were combined to form the comparator group. Subgroup analyses were performed on individual genotypes to explore the contribution of study design (prospective case-control, retrospective case-control, or cohort), sample size (>100 or <100 cases) and predominant ethnicity of study participants. Sensitivity analyses were performed, omitting those studies in which the definition of severe pre-eclampsia was unclear.

When more than 1 outcome of interest was available for the same population, we included the data for the “worst” outcome (ranked in order of importance by prior consensus, as follows: eclampsia; severe hypertension (systolic blood pressure above 160 mm Hg and/or diastolic blood pressure above 110 mm Hg); HELLP syndrome; and intrauterine growth restriction. Where more than 1 genotype was assessed in the same study, the genotype with the largest data set was included. Where there were equally important genotypes and complications, we undertook a sensitivity analysis, substituting the other available genotypes to evaluate the effects on the overall results.

Heterogeneity was assessed using the I² statistic. Publication bias was formally tested using Harbord’s modified test for small study effects (30). Analyses were carried out using RevMan, version 5.1, software (Cochrane Collaboration, Oxford, United Kingdom) and Stata, version 12, software (StataCorp LP, College Station, Texas) (31, 32).

RESULTS

Genetic association studies in severe preeclampsia

Fifty-seven studies were included, evaluating 50 genetic variants in 37 genes with 5,049 cases and 16,989 controls (Figure 1). Genetic variants included single nucleotide polymorphisms, microsatellite markers, and insertion/deletions (Table 1). Definitions of preeclampsia used in the studies are compared in Web Table 1. Four studies did not provide adequate information to determine whether criteria for severe preeclampsia were met (33–36). Exclusion criteria for cases were ignored in 13 studies, and only 1 study excluded the use of aspirin in pregnancy (37). Study characteristics including

![Figure 1. Study identification and selection process for meta-analysis of relationships between maternal genotype and severe pre-eclampsia, 1996–2012.](image-url)
population demographic characteristics, ethnicity, inclusion criteria (definitions of phenotype and each complication), exclusion criteria, and the genes and single nucleotide polymorphisms examined are provided in detail in Web Tables 1–3.

We included 51 (41 prospective and 10 retrospective) case-control studies and 6 cohort studies (33, 38–42) published between 1996 and 2012. Severe preeclampsia was not the primary outcome in the majority of studies. Of the 57 studies, 16 had more than 100 cases of severe preeclampsia, with the maximum number being 284 cases.

### Quality of the included studies

Of the 57 included studies, 17 (30%) had low risk of selection bias, 26 (46%) had medium risk, and 14 (24%) had high risk according to the Newcastle Ottawa Scale. Eleven (19%)...
had high risk of bias for comparability of cohorts, 21 (37%) had medium risk, and 25 (44%) had low risk. Poor documentation of assessment of exposure, outcome, and nonresponse rate resulted in high levels of bias for outcome ascertainment in 24 (42%) studies (Figure 2 and Web Table 3).

Assessment by the STREGA criteria (24) showed that the majority of studies (49/57, 86%) had homogeneous ethnic population groups. No studies used genomic methods to detect population stratification. Genotyping success was documented in only 5% (3/57) of studies. The methods of genotyping were clearly described in all studies, but only 5 studies described genotyping-quality-control policies, such as repeating a proportion of samples in positive tests or using negative controls.

There were sufficient data for reviewers to calculate Hardy-Weinberg equilibrium in 32 studies (56%), of which 22 genotypes were in equilibrium, and 10 deviated. Twenty-three (40%) studies reported that genotypes were in Hardy-Weinberg equilibrium; however, 5 of these genotypes deviated from equilibrium when the statistic was recalculated, and 3 had insufficient genotype frequencies for accurate assessment (<5 in any group).

Blinding of the laboratory staff to the clinical outcome was documented in 4 studies (43–46). Power calculations were performed a priori in 17 studies (30%), and of these, only 9 studies (37, 42, 45, 47–52) were designed with a priori power calculations for severe preeclampsia (Web Table 3).

**Individual genotypes and associations with severe preeclampsia**

Meta-analysis was possible for the following 11 genotypes: angiotensin-converting enzyme gene (ACE) DD (rs4646994) (4 studies) (53–56); angiotensinogen M235T (rs699) (3 studies) (56–58); estrogen receptor 1 gene (ESR1) PvuII (rs2234693) (2 studies) (48, 58); coagulation factor V (proaccelerin, labile factor) gene (F5) (rs6025) (23 studies) (37–40, 42, 43, 46, 51, 53, 58–71); leptin gene (LEP) TTTT(n) (2 studies) (34, 72); leptin receptor gene (LEPR) (rs1137100) (2 studies) (45, 73) lepin receptor gene (LEPR) R223Q (rs1137101) (4 studies) (34, 45, 73, 74); methylene tetrahydrofolate reductase gene (MTHFR) C677T (rs1801133) (10 studies) (37, 39, 42, 51, 53, 61–63, 65, 75); coagulation factor II (thrombin) gene (F2) (G20210A) (rs1799963) (9 studies) (37, 39, 51, 58, 60, 62, 64, 65, 69); transforming growth factor, beta 1 (TGFB1) codon 10 (rs1982073) (2 studies) (41, 76); and tumor necrosis factor gene (TNF) alpha (G03A) (rs1800629) (4 studies) (49, 76–78) (Web Figure 1).

Positive associations with severe preeclampsia were seen for F5 (rs6025) (odds ratio = 1.90, 95% confidence interval: 1.42, 2.54; 23 studies, I² = 29%); F2 G20210A (rs1799963) (odds ratio = 2.01, 95% confidence interval: 1.14, 3.55; 9 studies, I² = 0% (Web Figure 2), and LEPR rs1137100 (odds ratio = 1.75, 95% confidence interval: 1.15, 2.65; 2 studies, I² = 0%). The remaining genotypes were not associated.

There were no significant differences in subgroup estimates for F5 rs6025 and F2 G20210A when stratified by study design, ethnicity, or sample size (Web Tables 4 and 5). Sensitivity analysis excluding studies in which insufficient data were reported to confirm accurate assignment of the severe preeclampsia phenotype produced no significant differences in results.

**Associations between gene function groups and severe preeclampsia**

Thrombophilic genes were associated with severe preeclampsia (odds ratio = 1.87, 95% confidence interval: 1.43, 2.45; 24 studies, I² = 27%). There were no associations with vasoactive, immunogenetic, cell-signalling, or metabolic gene groups (Figure 3). Heterogeneity was high in the gene group meta-analyses for immune-related and metabolic genes. Sensitivity analyses of all possible combinations of the genotypes for each gene function group and for studies with uncertain phenotype assignment produced similar results. There was no evidence of publication bias in the thrombophilic gene group (Harbord’s modified test of small effects, P = 0.235) (Web Figure 3).

**Evaluating epidemiologic credibility of significant associations**

Associations between severe preeclampsia and 3 genotypes—F5 rs6025, F2 G20210A rs1799963, and LEPR rs1137100—were assessed using the Venice criteria. For F5 rs6025, there was moderate heterogeneity across studies (I² = 29%) and considerable potential for bias because of inconsistent phenotype definition, genotyping methods, population stratification, and restriction to only published data. For F2 G20210A rs1799963 and LEPR rs1137100, although heterogeneity was low, sample sizes were relatively small, and...
the potential for bias was high for similar reasons (Web Table 6).

DISCUSSION

Main findings

Relatively few genetic association studies have addressed severe preeclampsia as a primary outcome, and of these, the main genotypes evaluated are thrombophilic. Meta-analysis of studies reporting relevant data for this gene group showed positive associations with severe preeclampsia, as did meta-analyses for the individual thrombophilic genes (F5 G1691A rs6025 and F2 G20210A rs1799963) and LEPR rs1137100. There were no associations with other gene groups, although the numbers of studies examining vasoactive, metabolic, immune-related, and cell-signalling genes were relatively small. Given the levels of statistical heterogeneity between studies and the potential for bias, there is currently insufficient cumulative evidence of epidemiologic credibility to determine a causal association between thrombophilic genotypes and LEPR rs1137100 and severe preeclampsia.

Strengths and limitations of this review

This is the first systematic review to examine the association between maternal genotype and severe preeclampsia. We undertook a detailed search without language restrictions to identify all relevant publications. The quality of individual studies was assessed, and variations in definition of phenotype and in assessment and reporting of associations were taken into account using a standardized approach to data extraction. Potential bias in meta-analyses from design of the original studies, sample sizes, and ethnicity was explored where possible.

We took an a priori approach to use only a dominant genetic-effects model to limit the number of comparisons in the review. We considered restricting the analysis to homozygotes, but the numbers were too small to derive meaningful results.

Quality of included studies

There was consistently poor reporting of genotyping methods, including success rates, blinding of laboratory staff, use of internal controls, protocols for repeating samples, and whether there was deviation from Hardy-Weinberg equilibrium. Reported genotype data often deviated from equilibrium, suggesting that genotyping inaccuracies and data were frequently not reported in sufficient detail to allow extraction for secondary analysis.

Exclusion criteria for cases were ignored in 13 studies, and only 1 study excluded the use of aspirin in pregnancy. One of the major limitations is the lack of consistency in the definition of phenotype; in addition, the methods for assessing severity of the disease varied among studies. This is a common problem in obstetrical research and emphasizes the need to develop an internationally agreed-upon definition and a set of core adverse outcomes for preeclampsia.

Biological mechanisms

The key features in the pathogenesis of preeclampsia are abnormal placentation with shallow endovascular cytotrophoblast invasion in the spiral arteries, in addition to inappropriate endothelial cell activation and an exaggerated inflammatory response (79). The 2-stage model of preeclampsia proposes that the first stage consists of a triggering event of poor placental perfusion and abnormal placentation; this is then exacerbated by interaction with maternal constitutional factors and environmental factors in the second stage, resulting in the maternal syndrome of preeclampsia (80). In preeclampsia, there is evidence of increased apoptosis of endovascular cytotrophoblasts and highly procoagulant activity on the surface of apoptotic cells (81). These apoptotic cytotrophoblasts in spiral arteries may also be associated with fibrin deposition and platelet activation. Hence, an underlying thrombophilia is a potential exacerbating factor and would be likely to augment this process and, in particular, be associated with severe, recurrent, and early-onset preeclampsia (82).
With regard to the role of LEPR polymorphisms, serum leptin levels are elevated in women with preeclampsia compared to women with normotensive pregnancies; this is often the case early in pregnancy, preceding the development of the condition (83, 84). LEPR expression is higher in inflammation and plays a key role in immune response and T helper cell activation, which is a feature of preeclampsia (85). In addition to its actions on the immune system, LEPR may also affect blood pressure regulation by acting centrally on the sympathetic nervous system (86). This review lends qualified support to published systematic reviews evaluating the role of thrombophilia in preeclampsia. Consistent with our findings, the genotypes most commonly associated with preeclampsia have been F5 rs6025 and F2 G20210A rs1799963, with odds ratios of approximately 2 for both genotypes (14–16).

In our review, we focussed on severe preeclampsia as a primary outcome and included much larger numbers of studies and participants for F5 rs6025 (1,824 cases and 4,101 controls from 23 studies); methylenetetrahydrofolate reductase (NAD(P)H) gene (MTHFR) C677T rs1801133 (927 cases and 2,145 controls from 11 studies); and F2 G20210A rs1799963 (509 cases and 1,481 controls from 9 studies) than did previous systematic reviews of preeclampsia.

Sample sizes of thousands are necessary to detect true associations in genetic association studies for complex disorders, and although meta-analysis can potentially overcome this challenge, the heterogeneity among studies and across studies populations renders it difficult to give epidemiologic credibility to the associations that we observed (28).

**Role of non–hypothesis-driven genetic studies in research on severe preeclampsia**

Genome-wide studies may be a useful tool for future work because, unlike candidate gene studies, they take an unbiased approach to genetic hypotheses and allow the investigation of many genetic polymorphisms simultaneously. They are thus better designed to study the genetic basis of complex diseases such as preeclampsia, which are determined by a number of genetic and environmental factors. Because our understanding of the pathophysiology of preeclampsia and its complications is limited, a candidate gene approach may miss an important genetic factor. Thus non–hypothesis-driven genetic investigations may identify new biochemical and physiological mechanisms that might guide clinical and experimental investigations.

Genetic linkage analysis in families has been instrumental in the past in localizing and mapping “disease genes” to known “genetic markers” in other conditions. These studies have pointed to the following maternal preeclampsia susceptibility loci (87–91): 2p13 (log odds = 4.7) (87), 4q (log odds = 2.9) (88), 12q (log odds = 1.99) (89), 2q23 (log odds = 2.58), 11q23–24 (log odds = 2.02) (91), 2p25 (nonparametric linkage score = 3.74), and 9p13 (nonparametric linkage score = 3.74) (90). At the time of these studies, however, no candidate genes were identified in these regions, and there has been little success replicating these findings in population-based studies.

Genome-wide association studies have been a more promising approach, and 2 such investigations have identified biologically plausible candidate genes for preeclampsia (92, 93). A study of 293 white women from Iowa (177 cases and 116 controls) found copy number variants consisting of an enrichment of case deletions in 19q13.31, which encompasses the pregnancy specific beta-1-glycoprotein 11 gene (PSG11) (93). This study did not identify associations with any single nucleotide polymorphisms, possibly because of insufficient statistical power. Another genome-wide association study for preeclampsia with 538 cases and 540 controls in white women in Australia identified a risk locus on 2q14.2, an intergenic region near the inhibin, beta B gene (INHBB) (92). Three single nucleotide polymorphisms in this region—rs7579169, rs12711941, and rs7576192—were significant and in strong linkage disequilibrium with each other. INHBB encodes a protein that is a subunit of both inhibin and activin, 2 closely related glycoproteins with opposing effects on the action of follicle stimulating hormone and sex hormone synthesis. There is a body of substantive evidence to support the role of inhibins, activins, and other members of the transforming growth factor β family in the development of preeclampsia (94–101). Interestingly, genes coding for factor V (1q23) and F2 (11p11.2) have not been associated with preeclampsia or severe preeclampsia either in linkage analysis or genome-wide association studies.

**Implications for clinical practice and future research**

Identification of further genetic risk factors for complications of preeclampsia is an important research objective because it may lead to early identification and targeted management of high-risk pregnancies. Large international collaborations such as InterPregGen (www.interpreggen.org), with more than 13,000 persons currently recruited, bring us closer to achieving the sample sizes necessary for detecting robust and precise estimates of associations between genotypes and severe preeclampsia. A consortium approach may also enable the analysis of associations in different ethnic groups and the evaluation of the contribution of paternal and fetal genotypes to disease status and severity.

We urge researchers to consider severe preeclampsia, rather than preeclampsia, as the primary outcome of interest for future studies, because this will give the greatest insight into pathophysiological processes and yield the most important results for clinical practice.

**Conclusion**

These findings support, to an extent, the potential role for thrombophilia genes in severe preeclampsia, which is responsible for substantial maternal and fetal morbidity and death worldwide. There is, however, insufficient evidence currently available to establish causal inference. Further studies will be necessary to systematically examine the full repertoire of thrombophilia genes and their polymorphisms in large numbers of women with complications of preeclampsia and to replicate findings for other gene groups from recent genome-wide studies. This work is important not only because of the clinical relevance of complications, but because studying this rich phenotype is more likely to lead to a better understanding...
of the mechanisms underlying this major cause of maternal and neonatal morbidity.

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Author affiliations: Women’s Health Research Unit, Barts and the London School of Medicine and Dentistry, Queen Mary University London, London, United Kingdom (Fiona M. Fong, Shakila Thangaratinam); Centre of Primary Care and Public Health, Barts and the London School of Medicine and Dentistry, Queen Mary University London, London, United Kingdom (Manpreet K. Sahemey, Golnessa Hamedi, Rachel Eyitayo, Valerie Kuan, Robert T. Walton); Library and Information Service, Birmingham Women’s NHS Foundation Trust, Birmingham, United Kingdom (Derick Yates); and Multidisciplinary Evidence Synthesis Unit, Queen Mary University London, London, United Kingdom (Shakila Thangaratinam, Robert T. Walton).

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REFERENCES


