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ARTICLE

A genome-wide scan for preeclampsia in the Netherlands

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Preeclampsia, hallmarked by *de novo* hypertension and proteinuria in pregnancy, has a familial tendency. Recently, a large Icelandic genome-wide scan provided evidence for a maternal susceptibility locus for preeclampsia on chromosome 2p13 which was confirmed by a genome scan from Australia and New Zealand (NZ). The current study reports on a genome-wide scan of Dutch affected sib-pair families. In total 67 Dutch affected sib-pair families, comprising at least two siblings with proteinuric preeclampsia, eclampsia or HELLP-syndrome, were typed for 293 polymorphic markers throughout the genome and linkage analysis was performed. The highest allele sharing lod score of 1.99 was seen on chromosome 12q at 109.5 cM. Two peaks overlapped in the same regions between the Dutch and Icelandic genome-wide scan at chromosome 3p and chromosome 15q. No overlap was seen on 2p. Re-analysis in 38 families without HELLP-syndrome (preeclampsia families) and 34 families with at least one sibling with HELLP syndrome (HELLP families), revealed two peaks with suggestive evidence for linkage in the non-HELLP families on chromosome 10q (lod score 2.38, D10S1432, 93.9 cM) and 22q (lod score 2.41, D22S685, 32.4 cM). The peak on 12q appeared to be associated with HELLP syndrome; it increased to a lod score of 2.1 in the HELLP families and almost disappeared in the preeclampsia families. A nominal peak on chromosome 11 in the preeclampsia families showed overlap with the second highest peak in the Australian/NZ study. Results from our Dutch genome-wide scan indicate that HELLP syndrome might have a different genetic background than preeclampsia.
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Introduction

Preeclampsia is still a leading cause of pregnancy-related maternal and foetal morbidity and mortality in developed countries.[1] A strong familial factor in the aetiology of preeclampsia has been established[2–4] and to assess the underlying mode of inheritance, systematic family studies have been done. Proposed inheritance models range from a

maternal single recessive gene model,[2,3,5] to models involving influence of the foetal genotype,[6,7] homozygosity for a single recessive gene shared by mother and foetus,[8] and models involving a dominant maternal gene with reduced penetrance.[3] Summarising all systematic family studies, Arngrimsson *et al* concluded that a major dominant gene model with reduced penetrance, possibly depending on foetal genotype, or multi factorial inheritance, fitted the available data best.[9]

Finding susceptibility genes for preeclampsia has proved difficult. Several candidate gene studies have not provided consistent evidence for association between putative susceptibility genes and preeclampsia.[10–19]

Four genome-wide linkage studies have been performed so far. Hayward *et al* published the first genome-wide linkage study, designing an exclusion map for preeclampsia.[20] In the second genome scan Harrison *et al*[21] suggested the presence of a candidate region on chromosome 4q. The outcome of both these rather small studies were not in agreement with each other, nor did they confirm any of the previously published associations with candidate genes. Recently, Arngrímsson *et al*[22] published a genome-wide scan comprising a large number of affected women (*n*=343) from 124 pedigrees. Using 440 polymorphic micro satellite markers and parametric (model-dependent) as well as non-parametric (model-independent) linkage analyses, a maternal susceptibility locus for preeclampsia was revealed on chromosome 2p13 at 94.05 cM which met the criteria for genome-wide significance.[23] Evidence for a preeclampsia locus on chromosome 2 was confirmed by a similar genome-wide scan in 34 families from Australia and New Zealand from Moses *et al* who found suggestive evidence of linkage to chromosome 2q at 144.7 cM.[24] The current Dutch genome-wide scan was performed at the same laboratory as and in close collaboration with Arngrímsson and coworkers; 265 individuals from 67 Dutch families were genotyped at 293 microsatellite markers.

Materials and methods

Disease criteria

Affected women were recruited having suffered either from preeclampsia, HELLP syndrome or eclampsia during her first pregnancy ('strict' criteria), or from pregnancy induced hypertension only ('mild criteria') (Table 1).

Recruitment of the Dutch affected sib-pair families with preeclampsia

Between June 1995 and October 1997, 150 affected sib-pair families were recruited. Selection of 2940 women with a medical history of hypertension in pregnancy was through the medical records of the Vrije Universiteit Medical Centre in Amsterdam and the Academic Hospital in Groningen, The Netherlands, obstetrical databases of 20 other hospitals in the Netherlands and advertisements for the study. Medical family history questionnaires were sent out to all 2940 affected women. Medical records were examined for all women who responded; they had at least one affected sister (*n*=178). Their affected sibling's medical records were examined similarly. Finally 150 families fulfilled the study criteria and were included. All families contained 332 affected women of whom 233 met the strict criteria, and 241 unaffected relatives. For the current genome scan 67 families were selected with siblings only affected by the strict criteria. These 67 families contained 58 families with two affected siblings, nine families with three affected siblings and 122 unaffected relatives.

Genotyping

DNA was extracted in the Netherlands from the peripheral blood of all individuals in the 67 families. Facilities for genotyping were made available by deCODE Genetics in Reykjavík, Iceland. Genotyping was done using 293 fluorescently labelled primers randomly distributed throughout the genome with an average spacing of 11.8 cM. The markers typed came from the CALC./Weber Human Screening Set Version 8 from Research Genetics. Average heterozygosity of markers in this set is reported to be 76–78% in CEPH families. PCR's, genotyping and editing of the genotypes were similar to the Icelandic scan.[22] Marker orders and genetic distances used were obtained from publicly available genetic maps at the Marshfield Medical Clinic web site (www.marshmed.org).[25]

Statistical analyses

Data of the genome-wide scan were analysed using affected-only non-parametric/allele sharing methods.[26–28] Individ-

Table 1 Diagnostic criteria

Criteria	*Diagnosis*	*Definition*
Strict	Preeclampsia	*De novo* hypertension (diastolic BP[a] $\geqslant$ 90 mmHg with increment $\geqslant$ 20 mmHg from first trimester diastolic BP[b]) and proteinuria $\geqslant$ 300 mg/24 h or at least twice 1+ on dipstick
	Eclampsia	Seizures in a hypertensive pregnancy, with or without proteinuria
	HELLP-syndrome	LDH[c] $\geqslant$ 600 IU/l and ASAT[d] and ALAT[e] at least 70 IU/l and $\leqslant$ 100 platelets $\times 10^9$/l
Mild	PIH[f]	*De novo* hypertension in pregnancy without proteinuria

[a]BP=blood pressure; [b]BP levels observed on at least two occasions, more than 6 h apart; [c]Lactic dehydrogenase; [d]Aspartate amino transferase; [e]Alanine amino transferase; [f]PIH=pregnancy induced hypertension.

uals, including males, not known to be affected or individuals only affected according to the mild criteria, were classified as 'unknown'. Mendelian inheritance was verified by using the LINKAGE package's 'unknown' program.[26] All linkage results reported were produced by the ALLEGRO program[29] utilising the non-parametric method of linkage analysis.[28] Non-parametric linkage analysis assesses the amount of excess identity-by-descent (IBD) sharing among related affected as measured by a chosen scoring function. The scoring function used for this study was S_{all}, which is found to be quite powerful for a wide range of inheritance models.[28,30] All calculations were fully multipoint, ie all markers from the same chromosome were used simultaneously. The allele sharing lod scores reported here were, similar to the Icelandic scan,[22] all computed with respect to the exponential model.[27] Criteria for significance were based on guidelines published by Lander and Kruglyak;[23] a lod score >3.6 (P value <0.00002) indicates genome-wide significance, a lod score between 2.2 and 3.6 ($P<0.0007$) indicates suggestive linkage and lod scores between 0.6 ($P<0.05$) and 2.2 ($P<0.01$) are nominal.

We re-analysed our data for families with only preeclampsia cases (preeclampsia families; n=38) and families with at least one sibling with HELLP-syndrome (HELLP families; n=34) separately, since in the previous scans[22,24] no HELLP-syndrome cases had been recruited.

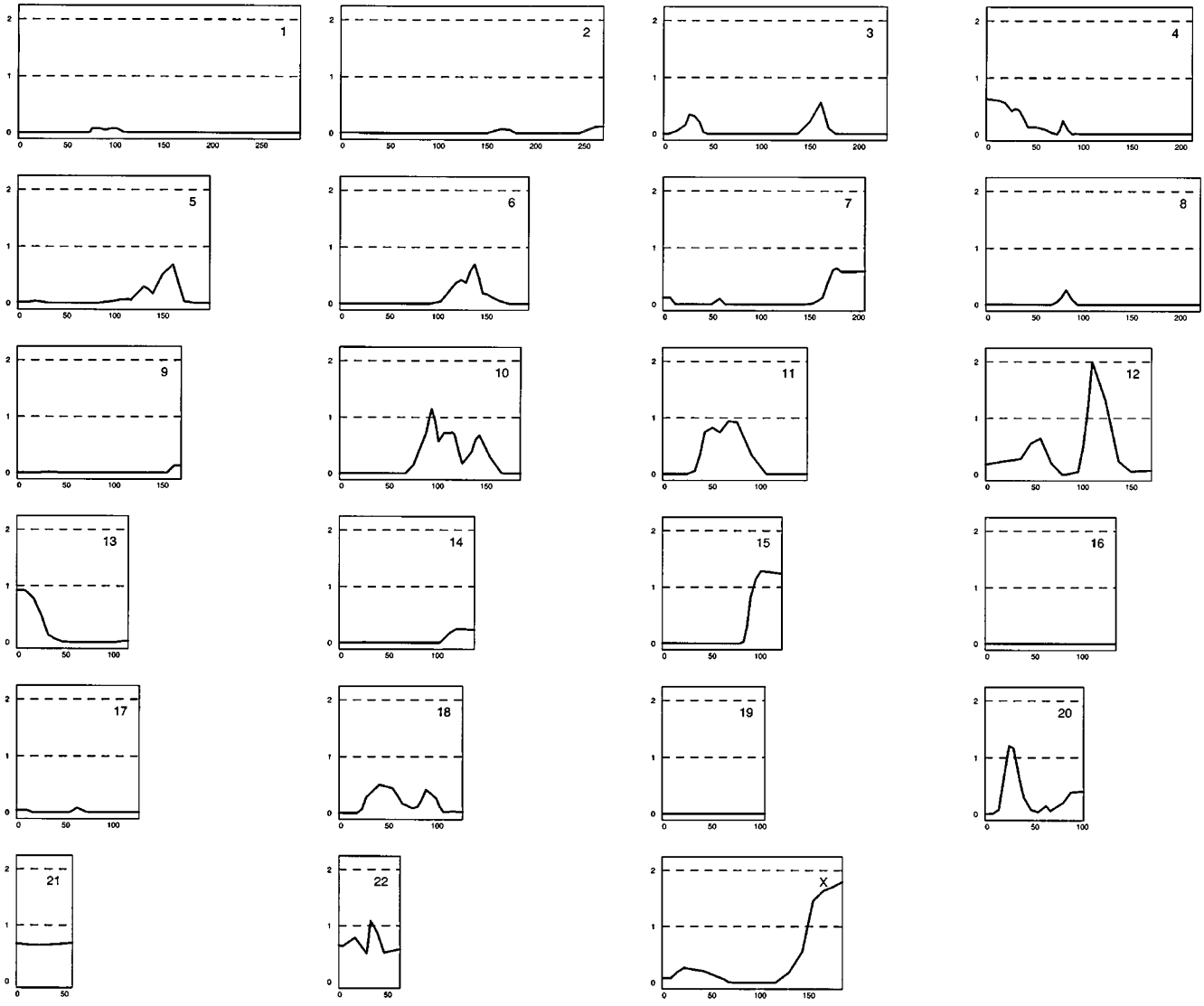

Figure 1 Results genome-wide scan in 67 Dutch families. Genome-wide scan for preeclampsia including HELLP-syndrome cases in 67 Dutch families, using a framework map of 293 polymorphic micro satellite markers. Each box represents a chromosome. The *y*-axis depicts the non-parametric multipoint lod score. The *x*-axis represents the cM distance of the markers used.

Results

Results are shown in Figure 1. According to the criteria of Lander and Kruglyak[23] 12 regions showed nominal lod score peaks. Six of these peaks with lod scores between 1.0 and 2.2 were seen on respectively chromosome 10q at 93.9 cM (D10S1432), 12q at 109.5 cM (PAH), 15q at 100.6 cM (D15S816), 20p at 24.7 cM (D20S851), 22q at 32.4 cM (D22S685) and Xq at 165.1 cM (DXS9908). The highest lod score was 1.99 (P=0.0012) on chromosome 12q (PAH) at 109.5 cM.

Because our strict disease criteria included HELLP-syndrome patients, in contrast to the Icelandic[22] and Australian/NZ[24] scan, our data was re-analysed for families with (HELLP families) and without HELLP-syndrome (preeclampsia families). Analysis in the preeclampsia families showed two areas with suggestive evidence for linkage, despite the smaller sample size (38 families; 37 with two affected, one with three affected and 72 relatives) (Figure 2). The highest lod score of 2.41 (P=0.00057) was seen on chromosome 22q at 32.4 cM (D22S685), the second highest peak of 2.38 (P=0.00057) was located on chromosome 10q at 93.9 cM (D10S1432). The two highest nominal peaks were seen on chromosome 11 (lod score 1.84, P=0.0015; D11S2371, 76.1 cM) and chromosome 18 (lod score 1.65, P=0.0027; D18S843, 28.1 cM). In the analysis of the HELLP families (34 families; 10 with at least two HELLP-syndrome siblings, 24 with one HELLP-syndrome

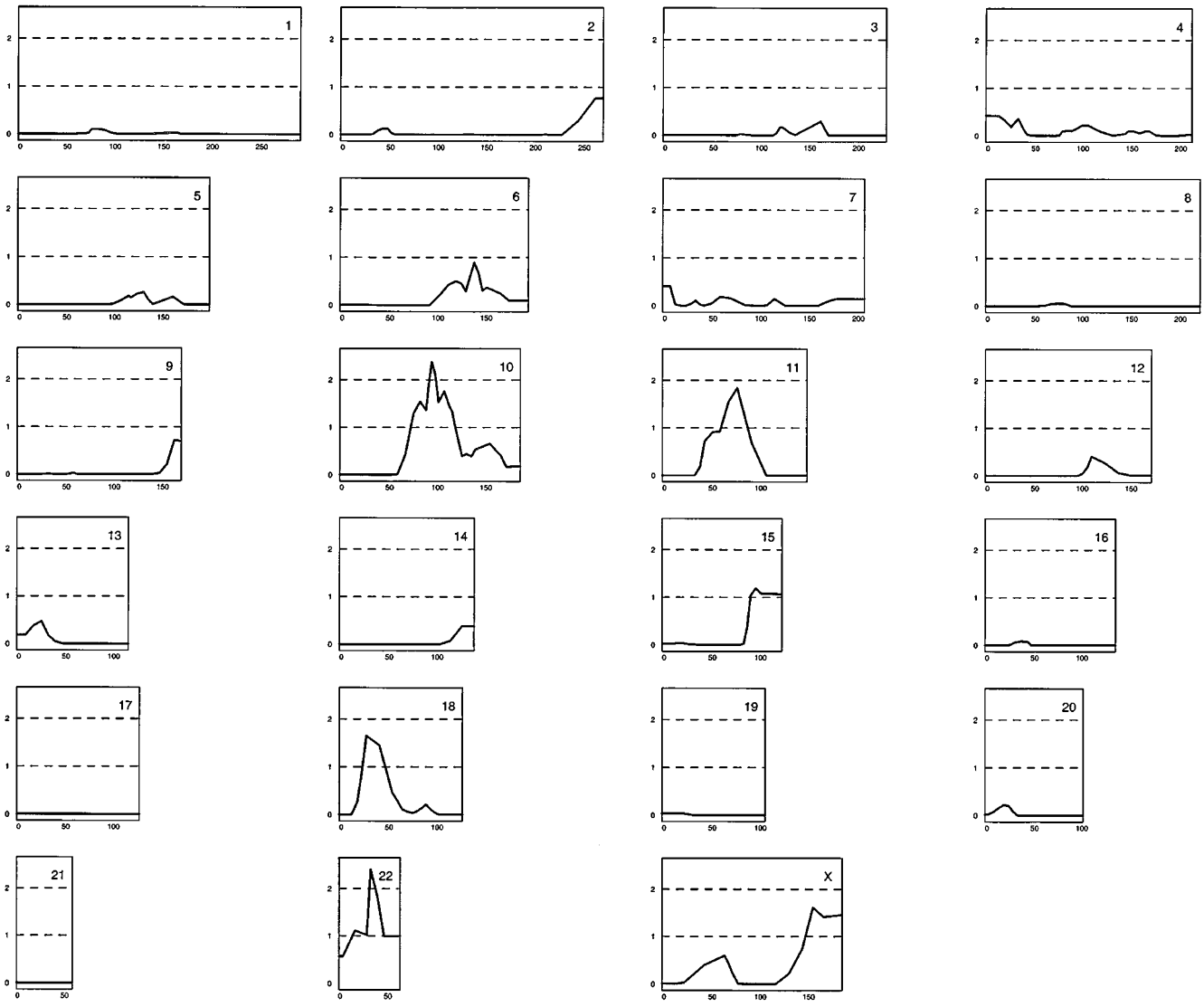

Figure 2 Results genome-wide scan in 38 Dutch families without HELLP-syndrome cases (preeclampsia families). Genome-wide scan for preeclampsia in 38 Dutch families, using a framework map of 293 polymorphic micro satellite markers. Each box represents a chromosome. The y-axis depicts the non-parametric multipoint lod score. The x-axis represents the cM distance of the markers used.

sibling and at least one preeclamptic sibling and 65 relatives), these peaks were absent (data not shown). The peak on 12q, however, increased to a lod score of 2.1. The peak on Xq appeared to be additive in the preeclampsia and HELLP families analyses.

In none of the Dutch analyses relevant peaks were seen at previously published candidate loci, like angiotensinogen (1q42-43), endothelial nitric oxide synthase (7q36), methylenetetrahydrofolate reductase (1p36.3), TNF-α (6p21) or chromosome 4q.[10,12,14,18,21]

Discussion

Preeclampsia remains a puzzling disorder and this study reconfirms its complex genetic background. Although much effort was put into the recruitment of sib-pairs classifying to very stringent disease criteria, only nominal peaks came up in our initial analysis. The highest peak was found on chromosome 12q with a lod score of 1.99. Interestingly, omission of the HELLP-syndrome cases from the analyses resulted in two peaks with evidence for suggestive linkage on chromosome 10q and 22q, whereas the peak on chromosome 12q diminished considerably. In contrast, the chromosome 10 and 22 peaks were absent in the HELLP families, whereas the peak on 12q increased to 2.1. This may indicate that HELLP syndrome is a separate genetic entity, in contrast to the latest full consensus statement of the Australasian Society of the Study of Hypertension in Pregnancy,[31] which states that HELLP-syndrome is a severe form of preeclampsia and therefore an even stronger strict disease criterium for the 'preeclampsia-phenotype' than preeclampsia alone. The only peak that appeared to contain contributions from both preeclampsia and HELLP families, was on Xq.

When we compare the results of our Dutch genome scan with the four genome-wide scans on preeclampsia published so far[20–22,24] the following picture emerges. The first genome-wide exclusion map was published in 1992 by Hayward *et al*.[20] Linkage analysis was based on a simple single recessive gene model using a limited number of families and only 43 markers which makes comparison with our current data difficult. In the second genome-wide scan from Australia[21] parametric (recessive and dominant models) as well as non-parametric analyses revealed an interesting candidate region on chromosome 4q (lod score=2.9). Results of our initial scan and the subsequent analyses with and without HELLP-syndrome cases did not replicate this finding. Neither could we confirm the significant chromosome 2p13 peak found in the recent large Icelandic genome-wide scan.[22]

Since our Dutch genome-wide scan was set up similar to and performed in collaboration with the Icelandic group, close comparison between both scans was feasible and rendered two interesting overlapping regions on chromosome 3p (Dutch lod score 0.34, Icelandic lod score 1.02; D3S4545) and another on chromosome 15q (Dutch lod score 1.14, Icelandic lod score 0.95; D15S652-D15S816). The Icelandic lod scores were those for the strict criteria. Removing HELLP-cases from our analyses reduced the overlap on chromosome 3p but did not change the shared region on 15q. Other regions where both scans had nominal peaks were 2q, 13p and 18p. The most recent large genome-wide scan using Australian and New Zealand (NZ) families[24] had its highest peak on chromosome 2q (lod score=2.58 at 144.7 cM) with a broad basis overlapping the broad basis of the significant peak in the Icelandic scan on chromosome 2p (lod score 4.77 at 94.05 cM). The area harbouring the two peaks was designated the 'PREG1' locus. Since the distance between the maximum lod score peaks was still quite large (51 cM) it is possible that both scans revealed two separate loci instead of one. We did not see any overlapping peaks on chromosome 2 in our scan. After re-analysis without the HELLP-cases a new peak was revealed on chromosome 11 (lod score 1.84, 76.1 cM). The Australian/NZ scan had its second highest peak on the same chromosome (lod score 2.02, 121.3 cM). As with the PREG1 locus, given the limited precision of estimates of the map location of disease genes contributing to complex traits as preeclampsia, these two peaks might suggest the presence of a second preeclampsia risk locus. Their mutual distance (45 cM) might, however, also indicate two loci instead of one.

The two peaks with suggestive lod scores in our data were not found in the Icelandic[22] and Australia/NZ[24] scans. Their importance therefore remains to be elucidated.

Apart from the obvious population differences, several other factors may have influenced differences in outcome between our study and the Icelandic[22] and Australian/NZ[24] scan. Both previous scans were performed in larger pedigrees using more markers (n=400) than the Dutch (n=293). Nonetheless, our 67 Dutch families contained 143 strictly-defined affected women compared to 186 strictly affected women in the 124 Icelandic pedigrees and 87 in the Australian/NZ scan. It is possible that expanding our Dutch scan to all 150 Dutch families with in total 332 affected including 233 strictly affected women, and using more markers, might reveal more similarities between the scans.

Furthermore, it is likely that preeclampsia is a genetically heterogeneous disorder which doesn't fit into simple recessive or dominant inheritance models. Numerous susceptibility genes with their individual mode of inheritance and penetrance may be involved, which emphasises the importance of using non-parametric linkage analyses in genome scans. Preeclampsia's heterogeneity is illustrated by the fact that the impressive significant peak on chromosome 2p13 in the Icelandic scan[22] came largely from two big families. These two families contained 17 affected of the total 343 affected in all Icelandic families; three of the total 157 mildly affected (2%) and 14 of the total 186 strictly affected (7.5%). This important locus might therefore be involved in only a part of the familial preeclampsia cases. Overlapping peaks like those on chromosome 3p and 15q with the Icelandic and on chromosome 11 with the Australian/NZ[24]

scan may indicate loci that add to the overall risk profile of preeclampsia in a considerable number of patients worldwide.

Complicating the elucidation of genetic factors involved in preeclampsia further, there is substantial evidence that the foetal genotype plays a role in susceptibility for preeclampsia.[7,32–39] The foetal genotype is likely to affect penetrance of maternal susceptibility genes. Our study focused on maternal susceptibility genes using the affected-only approach by analysing maternal DNA from affected sib-pairs, thereby minimising effects of the foetal genotype on penetrance. Nevertheless, although the 'preeclampsia-phenotype' was fully penetrant in these sib-pairs, this phenotype still might originate from different combinations and numbers of maternal susceptibility genes. When this is a true phenomenon, it will have substantially reduced the power of all five genome scans so far.

Adding to the reduction of power to find maternal susceptibility genes, in support of the Barker hypothesis[40] there is evidence that non-genetic environmental factors are involved in the pathogenesis of preeclampsia; being born small or premature of a woman seems to increase her risk of developing preeclampsia in later life.[41]

In view of the complexity of the preeclampsia-trait, finding relatively little overlap between the five genome scans is maybe not particularly surprising. It is a fairly typical finding for genome-wide scans of complex traits performed in relatively small sample sizes. Merging raw data of published scans on a web-site and joining forces between groups studying the genetic background of preeclampsia might therefore greatly enhance the power of linkage studies to find maternal susceptibility genes in the future.

Finding maternal susceptibility genes that increase a woman's risk of preeclampsia would greatly enhance the possibilities for predicting risk pregnancies. The Dutch-Icelandic and Dutch-Australian/NZ overlapping areas may harbour such maternal risk loci. Our study suggests that these risk loci may be different for HELLP-syndrome than for preeclampsia.

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