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# LINKAGE AND ASSOCIATION STUDIES OF *IL1B* AND *IL1RN* GENE POLYMORPHISMS IN PREECLAMPSIA

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## ABSTRACT

*Objective*: To determine whether preeclampsia is either associated with or linked to two polymorphisms in the *IL1B* gene (*IL1B-Taq*I and *IL1B-511*) and one polymorphism in the *IL1RN* gene (*IL1RN-IVS*2). *Methods*: Genotyping was performed in 150 affected sib-pair families and 104 healthy Dutch blood donors. Genotype and allele frequencies as well as allelic associations were

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assessed in three groups of unrelated women from these 150 families; 133 with either eclampsia, preeclampsia or the haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, 101 with preeclampsia only, and 63 with HELLP syndrome only. These frequencies were compared to those in controls. Frequencies of transmitted and nontransmitted haplotypes, inferred from the three polymorphisms, were compared. Allele sharing between affected siblings from all 150 families was assessed by means of multipoint nonparametric affected sib-pair analyses. *Results*: No significant differences in genotype and allele frequencies were found between the unrelated study groups and controls. No allelic associations were apparent, nor were there differences in frequencies of transmitted and nontransmitted haplotypes within affected families. Excess allele sharing for any of the three polymorphic markers was absent in affected sib-pairs. *Conclusions*: None of the *IL1B* and *IL1RN* polymorphisms provided evidence for either association or linkage with the risk for (pre)eclampsia/HELLP syndrome, preeclampsia only, or HELLP syndrome only.

*Key Words*: Preeclampsia; Genetics; Polymorphisms; *IL1B*; *IL1RN*

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## INTRODUCTION

Preeclampsia is one of the most extensively studied disorders of pregnancy. Nonetheless, its etiology and pathogenesis have not been established so far. Studies on the prevalence of familial preeclampsia have provided considerable but indirect evidence that genetic susceptibility is an important risk factor for preeclampsia (1–6). It seems likely that preeclampsia is not monogenic in origin but that concurrent genetic and environmental influences, like interactions between maternal and fetal genes, are important determinants of maternal disease susceptibility (7) and phenotype (4, 5, 8, 9).

Generalized activation of the endothelium (10) is one of the common features in preeclampsia and might be the result of maternal immune maladaptation to the fetal allograft (11). Recently, Redman et al. (12) postulated that preeclampsia develops if maternal inflammatory responses to normal pregnancy decompensate through either an environmental stimulus (e.g., fetal factors) or through a maternal response that is too strong. A wide variety of proinflammatory cytokines are increased in preeclampsia and might trigger endothelial dysfunction. Genetic associations with polymorphisms in the HLA region have been investigated, but results have been conflicting so far (9, 13, 14). Genes encoding proinflammatory cytokines and genes participating in the regulation of the immune response are therefore good candidates for further study of maternal genetic susceptibility to preeclampsia.

In this study, three polymorphisms in the *IL1B*/*IL1RN* gene region on chromosome 2q12–14 were investigated. Two di-allelic polymorphisms in the *IL1B* gene; the *Taq*I restriction fragment length polymorphism (RFLP) at position +3953 in exon 5 (*IL1B-TaqI*) and the *Ava*I RFLP at position −511 in the promoter region (*IL1B-511*), and one penta-allelic polymorphism in the *IL1RN* gene of its antagonist IL-1ra (*IL1RN-IVS2*). The cytokines IL-1$\alpha$ and IL-1$\beta$ are prototypes of proinflammatory cytokines in that they

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induce the expression of a wide variety of genes and the synthesis of several proteins such as IL-6, prostaglandins, nitric oxide synthase, and platelet-activating factor (15, 16). These, in turn, induce a variety of defense mechanisms (17). It is thus an important mediator of inflammatory and immune responses as well as of host defense (18). IL-1ra, the naturally occurring inhibitor of IL-1, fine tunes the inflammatory responses to IL-1β's action. It competes with IL-1β for the IL-1 receptors, but by itself does not result in signal transduction and is therefore regarded as a pure antagonist (19). Although several studies in which plasma levels of IL-1β were compared between preeclamptic patients and controls failed to show a rise in IL-1β levels in preeclampsia (20–23), two studies showed that levels of IL-1ra were increased in preeclampsia compared to controls (21, 23). Hurme and Santtila (24) recently found that Finnish blood donors homo- or heterozygous for allele 2 of the *IL1RN* gene (*IL1RN-IVS*2 * 2) had significantly higher plasma IL-1ra levels than allele 2 negative donors. Moreover, this enhancing effect of *IL1RN-IVS*2 * 2 required the presence of allele 2 of the *IL1B-511* polymorphism (*IL1B-511* * 2) or absence of allele 2 in the *IL1B – Taq*I polymorphism. The *IL1B* gene thus participates in the regulation of in vivo production of IL-1ra. Bioque et al. (25) observed that *IL1RN-IVS*2 * 2 is more often associated with *IL1B-Taq*I * 1 in inflammatory bowel disease patients than in controls (25).

To our knowledge, so far, no association studies between genes in the IL-1 region and preeclampsia have been reported. In the current study, the *IL1B-Taq*I, *IL1B-511*, and *IL1RN-IVS*2 polymorphisms were analyzed in 150 affected sib-pair families with preeclampsia. Genotype and allele frequencies, and allelic associations were compared between preeclamptic women and 104 healthy controls. Comparison between transmitted and nontransmitted haplotypes within the 150 preeclamptic families was made and allele sharing for the three markers within sib-pairs was studied.

## MATERIAL AND METHODS

### Disease Criteria

Affected women were recruited according to ''strict'' or ''mild'' disease criteria. Strict disease criteria were met if a woman had suffered either from (severe) preeclampsia, hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, or eclampsia during pregnancy. Mild criteria were met if she had only pregnancy-induced hypertension (PIH). Preeclampsia was defined as a diastolic blood pressure (BP) of at least 90 mm Hg with an increment of at least 20 mm Hg from a first trimester diastolic BP measurement and significant proteinuria ($\geq 300$ mg/24 hr or at least twice 1+ on dipstick). Preeclampsia was called ''severe'' when the diastolic BP was $\geq 110$ mm Hg and proteinuria $\geq 1000$ mg/24 hr. The absolute BP levels had to be observed on at least two occasions, more than 6 hr apart. To diagnose HELLP syndrome, the following criteria had to be met: hemolysis, defined by increased lactic dehydrogenase ($\geq 600$ IU/L); elevated liver enzyme levels (ASAT and ALAT $\geq 70$ IU/L); and thrombocytopenia ($\leq 100$ platelets per $10^9$/L). Eclampsia was defined as seizures occurring in a hypertensive pregnancy, with or without proteinuria. PIH was defined as a diastolic BP of at least 90 mm Hg with an increment of at least 20 mm Hg

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from a first trimester diastolic BP measurement without significant proteinuria ($\leq 300$ mg/24 hr).

### Recruitment of Affected Sib-Pair Families with Preeclampsia and Controls

In the period between June 1995 and October 1997, 150 affected sisters and their parents were recruited for a genome-wide DNA marker analysis (genome-wide scan) and candidate gene studies for preeclampsia.

Recruitment of these 150 families was achieved through three routes of ascertainment, yielding 2940 index women. Covering the period between 1985 and 1996, 448 affected index women fulfilling the strict criteria were selected from the medical records of the Vrije Universiteit Medical Center in Amsterdam and the Academic Hospital in Groningen, The Netherlands. Another 2443 affected index women were recruited from obstetrical data bases of 20 other hospitals in The Netherlands, using only the crude search criterium of a diastolic BP of at least 100 mm Hg in pregnancy. Their records covered the period between 1985 and 1997. Another 49 index women responded to advertisements for the study. Questionnaires inquiring about affected sisters were sent out to all of the 2940 index women. A positive family history for preeclampsia was reported by 178 index women. After verification of the diagnoses in the medical records of these 178 index women and their sisters, excluding women with multiple pregnancies, a history of essential hypertension, diabetes or renal disease, 150 families fulfilled the study criteria and could be included in the study.

The sib-pairs in the 150 families contained 332 affected women, of whom 233 met the strict criteria. Of the 332 affected women, 298 (90%) were nulliparous. Families were classified into 79 strict and 71 mild families. In strict families, at least two siblings met the strict criteria. In 63 mild families, only one of the siblings met strict criteria, the other sibling(s) met mild criteria. In the remaining eight mild families, all siblings met only mild criteria. The largest family included six affected siblings. Both parents were available for genotyping in 88 families, in 50 families only one parent was available and in 12 families both parents were unavailable.

Comparisons of genotype and allele frequencies were made between the control group and three separate study groups: a study group of 133 unrelated women with either preeclampsia, eclampsia, or HELLP syndrome [the (pre)eclampsia/HELLP group], a study group of 101 unrelated women with preeclampsia or eclampsia only (the preeclamptic group), and a study group of 63 unrelated women with HELLP syndrome only (the HELLP group). Unrelated means that when in one family more than one sibling met a group's specific criterium, just one of the siblings was included in the study group. Therefore, the unrelated preeclamptic ($n = 101$) and unrelated HELLP group ($n = 63$) separately contained more affected women ($n = 164$) than the combined (pre)eclampsia/HELLP group ($n = 133$).

For comparisons between transmitted and nontransmitted haplotypes and the affected sib-pair analyses, all affected siblings were included.

The control group consisted of 104 unrelated healthy native Dutch students and staff members of the Vrije Universiteit Amsterdam, The Netherlands, of whom 57 were male and 47 were female. IL1b and IL1ra genotype and allele frequencies of 98 of these 104 controls were previously published (26). This control group was used to represent the

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typical genotype and allele frequencies in the general Dutch population. Frequencies in males and females were equal.

### The *IL1B* and *IL1RN* Gene Region

The genes for IL-1β and IL-1ra are located on the long arm of chromosome 2 at position 2q12–14. The *IL1RN* gene is in close linkage with the *IL1B* gene (27, 28). Two of the three polymorphisms are located in the *IL1B* gene and are di-allelic; the *Taq*I polymorphic site at position +3953 within exon 5 (29) and the *Ava*I polymorphism at position −511 in the promoter region (30). The third polymorphism (ILra-*IVS*2) is penta-allelic and located within the second intron of the *IL1RN* gene (31).

### Typing of the Three Polymorphisms in the *IL1B* and *IL1RN* Genes

Genomic DNA was extracted from isolated peripheral leucocytes using DNAzol™ reagent (Life Technologies™, GibcoBRL, Molecular Research Center, Cincinatti, OH).

#### *IL1B-Taq*I Polymorphism

The region containing the polymorphic *Taq*I site within exon 5 of the gene (29) was amplified by PCR using primers and conditions previously described by Bioque et al. (25). The 249 base pair long polymerase chain reaction (PCR) products were digested with the restriction enzyme *Taq*I, resulting in fragments which were either cut into two fragments of 135 and 114 base pairs (*IL1B-Taq*I * 1) or remained intact (*IL1B-Taq*I * 2). Digestion products were separated by electrophoresis on 3% agarose gels containing 0.1% ethidium bromide.

#### *IL1B-511* Polymorphism

A 305 base pair region containing the *Ava*I restriction site at position −511 of the promoter region of the *IL1B* gene was amplified by polymerase chain reaction (PCR) and using primers described by Di Giovine et al. (30). PCR products were subsequently digested with the restriction enzyme *Ava*I, resulting in fragments which were either cut into two fragments of 199 and 106 base pairs (*IL1B-511* * 1) or remained intact (*IL1B-511* * 2). Digestion products were separated by electrophoresis on 2% agarose gels containing 0.1% ethidium bromide.

#### *IL1RN-IVS*2 Polymorphism

The *IL1RN* gene contains variable numbers of a tandem repeat of 86 base pairs within intron 2. A fragment containing this polymorphism was amplified by PCR using primers described by Mansfield et al. (31). PCR conditions were previously described by

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Bioque et al. (25). PCR fragments containing four repeats (*IL1RN-IVS*2 * 1), two repeats (*IL1RN-IVS*2 * 2), five repeats (*IL1RN-IVS*2 * 3), three repeats (*IL1RN-IVS*2 * 4), and six repeats (*IL1RN-IVS*2 * 5) were analyzed by electrophoresis on standard 2% agarose gels stained with 0.1% ethidium bromide.

### Haplotypes Inferred from the Three Polymorphisms in the *IL1B* and *IL1RN* Genes

Each individual of the 150 affected sib-pair families was typed for the three polymorphisms. Using allelic information of all available parents, haplotypes of 126 index women and 126 sisters could be derived (transmitted haplotypes). It was not possible to derive haplotypes in the remaining siblings, either due to information missing from parents, or because the combination of an individual's alleles gave rise to more than two possible haplotypes, which therefore remained inconclusive. In only 80 families,

***Table 1.*** Observed and Expected Distribution of 160 Nontransmitted Haplotypes in the *IL1B* and *IL1RN* Region, by 160 Parents from 80 Fully Typed Affected Sib-pair Families with Preeclampsia

| Haplotypes | *IL1B-Taq*I | *IL1B-511* | *IL1RN-IVS*2 | Observed *N* (%) | Expected *N* (%) | $\chi^2$ |
|---|---|---|---|---|---|---|
| A | 1 | 1 | 1 | 60 (37.5) | 57.0 (35.6) | 158 |
| **B** | **1** | **1** | **2** | **14 (8.8)** | **23.0 (14.4)** | **352** |
| C | 1 | 1 | 3 | 1 (0.63) | 2.6 (1.6) | $\mathscr{L}$ |
| D | 1 | 1 | 4 | 1 (0.63) | 1.0 (0.65) | $\mathscr{L}$ |
| E | 1 | 1 | 5 | – | – | $\mathscr{L}$ |
| F | 1 | 2 | 1 | 22 (13.8) | 27.4 (17.2) | 106 |
| **G** | **1** | **2** | **2** | **25 (15.6)** | **11.1 (6.9)** | **174** |
| H | 1 | 2 | 3 | – | 1.3 (0.79) | $\mathscr{L}$ |
| I | 1 | 2 | 4 | 1 (0.63) | 0.50 (0.31) | $\mathscr{L}$ |
| J | 1 | 2 | 5 | – | – | $\mathscr{L}$ |
| **K** | **2** | **1** | **1** | **24 (15.0)** | **16.5 (10.3)** | **341** |
| L | 2 | 1 | 2 | 4 (2.5) | 6.7 (4.2) | 109 |
| M | 2 | 1 | 3 | 4 (2.5) | 0.76 (0.47) | $\mathscr{L}$ |
| N | 2 | 1 | 4 | – | 0.30 (0.19) | $\mathscr{L}$ |
| O | 2 | 1 | 5 | – | – | $\mathscr{L}$ |
| **P** | **2** | **2** | **1** | **3 (1.9)** | **8.0 (5.0)** | **313** |
| Q | 2 | 2 | 2 | 1 (0.63) | 3.2 (2.0) | $\mathscr{L}$ |
| R | 2 | 2 | 3 | – | 0.37 (0.23) | $\mathscr{L}$ |
| S | 2 | 2 | 4 | – | 0.15 (0.09) | $\mathscr{L}$ |
| T | 2 | 2 | 5 | – | – | $\mathscr{L}$ |
| $\mathscr{L}$ Haplotypes lumped for $\chi^2$-analysis | | | | | | 475 |
| Sum | | | | 160 (100.0) | 160.0 (100.0) | **302** |
| Subtotal of lumped haplotypes | | | | 8 (5.02) | 10.2 (6.3) | |

$\chi^2$ for difference between observed and expected frequencies = 30.2, $p < 0.0001$, 7 *df*.
Allele frequencies: *IL1B-Taq*I, allele 1 (0.775), allele 2 (0.225); *IL1B-511*, allele 1 (0.675), allele 2 (0.325); *IL1RN-IVS*2 allele 1 (0.681), allele 2 (0.275), allele 3 (0.0312), allele 4 (0.0125), allele 5 (0.0).

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both parents and two siblings were fully informative on their haplotypes. In these families, the observed and expected distribution of 160 nontransmitted haplotypes in the *IL1B* and *IL1RN* region were calculated. In total, 12 different haplotypes were observed (Table 1).

## Statistical Analysis

Differences in genotype and allele frequencies of the separate polymorphisms between the studied groups were calculated by means of Fisher's exact test ($2 \times 2$ contingency tables) and expressed as odds ratios (OR), using Instat® version 2.02 (Graphpad Software, San Diego, CA). In order to determine whether presence of allele 2 of the *IL1B-511* or absence of allele 2 of the *IL1B-Taq*I polymorphism were associated with allele 2 of *IL1RN-IVS*2, as suggested by Hurme and Santtila (24), for each polymorphism, individuals were divided into two groups: those homo- or heterozygous for the allele of interest ($+-$/++) and those without this allele in their genotype ($--$). The strength of association between each pair of alleles within each of the unrelated groups and the control group was estimated by OR after performing Fisher's exact test ($2 \times 2$ contingency tables). By performing exact homogeneity tests, the strength of the allelic associations within each unrelated study group was compared with the strength of these allelic associations within the control group (StatXact software package, Cytel Software, Cambridge, MA). A two-sided $p$ value $<0.05$ was considered significant.

Using the Affected Family Based Controls (AFBAC) program (32, 33) (http://www.alleleb.biol.berkeley.edu/~mpn/afbac.html) and the Transmission/Disequilibrium Test for Siblings (S-TDT) program (34), calculations for comparisons between frequencies of transmitted and nontransmitted haplotypes in the 150 families were carried out. S-TDT was also performed for all three polymorphisms separately.

Nonparametric multipoint affected sib-pair analyses (35) were performed using the Mapmaker/Sibs program (Whitehead Institute for Biomedical Research in Cambridge, MA) (36). Since siblings will normally share 50% of their genetic information, the null hypothesis of this analysis, assuming no linkage between disease genes and tested markers, is that 25% of sibs will share zero alleles, 50% will share one allele, and 25% will share two alleles identical by descent (IBD) from their parents for each marker. Positive linkage will be reflected by a deviation from these percentages with an excess of sharing of one or two alleles between the affected sibs, irrespective of the mode of inheritance. For the multipoint analyses, all three markers were used simultaneously and analyses were carried out on three levels. In the first, broad analysis, all women in the 150 pedigrees with a disease status according to either the strict or mild criteria were marked as ''affected.'' In the second, intermediate analysis, only women satisfying strict criteria were marked as ''affected;'' the disease status of the mildly affected relatives was marked as ''unknown.'' In the final, narrow analysis, only women affected with a very severe form of the disease, those with eclampsia, severe preeclampsia, or HELLP syndrome, were marked as ''affected,'' and all other relatives were marked as ''unknown.'' All analyses were nonparametric (e.g., did not require any assumption about the underlying genetic model). Results are given as the distribution of sharing zero, one or two parental alleles IBD between siblings and as multipoint lod scores. A lod score above 3.6 is considered significant for linkage (37). In sib-pair analyses, it is possible to exclude a locus of a

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***Table 2.*** Genotype Frequencies of the *IL1B-Taq*I, *IL1B-511* and *IL1RN-IVS2* Polymorphisms in Unrelated Women with (Pre)Eclampsia or HELLP Syndrome, Preeclampsia Only, HELLP Syndrome Only, and Healthy Controls

| | *IL1B-Taq*I Genotypes, *n* (%) | | | *IL1B-511* Genotypes, *n* (%) | | | *IL1RN-IVS2* Genotypes, *n* (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11 | 12 | 22 | 11 | 12 | 22 | 11 | 13 | 22 | 14 | 15 |
| (Pre)eclampsia/HELLP ($n = 133$) | 69 (52) | 55 (41) | 9 (7) | 59 (44) | 64 (48) | 10 (8) | 72 (54) | 3 (2) | 4 (3) | 1 (1) | 1 (1) |
| Preeclampsia ($n = 101$) | 59 (58) | 38 (38) | 4 (4) | 38 (38) | 53 (52) | 10 (10) | 49 (49) | 2 (2) | 4 (4) | 1 (1) | 1 (1) |
| HELLP ($n = 63$) | 31 (49) | 27 (43) | 5 (8) | 33 (52) | 27 (43) | 3 (5) | 36 (57) | 2 (3) | 3 (5) | – | – |
| Controls ($n = 104$) | 56 (54) | 44 (42) | 4 (4) | 44 (42) | 48 (46) | 12 (12) | 60 (58) | 3 (3) | 10 (10) | – | – |

Genotype frequencies in each of the study groups were not significantly different from those in the controls.

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***Table 3.*** Combinations of Presence (+ − /++) or Absence (− −) of Allele 2 in the *IL1B-Taq*I, *IL1B-511,* and *IL1RN-IVS*2 Genotypes Are Given for the Unrelated (Pre)Eclampsia/HELLP Group and Controls

| | *IL1B-Taq*I | | | *IL1B-511* | | |
|---|---|---|---|---|---|---|
| | Allele 2 (+ − /++) *n* (%) | Allele 2 (− −) *n* (%) | OR [95% CI] | Allele 2 (+ − /++) *n* (%) | Allele 2 (− −) *n* (%) | OR [95% CI] |
| *(Pre)eclampsia/HELLP (n = 133)* | | | | | | |
| *IL1RN-IVS*2, allele 2 (+ − /++) | 23 (17) | 33 (25) | 0.61 [0.31–1.2] | 41 (31) | 15 (11) | 3.6 [1.7–7.7] |
| *IL1RN-IVS*2, allele 2 (− −) | 41 (31) | 36 (36) | | 33 (25) | 44 (33) | |
| *Controls (n = 104)* | | | | | | |
| *IL1RN-IVS*2, allele 2 (+ − /++) | 18 (17) | 23 (22) | 0.86 [0.39–1.9] | 31 (30) | 10 (10) | 3.6 [1.5–8.7] |
| *IL1RN-IVS*2, allele 2 (− −) | 30 (29) | 33 (32) | | 29 (28) | 34 (33) | |
| Exact homogeneity test, comparing strength of allelic association (ORs) between the two above study groups | | | $p = 1.000$ | | | $p = 0.59$ |

Within both study groups, the strength of the allelic associations is presented as ORs. These allelic associations (ORs) were subsequently compared between both study groups by means of the exact homogeneity test ($p$-values given).

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specific effect. In our analyses, we computed lod scores assuming a locus that contributes to a relative risk for siblings ($\lambda_s$), for preeclampsia, of 2. A $\lambda_s$ lod score of lower than $-2$ was considered significant evidence for exclusion of linkage.

### Ethical Aspects

This study was approved by the Medical Ethical Committee of the University Hospital, Vrije Universiteit, Amsterdam. Appropriate informed consent was obtained from all participants.

## RESULTS

As Table 1 shows, alleles composing the different haplotypes are in linkage disequilibrium.

In Table 2, genotype frequencies of the *IL1B-Taq*I, *IL1B-511* and *IL1RN-IVS*2 polymorphisms are shown for the unrelated (pre)eclampsia/HELLP group, the unrelated preeclamptic group, the unrelated HELLP group, and the control group. Genotype frequencies of the *IL1B-Taq*I and *IL1B-511* polymorphisms were not significantly different between each of the affected groups and the control group. The *IL1RN-IVS*2 * 2.2 genotype, however, appeared to be significantly less frequent in the (pre)eclampsia/HELLP group than in the controls; 3% compared to 10%, respectively (OR = 0.29; 95% CI = 0.089–0.96), and the *IL1RN-IVS*2 * 1.2 genotype appeared to be more frequent in the preeclamptic group than in the controls; 44% and 30%, respectively (OR = 1.8; 95% CI = 1.02–3.2). However, after correction for multiple comparisons, these differences did not reach significance. There also were no significant differences in allele frequencies between the unrelated study groups and controls (data not shown). Associations between presence (+ −/++) of *IL1B-511* * 2 and *IL1RN-IVS*2 * 2, or absence (− −) of *IL1B-Taq*I

***Table 4.*** Allele Sharing Distribution and Lod Scores of the Affected Siblings in 150 Sib-pair Families

| Markers ($n$)[a] | Analysis[b] | Families ($n$)[c] | z0[d] | z1 | z2 | Maximum Lod Score | Information (range/median)[e] |
|---|---|---|---|---|---|---|---|
| 3 | Broad | 143 | 23 | 50 | 27 | 16 | 0.68–0.70/0.69 |
| 3 | Intermediate | 75 | 25 | 50 | 25 | 0 | 0.69–0.70/0.69 |
| 3 | Narrow | 25 | 25 | 50 | 25 | 0 | 0.69–0.71/0.69 |

[a] Markers: *IL1B-Taq*I (di-allelic), *IL1B-511* (di-allelic) and *IL1RN-IVS*2 (penta-allelic).
[b] Broad: women satisfying strict and mild criteria marked as ''affected.'' Intermediate: women satisfying strict criteria ''affected,'' mild relatives ''unknown.'' Narrow: only women with eclampsia/severe preeclampsia/HELLP syndrome ''affected,'' other relatives ''unknown.''
[c] Number of informative families used in the three analyses.
[d] Observed distribution of sharing z0 = 0, z1 = 1 or z2 = 2 alleles. Expected distribution under assumption of no linkage is 0.25–0.5–0.25.
[e] Information content for all three markers.

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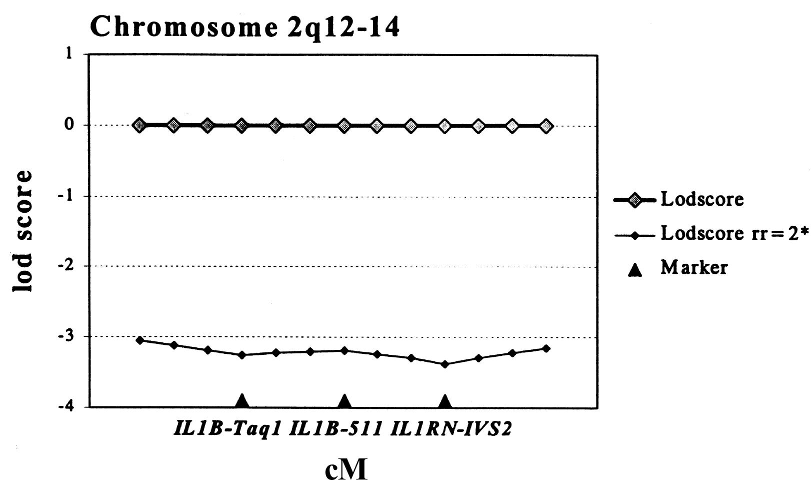


***Figure 1.*** Plot of the maximum multipoint lod scores using all three markers in the *IL1B* and *IL1RN* region simultaneously. Strict and mild sibs are marked as ''affected.'' No assumptions are made on the mode of inheritance. Filled triangles indicate the position of the three markers on the chromosome. *Lod scores computed assuming a locus which contributes to a relative risk (rr) for siblings ($\lambda_s$) of 2.

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allele 2 and presence (+ −/++) of *IL1RN-IVS*2*2 in the genotypes were very similar within each of the three unrelated groups and the controls. Hence, the exact homogeneity test for differences in these associations between the study groups and control group was never significant. In Table 3, the results are displayed for the (pre)eclamptic/HELLP group and controls.

No differences between frequencies of transmitted and nontransmitted haplotypes were seen. The AFBAC and the S-TDT haplotype analyses had too little power, since in only 10 families transmission of haplotypes was completely informative. The S-TDT was nonsignificant for transmission of any polymorphism allele tested.

Results of the nonparametric affected sib-pair analyses in the 150 affected sib-pair families are shown in Table 4. Information content for all markers was at least 0.68. The observed distribution for sharing zero, one, or two parental alleles between all sibs was 0.23, 0.50, and 0.27 for the broad analysis, and 0.25, 0.50, and 0.25 for the intermediate and narrow analyses, respectively. The corresponding multipoint lod scores were close to zero for all three markers in all analyses. A plot is made from the broad analysis (Figure 1). Included in the plot are also the nonparametric multipoint lod scores under the assumption that the $\lambda_s$ of variations at the *IL1B/IL1RN* locus for preeclampsia is 2. The latter lod scores were lower than −3 on all marker positions.

## DISCUSSION

In this study, we have investigated whether an association between preeclampsia and the *IL1B-Taq*I, *IL1B-511*, and *IL1RN-IVS*2 polymorphisms exists by assessing genotype and allele frequencies, allelic associations, frequencies of transmitted and

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nontransmitted haplotypes, and allele sharing in affected sib-pairs. All results based on these approaches have remained nonsignificant. Allelic associations found in our study were very similar to those in Hurme and Santtila's (24) study (Table 3). In their study, these associations, presence of allele *IL1RN-IVS2* * 2 combined with either presence of allele *IL1B-511* * 2 or absence of allele *IL1B-Taq*I * 2 in the genotype, correlated with higher plasma levels of IL-1ra in 200 healthy Finnish blood donors. In our study, the association between absence of allele *IL1B-Taq*I * 2 and presence of allele *IL1RN-IVS2* * 2 in the genotype was also similar to that in Hurme and Santtila's (24) study but weaker and not significant (Table 3). However, these associations were of equal strength in our study groups and control group. It is therefore unlikely that the higher levels of IL-1ra in preeclampsia compared to controls as described in the studies of Greer et al. (21) and Kimya et al. (23) can be explained by these allelic associations of the three polymorphisms in the *IL1B*/*IL1RN* genes alone.

The control group used in this study consisted of healthy males and females representing Dutch population genotype and allele frequencies. Since the loci studied here are autosomal, gender has no influence on these frequencies. Considering that the incidence of preeclampsia in pregnant women is around 5%, the maximum contribution of potential preeclampsia-associated genotypes to the control group is 5%. The chance of masking a possible gene effect using this control group is therefore negligible.

Haplotype analyses lacked power, since two of the three polymorphisms were di-allelic, thus not very informative. Consequently, a large number of parents in the 80 fully typed families were homozygous for a haplotype or shared one or two haplotypes with their partner, leaving only 10 families informative for the calculations. Comparison of the observed haplotype frequencies in our study population with those in the general Dutch population was not possible, since the latter frequencies were not available. Our control group did not supply these frequencies either, since it did not contain families that were indispensable for haplotype identification. Since all other comparisons with the control group remained negative, no major differences with the Dutch population frequencies are to be expected.

The outcomes of the nonparametric multipoint allele sharing analyses were negative as well. The observed distributions of allele sharing were very similar to those expected under the null-hypothesis in each of the three analyses (see Table 4). Moreover, when the broad analysis was repeated assuming that a polymorphism in the *IL1B*/*IL1RN* genes would contribute to the risk for preeclampsia with a relative risk for siblings ($\lambda_s$) of 2, the multipoint lod scores showed significant exclusion of both regions. This rules out any impact of importance of these loci on the risk for preeclampsia. It would have been interesting to perform these sib-pair analyses in subgroups of sibs with both HELLP syndrome or both preeclampsia. Since families with such siblings are few in number in our data set (35 sib-pairs with both preeclampsia and 11 sib-pairs with both HELLP syndrome), such analyses would have limited power to detect excess sharing in either group, if present.

A minor contribution of genes in the *IL1B*/*IL1RN* region to preeclampsia cannot be excluded in view of the likely assumption that preeclampsia is possibly heterogeneic or multifactorial in origin (4). Preeclampsia might be the result of a decompensation of the balance between the maternal genetically determined susceptibility to preeclampsia and the genetic load of the fetal allograft, causing adverse maternal immune adaptation (9, 11, 12). If this maternal susceptibility for preeclampsia is genetically determined by a combination of multiple maternal susceptibility genes, and maybe by variable combina-

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tions of different susceptibility genes, candidate gene studies like ours here will generally not have enough power to show significant association or linkage. Although several positive genetic associations have been found so far for several risk groups (38–44), none of these associations by themselves will have enough positive predictive power to foretell the risk for preeclampsia. It is thus possible that the *IL1B/IL1RN* polymorphisms investigated in this study do have a minor impact on the overall risk for preeclampsia, but this effect is only seen when certain allelic combinations with other candidate genes, or fetal genes, are present.

Genome-wide scans in preeclamptic families may turn out to be more informative, since effects of multiple loci can be detected, given that the influence of the loci involved is strong enough. The first genome scan, performed in the homogeneic population of Iceland, has recently been published (45) followed by a similar scan from Australia/New Zealand (46). A genome scan in 67 of our 150 Dutch affected sib-pair families is rounded off and will be published in due time as well.

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