Searching for genetic clues to the causes of pre-eclampsia

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ABSTRACT
Pre-eclampsia and its related syndromes are significant causes of maternal and fetal death, but much remains unclear about the underlying disease mechanisms. Epidemiological research has consistently demonstrated a familial predisposition to pre-eclampsia, which has encouraged genetic research in this area. The goal is the discovery of susceptibility genes which will inform understanding of the pathophysiology of pre-eclampsia, and may prove to be targets for therapeutic or preventative strategies. This review examines the application of molecular technologies to the search for genetic clues in pre-eclampsia and emphasizes the importance of integrative approaches. The results of recent genome-wide linkage studies have been particularly encouraging, identifying a number of loci which merit closer examination. Candidate gene studies have proved less fruitful, generating conflicting and inconclusive results. Possible explanations and remedies for this deficiency are discussed with a view to stimulating closer collaboration between researchers in this field.

INTRODUCTION
Pre-eclampsia is a potentially life-threatening multi-system disorder which affects approx. 3% of pregnant women in the Western world [1]. Recognized clinically by the onset of hypertension and loss of protein in the urine, pre-eclampsia is nevertheless phenotypically diverse and much remains to be understood about its causes. The imperative for research into pre-eclampsia is its unacceptable burden on maternal and fetal health, much of which is borne by the developing world. Globally, the hypertensive disorders of pregnancy are responsible for up to 50 000 maternal deaths and as many as 900 000 perinatal deaths annually [2]. Prompt diagnosis and intervention are of vital importance in reducing maternal mortality and have guided the development of antenatal care facilities for regular monitoring of maternal blood pressure and early detection of proteinuria. Because pre-eclampsia resolves postpartum, premature delivery of the baby may be essential to safeguard the mother’s life. Consequently, many of the infants born of pre-eclamptic pregnancies require costly support on special care baby units. The burden of pre-eclampsia on health care resources is therefore substantial. Progress in the prevention and treatment of this condition will require advances in our understanding of the pathophysiology of the disorder at the molecular level.

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. Increasingly, researchers of complex disorders are turning to integrated approaches, which must include an appreciation of underlying biological processes, but can also utilize the power of genome-wide screening, RNA

Key words: fetal genotype, genome-wide screen, hypertension, maternal genotype, pre-eclampsia, pregnancy, susceptibility gene.
Abbreviations: Ang II, angiotensin II; AT1, Ang II type 1; CI, confidence interval; eNOS, endothelial nitric oxide synthase; GOPEC, Genetics Of Pre-EClampsia; IGF, insulin-like growth factor; IGFBP-1, IGF-binding protein-1; KIR, killer Ig-like receptor; NK, natural killer; PlGF, placental growth factor; sFlt-1, soluble Flt-1; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium testing; TNFa, tumour necrosis factor α; uNK, uterine NK; VEGF, vascular endothelial growth factor.
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microarray technology and proteomics to generate novel hypotheses.

Comprehensive reviews of genetic studies in pre-eclampsia are available [3,4], and no attempt will be made to reproduce these excellent articles in detail here. The aim of this review is to present some of the recent genetic studies in the context of earlier research, and to explore the impact of developing technologies and bioinformatic resources on evolving research strategies.

**PRE-ECLAMPSIA PHENOTYPES**

Pre-eclampsia is a syndrome observed only at the time of pregnancy. The condition resolves following delivery and there is no recognized counterpart in males or non-parous women. Pre-eclampsia has been defined as the onset of persistent hypertension exceeding 140/90 mmHg associated with proteinuria exceeding 300 mg/day after the 20th week of gestation [5]. Although this definition is useful for research purposes, it forces diagnostic thresholds on the continuous distributions of blood pressure and proteinuria, and masks the phenotypic diversity which characterizes clinical practice. Additional features affecting sub-groups of women include thrombocytopenia, deranged liver function, renal impairment and pulmonary oedema. A cluster of three features, Haemolysis, Elevated Liver enzymes and Low Platelets, usually but not invariably associated with hypertension and proteinuria, is known as HELLP syndrome [6]. Eclampsia, which affects a minority of women, includes some or all of the features of pre-eclampsia, in association with convulsions. Pre-eclampsia may run a fulminating course, necessitating swift, frequently premature, delivery of the child. In other cases, the disorder progresses more slowly, allowing time to control maternal blood pressure and deliver the child at a later gestational age. Up to one-third of infants born of pre-eclamptic pregnancies are affected by intrauterine growth restriction; in other pregnancies, fetal growth does not appear to be seriously impaired [7]. It is clear that pre-eclampsia, eclampsia and HELLP are related but phenotypically varied syndromes.

In clinical practice, a substantial proportion of the women affected by pre-eclampsia have predisposing medical conditions, which include chronic hypertension [8], diabetes [9], renal disease [10] and dyslipidaemia [11]. Furthermore, long-term follow-up studies have shown that women who were unaffected by these conditions prior to conception are nevertheless more likely to develop them later in life following an episode of pre-eclampsia [12]. It is worth noting that inherited liability to essential hypertension, diabetes and dyslipidaemia is well-recognized, raising the possibility that pre-eclampsia may share susceptibility genes with these conditions.

Treatment is directed initially towards stabilization of blood pressure and prevention of convulsions. Methyl-dopa and labetolol are widely used to control blood pressure in pre-eclampsia, supplemented by nifedipine. Magnesium sulphate is the drug of choice for the treatment and prevention of eclamptic convulsions. There is now good evidence that it is superior to the anticonvulsants phenytoin and diazepam [2]. If the mother’s condition can be stabilized, it may be possible to prolong pregnancy to allow further maturation of the fetus. However, pre-eclampsia does not begin to resolve until after delivery, and rapidly progressive disease may demand premature delivery to protect the life and health of both mother and baby.

**PATHOPHYSIOLOGY OF PRE-ECLAMPSIA**

Established pre-eclampsia is characterized by reduced vascular perfusion of major organs resulting from widespread maternal vascular endothelial dysfunction, which antedates the onset of clinical disease [13]. Although pre-eclampsia presents clinically after the 20th week of gestation, in many cases it has its origins much earlier in pregnancy, at the time of trophoblastic invasion into maternal tissues. In normal pregnancy, specialized extravillous trophoblast cells infiltrate the maternal spiral arteries supplying the placental bed, converting them from muscular vessels responsive to vasopressor stimuli into flaccid high-capacity sinuses, ideal for the exchange of nutrients and gases between maternal and fetal blood [14]. The process of spiral artery remodelling is incomplete in many pre-eclamptic pregnancies, pointing to the importance of the interaction between fetal and maternal factors in the origin of the disease. The resulting impairment of placental perfusion triggers maternal endothelial activation in those women who subsequently develop pre-eclampsia. It is worth noting that impaired spiral artery remodelling does not invariably lead to pre-eclampsia; similar features are observed in growth-restricted pregnancies without maternal disease. The implication is that maternal factors, including genetic predisposers, determine whether impaired placentalisation results in pre-eclampsia (Figure 1).

Research into the link between placental ischaemia and maternal endothelial activation is on-going, but it is clear that oxidative stress, both in the placenta and within the maternal systemic circulation, is an important component of the pathophysiology [15]. There is evidence for increased trafficking of syncytiotrophoblast microfragments into maternal plasma in pre-eclamptic pregnancies, with the release of inflammatory cytokines [16]. The ability of the mother to mount an adequate response to released pro-oxidant molecules may be of key importance. This view is endorsed by the increased risk of pre-eclampsia in women with pre-existing
conditions associated with oxidative stress, including obesity [17], chronic hypertension [8] and diabetes [9], where antioxidant reserves may be inadequate. These observations have led to trials of the prophylactic effects of antioxidants, including vitamins C and E and selenium. A recent Cochrane review of seven randomized trials [18] reported a 39% reduction in the risk of pre-eclampsia associated with oral antioxidant supplements. The authors stress that these findings should be interpreted with caution as some data were from poor quality studies. The results of large randomized controlled trials are awaited.

A promising proposal for a placental factor which might trigger endothelial dysfunction is the soluble form of the VEGF (vascular endothelial growth factor) receptor sFlt-1. Fli-1 is encoded by VEGF1; the soluble form is a splice variant lacking the cytoplasmic and transmembrane domains. VEGF and PlGF (placental growth factor), which play a role in maintaining vascular endothelial integrity, are both bound by circulating sFlt-1 (soluble Flt-1), which thus acts as an antagonist for these factors. In a study of women with established pre-eclampsia, placental sFlt-1 production was up-regulated, and sFlt-1 was detectable at increased concentrations in maternal plasma, with a corresponding reduction in circulating free VEGF and PlGF [19]. Furthermore, injection of sFlt-1 into pregnant and non-pregnant rats was followed by the development of hypertension and proteinuria. sFlt-1 production is induced by hypoxia, providing a plausible link between placental ischaemia and maternal disease. It is also possible that primary dysfunction of the VEGF system contributes to reduced trophoblastic invasion, and genetic approaches may be appropriate for testing this hypothesis.

EVIDENCE FOR A GENETIC BASIS TO PRE-ECLAMPSIA

A familial predisposition to pre-eclampsia has been confirmed in numerous studies, from the U.S.A., Scotland, Iceland, Scandinavia and Australia, which record a 2–5-fold increase in risk to first-degree relatives of women with pre-eclampsia [1,20–23]. Segregation analysis assuming a Mendelian mode of inheritance has yielded a number of possible models, including maternal recessive or maternal dominant with partial penetrance [22]. It was noted that factors which modify the penetrance of maternal genes could include fetal genetic effects.

Alternative suggested genetic mechanisms include a mitochondrial mode of inheritance [24], although epidemiological data indicate that mitochondrial genes do not contribute to the inheritance of pre-eclampsia in the majority of families [1]. Graves [25] proposed a susceptibility gene which is paternally imprinted; only the maternally inherited allele is active. Inheritance of a mutated copy of the gene from a heterozygous mother would effectively result in loss of gene activity in the fetus (Figure 2). This proposal combines both maternal and fetal gene effects, and is prescient in the light of the results of linkage studies recently reported by Dutch researchers [26], discussed below. In view of the diversity of inheritance models which have been suggested, it is

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common to regard pre-eclampsia as a complex disorder, which allows for Mendelian inheritance in a subgroup of pedigrees and interactions of multiple susceptibility genes in other families.

The classic approach to dissecting the contribution of environmental and inherited components to disease susceptibility is to compare the concordance rates in monozygotic and dizygotic twins, who share 100% and 50% respectively of their genetic makeup. Early studies cast doubt on the existence of maternal genetic factors, as concordance rates for pre-eclampsia among parous monozygotic twin sisters were low [27]. Estimates of heritability — the proportion of phenotypic variability attributable to genetic causes — had wide CIs (confidence intervals; 95% CI, 0–0.49) due to the relatively small number of affected women ascertained. The largest published twin study cross-linked data from the Swedish Twin Register and the Swedish Medical Birth Register [28]: 917 pairs of parous monozygotic and 1199 pairs of dizygotic twin sisters were identified. The estimate of the heritability of pre-eclampsia was 0.54, but even with this large sample the CIs were wide (95% CI, 0–0.71). When non-proteinuric gestational hypertension was considered as a mild form of pre-eclampsia, the heritability was 0.47 (95% CI, 0.13–0.61).

Evidence of a role for paternally inherited fetal genes comes from an analysis of data from the Norwegian Medical Birth Registry from 1967 to 1992, which identified almost 400,000 women who had had at least two pregnancies [1]. It was possible from the records to distinguish between pregnancies which had the same parents, and those where either the mother or the father differed in the second pregnancy. Only 1.3% of women who had not had pre-eclampsia in their first pregnancy developed pre-eclampsia in the second pregnancy. This risk was significantly increased (2.9%; P = 0.005) if they had a new partner who had previously fathered a pre-eclamptic pregnancy, consistent with an effect of paternal genes acting via the fetus. Further support for a role for paternal genes comes from a study which used records on the Utah Population Database to identify men and women born of pre-eclamptic pregnancies and to match them with controls born of healthy pregnancies [29]. Men who were born of a pre-eclamptic pregnancy were at increased risk of fathering a pre-eclamptic pregnancy [odds ratio 2.1 (95% CI, 1.0–4.3)], suggesting that susceptibility can be transmitted via paternal genes, presumably acting in the fetus. A recent analysis of 438,597 mother–baby pairs and 286,945 father–baby pairs from the Norwegian birth registry confirmed a 1.5-fold increase in risk of fathering an affected pregnancy to men born of a pre-eclamptic pregnancy, and a 2.2-fold increase in risk to women born of an affected pregnancy [30]. The authors reasoned that the risk to women is influenced by genes active both in the mother and in the fetus, whilst paternal genes can act only via the fetus. In support of this hypothesis, they showed that sisters who were not born of pre-eclamptic pregnancies nevertheless were at increased risk in their own pregnancies, due to maternally active genes. In contrast, brothers born of unaffected pregnancies, who presumably had not inherited fetal susceptibility genes, had a similar risk to men with no family history of fathering pre-eclamptic pregnancies. The data also demonstrated an increased risk of pre-eclampsia presenting before the 34th week of gestation if the father or mother had been born of an affected pregnancy, suggesting that genetic factors are predictive of severe disease.

A large study using data from the population-based Swedish Birth Register, which prospectively records antenatal, obstetric and neonatal data, estimated heritability due to maternal genes as 0.35 (95% CI, 0.33–0.36) and that due to fetal genes as 0.20 (95% CI, 0.11–0.24) [31]. Paternally and maternally inherited genes contributed equally to fetal genetic effects. Overall, these data suggest that genetic factors are responsible for over half of the liability to pre-eclampsia.

**CANDIDATE GENE STUDIES**

A PubMed search conducted in September 2005 using the search terms ‘pre-eclampsia, pregnancy hypertension,
gene, genetic, polymorphism and molecular’ identified over 130 English language reports of candidate gene studies in pre-eclampsia (Table 1, and Supplementary Table 1, at http://www.clinsci.org/cs/110/cs1100443add.htm). The majority of candidate gene studies adopted the simplest approach to the identification of genetic association, comparing the frequency of genetic variants in cases and controls. Many investigated a single polymorphism in a single candidate gene; a minority tested several genes, or multiple polymorphisms in one or more genes.

Although studies of over 50 candidate genes have been reported, eight genes account for 70% of published research into candidate genes for pre-eclampsia. These have been suggested by the features of established disease: genes encoding elements of the renin–angiotensin system, which regulates blood pressure (angiotensinogen, angiotensin-converting enzyme and angiotensin receptors); inherited thrombophilias (coagulation factor V Leiden variant, prothrombin and methylene tetrahydrofolate reductase); the NOS3 gene regulating the synthesis of the vasorelaxant eNOS (endothelial nitric oxide synthase); and the gene encoding the cytokine TNF (tumour necrosis factor) α.

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 consistent reproducibility of results, as has been the experience with many complex disorders. Possible explanations have been discussed widely [73], and studies of pre-eclampsia share some of these generic problems. Progress in this field will require attention to both study design and choice of candidate genes, and these aspects are considered in this review.

<table>
<thead>
<tr>
<th>Table 1 Candidate gene studies in pre-eclampsia</th>
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<td>AT2, Ang II type 2.</td>
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<tr>
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<th>Gene name</th>
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<td>[32–35]</td>
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<td>[34,36]</td>
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<td>[33]</td>
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<td>[34,38]</td>
<td>Oxidative stress</td>
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<td>β2-Fibrinogen</td>
<td>FGB</td>
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<td>EPHX1</td>
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<td>ITGB3</td>
<td>[36]</td>
<td>Glutathione S-transferase π</td>
<td>GSTP1</td>
<td>[38,59]</td>
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<td>[48]</td>
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<td>[52]</td>
<td>Matrix metalloproteinase 1</td>
<td>MMP1</td>
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Phenotype
Considerable effort is required to minimize phenotypic heterogeneity in both case and control groups in genetic studies, in the belief that this will ensure greater genetic homogeneity and thus maximize the power of the study. Internationally recognized definitions of pre-eclampsia are available [5], but strict application of the definition can be difficult as pre-eclampsia has a transient phenotype. Retrieving data from incomplete medical records in order to recruit retrospectively may be unreliable, and recruitment at the time of diagnosis is likely to lead to more reliable phenotyping.

Ethnicity
Allele frequencies in many polymorphisms differ between ethnic groups. If disease prevalence differs between subgroups of differing ancestry, population admixture may result in differences in allele frequencies between cases and controls, which may be wrongly interpreted as indicating genetic susceptibility. Typing of multiple unlinked genetic markers has been advocated as a means of detecting and correcting for population stratification [74], but these have not been applied to studies of pre-eclampsia.

The overwhelming majority of candidate gene studies in pre-eclampsia have investigated women of white Western European descent. Very few studies have included black women, which is both surprising and regrettable in view of the high risk of pre-eclampsia in black women and the high incidence of eclampsia in Africa.

Maternal or fetal genes?
One challenge which is a specific feature of disorders of pregnancy is that both maternal and fetal genes may play a role, but it is not possible to distinguish between these using a case-control design; genes which are active in the mother will also be over-represented in the fetus, and vice versa. In fact, remarkably few of the published molecular genetic studies of pre-eclampsia have investigated the fetal genotype.

Genotype or haplotype?
In the early years of molecular genetics, researchers focused on previously reported polymorphisms, often one or two per candidate gene, for which assays were readily available. More recently, 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 identified multiple SNPs (single nucleotide polymorphisms) in each gene. The combination of alleles at each polymorphic site determines the gene haplotype.

Comprehensive genetic analysis demands a thorough evaluation of haplotype associations with disease through testing of numerous polymorphisms in each gene [75]. Technologies are now available to meet the requirements for high-throughput genotyping at multiple SNPs. This approach has only recently been applied to genetic studies in pre-eclampsia and is mandatory if genes are to be confidently excluded from the list of possible candidates.

Statistical significance and power
There is ongoing debate about the level of statistical significance which is required to declare significant association in genetic studies. Over 4 million verified SNPs have been identified in the human genome, but there is no consensus on an appropriate statistical correction for multiple testing. A Bayesian approach has been suggested [73], which recognizes that a gene selected at random has a lower prior probability of association than a candidate gene supported by biochemical, pathophysiological or linkage data. Using this approach, the level of statistical significance required in a study of a gene with a moderate prior probability of association would be of the order of $5 \times 10^{-4}$, whereas the equivalent value for a gene selected at random would be $5 \times 10^{-5}$ or lower. It is apparent that only large studies including hundreds of cases and controls are adequately powered to meet this requirement. For example, testing of a biallelic polymorphism with a minor allele frequency of 0.3 would require over 300 cases and 300 controls to achieve 80% power to detect a doubling of risk of disease associated with the minor allele at a significance level of $5 \times 10^{-4}$.

To detect a genetic risk ratio as low as 1.3, which is not unrealistic in complex disorders, the required number of cases and controls is over 2000. From over 130 case-control studies identified by PubMed searching, only five included more than 300 cases. Three yielded negative results [34,42,45]. One large study reported an association with the platelet glycoprotein IIIa gene ITGB3 ($P < 0.01$) [36], which has not yet been re-tested in an adequately powered study. Another large study from Colombia found an association with the eNOS gene NOS3 ($P = 0.001$) [46]. The mixed ethnic background of subjects in this study is a limitation acknowledged by the authors, who advocated the use of unlinked polymorphic markers to assess confounding by ethnicity in future studies. Their positive findings were not replicated in a large study from the U.K. using TDT (transmission disequilibrium testing) [35].

Meta-analysis
It has been argued that such stringent criteria may be impractical and that combining the results of several smaller studies in a meta-analysis is an alternative powerful approach. One recent meta-analysis 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)) [76]; an earlier meta-analysis suggested that 677C $>$ T may be associated with severe pre-eclampsia only [diastolic blood
pressure $\geq 110$ mm Hg; odds ratio, 1.41 (95% CI, 1.03–1.73)] [77]. Meta-analysis of prothrombin 20210G $\geq$ A did not support a role for this polymorphism in pre-eclampsia [odds ratio, 1.37 (95% CI, 0.72–2.57)] [76]. Factor V Leiden was associated with an approx. 2-fold increase in risk of pre-eclampsia in all of three meta-analyses incorporating many of the same studies [76,78,79]. Some important general points 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 smaller studies.

**The GOPEC (Genetics Of Pre-EClampsia) study**

The U.K. GOPEC study was designed to address some of the problems inherent in genetic studies of pre-eclampsia [35]. Involving a consortium of researchers from ten U.K. universities, GOPEC recruited over 1000 women affected by pre-eclampsia and their families. Importantly, all were recruited at the time of their illness using rigorously defined criteria. A TDT approach to genetic analysis was planned [80]. TDT evaluates the transmission of allelic variants from heterozygous parents; under the null hypothesis of no association, the expected frequency of transmission of either allele to an affected offspring is 50%. Significant deviations from this expected frequency across affected families indicate the presence of a genetic variant associated with disease. By genotyping affected women and their parents, it is possible to conduct TDT of the maternal genotype. Genotyping the affected woman, her partner and baby enables independent testing of the fetal genotype. Furthermore, TDT is not affected by unsuspected population admixture, in contrast with case-control studies. As is increasingly the case in studies of complex disorders, the GOPEC study is designed to test multiple polymorphisms in each candidate gene in order to capture the majority of haplotypic diversity within the population.

The first phase of the GOPEC study sought to provide definitive results for candidate genes which have yielded conflicting data from earlier studies [35]. These included AGT, AGTR1, AGTR2, NOS3, MTHFR, TNF and the Factor V Leiden variant. A haplotyping approach was adopted for all genes with the exceptions of the Factor V gene and MTHFR, where analysis was restricted to variants of known functionality. Twenty-eight SNPs were analysed in 657 families, providing 85% power to detect a genotype relative risk of 1.6 at a significance level of $5 \times 10^{-4}$. There was no evidence in either maternal or fetal genotypes or haplotypes for association with pre-eclampsia for any of the genes tested. The CIs were narrow and suggested that these genes contribute little or nothing to the risk of pre-eclampsia.

**Immunogenetics**

The fetus expresses both paternal and maternal genes, and mechanisms have evolved to protect the fetal semi-allograft from immune rejection by the mother. There has been speculation that an inappropriate maternal immune response might lead to impaired trophoblast invasion and pre-eclampsia. Studies of maternal-fetal immunological interactions must take into account the unusual repertoire of HLA antigens expressed by trophoblastic cells. Trophoblasts at the maternal fetal junction do not express classical HLA antigens (HLA-A, -B and -DR), rather they express a distinctive repertoire of HLA-C, HLA-E and HLA-G antigens. HLA-G has attracted particular attention in the investigation of pre-eclampsia and other pregnancy-related disorders, as HLA-G expression may protect trophoblast cells from NK (natural killer)-mediated cell lysis [81].

Studies of HLA antigens in pre-eclampsia, which have been comprehensively reviewed recently [82], have yielded conflicting results. Such studies are inherently difficult to perform; the extensive polymorphism within HLA-A, -B and -DR classes results in many small subgroups and loss of statistical power. In a review of this topic, Saftlas and co-workers [82] highlight the urgent need for large studies examining interactions between maternal, paternal and fetal genotypes in order to adequately assess the role of HLA in pre-eclampsia [82].

A novel hypothesis has been advanced in a recent study focusing on fetal HLA-C genes and their interaction with maternal KIRs (killer Ig-like receptors) expressed by specialized maternal uNK (uterine NK) cells at the maternal-fetal interface. uNK cells are believed to facilitate and regulate trophoblast invasion through the secretion of cytokines. HLA-C are ligands for KIRs, and binding results in activation or inhibition of cytokine production depending on the repertoire of KIR genes possessed by the NK cell. KIR haplotypes are grouped into haplotype A, which mediates predominantly inhibitory signals, and haplotype B, which contains additional KIR genes with an activating profile. HLA-C antigens are expressed by invasive trophoblasts and the different alleles can conveniently be classified as C1 or C2 based on their interaction with KIRs. A case-control study of mother-child pairs detected a significant increase in maternal homozygosity for the KIR A (inhibitory) haplotype in pre-eclamptic pregnancies where the fetus possessed one or two copies of an HLA-C2 allotype [83]. This combination of maternal KIR and fetal HLA-C genotypes might possibly result in inhibition of uNK cytokine production, a mechanism that could explain the
impaired trophoblast invasion which can lead to pre-eclampsia.

CLUES TO NOVEL CANDIDATE GENES

Where should researchers be looking for novel susceptibility genes? Some important pathways suggested by the disease pathophysiology remain relatively unexplored. However, the identification of candidate genes from biological hypotheses is limited by our current understanding of the pathways involved. Molecular biology is now providing global screening strategies which generate biological hypotheses (Figure 3). Genome-wide scans are vital weapons in the molecular genetics armoury, and screening of pre-eclamptic pedigrees has pointed to several loci which merit fine mapping with a view to the identification of positional candidates. Rapidly developing genotyping technologies and the availability of haplotype maps have made genome-wide screening using dense SNP markers a reality which could be applied to large carefully phenotyped case-control collections. Other molecular strategies which do not require a priori aetiological hypotheses include the study of gene expression profiles (transcriptomics) and proteomics. These techniques hold great promise, but have been applied only relatively recently to the study of pre-eclampsia and other disorders of pregnancy.

Clues from pathophysiology

Current pathogenetic models of pre-eclampsia highlight two mechanisms which merit detailed genetic analysis: impaired placentation and oxidative stress [13]. Placentation requires the orchestrated expression of a range of proteinases, cytokines, angiogenic factors, adhesion molecules and their receptors which have barely figured in genetic studies to date. A handful of genes involved in antioxidant response mechanisms have been considered (Table 1), but these have mostly been relatively small studies of one or two polymorphisms per gene. There has, as yet, been no attempt to undertake comprehensive haplotype screening of antioxidant pathways in a large candidate gene study.

Linkage studies

Until recently, genetic linkage analysis has been the only approach available to geneticists for screening the entire genome for susceptibility loci. 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 the Netherlands have undertaken genome-wide linkage screens which have yielded encouraging results (Figure 4).

Chromosome 2

A study of 124 Icelandic pedigrees was the first to report linkage to chromosome 2p [84]. Within these families, affected women were classified as having mild disease (gestational hypertension with no proteinuria) or severe disease (hypertension with proteinuria and/or seizures, or severe gestational hypertension). The Icelandic group analysed the linkage data both with and without inclusion...
of women with mild pre-eclampsia, which they termed general and strict criteria respectively. This dual approach to pedigree analysis has been adopted in a number of published linkage studies of pre-eclampsia. Linkage to the locus on chromosome 2p was apparent under both general and strict criteria, and was chiefly attributable to two large multicae pedigrees. When these families were removed from the analysis, a different locus on chromosome 2q demonstrated suggestive linkage under the general criteria. A subsequent study of 34 pedigrees from Australasia and New Zealand also demonstrated two linkage peaks on chromosome 2p and 2q close to the regions identified in the Icelandic study [85]. It is possible that these two studies have identified identical loci in the pericentromeric region of chromosome 2, strengthening the case for a susceptibility gene in this region. Interestingly, suggestive linkage to the 2q region was reported in a genome-wide screen of British families affected by essential hypertension [86], a reminder that gestational hypertension and non-pregnancy hypertension may share some inherited features.

Bioinformatics databases record over 400 genes, including many of unknown function, within the 2p peak region and over 100 at the 2q peak, indicating the magnitude of the challenge to identify individual susceptibility genes [87]. The Australian group tested multiple SNPs within two positional candidate genes: TACR1, which encodes the neurokinin B receptor, and TCF7L1, which encodes a transcription factor with a possible role in embryo implantation. There was no evidence that any of the SNPs tested was associated with pre-eclampsia.

A genome-wide screen of 15 Finnish pedigrees [88] has implicated a susceptibility locus on chromosome 2p distinct from the loci identified by the Australian and Icelandic groups. As a possible explanation for these findings, the authors raise the interesting possibility that members of the same gene family with overlapping functions, arising from historical gene duplication events, may co-exist on chromosome 2.

**Chromosome 4**

A study of 15 Australian pedigrees identified a locus with suggestive linkage on chromosome 4q [89]. A subsequent genome-wide scan of 34 families [85], including those screened in the earlier report [89], used a denser panel of markers. This implicated chromosome 2, as discussed above, but linkage to chromosome 4q was less significant, and there was no evidence of linkage to this region in the Icelandic pedigrees. More recently, the genome-wide screen of Finnish pedigrees [88] has demonstrated suggestive linkage to chromosome 4q, very
close to the linked markers identified in the Australian study.

**Chromosome 9**
The Finnish study demonstrated linkage to chromosome 9p under both the general and strict diagnostic criteria [88]. In view of the increased risk of pre-eclampsia associated with diabetes, it is of interest that linkage to this region of chromosome 9p has also been demonstrated in Finnish families affected by Type II diabetes [90]. Two other markers on chromosome 9q displayed weak linkage under the general diagnostic criteria only.

**Chromosome 10**
A genome-wide screen undertaken on Dutch women yielded evidence for linkage with pre-eclampsia on chromosome 10q [91]. Interestingly, in families affected by pre-eclampsia associated with the HELLP syndrome, there was suggestive evidence for a separate susceptibility locus on chromosome 12q. In order to reduce the 132 positional candidate genes within the linked locus on chromosome 10q to a manageable number, this group adopted an approach which illustrates the combined power of bioinformatics and integrative biology. They hypothesized that pre-eclampsia may be associated with genetic imprinting. Re-analysis of their data under this model confirmed excess sharing of maternally inherited alleles at the 10q locus between sisters affected by pre-eclampsia, suggesting a paternally imprinted maternally active susceptibility gene [92]. They subjected the 132 genes at the 10q locus to homology (BLAST) searches to detect DNA sequence features characteristic of imprinted genes. As a further strategy to identify the most likely positional candidate genes, homology searches were conducted between the 10q locus and the genomic regions on chromosomes 2p and 9p, which have demonstrated significant linkage in genome-wide screens, on the assumption that similar genes on different chromosomes may cause similar disease phenotypes in different populations. Fifty-five genes met one or more of the specified criteria for further investigation. To test whether these genes are imprinted in trophoblast, mRNA expression was compared between first trimester placental tissue from terminated pregnancies and tissue obtained from first trimester hydatidiform molar pregnancies, which contain solely paternally derived nuclear DNA. Two clusters of genes were shown to be transcribed in normal trophoblast, but not in androgenic placenta, implying preferential expression of maternally derived genes [92]. DNA sequencing of 17 genes identified 55 SNPs and enabled the group to identify a minimum critical region of allele sharing between affected sisters, which included the paternally imprinted gene STOX1 [26]. This encodes a putative DNA-binding protein, which appears to function in the transition of invasive diploid extravillous trophoblast into non-invasive polyploid syncytiotrophoblast. A common tyrosine > histidine polymorphism at codon 153 in the DNA-binding domain of STOX1 was consistently shared by affected sisters in pre-eclamptic pedigrees, and importantly was almost invariably transmitted to the child born of the affected pregnancy. As the trophoblast is fetal tissue, the possession of a mutant maternally derived STOX1 allele, together with a silenced paternally imprinted allele, would lead to loss of function. The number of informative pregnancies in this fascinating study was limited, but if the findings can be independently confirmed they have far-reaching implications for research into the pathogenesis of pre-eclampsia. Interestingly, a paralogue of the gene, STOX2, is located on chromosome 4q close to the suggestive region identified in genome-wide screens in Australia and Finland, and merits further investigation.

**Transcriptomics**
Microarray technology is widely used to study the expression patterns of thousands of genes simultaneously. Investigating the expression of genes in both diseased and healthy tissue may provide clues to the cause of the disease and allow further research to focus on specific genes or pathways. Although this may be reasonably straightforward for diseases such as cancer, where samples of affected and healthy tissue can be taken from the same patient, conditions such as pre-eclampsia are more difficult, since variation between individuals has also to be taken into account. A PubMed search identified nine studies published between 2002 and 2005 which have used microarrays to compare placental gene expression in pre-eclamptic and normotensive pregnancy [93–101]. However, the lists of genes produced by each study show a discouraging lack of overlap.

The first problem with using this technology for the study of pre-eclampsia is tissue availability. It is clear that the placenta plays an integral role in the disease, but the disease is established early in pregnancy with a failure of trophoblast invasion into the uterine tissue. Since microarray studies use placental tissue which is obtained following delivery, usually early in the third trimester, it is difficult to establish whether changes in gene expression are a cause or a consequence of the disease. A related problem is obtaining control tissue matched for gestational age, as patients with pre-eclampsia often require premature delivery. Although it is possible to obtain samples from normotensive pregnancies which deliver before term, it is possible that the factors which resulted in premature delivery might themselves alter gene expression. For example, in one microarray study three out of six controls delivered early due to premature rupture of membranes, which is often associated with infection. This may lead to an increased expression of genes involved in inflammation or the immune response. As it seems likely that onset of labour will affect gene
expression, cases and controls should be matched for mode of delivery. The majority of studies included in the present review used samples obtained following Caesarean section, with one exception, which used both pre-eclamptic and control samples obtained after vaginal delivery [97].

There has been variation in the microarray design used for expression studies of pre-eclampsia. Two types of array have been used: cDNA arrays [93–98,100,101] and oligonucleotide arrays [99]. This terminology refers to the type of DNA probe that is immobilized on to the solid support. cDNA arrays have spots of cDNA ranging from 500–5000 bp, whereas the oligonucleotide arrays use shorter molecules of around 15–70 bp. Furthermore, the number of genes represented on an array has ranged from a selected group, e.g. cytokines [93], to much larger chips, such as the Affymetrix U133A array which contains 14 500 well-characterized genes [99]. Lack of replication of results between studies may therefore be due to the absence of a gene from the array used in some studies. A third variable is the method of labelling and detection. Many studies have used radioactive labelling to detect hybridization [97,98,101] which mandates hybridization of samples from pre-eclamptic and normal patients to separate copies of the array. Labelling of samples from cases and controls with different fluorescent dyes enables hybridization of both to a single array, reducing the experimental variability [93–96,99,100].

Pooling of RNA from a number of subjects is commonly used in hybridization experiments to reduce costs, to compensate for low amounts of starting material or to reduce variation between individual samples. Pooling of all samples from one group has been used in a number of studies [93–96,100,101], an approach which cannot provide an estimate of variability among arrays. Sub-pooling, the pooling and hybridization of multiple subsets of samples selected at random, is preferable, as the variation between arrays can be considered, although obviously a larger number of patient samples will be required [103]. Pooling RNA from different subjects results in a permanent loss of information and has also been shown to result in a loss of sensitivity and an increase in false-positive results. In spite of the financial implications, hybridization of individual RNA samples and pooling of results for statistical analysis was the approach used in two studies [97,98].

Although it is now widely accepted that microarray experiments should be replicated in independent experiments to confirm changes in gene expression, it appears that the majority of experiments using placental tissue have been performed only once or repeated using the same samples and the same membrane-based array [101].

After considering all the differences between the nine array experiments included in the present review, it is disappointing, but perhaps not surprising, that there is so little agreement between them. However, the expression patterns of genes shown in Table 2 were similar in at least two independent studies. Some encode proteins which have been associated previously with pre-eclampsia; however, two of the genes in Table 2 have not been implicated previously in pre-eclampsia. TNF receptor (ligand) superfamily member 10 (TRAIL [TNF-related apoptosis-inducing ligand]) is expressed at a significant level in most normal tissues. It has been shown to preferentially induce apoptosis in transformed and tumour cells, but does not appear to kill normal cells (reviewed in [104]). AXL receptor tyrosine kinase is a member of the receptor tyrosine kinase subfamily. Members of this subfamily play an important role in cellular proliferation and differentiation, and may mediate the effects of Ang II (angiotensin II) via the AT1 (Ang II type 1) receptor, although the exact mechanisms are unclear [105].

**Proteomics**

Evolving MS-based technologies now facilitate screening and identification of multiple proteins in tissues and biofluids. The application of these techniques to disorders of pregnancy is in its early stages [115] and a detailed discussion of these methodologies is beyond the scope of this review, but the integration of proteomic and genetic approaches to the study of pre-eclampsia holds much promise. The spatial and temporal expression patterns of molecules at the maternal–fetal interface in early pregnancy will be of particular interest. Screening of maternal plasma collected prospectively from early pregnancy may detect biomarkers which predate the onset of clinical pre-eclampsia. Genetic testing of novel candidates suggested by the results of proteomic screening will be a powerful complementary strategy in the identification of causative mechanisms of disease.

**Transgenic animal models**

There is no naturally occurring animal model of pre-eclampsia; however, recent advances have enabled the use of transgenic methods to study the impact of specific genes.

One of the first transgenic models of pre-eclampsia arose from the generation of mice expressing human angiotensinogen or human renin [116]. When female mice carrying the human angiotensinogen gene were mated with males carrying the human renin gene, the females developed hypertension and proteinuria which resolved after delivery. Pregnant females derived from all other mating combinations did not develop these symptoms. In affected females, renin was localized to the trophoblast cells in the placenta and was secreted into the maternal circulation. It was suggested that the secretion of placent (paternal) human renin contributes to the regulation of maternal blood pressure, providing the female was expressing human angiotensinogen. This experiment has also been carried out in rats with similar results [117].
Table 2  Genes showing altered placental expression in pre-eclampsia in at least two independent studies

<table>
<thead>
<tr>
<th>Genes with altered expression in pre-eclampsia</th>
<th>Gene symbol</th>
<th>Fold increase in expression in pre-eclampsia</th>
<th>Previous association with pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercellular adhesion molecule 1</td>
<td>ICAM1</td>
<td>21.2 [94]</td>
<td>Serum level increased prior to onset of clinical symptoms [106].</td>
</tr>
<tr>
<td>Vascular cell-adhesion molecule 1</td>
<td>VCAM1</td>
<td>39.6 [94]</td>
<td>Serum level increased prior to onset of clinical symptoms [106].</td>
</tr>
<tr>
<td>Integrin α1</td>
<td>ITGA1</td>
<td>43.7 [100]</td>
<td>Expected up-regulation of α1β1 integrin is not seen in pre-eclampsia [107]. This contrasts with the array results, but the α1 integrin monomer could be forming other dimers.</td>
</tr>
<tr>
<td>Integrin αL</td>
<td>ITGAL</td>
<td>1.5 [94]</td>
<td>LFA-1 has αL and β1 subunits. Higher levels of LFA-1 observed within the uteroplacental circulation compared with peripheral venous blood in pre-eclamptic patients [108].</td>
</tr>
<tr>
<td>Glycoprotein hormones α polypeptide</td>
<td>CGA</td>
<td>2.1 [101]</td>
<td>Subunit of hCG, FSH, LH, TSH. Serum hCG concentration is increased in pre-eclampsia [109].</td>
</tr>
<tr>
<td>Inhibin βA</td>
<td>INHBA</td>
<td>4.9 [100]</td>
<td>Subunit of inhibin A and activin A. Serum concentrations of inhibin A (αβA) and activin A(βAβA) are increased in pre-eclampsia [111].</td>
</tr>
<tr>
<td>IFNγ receptor 2</td>
<td>IFNGR2</td>
<td>6.9 [93]</td>
<td>Normal up-regulation of the receptor does not occur in pre-eclampsia [112] in contrast with the microarray results.</td>
</tr>
<tr>
<td>Secreted phosphoprotein 1 (osteopontin)</td>
<td>SPP1</td>
<td>1.6 [101]</td>
<td>Increased production of osteopontin in cytrophoblasts from pre-eclamptic placentas [113].</td>
</tr>
<tr>
<td>Prostaglandin I2 (prostacyclin) synthase</td>
<td>PTGIS</td>
<td>1.9 [96]</td>
<td>Reduced placental prostacyclin secretion in pre-eclampsia antedates clinical disease [114].</td>
</tr>
<tr>
<td>TNF-receptor (ligand) superfamily member 10 (TRAIL)</td>
<td>TNFSF10</td>
<td>3.2 [93]</td>
<td>Novel candidate.</td>
</tr>
<tr>
<td>AXL receptor tyrosine kinase</td>
<td>AXL</td>
<td>4.1 [100]</td>
<td>Novel candidate.</td>
</tr>
</tbody>
</table>

In addition to developing hypertension and proteinuria, the female rats also produce auto-antibodies which bind to and activate the AT1 receptor. Women with pre-eclampsia produce anti-(AT1 receptor) antibodies [118] and it has been suggested that these may play a role in the pathogenesis of pre-eclampsia, making this a potentially valuable animal model.

Transgenic mice have also been used to assess the contribution of the IGFBP-1 [IGF (insulin-like growth factor)-binding protein-1] to placental development and intrauterine growth restriction [119]. These mice can be used to gain a better understanding of the factors important for correct placentaion, which is thought to be dysfunctional in pre-eclampsia. The contribution of fetal genotype to placental and fetal development was assessed by studying the characteristics of transgenic and wild-type fetuses derived from a female wild-type mouse. Similarly, the effect of maternal genotype was studied using the characteristics of wild-type fetuses derived from both wild-type and transgenic females. These experiments revealed that the fetal IGFBP-1 contribution to placental and fetal growth appears to be minimal. Although there is a modest reduction of fetal weight early in pregnancy in transgenic fetuses, by late pregnancy the wild-type and transgenic fetuses had identical growth characteristics. In addition, placental growth was similar for both throughout gestation. However, maternal IGFBP-1 genotype appears to have some effect on placental growth, with a greater placental weight in transgenic females at all time points irrespective of fetal genotype. In addition, trophoblast invasion is less pronounced in transgenic females compared with wild-type. Fetal growth in early pregnancy is impaired in transgenic females, but growth is similar in both wild-type and transgenic females by later time points. There also appears to be an increase in fetal death from matings involving a transgenic female.

The suggestion that pre-eclampsia may be caused by a paternally imprinted gene [25] is supported by the results from mice deficient in p57Kip2, which is a paternally imprinted gene in both humans and mice, i.e. only expressed from the maternally derived chromosome. A decrease in expression has been associated with abnormal proliferation of trophoblast cells [120], which may be
relevant to pre-eclampsia. Heterozygous female mice that were mated with heterozygous males showed signs of pre-eclampsia, including hypertension, proteinuria and increased endothelin levels in late pregnancy. These features were also noted in heterozygous females which were mated with wild-type males, but not in wild-type females mated with either heterozygous or wild-type males, i.e. symptoms were only seen in females carrying p57Kip2-deficient pups regardless of their own p57Kip2-expression status. This indicates that the fetal genotype may play a role in the development of pre-eclampsia.

The results of animal models reveal that symptoms similar to those exhibited by women with pre-eclampsia can be initiated either by maternal factors (IGFBP-1 mice), by factors originating in the fetus (p57Kip2 mice) or by an interaction of both maternal and fetal factors (renin/angiotensinogen rodents). If the same holds true for humans, with patterns of genetic influences differing between pedigrees, this will complicate the analysis of the underlying genetics of the disorder further.

CONCLUSIONS

A major challenge in the study of pre-eclampsia is to disentangle the causes of the disease from its consequences. In this respect, genetic studies are particularly valuable, as genetic variation may be the cause of a pathophysiology, but not its consequence. Genetics has a dual role in the search for disease mechanisms: genome-wide screens can suggest novel hypotheses, and candidate gene studies can be used to test hypotheses suggested by pathophysiology or global screening strategies. Recent genome-wide screens of families affected by pre-eclampsia have yielded encouraging results. By contrast, the credibility of candidate gene studies has been undermined by conflicting and inconclusive results. It is apparent that larger studies of 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 which will lead to the establishment of large DNA resources, or at least enable statisticians to conduct meaningful meta-analyses. Continuing epidemiological research indicates that both the maternal and fetal genotypes are valid targets for study. The adoption of current global screening technologies will challenge bioinformaticians to produce software to interpret and integrate massive amounts of data. The role of the statistical geneticist is vital in developing sophisticated analytical tools which will tackle the complexities of haplotype estimation, gene–gene interactions and the interplay of fetal and maternal genotypes. Clinicians, geneticists, epidemiologists, bioinformaticians and statisticians therefore all have a part to play in finding genetic clues to the aetiology of pre-eclampsia; truly a task for dedicated teamwork. The goal is the identification of pathophysiological mechanisms which will inform future strategies for the prevention and treatment of this devastating condition.

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REFERENCES

14 Pijnenborg, R., Dixon, G., Robertson, W. B. and Brosens, I. (1980) Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. Placenta 1, 3–19


102 Reference deleted
104 Reference deleted
110 Reference deleted

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