Genetics of Pre-eclampsia

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Pre-eclampsia is a major cause of maternal and perinatal mortality and morbidity worldwide, but remains unclear about the underlying disease mechanisms. Pre-eclampsia is currently believed to be a two-stage disease. The first stage involves shallow cytotrophoblast invasion of maternal spiral arteriole, resulting in placental insufficiency. The hypoxic placenta release soluble factors, cytokines, and trophoblastic debris into maternal circulation, which induce systemic endothelial damage and dysfunction. This cause the second stage of the disease: maternal syndrome. Epidemiological research has consistently demonstrated a familial predisposition to pre-eclampsia. Intensive research efforts have been made to discover susceptibility genes that will inform our understanding of the pathophysiology of pre-eclampsia and that may provide direction for therapeutic or preventative strategies. In this review, we summarize the current understanding of the role of genetic factors in the pathophysiology of pre-eclampsia and explain the molecular approach to search for genetic clues in pre-eclampsia.

Key Words: Pre-eclampsia, Genetics, Susceptibility gene

Introduction

Pre-eclampsia is a pregnancy-specific syndrome that carries a high risk of maternal and perinatal mortality and morbidity. Pre-eclampsia affects 5 to 10% of all pregnancies and is characterized by the onset of hypertension and proteinuria after 20 weeks of gestation. It can progress to HELLP (hemolysis, elevated liver enzymes, and low platelet counts) syndrome and seizures (eclampsia). Despite decades of intense research on the problem, the mechanisms of the disease onset remains unclear.

Despite extensive clinical trials, no therapeutic approaches are currently available to either treat or prevent pre-eclampsia. Anti-hypertensive drugs, corticosteroids for lung maturation, and/or magnesium sulfate to prevent eclampsia are administered to handle (or prevent the worsening of) symptoms as a temporization strategy to allow safe delivery of a more mature fetus. However, when temporizing management, the maternal risks must be carefully weighed against the possible fetal benefits, as the risk of fatal deterioration of the health of the mother and/or fetus is high. Several prophylactic therapies (anti-oxidant vitamins, calcium or folic acid supplementation, aspirin) have so far not proved efficacious at preventing pre-eclampsia in healthy, nulliparous subjects, although these therapies have shown some benefits in groups1-4. As a consequence, the sole, albeit radical, way to resolve pre-eclampsia is to remove the placenta; in the case of prematurity, this results in delivery of a preterm baby.
Therefore, pre-eclampsia, with or without intrauterine growth retardation (IUGR), remains a major cause of maternal and neonatal mortality and morbidity worldwide.

A number of epidemiological studies have confirmed that preeclampsia has a maternal genetic component. Daughters of pre-eclamptic women have a higher chance of themselves developing pre-eclampsia\(^5\). Phenotyping patients with pre-eclampsia are vital for any genetic study. Furthermore, previous studies have provided compelling evidence that nulliparity, a family history of pre-eclampsia (sister/mother who suffered pre-eclampsia), a personal history of a previous pregnancy with pre-eclampsia, an increase in the trophoblastic mass (multiple pregnancy, molar pregnancy), paternity changes between pregnancies, age over 40 years, obesity, and some maternal chronic conditions such as diabetes, chronic hypertension, renal disease, autoimmune disease, and antiphospholipid syndrome are risk factors for pre-eclampsia\(^6, 7\).

It has been recognized for many years that pre-eclampsia has a familial component, and the identification of susceptibility genes is one of a number of strategies designed to elucidate the underlying pathogenetic mechanisms of this condition. The objective of this review is to summarize our understanding of what role genetic factors play in the pathophysiology of pre-eclampsia and to describe the molecular approach to search for genetic clues in pre-eclampsia.

**Diagnosis**

Pre-eclampsia is usually diagnosed by the presence of hypertension associated with proteinuria. Hypertension is defined as a blood pressure of at least 140 mm Hg (systolic) or at least 90 mm Hg (diastolic) on at least two occasions and at least 4-6 hrs apart after the 20 weeks of gestation in women known to be normotensive beforehand\(^8-10\). Blood-pressure recordings to establish the diagnosis should be no more than 7 days apart\(^8, 9, 11\). Hypertension is regarded as severe if there are sustained rises in blood pressure to at least 160 mm Hg (systolic), at least 110 mm Hg (diastolic), or both\(^8, 9, 11, 12\). Proteinuria is defined as excretion of 300 mg or more of protein every 24 hrs. If 24 hrs urine samples are not available, proteinuria is defined as a protein concentration of 300 mg/L or more (≥1+ on dipstick) in at least two random urine samples taken at least 4-6 hrs apart\(^8, 9\). The urine dipstick measurements used to establish proteinuria should be performed no more than 7 days apart\(^8, 9, 11\).

Dependent on the amount of systemic involvement, several other symptoms, such as edema, disturbance of hemostasis, renal or liver failure, and HELLP syndrome also complicate the clinical picture. Pre-eclampsia can be early onset (pre-eclampsia starting before 34 weeks of gestation) or late onset (pre-eclampsia starting after 34 weeks of gestation), can show mild or severe symptoms (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg, proteinuria >5 g/24 hrs, oliguria, neurological symptoms, other clinical symptoms such as deranged liver function, thrombocytopenia <100,000 mm\(^3\), HELLP syndrome), and can evolve into eclampsia in the most severe cases (Table 1)\(^13\). In addition, it can manifest as a maternal disorder only, with normal fetal growth, or intrauterine growth restric-

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Nonsevere</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic blood pressure</td>
<td>&lt;110 mmHg</td>
<td>≥110 mmHg</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>&lt;160 mmHg</td>
<td>≥160 mmHg</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>≤2+</td>
<td>≥3+</td>
</tr>
<tr>
<td>Headache</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Upper abdominal pain</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Oliguria</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Convulsion (eclampsia)</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>Normal</td>
<td>Elevated</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Serum transaminase elevation</td>
<td>Minimal</td>
<td>Marked</td>
</tr>
<tr>
<td>Fetal-growth restriction</td>
<td>Absent</td>
<td>Obvious</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>
tion of the fetus (IUGR) or sudden fetal distress may occur.

The traditional criteria used to confirm a diagnosis of pre-eclampsia (new onset of both hypertension and proteinuria after 20 weeks’ gestation) are applicable to most healthy, nulliparous women. However, the criteria mentioned so far are not reliable in women who have either hypertension or proteinuria before 20 weeks’ gestation, especially those receiving antihypertensive drugs\(^{10, 14, 15}\). Because of the physiological changes leading to raised maternal blood pressure and increased protein excretion with advanced gestation in such women, more stringent criteria should be used to diagnose pre-eclampsia in those with microvascular disease\(^{10, 14, 15}\). Consequently, markers to predict and methods to prevent pre-eclampsia in these women are probably different from those in healthy nulliparous women.

### Pathophysiology

The precise origin of pre-eclampsia remains elusive, but it is believed to be multifactorial. A certainty is the central role played by the placenta\(^{16, 17}\). A long standing hypothesis has been that pre-eclampsia develops as a consequence of some kind of immune maladaptation between the mother and the fetus during the very first weeks of pregnancy, leading to the following two-stage progression (Fig. 1)\(^{18}\). In the first asymptomatic stage, local aberrant feto-maternal immune interactions within the uterine wall lead to impaired tissue and arterial invasion by trophoblast cells (Fig. 2)\(^{19}\). This results in failed transformation of the uterine spiral arteries and subsequently poor placental perfusion. Chronic hypoxia or alternate periods of hypoxia/re-oxygenation within the intervillous space are expected to trigger tissue oxidative stress and increase placental apoptosis and

Fig. 1. Pathophysiological mechanisms in pre-eclampsia\(^{10}\).

![Fig. 1. Pathophysiological mechanisms in pre-eclampsia](image-url)

![Fig. 2. Abnormal placentation in pre-eclampsia](image-url)
necrosis\textsuperscript{20, 21}. In the second stage, the clinical disorder arises, when the maternal vascular and immune systems can no longer handle the high levels of shedding of placentally produced debris and the aberrant expression of pro-inflammatory, anti-angiogenic and angiogenic factors, leading to systemic endothelial cell dysfunction and an exaggerated inflammatory response\textsuperscript{22-24}. Recently, this hypothesis has been challenged\textsuperscript{25}. It was proposed instead that an intrinsic failure of trophoblast differentiation at different time points of ontogeny may lead to either a mild disorder with late-onset appearance, or IUGR, with or without maternal symptoms. However, the origin of pre-eclampsia might not be restricted to an alteration in trophoblast differentiation, but may also in some cases depend on underlying maternal constitutional factors such as genetic factors, obesity, dysfunctional maternal clearance, or a dysfunctional maternal inflammatory system\textsuperscript{26}.

Candidate genes

Various candidate gene studies have been performed to identify associations between pre-eclampsia and genetic polymorphisms in specific genes between cases and controls. Candidate genes are genes with documented biological actions in pathways known to be active in pre-eclampsia that are polymorphic. Single nucleotide polymorphisms (SNPs) are the markers most commonly used to determine genetic associations. Recent initiatives such as the HapMap project (http://www.hapmap.org/) and the establishment of the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) have resulted in the identification of multiple SNPs in all human genes.

In association studies, the frequency of alleles and genotypes (homozygous and heterozygous) in the disease group and a healthy population are compared. The most advanced type of association study is a case-control study, where subjects are matched for age and ethnicity. Association studies involving unrelated subjects allow the detection of common variants or common alleles of small effect. In these types of studies, it is also possible to determine those genes that have a weak influence on disease risk. Currently, more than 60 candidate genes related to pre-eclampsia development have been identified, and several groups of candidate genes have been distinguished based on the pathophysiology of pre-eclampsia. The genes that have been most frequently investigated are those involved in blood pressure regulation (angiotensinogen, angiotensin-converting enzyme, and angiotensin receptors), inherited thrombophilias (coagulation factor V Leiden variant, prothrombin, and methylene tetrahydrofolate reductase), vasodilation regulating genes (endothelial nitric oxide synthase, eNOS), and the gene encoding the cytokine tumor necrosis factor alpha (TNF\(\alpha\)) (Table 2)\textsuperscript{27-69}. However, our studies based on examinations in Korean population have suggested that the contribution of genetic polymorphism of \(\beta\)-adrenoceptor, EDN1, ICAM-1, INHA, STOX1, and TNF genes to the occurrence of pre-eclampsia is few. It seems that pre-eclampsia is a disease with polygenic inheritance patterns, influenced by environmental factors, gene-gene and gene-environmental interactions\textsuperscript{70-75}.

It must be conceded that over a decade of molecular genetic research has failed to identify a single universally accepted susceptibility gene for pre-eclampsia. There has been a lack of reproducibility of results, as has been the experience with many complex disorders. Possible explanations have been discussed widely\textsuperscript{76}, and studies of pre-eclampsia share some of these generic problems. Progress in this field will require attention to both study design and the choice of candidate genes.

Meta-analyses

It has been argued that the stringent criteria used in association studies may be impractical and that more insight might be gained by combining the results of
several smaller studies in a Meta-analyses. One recent Meta-analyses failed to find any evidence for an increased risk of pre-eclampsia associated with the MTHFR 677C>T variant [pooled odds ratio, 1.01 (95% CI, 0.79-1.29)]\(^{77}\); an earlier Meta-analyses suggested that 677C>T may be associated with severe pre-eclampsia only \([\text{diastolic blood pressure} \geq 110 \text{ mm Hg}; \text{odds ratio}, 1.41 (95\% \text{CI}, 1.03-1.73)]\(^{78}\). Meta-analyses of prothrombin 20210G>A did not support a role for this polymorphism in pre-eclampsia \([\text{odds ratio}, 1.37 (95\% \text{CI}, 0.72-2.57)]\(^{77}\). Factor V Leiden was associated with an approx. 2-fold increase in risk of pre-eclampsia in three separate meta-analyses, although many of the same studies were incorporated in these particular meta-analyses\(^{77,79,80}\). Some important general points have emerged from these meta-analyses. There was considerable variation in the recruitment protocols for case-control studies and in the phenotypic profile of affected women. Furthermore, there was statistical evidence of heterogeneity between the results of studies: in particular, large studies and studies published within the last few years tended to yield negative results, whereas the majority of positive results came from earlier and smaller studies.

### Table 2. Candidate Gene Studies in Pre-eclampsia

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>References</th>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophilia</td>
<td></td>
<td></td>
<td>Interleukin 1β</td>
<td>IL1B</td>
<td>48</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>F5</td>
<td>27-29</td>
<td>Interleukin 1 receptor antagonist</td>
<td>IL1RN</td>
<td>49</td>
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<tr>
<td>Prothrombin 20210</td>
<td>F2</td>
<td>29,30</td>
<td>Interleukin 10</td>
<td>IL10</td>
<td>50</td>
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<tr>
<td>Methylene tetrahydrofolate reductase</td>
<td>MTHFR</td>
<td>27-29,31,32</td>
<td>T-lymphocyte-associated protein 4</td>
<td>CTLA4</td>
<td>51</td>
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<tr>
<td>Cystathione β-synthase</td>
<td>CBS</td>
<td>28</td>
<td>TNF-receptor supergammilt member 6</td>
<td>FAS</td>
<td>52</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>SERPINE1</td>
<td>29,32</td>
<td>Oxidative stress</td>
<td></td>
<td></td>
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<tr>
<td>β-Fibrinogen</td>
<td>FGB</td>
<td>33</td>
<td>Microsomal epoxide hydrolase</td>
<td>EPHX1</td>
<td>53</td>
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<tr>
<td>Platelet glycoprotein lila</td>
<td>ITGB3</td>
<td>30</td>
<td>Glutathione S-transferase pi</td>
<td>GSTP1</td>
<td>41,54</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>THBD</td>
<td>34</td>
<td>Glutathione S-transferase mu 1</td>
<td>GSTM1</td>
<td>54,55</td>
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<td>Factor VII</td>
<td>F7</td>
<td>33</td>
<td>Glutathione S-transferase theta 1</td>
<td>GSTT1</td>
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<tr>
<td>Platelet collafen receptor α2β1</td>
<td>ITGA2</td>
<td>29</td>
<td>Myeloperoxidase</td>
<td>MPO</td>
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<td>Factor XIII A-subunit</td>
<td>F13A1</td>
<td>35</td>
<td>Manganese superoxidase dismutase</td>
<td>SOD2</td>
<td>55</td>
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<td>Haemodtnamics</td>
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<tr>
<td>Angiotensinoten</td>
<td>AGT</td>
<td>32,36</td>
<td>Haptoglobin</td>
<td>HP</td>
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<td>Renin</td>
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<td>37</td>
<td>p22(^{\text{phox}})</td>
<td>CYBA</td>
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<tr>
<td>Angiotensin-converting enzyme</td>
<td>ACE</td>
<td>32,36</td>
<td>Lipid metabolism</td>
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<td>AT1 receptor</td>
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<td>Lipoprotein lipase</td>
<td>LPL</td>
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<td>AT2 receptor</td>
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<td>38</td>
<td>Apolipoprotein E</td>
<td>APOE</td>
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<td>Epithelial sodium channel</td>
<td>SCN1B</td>
<td>39</td>
<td>Peroxosme-proliferator-activated receptor γ</td>
<td>PRARG</td>
<td>60</td>
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<tr>
<td>Endothelial function</td>
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<tr>
<td>Enos</td>
<td>NOS3</td>
<td>40</td>
<td>Cholesteryl ester transfer protein</td>
<td>CETP</td>
<td>61</td>
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<tr>
<td>Endothelin 1</td>
<td>EDN1</td>
<td>41</td>
<td>β3-Adenergicreceptor</td>
<td>ADRB3</td>
<td>62</td>
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<tr>
<td>Dimethylarginine dimethylaminohydrolase 1</td>
<td>DDAH1</td>
<td>42</td>
<td>Endocrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethylarginine dimethylaminohydrolase 2</td>
<td>DDAH2</td>
<td>42</td>
<td>Oestrogen receptor</td>
<td>ESR1</td>
<td>63</td>
</tr>
<tr>
<td>G-protein β3</td>
<td>GNB3</td>
<td>43</td>
<td>Oestrogen receptor</td>
<td>ESR2</td>
<td>64</td>
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<tr>
<td>Cytokines</td>
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<tr>
<td>TNFα</td>
<td>TNF</td>
<td>32,44</td>
<td>Angiogenesis</td>
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<td></td>
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<tr>
<td>TGF β1</td>
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<td>45</td>
<td>VEGF</td>
<td>VEGF</td>
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<tr>
<td>IGF II</td>
<td>IGF2</td>
<td>46</td>
<td>Matrix metallopeptidase 1</td>
<td>MMP1</td>
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<tr>
<td>Interleukin 1α</td>
<td>IL1A</td>
<td>47</td>
<td>Catechol-methyltransferase</td>
<td>COMT</td>
<td>69</td>
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</table>

\(^{77}\) Genetics of Pre-eclampsia
**Genome-wide linkage analysis**

Genome-wide linkage analysis is the study of genetic variations across the entire human genome. Genome-wide screening has the potential to detect genetic loci that contribute to susceptibility to pre-eclampsia and identify susceptibility loci for development of this disease. Linkage analysis has proved its worth in identifying the molecular basis of many Mendelian disorders, but has been less successful in guiding the search for susceptibility genes for complex disorders. This may be due to underlying genetic heterogeneity (susceptibility loci may differ between affected pedigrees) or to the relatively low genetic risk of disease conferred by individual genes in a complex mode of inheritance. Ascertainment of multicase families presents difficulties in studies of pre-eclampsia, as there is no known male phenotype, and susceptibility in females is apparent only during pregnancy. Nevertheless, research groups from Iceland, Australia, Finland, and Netherlands have undertaken genome-wide linkage screens which have yielded encouraging results (Fig. 3)\(^{81}\). The studies in Australia indicated the \textit{PREG1} gene as a contributor of pre-eclampsia development\(^{82}\). In a genome-wide linkage analysis of Icelandic families representing 343 affected women, a significant locus was found on chromosome 2 (2p13)\(^{83}\), while in another genome-wide linkage study of pre-eclampsia, evidence was found for a candidate region on 4q\(^{84}\). In a large Norwegian population (case-control study, 1139 cases, 2269 controls), 71 SNPs within candidate genes in the region 2q22-23 were genotyped. The gene encoding activin receptor 2 (ACVR2A) lies within this region. Activin is a possible autocrine/paracrine regulator of the human placenta\(^{85}\). The type II activin receptor has a molecular weight of 85 kD, and three are two receptor isoforms (ACVR2A and ACVR2B) that have tissue- and temporal-specific differences. The available evidence suggests that ACVR2A may play a role in the etiology of pre-eclampsia\(^{86,87}\).

Epigenetic markers and gene-gene interactions have also been investigated in genome-wide association studies to elucidate the pathophysiological processes involved in pre-eclampsia. It has been proposed that SERPIN (serine protease inhibitor) proteins could contribute to the development of pre-eclampsia. **SERPIN** genes encode more than 36 proteins with various functions ranging from protease inhibitors, storage proteins, carrier proteins, to hormone precursors without an inhibitory function. SERPIN family proteins are involved in the coagulation and fibrinolysis cascade, inflammatory processes, complement activation, and phagocytosis. Alterations in promoter methylation (hypomethylation or total methylation) of the genes encoding SERPIN proteins could play role in transcriptional regulation (alterations in CpG methylation can influence the binding of transcription factors) and activity of **SERPIN** genes.

Much attention has been paid to the gene coding for SERPINA3 (alpha-1-antichymotrypsin), which is thought to be involved in pre-eclampsia\(^{88}\). Recently, **STOX1** (storkhead box 1) missense mutations have been suggested to predispose to pre-eclampsia in Dutch families \(^{89}\). **STOX1** is a transcriptional factor that is encoded by...
a gene on chromosome 10 (10q22). This factor is placenta-ently expressed and controls polyploidization of extravillous trophoblasts. Four STOX1 transcripts are expressed in the early placenta (including extravillous trophoblasts), and alterations in STOX1 activity have been implicated in pre-eclampsia susceptibility. Several recent studies, however, have contested the generality of the involvement of this gene\(^{20}\). Further investigation into the function of STOX1 is therefore required to determine if this gene is indeed involved in the pathophysiological cascades that lead to the onset of pre-eclampsia.

It should be noted that there is little overlap between the loci identified in different populations in these analyses, possibly because of genetic differences between populations, highlighting the importance of carrying out genetic studies in different parts of the world.

**Conclusion**

Genetic screening could potentially be the initial step in pre-eclampsia prevention/treatment and could increase our understanding of the pathophysiological mechanisms underlying pre-eclampsia. Where should genetic studies of pre-eclampsia be directed? Recent genome-wide linkage analysis of families affected by pre-eclampsia has yielded encouraging results. In contrast, the credibility of candidate gene studies has been undermined by conflicting and inconclusive results. It is clear that large studies that have adequate statistical power to detect small genetic effects are needed to reliably identify or exclude susceptibility genes. This invites a multicentre collaborative approach between clinicians and geneticists to develop common recruitment protocols for the establishment of large DNA resource databases that can be used by statisticians to conduct meaningful meta-analyses. The results of pathophysiological and genetic studies have provided some insights into the nature of pre-eclampsia, but an understanding of the fundamental causes of pre-eclampsia remains tantalizingly elusive.

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