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Report

A Genome Scan in Families from Australia and New Zealand Confirms the Presence of a Maternal Susceptibility Locus for Pre-Eclampsia, on Chromosome 2

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Epidemiological studies have shown that genetic factors contribute to the etiology of the common and serious pregnancy-specific disorder pre-eclampsia (PE)/eclampsia (E). Candidate-gene studies have provided evidence (albeit controversial) of linkage to several genes, including angiotensinogen on 1q42-43 and eNOS on 7q36. A recent medium-density genome scan in Icelandic families identified significant linkage to D2S286 (at 94.05 cM) on chromosome 2p12 and suggestive linkage to D2S321 (at 157.5 cM) on chromosome 2q23. In the present article, the authors report the results of a medium-density genome scan in 34 families, representing 121 affected women, from Australia and New Zealand. Multipoint nonparametric linkage analysis, using the GENEHUNTER-PLUS program, showed suggestive evidence of linkage to chromosome 2 (LOD = 2.58), at 144.7 cM, between D2S112 and D2S151, and to chromosome 11q23-24, between D11S925 and D11S4151 (LOD = 2.02 at 121.3 cM). Given the limited precision of estimates of the map location of disease-predisposing loci for complex traits, the present finding on chromosome 2 is consistent with the finding from the Icelandic study, and it may represent evidence of the same locus segregating in the population from Australia and New Zealand. The authors propose that the PE/E-linked locus on chromosome 2p should be designated the *"PREG1"* (pre-eclampsia, eclampsia gene 1) locus.

Pre-eclampsia (PE [MIM 189800]) is a complex and serious disorder of human pregnancy, with a worldwide incidence of 2%–5%. It is characterized by the development, in the latter half of pregnancy, of new-onset hypertension that resolves postpartum (Roberts and Redman 1993; Witlin and Sibai 1997; Davey and MacGillivray 1988). In its severe form the disorder produces significant proteinuria, edema, and multiorgan dysfunction. There is no reliable predictive test, nor is there an effective medical therapy to treat this serious condition. The most worrisome complication of PE is its unpredictable progression to eclampsia (E), a convulsive condition that is life threatening for both mother and infant.

Although the full etiology and pathogenesis of PE/E are unknown, it is accepted that the placenta is involved in a fundamental manner. The evidence to date suggests that abnormal interaction between maternal and fetal tissues during placentation may be the initiating factor in the complex chain of events that constitute the syndrome of PE/E. In a normotensive pregnancy, cytotrophoblasts invade the spiral arterioles, replacing the endothelium and removing the muscular covering of the arterioles (Robertson et al. 1967; Zhou et al. 1997). The arterioles therefore dilate, and the decidual-trophoblast blood flow is increased. These events take place at 15–18 wk of pregnancy. In PE/E pregnancies the invasion of the spiral arterioles by the cytotrophoblasts is limited. They remain small in diameter and retain their muscular

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covering, with a consequent relative restriction of maternal blood flow to the placenta (Brosens et al. 1972; Gerretsen et al. 1981; Khong et al. 1986; Redman 1991; Zhou et al. 1997). This histopathological defect in PE/E pregnancies precedes the onset of clinical symptoms. A widely proposed hypothesis attempts to link the defect to the eventual onset of symptoms by postulating that the reduced maternal placental blood flow leads to placental hypoxia, with consequent release of toxic factors from the placenta into the maternal circulation, which causes a generalized dysfunction of maternal vascular endothelial cells. In turn, this dysfunction perturbs the balance of endothelial cell–derived vasoactive autacoids (e.g., prostacyclin, nitric oxide, and endothelin) and thereby leads to the generalized vasospasm, diminished tissue perfusion, and hypoxia of organs (e.g., the brain, kidneys and liver) that are characteristic of PE/E (Redman 1991; Roberts and Redman 1993; Arbogast et al. 1994; Lim et al. 1997; Taylor 1997; Higgins and Brennecke 1998; Page et al. 2000).

It is widely accepted that genetic factors play a major role in susceptibility to PE/E (Cooper et al. 1993; Pipkin 1999; Ros et al. 2000), and several models of inheritance have been proposed (Cooper and Liston 1979; Arngrímsson et al. 1995; Graves 1998). Although none of these has been proved, it is likely that paternal genes acting through the fetus contribute in some way to the mother's risk (Thornton and Onwude 1991; Lie et al. 1998; Ros et al. 2000). PE/E, like many common human diseases (e.g., heart disease, diabetes, cancer, and hypertension) is a multifactorial trait that is probably influenced by combinations of genetic and nongenetic risk factors, complicating the process of identifying them (see Terwilliger and Göring 2000). There have been concerted efforts to identify the maternal risk genes, mostly focused on candidate genes (Cooper et al. 1993; Roberts and Cooper 2000), but results to date have been ambiguous at best. Genome-scan studies have reported findings on chromosomes 4q (Harrison et al. 1997) and 2p13 (Arngrímsson et al. 1999), for the latter of which we find strong confirmatory evidence in our current genome scan, as indicated in the present report.

We have performed a genome scan to identify maternal genes whose genotypes may influence the risk of developing PE; 366 individuals from 26 Australian and 8 New Zealand families were genotyped at 400 microsatellite markers, with fluorescence-labeled primer pairs from the ABI PRISM Human Linkage Mapping Set, version 2 (PE Biosystems). The families were recruited over a 15-year period (1984–99), through the Royal Women's Hospital and the Monash Medical Centre in Melbourne and via print and electronic media advertisements in Sydney and through the National Women's Hospital, Auckland. Subsets of the 26 Australian families have been used in earlier studies (Wilton et al. 1990, 1991, 1995; Humphrey et al. 1995; Guo et al. 1997, 1999; Harrison et al. 1997; Lade et al. 1999). The 34 families included 13 women with E, 74 women with severe PE, 34 women who had hypertensive first pregnancies without proteinuria (mild PE), and 71 women who had normotensive first pregnancies. Diagnosis was based on clinical assessment, using the criteria of the Australasian Society for the Study of Hypertension in Pregnancy (Brown et al. 1993), which are very similar to those used in the recently reported genome scan from Iceland (Arngrímsson et al. 1999). Pregnant women were considered to have severe PE if they had either (1) an increase of $\geqslant$25 mm Hg above baseline systolic blood pressure and/or an increase of $\geqslant$15 mm Hg above baseline diastolic blood pressure or (2) a persistent systolic blood pressure of $\geqslant$140 mm Hg and/or a diastolic blood pressure of $\geqslant$90 mm Hg. These levels had to occur on at least two occasions $\geqslant$6 h apart. The level of proteinuria had to be >0.3 g/liter in a 24-h specimen, or the dipstick proteinuria score had to be $\geqslant$2+ in a random urine collection. Women who met these criteria and who had experienced either convulsions or unconsciousness in the perinatal period were classified as having had E. Because PE/E is typically a disease of first pregnancies (Chesley et al. 1968; Fisher et al. 1981), only those women with the above-mentioned features in their first and not in their subsequent pregnancies were included in this study. Women with preexisting hypertension or other medical diseases known to predispose them to PE were excluded. Approval for this study was obtained from the ethics-review committees of all relevant institutions, and informed written consent was obtained from family members before peripheral blood samples were taken.

The genotyping for chromosomes 2, 5, 6, 15, 16, and 19–22 was performed by the Australian Genome Research Facility. The remaining autosomes and chromosome X were genotyped by the authors. For genotyping, genomic DNA was extracted from the blood samples, with a commercial kit (Blood and Cell Culture DNA Midi Kit; Qiagen). PCR reactions (10 μl) were set up in 384-well polypropylene microplates (MJ Research), with a HYDRA-96 micropipetting robot (Robbins Scientific), and contained 34 ng of genomic DNA, 0.33 μM of each primer, 250 μM of each deoxynucleotide and 2.5 mM $MgCl_2$, together with 0.4 U of Ampli*Taq* Gold DNA polymerase and 1 × GeneAmp PCR Buffer II (both from PE Biosystems). The reactions were carried out using FTS-960 thermal sequencers modified to accommodate 384-well plates (Corbett Research) and were programmed to deliver the following cycling profile: 1 cycle at 95°C for 10 min, to activate the DNA polymerase; 10 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 30 s; followed by 20 additional cycles that were identical, apart from a reduced denaturation temperature of 89°C. The re-

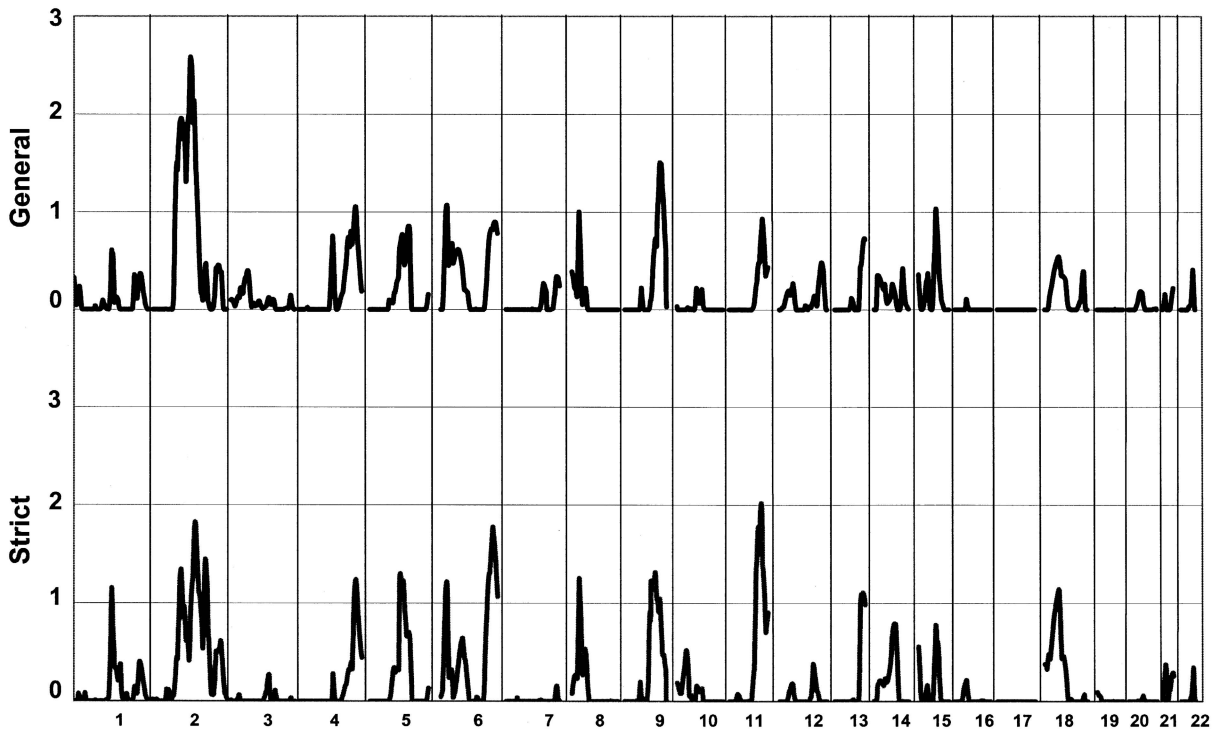

Figure 1 Multipoint nonparametric linkage analysis using the S_{all} scoring function in the GENEHUNTER-PLUS modification of the GENEHUNTER package. The GENEHUNTER-PLUS LOD scores on the *Y* axis are plotted against the genetic distance, in cM, on the *X* axis.

actions were then finished with a final extension step at 72°C for 10 min. The PCR products were pooled using the HYDRA-96, and, after the addition of fluorescence-labeled internal size standards, were separated by electrophoresis and were automatically detected on an ABI PRISM 377 DNA sequencer equipped with GENESCAN software, version 3.1 (PE Biosystems). The subsequent assignment of genotypes was performed using GENOTYPER software, version 2.5 (PE Biosystems).

Linkage analysis was performed using the GENEHUNTER-PLUS package (Kruglyak et al. 1996; Kong and Cox 1997), with the PEDMANAGER program, version 0.9, to test for Mendelian inconsistencies. PEDMANAGER was also used to calculate the allele frequencies for each marker locus, by use of the genotypes of the founder individuals from the 34 families with PE/E and the genotypes of 50 additional unrelated Australian individuals. The markers were assumed to be positioned as indicated in the Sex-Averaged CHLC/ABI Prism Reference Maps, version 2.0. Two diagnostic schemes were used in the analysis—a strict diagnostic model, in which women with E and women with severe PE were classified as affected, and a general diagnostic model, in which women with mild PE were also classified as affected. An affecteds-only strategy was used, in which we considered all men and women who had not reproduced to be phenotypically unknown. This was identical to the analysis performed by the Icelandic group in their genome scan (Arngrímsson et al. 1999). The resulting LOD scores based on the S_{all} scoring function are presented in figure 1 for both the strict diagnostic model and the general diagnostic model. When the general classification criteria were used, the maximum multipoint LOD score in the present study was observed on chromosome 2q, between D2S112 and D2S151 (LOD = 2.58 at 144.7 cM), which is not far from the location where Arngrímsson et al. (1999) reported a LOD score (with the same statistical analysis) of 4.7 in their Icelandic study, also based on their general diagnostic model. (In their initial genome scan, with a density similar to that in our scan, the maximum LOD score was 3.14.) The peak due to this linkage finding extended over a very wide region, with significant ($P <$ .05) markers over an 85-cM region, adding support to the idea that this may be a true-positive result (see Terwilliger et al. 1997). Furthermore, although the peaks do not occur at exactly the same position on the chromosome, the precision of genome scans for complex traits is poor, and the estimated map locations of this locus are sufficiently close that they are consistent with a single risk locus in both studies, as shown in the large simulation study by Hovatta et al. (1998). Furthermore, as shown in figure 1, the peak for chromosome 2 rises far above the background signals on the other chro-

mosomes, at least under the general diagnostic model, for which the next-highest LOD score over the whole genome was 1 LOD unit smaller. Under the strict diagnostic category, the sample size was notably smaller, and the number of regions with LOD scores approaching 2 was much larger than that under the general diagnostic model. This is consistent with the expectation that a higher false-positive rate would be found when a much smaller data set is studied by linkage analysis. The best finding under the strict diagnostic model was on chromosome 11, but this does not stand out much above the background noise. Although the LOD score on chromosome 2 is somewhat lower under the strict diagnostic model, chromosome 2 is still the second-most-significant region in the genome scan, and the finding is consistent with the hypothesis that a locus that influences PE/E, irrespective of severity, may be found in the chromosome 2 region. It is noteworthy that the shape of the multipoint LOD-score curve in our study is also similar to that of the study by Arngrímsson et al. (1999), with a broad region identified in the initial scan and with minor peaks in similar locations. We conclude that it is likely that we and Arngrímsson et al. (1999) have detected the same locus on chromosome 2, and therefore we propose that it should be designated the *PREG1* (pre-eclampsia/eclampsia gene 1) locus.

In earlier linkage studies performed using subsets of the families used in the present study, evidence suggestive of linkage was found for regions on chromosomes 4q (Harrison et al. 1997) and 7q36 (Guo et al. 1999). In the present study, under the severe classification criteria, nominal evidence of linkage, with multipoint P values $<.05$, was observed in the same region of chromosome 4q (LOD = 1.24 at 217.3 cM), but there was no evidence of linkage to 7q. In this context, it should be noted that the 7q36 marker for which suggestive evidence of linkage was previously found (D7S1805) was not genotyped as part of the scan described in the present report. It is also noteworthy that, although Arngrímsson et al. (1999) found suggestive evidence of linkage to 7q36 in 50 families from Scotland and Iceland, they found no evidence for linkage to this (or any other reported candidate region) in their subsequent genome scan in 134 Icelandic families (Arngrímsson et al. 1999).

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for pre-elampsia/eclampsia [MIM 189800])

Sex averaged CHLC/ABI Prism Reference Maps (version 2.0), http://lpg.nci.nih.gov/data/ABIMaps/

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