Identification of two novel quantitative trait loci for pre-eclampsia susceptibility on chromosomes 5q and 13q using a variance components-based linkage approach

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Pre-eclampsia/eclampsia (PE/E) is a common and serious disorder of human pregnancy that is associated with substantial maternal and perinatal morbidity and mortality. The suspected aetiology of PE/E is complex, with susceptibility being attributable to multiple environmental factors and a large genetic component. By assuming that the underlying liability towards PE/E susceptibility is inherently quantitative, any PE/E susceptibility gene would represent a quantitative trait locus (QTL). This assumption enables a more refined and powerful variance components procedure using a threshold model for our PE/E statistical analysis. Using this more efficient linkage approach, we have now re-analysed our previously completed Australian/New Zealand genome scan data to identify two novel PE/E susceptibility QTLs on chromosomes 5q and 13q. We have obtained strong evidence of linkage on 5q with a peak logarithm-of-odds (LOD) score of 3.12 between D5S644 and D5S433 [at ~121 centimorgan (cM)] and strong evidence of linkage on 13q with a peak LOD score of 3.10 between D13S1265 and D13S173 (at ~123 cm). Objective identification and prioritization of positional candidate genes using the quantitative bioinformatics program GeneSniffer revealed highly plausible PE/E candidate genes encoding aminopeptidase enzymes and a placental peptide hormone on the 5q QTL and two type IV collagens on the 13q QTL regions, respectively.

Introduction

Pre-eclampsia/eclampsia (PE/E) is the most common and serious disorder of human pregnancy displaying heritable characteristics and susceptibility to the PE/E syndrome being attributable to a large genetic component (Salonen Ros et al., 2000; Nilsson et al., 2004; Carr et al., 2005). Associated with substantial maternal and perinatal morbidity and mortality, PE is characterized by the development in the latter half of pregnancy (>20 weeks gestation) of new onset hypertension that generally resolves post-partum. In its most severe form, there is evidence of proteinuria, oedema and multi-(internal) organ dysfunction. Of most concern is the unpredictable progression of PE to E, a convulsive condition superimposed over PE symptoms that is life threatening to both mother and baby. Worldwide prevalence of PE/E, ethnic background aside, is estimated at 10% of all pregnancies and subsequently accounts for ~12% (the fourth highest cause) of all maternal deaths (World Health Organization, 2002). Currently, there is no reliable diagnostic test or any efficacious prophylactic intervention for PE/E, with the only effective means of definitively managing PE being delivery irrespective of gestation. Should preterm delivery be required to manage PE, then this intervention itself may increase the incidence of short-term and long-term complications from prematurity for the neonate. Approximately 12% of infants born to mothers with PE/E will die within the first month of life, while the remaining 88% are at an elevated risk of impaired mental development and/or cardiovascular-related diseases later in life (World Health Organization, 2002).

Despite the concordant clinical characterization of endothelial cell dysfunction and the vascular and immune complications associated with abnormal placentation "vis-à-vis" normal pregnancy (Sibai et al., 2005), the exact aetiology of PE/E still remains largely undetermined. Several hypotheses have however addressed the underlying involvement of the maladaptation of maternal immune responses to early placentation, oxidative stresses and dysfunctional endothelial cell activation in the PE/E clinical phenotype. These hypotheses are thought not to be mutually exclusive but interact, possibly synergistically, with each other to result in a PE/E pregnancy in what may be described as a three-tier disorder (Jauniaux et al., 2006). The development of PE/E is further exacerbated by several risk factors including, but not limited to, nulliparity (Odegard et al., 2000), primipaternity (Dekker and Sibai, 2001), recurrent pre-eclamptic pregnancies (Hnat et al., 2002), maternal age extremities (<20 or >40 years of age) (Dekker and Sibai, 2001), obesity (O’Brien et al., 2003), chronic hypertension (Sibai, 2002) or a family history of PE (Salonen Ros et al., 2000; Carr et al., 2005) with genome-wide linkage studies providing strong evidence leading to the implication of maternal susceptibility genes (Fitzpatrick et al., 2004; van Dijk et al., 2005; Moses et al., 2006).
Although there is evidence for the interaction of maternal and paternal genetic effects contributing to PE susceptibility (Lie et al., 1998; Oudejans et al., 2004; van Dijk et al., 2005), the focus of maternal genetic studies has been on the identification of maternal susceptibility genes. Given the disparate nature of reported models of PE inheritance (summarized in Lachmeijer et al., 2002), previous investigations to identify maternal genetic susceptibility have in most part employed genetic linkage analysis algorithms nullifying any inheritance model. With the exception of Hayward et al. (1992) and Harrison et al. (1997), current PE genome-wide linkage studies have neglected analyses using a PE/E inheritance model in favour for determining the extent of allele sharing amongst affected women only (Arrigimson et al., 1999; Moses et al., 2000; Lachmeijer et al., 2001; Laivuori et al., 2003; Fitzpatrick et al., 2004). These genome scan linkage studies have currently mapped putative pre-eclamptic susceptibility loci to chromosomal regions 4q (Harrison et al., 1997), 2p13 (Arrigimson et al., 1999), 2p11–p12, 2q23 and 11q23–q24 (Moses et al., 2000; Fitzpatrick et al., 2004), 10q, 11q13 and 22q (Lachmeijer et al., 2001), and 2p25, 4q32 and 9p13 (Laivuori et al., 2003).

A previous genome-wide scan and subsequent fine mapping studies of Australian and New Zealand pedigrees adopting a less efficient genetic linkage approach focusing on affected females identified putative pre-eclamptic susceptibility loci on chromosomes 2p11–p12, 2q23 and 11q23–q24 (Moses et al., 2000; Fitzpatrick et al., 2004). The putative susceptibility locus on 2q has since been strengthened and resolved further to 2q22 [at 155 centimorgan (cM)] by the application of a more efficient genetic linkage method assuming that the underlying liability of pre-eclamptic susceptibility is inherently quantitative (Moses et al., 2006). Under this assumption, any PE/E susceptibility gene represents a quantitative trait locus (QTL), thus leading to a more refined and novel variance components-based procedure that uses a biological threshold model for PE in the statistical analysis procedures. This approach is more efficient than our previous method as it utilizes all the available pedigree data from both affected and unaffected females. In this study, using this quantitative trait linkage approach, we have now re-analysed the remainder of our previously completed genome scan data (Moses et al., 2000) to identify two additional and novel pre-eclamptic susceptibility (QTL) regions on chromosomes 5q and 13q.

Materials and methods

PE/E pedigrees

Over a 15-year period (1984–99), 34 PE/E pedigrees (26 Australian and 8 New Zealand) were ascertained through the Monash Medical Centre and the Royal Women’s Hospital, Melbourne, Australia, via media advertisements in Sydney, Australia and through the National Women’s Hospital Auckland, New Zealand. These pedigrees were initially utilized to localize a PE/E susceptibility locus on chromosome 2q23 (Moses et al., 2000; Fitzpatrick et al., 2004). The 34 families included 13 women with E, 74 women with severe PE, 34 women who were hypertensive during their first pregnancy and no proteinuria (mild PE) and 71 women who were normotensive during their first pregnancy and no proteinuria. Pregnancy was considered severely pre-eclamptic if they, on at least two occasions six or more hours apart, had (i) a rise from baseline systolic blood pressure (SBP) of at least 25 mmHg and/or a rise from baseline diastolic blood pressure (DBP) of at least 15 mmHg or (ii) SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. Additionally, proteinuric levels were >0.3 g/l in a 24-h specimen or at least a 2+ proteinuria dipstick reading from a random urine collection. Women who satisfied these criteria and experienced convulsions or unconsciousness in their perinatal period were diagnosed as having eclampsia. Only women with the aforementioned criteria within their first and not subsequent pregnancies were included for the study (Moses et al., 2000; Fitzpatrick et al., 2004). Ethical approval for the recruitment of Australian/New Zealand PE/E pedigrees was granted by the Royal Women’s Hospital Research and Ethics Committees, Melbourne, Australia. Written informed consent was obtained from study participants prior to them being phlebotomized. Ethical approval for the additional quantitative genetic linkage analyses conducted on the PE/E familial cohort was obtained from The University of Texas Health Science Center at San Antonio, Institutional Review Board.

Genotyping

Extraction of genomic DNA from peripheral blood samples, amplification conditions for 400 microsatellite markers from the Applied Biosystems Human Linkage Mapping Set (version 2), electrophoretic separation, detection and assignment of sample genotypes have been previously described in detail (Moses et al., 2000).

Mendelian inconsistency checking

Pedigree genotypes not conforming to Mendelian inheritance patterns were identified by the PEDSYS (Dyke, 1989) programs INFER and GENTEST. A mistyping analysis was conducted using the Markov chain Monte Carlo (MCMC)-based program SimWalk2 (Sobel and Lange, 1996). Mendelian discrepancies were removed by blanking genotypes identified by SimWalk2 as having a high probability of being in error.

Variance components-based linkage analysis

Variance components-based linkage analyses were performed using the statistical genetics package SOLAR (Almasy and Blangero, 1998). Using this approach, all the linkage information is computed as a function of estimated identical by descent (IBD) matrices. For our PE/E pedigrees, we calculated an exact estimate of location-specific IBD probability matrices using the computer program Loki (Heath, 1997; Heath et al., 1997) for subsequent importation and use in SOLAR.

The basic premise behind the variance components linkage algorithm is the ability to reduce common complex phenotypes in terms of their quantitative architecture. While most obviously used for classical quantitative traits, variance component procedures can be applied to reduce dichotomous traits pertaining to affection status, as applied in our PE/E diagnoses, by assuming an underlying continuous distribution of liability or risk (Williams et al., 1999). The use of variance components procedures allows multipoint IBD analyses to be performed on pedigrees of arbitrary size and complexity to infer the localization and effect of all QTLs. This then eliminates the need to remove individuals from the pedigree(s) to satisfy computational constraints. Retention of all individuals in their respective pedigree structures thus maintains the power to detect true linkage signals. In estimating the genetic variance attributable to chromosomal regions specific to genetic loci, the variance components model tests the null hypothesis of the additive genetic variance of the i-th QTL equalling zero (i.e. no linkage). The null hypothesis then assesses the likelihood of this restricted model against a model whereby the variance of the i-th QTL is estimated. The difference between the two log10 likelihoods thus provides a logarithm-of-odds (LOD) score which measures the support for the alternative hypothesis of linkage over the null hypothesis of ‘no linkage’ at a specific chromosomal location. Empirically, testing for the existence of a locus influencing the phenotype at each location in the genome is generally performed by tests of linkage at every cM. P values are obtained by considering twice the difference in log10 likelihoods of these two models yielding a test statistic that is asymptotically distributed as a $\chi^2$ variable and a point mass at zero (Self and Liang, 1987). Inflated type I errors may be evident when assuming normality of a trait when in fact its distribution is truly non-normal (Allison et al., 1999). Several methods have thus been developed, in SOLAR, to handle non-normal distributed traits including the use of the multivariate t-distribution (Blangero et al., 2001) and the robust LOD score method that can be used for any trait distribution (Blangero et al., 2000, 2001). For a dichotomous trait, as applied in our PE/E diagnoses, the robust LOD score method is preferential and has been applied in the current analyses. Reported results are effectively equivalent of empirical P values.

Multistep linkage analysis for the X chromosome is not currently implemented in SOLAR. To negate the trimming of PE/E pedigrees via use of alternative variance components programs (e.g. GENEHUNTER), and thus maintain our pedigree structures, two-point linkage analysis was performed on all X-linked loci only.
Candidate gene prioritization

Objective prioritization of positional candidate genes residing within the bounds of putative QTL susceptibility region(s) was performed using the quantitative bioinformatics program GeneSniffer (www.genesniffer.org). GeneSniffer is a tool that automates bioinformatic database mining from NCBI's Entrez Gene, OMIM and PubMed databases to objectively identify plausible positional candidate genes residing under defined linkage (QTL) regions. Interrogation of these databases is performed using a set of disease-specific key words inputted by the end user. Inputted key words are assigned a score (scale 1–10, 10 being greatest) based on their relevance and significance to the studied disease. Homologues of each gene, under the linkage peak, are identified by BLAST and scored for content of their Entrez Gene, OMIM and PubMed entries. Each score is weighted in accordance with the degree of homology, and a cumulative ‘hit-score’ is calculated and outputted for each gene. Output is provided in an HTML format documenting all genes under an observed QTL region with the source of database hits and links to external databases for additional information. GeneSniffer can also incorporate the observed LOD score function within the QTL region to use the localization data as an additional weighting function in which genes are considered more relevant the closer they are to the observed QTL peak.

Results

Heritability estimates

Using the statistical genetics analysis package SOLAR, both the severe and the mild PE phenotypes were computed to be significantly heritable in our Australian/New Zealand pedigrees. Heritability estimates of the severe and mild PE phenotypes were 0.51 ± 0.06 ($P = 1.13 \times 10^{-20}$) and 0.58 ± 0.13 ($P = 2.48 \times 10^{-24}$), respectively.

Variance components-based linkage analysis

Multipoint linkage analysis using SOLAR was performed on both the severe (Figure 1) and the mild (Figure 2) PE phenotypes for all autosomal data (Chromosomes 1, 3–22). Strong evidence of linkage for the severe PE phenotype was observed on 5q with a peak LOD score of 3.12 between markers D5S644 and D5S433 at $\sim$121 cM (Figure 3). Strong evidence of linkage was also observed on 13q with a peak LOD score of 3.10 between markers D13S1265 and D13S173 at $\sim$123 cM (Figure 4). Suggestive evidence of linkage for the mild PE phenotype was observed on 5q with a peak LOD score of 2.57 between markers D5S428 and D5S644 at $\sim$116 cM (Figure 3). Nominal evidence of linkage for the severe PE phenotype was observed on chromosomes 1, 6 and 9–12 (Table I), and for the mild PE phenotype, nominal evidence of linkage was observed on chromosomes 12 and 13 (Table I).

Two-point linkage analysis was performed on both the severe and the mild PE phenotypes for all X-linked loci only. For the severe PE phenotype, nominal evidence of linkage was observed on Xq with a LOD score of 1.33 at marker DXS8091 (167 cM) (Table I). For the mild PE phenotype, nominal evidence of linkage was observed at the same DXS8091 marker with a LOD score of 1.89 (Table I).

Prioritization of positional candidate genes under the 5q and 13q QTL loci

In linkage mapping studies, a 1-LOD drop flanking the highest linkage signal is used to define a 95% confidence interval. For our GeneSniffer analysis, we were able to interrogate the entire chromosomal region under both the 5q and the 13q linkage peaks approximating a 3-LOD drop, 99% confidence interval. Under these criteria, GeneSniffer interrogated 299 genes in the critical region on 5q from D5S424 (90.87 cM) to D5S2115 (136.75 cM) using NCBI Human Build 35.1. These included 222 confirmed genes, 30 expressed sequence tags (ESTs), 19 predicted genes, 8 interim locus IDs and 20 conflicting gene models. GeneSniffer was also used to interrogate 31 genes in the critical region on 13q from D13S424 (90.87 cM) to D13S2115 (136.75 cM) using NCBI Human Build 35.1. These included 23 confirmed genes, 3 ESTs and 5 predicted genes. Positional candidates with major

Figure 1. Multipoint genome-wide linkage scan for the severe pre-eclampsia (PE) phenotype (chromosomes 1, 3–22). Linkage scan results pertaining to chromosome 2 for the same phenotype have been reported previously (Moses et al., 2006).
‘hit-scores’ on 5q were the aminopeptidase enzymes adipocyte-derived leucine aminopeptidase (ARTS-1) and leucyl-cystinyl aminopeptidase (LNPEP) and a placenta l peptide hormone corticotrophin-releasing hormone-binding protein (CRHBP). Positional candidates with major ‘hit-scores’ on 13q were the type IV collagens, alpha-1 (COL4A1) and alpha-2 (COL4A2).

Discussion
In this study, we have used a novel variance components-based linkage analysis method in the quest to identify maternal susceptibility loci for PE/E. We have previously used this approach to both strengthen and resolve a previously reported chromosome 2 linkage signal (Moses et al., 2000; Fitzpatrick et al., 2004) to now provide comprehensive evidence of a PE susceptibility QTL on 2q22 at 155 cM (Moses et al., 2006). This QTL has been evaluated further by an objective prioritization strategy implementing quantitative bioinformatics, differential gene expression, single-nucleotide polymorphisms (SNP) association and linkage disequilibrium analyses to identify two putative PE/E susceptibility genes ACVR2 and ACVR1C (Moses et al., 2006). As an extension to the recent resolution of the 2q22 QTL, we have re-assessed the remaining autosomes (1, 3–22) and sex
chromosome (X) using this same quantitative variance components-based linkage method to identify a further two, novel PE/E susceptibility QTLs on 5q (at ∼121 cM) and 13q (at ∼123 cM).

Even though the PE diagnostic model is dichotomous in nature, the hypothesized rationale for this study in conjunction with our previously reported 2q22 QTL (Moses et al., 2006) is that the underlying liability to PE susceptibility is inherently quantitative. In what could be regarded as a departure from the conventional means of PE whole genome-scan linkage analyses (Arrgrimson et al., 1999; Moses et al., 2000; Lachmeijer et al., 2001; Laivuori et al., 2003; Fitzpatrick et al., 2004), application of a quantitative variance components procedure enables a biological mean threshold to be set, independent of PE severity (i.e. severe or mild), within a continual liability (or risk) distribution of the population. This has one of three advantages: first, all pedigree information is utilized as no pedigree members are excluded because of computational constraints. This therefore eliminates any loss in power, and IBD matrix calculations are thus maximized over all pedigreed individuals. Secondly, realistic models pertaining to a biological threshold, as for PE severity (severe or mild), can be directly considered, and thirdly, trait prevalence differences such as age and other covariate information can be accommodated for.

One of the most pronounced differences in our application of the variance components linkage analysis method as opposed to a ‘model-free’ linkage analysis method was the means of calculating IBD matrices that are used to estimate a QTL(s) along any chromosomal position. It is apparent that accurate multipoint linkage analysis elevates the power to detect linkage while reducing the false-positive rate. However, initial multipoint IBD estimation utilized the Lander–Green Hidden Markov Model (Lander and Green, 1987) which, while exact, is restricted to pedigrees of a small number of non-founders (∼12) (Kruglyak et al., 1996; Kong and Cox, 1997). The limitation to a small finite number of non-founding members associated with using this algorithmic approach is because of the requirement of computing all possible inheritance vectors within a pedigree across all genotyped loci (Sobel et al., 2001). As pedigree members are added, there is an exponential increase in the computation required to estimate IBD values. A restriction of pedigree size using this method therefore places limitations on the power of a pedigree(s) to detect true linkage signals. In contrast, while inheritance vectors are again required to be computed, the MCMC algorithm utilized for the variance components-based analysis samples the set of all possible inheritance vectors in a way that is proportional to each vector’s likelihood (Sobel et al., 2001).

Therefore, as implemented in the program Loki (Heath, 1997; Heath et al., 1997), an average over sampled inheritance vectors is used to estimate the IBD values. Additionally, these MCMC IBD calculations are more precise than those obtained using the extended Fulker–Cardon technique (Fulker and Cardon, 1994; Fulker et al., 1995), which is the default procedure implemented in SOLAR. The accurate MCMC method makes it feasible to compute IBD matrices for pedigrees of arbitrary size and complexity, thus maximizing the power of each pedigree to detect true linkage signals.

The resolution of the 5q and 13q PE/E QTLs posits the next challenge of positional candidate gene selection for further, more in depth, genetic and (potential) functional examination. Use of the bioinformatics tool GeneSniffer has enabled us to identify a plausible set of positional candidate genes under both the 5q QTL (CRHBP, ARTS-1 and LNPEP) and the 13q QTL (COL4A1 and COL4A2) regions, respectively.

The placental peptide hormone CRHBP (OMIM 122559) exhibits a specific affinity to CRH with a resultant negation in the bioactivity of CRH. Hormonal actions of CRH are instigated by its synthesis from neuroendocrine cells in the paraventricular nucleus of the hypothalamus. Additionally, CRH employs a role in parturition where it is reported to govern a role in pregnancy duration (McLean et al., 1995). During the ante-partum period, in particular the third trimester, the human placenta is a major source of CRH production as synthesized by syncytiotrophoblasts (Sasaki et al., 1984; Campbell et al., 1987). Under normal pregnancy conditions, elevated amounts (∼1000-fold increase after 20 weeks gestation) of CRH are released from the placenta into the maternal circulation. In direct contrast, during this same period, there is a subsequent drop in maternal circulating levels of CRHBP. The indirect relationship between CRH and CRHBP levels as witnessed from the third trimester until parturition is evident in normal, uncomplicated, human pregnancy (Perkins et al., 1993). This indirect relationship is exacerbated however in complications associated with human pregnancy (e.g. PE) where there are significantly higher levels of CRH and significantly lower levels of CRHBP (Perkins et al., 1993, 1995). Characteristic of pre-eclamptic pathogenesis is a shallow invasion of trophoblast cells into the placental bed (Kauffmann et al., 2003). It has thus been speculated that measurement of placental peptide or steroid hormones may act as an efficacious biomarker for pre-eclamptic pregnancies (Myatt and Miodovnik, 1999). It is also interesting to note that another placental peptide, activin A, is implied in the pathophysiology of PE (Petraglia et al., 1995) for which we have previously reported plausible evidence of the role of the activin receptor genes ACVR2 and ACVR1C in PE (Moses et al., 2006).

Exhibiting a high degree of homology (Hattori et al., 2001), both the secretory ARTS-1 (OMIM 606832) and the membrane-bound LNPEP (OMIM 151300) enzymes belong to the M1 family of zinc-containing aminopeptidases. The enzymatic activity of ARTS-1 to inactivate angiotensin II and convert kallidin to bradykinin within the kidney has implicated the importance of this enzyme in blood pressure regulation (Hattori et al., 2000). Similarly, LNPEP, or placental leucine aminopeptidase, has an implicated role in blood pressure regulation via the hydrolysis of oxytocin, vasopressin and angiotensin III (Tsujimoto et al., 1992; Matsumoto et al., 2000). Transcriptional profiling of normal placental tissue by Ino et al. (2003) identified ARTS-1 to be highly expressed in extravillous trophoblasts infiltrating the decidua of spiral arteries during the second trimester of pregnancy. Additionally, Ino et al. (2003) identified LNPEP to be highly expressed in the villous syncytiotrophoblasts and also in the extravillous trophoblasts infiltrating the decidua or maternal spiral arteries during the late first

### Table 1. Empirical logarithm-of-odds (LOD) scores (>1.00) displaying nominal evidence of linkage for the severe and mild pre-eclampsia phenotypes

<table>
<thead>
<tr>
<th>Pre-eclampsia phenotype</th>
<th>Chromosome</th>
<th>LOD</th>
<th>cM</th>
<th>Locus</th>
</tr>
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<td>144</td>
<td>D1S498</td>
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<tr>
<td></td>
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<td>6q</td>
<td>1.27</td>
<td>209</td>
<td>D6S446</td>
</tr>
<tr>
<td></td>
<td>9q</td>
<td>1.73</td>
<td>124</td>
<td>D9S1776-D9S1682</td>
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<tr>
<td></td>
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<td>1.88</td>
<td>36</td>
<td>D10S1653</td>
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<td>1.48</td>
<td>128</td>
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</tr>
<tr>
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</tr>
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*Two-point analysis performed on X-linked loci only.*
to second trimesters of pregnancy, LNPEP expression levels decreased during the third trimester of normal pregnancy. Of particular note, LNPEP activity has been shown to significantly decrease in severe pre-eclamptic pregnancies (Mizutani et al., 1998), thus suggesting that LNPEP expression may be reliant upon differentiation of trophoblastic cells and subsequent infiltration into the decidua tissue.

Poor placentaion is considered a strong predisposing risk factor for PE (Sibai et al., 2005). Poor placentaion can result from an initial ineffective re-modelling of cell basement membranes required for cell invasion (Weber et al., 1985; Bjorn et al., 1997). A major component of the cell basement membrane is the type IV collagens, the genes for two of which, collagen, type IV, alpha-1 (COL4A1; OMIM 120130) and collagen, type IV, alpha-2 (COL4A2; OMIM 120090) reside in our chromosome 13q QTL region. The type IV collagens have been demonstrated to be highly expressed and co-localised in the extravilous cytotrophoblasts of anchoring villi and in cytotrophoblasts having infiltrated the placental bed (Bjorn et al., 1997). With particular reference to COL4A2, expression levels of this basement membrane collagen have also been reported to be up-regulated in pre-eclamptic placental tissue (Pang and Xing, 2003). The abnormal expression of collagen genes is thought to play a role in the ineffective base-

In our original genome scan analysis (Moses et al., 2000), we identified suggestive evidence of linkage to chromosome 11q23–q24 between D11S925 and D11S1415 (LOD = 2.02 at 121.3 cm). In this study, using SOLAR to re-analyse this same genome scan data, we have also identified a nominal linkage signal to chromosome 11q25 at 144 cm near D11S1320 (LOD = 1.59). Although this most recent result is ~23 cm telomeric to our previous linkage signal, it may represent the same putative susceptibility locus segregating in our Australian/New Zealand PE pedigrees. In a Dutch genome scan, Lachmeijer et al. (2001) also reported a nominal linkage signal for PE susceptibility on 11q. However, given that this signal at 76.1 cm is ~68 cm centromeric to our nominal 11q localization evidence, it would seem unlikely that it could represent the same susceptibility QTL segregating in this population.

The 9q nominal linkage signal observed in this study between D9S1776 and D9S1682 (LOD = 1.79 at 124 cm) lies in proximity to a nominal linkage signal for PE susceptibility independently identified in a Finnish cohort at 120.8 cm near D9S1811 (Laivuori et al., 2003). These concordant 9q (nominal) linkage signals may well be the same PE susceptibility locus, segregating in Australian/New Zealand and Finnish PE pedigrees, respectively.

In conclusion, we have applied a novel variance components-based linkage analysis method to identify two novel pre-eclamptic susceptibility QTLs on chromosomes 5q and 13q. These results follow our previous application of this linkage analysis method to resolve a pre-eclamptic susceptibility QTL on 2q22 (Moses et al., 2006). The use of the bioinformatic tool GeneSniffer has also enabled us to objectively identify several plausible positional PE/E candidate genes in the 5q and 13q QTL regions. Our strongest positional candidates will now be interrogated using known gene-centric SNPs for preliminary association and linkage disequilibrium analyses in our 34 PE/E pedigrees used to map the 5q and 13q QTLs. On the basis of (potential) putative association findings, independent cohort replication of these potential results will assist in the resolution of our novel PE/E QTL regions.

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