Susceptibility of Preeclampsia

Lipoprotein Lipase Gene Mutations and the Genetic Susceptibility of Preeclampsia

Young J. Kim, Roger A. Williamson, K. Chen, Jennifer L. Smith, Jeffrey C. Murray, David C. Merrill

Abstract—In the pathogenesis of preeclampsia, endothelial cell activation or dysfunction is a central theme, and marked dyslipidemia may contribute to endothelial cell dysfunction. The objective of this study was to evaluate the association between preeclampsia and mutations within the lipoprotein lipase (LPL) gene. DNA was extracted from whole blood or cheek swabs of 250 preeclamptic patients, 265 control subjects, and 106 offspring of preeclamptic patients (all white). Control subjects were women who had undergone ≥2 term pregnancies unaffected by preeclampsia. All samples were genotyped for 3 LPL polymorphisms with the use of polymerase chain reaction of known allelic variants. The 3 mutations studied were the following: (1) Asp9Asn substitution in exon 2, (2) T-to-G substitution at position −93 of the proximal promotor region (−93T/G), and (3) Asn291Ser substitution in exon 6. Results were analyzed with an χ² contingency table. The prevalences of the Asp9Asn mutation, −93T/G promoter mutation, and Asn291Ser mutation were not significantly different among the preeclamptic patients and control subjects (Asp9Asn: patients, 2.8%; control subjects, 4.0%; −93T/G: patients, 4.5%; control subjects, 5.5%; Asn291Ser: patients, 4.0%; control subject, 3.0%). In addition, there was no difference in the frequency of any of the mutations in the offspring of preeclamptic women compared with that observed in the control population. Between a small group of patients with nulliparous HELLP syndrome (a variant of severe preeclampsia: hemolysis, elevated liver enzyme, low platelets) patients (n = 12) and control subjects, there was a significant difference in the prevalence of the Asn291Ser mutation (16.7% versus 3.0%, P = 0.01). In this large white population, the Asp9Asn mutation, −93T/G promoter mutation, and Asn291Ser mutation were not associated with an increased risk for preeclampsia. In a small subgroup of patients, the Asn291Ser mutation was associated with an increased risk for nulliparous HELLP syndrome. (Hypertension. 2001;38:992-996.)

Key Words: preeclampsia ■ lipoprotein lipase ■ mutations ■ genetic susceptibility

Preeclampsia is a serious complication of the second half of human pregnancy that occurs at frequencies of 1% to 5% worldwide. Although it is a leading cause of maternal and perinatal morbidity and mortality, the pathophysiology of preeclampsia remains unknown.

In the pathogenesis of preeclampsia, endothelial cell activation or dysfunction appears to be the central theme.1 At present, 4 hypotheses are the subject of extensive investigation: placental ischemia, altered endothelial cell function possibly secondary to altered lipid metabolism, immune maladaptation, and genetic imprinting.1 In preeclampsia, circulating free fatty acid triglycerides are increased 15 to 20 weeks before the onset of clinical disease.2,3

Clinical studies have documented a familial tendency to develop preeclampsia, although the pattern of inheritance is unclear.4,5 Candidate genes for preeclampsia susceptibility include those involved in blood pressure control, placental function, vascular disease, and dyslipidemia.6,7

Marked dyslipidemia may contribute to endothelial cell dysfunction in preeclampsia. Mutations in the lipoprotein lipase (LPL) gene show a reduction in LPL activity and may predispose to dyslipidemia and cardiovascular disease.7 There are 2 common mutations in LPL gene, an Asp9Asn substitution in exon 2 and a T-to-G transition at position 3 of the proximal promoter region (−93T/G).5 The LPL Asp9Asn mutation is in nonrandom association with a T-to-G substitution at position −93 of the proximal promoter region, and the combined −93G/Asn9 genotype predisposes to decreased HDL cholesterol and an increased risk of coronary artery disease.8

Recently, a more common serine for asparagine substitution at residue 291 (Asn291Ser) in exon 6 of the LPL gene has been described that is observed with high frequency, ranging from 2% to 5% in different populations.9 This common Asn291Ser LPL mutation significantly influences the risk for cardiovascular disease in patients with familial hypercholesterolemia.9 A fourth LPL variant, Ser447Ter nonsense mutation, does not impair LPL enzyme activity.10

The purpose of this study was to evaluate the association between preeclampsia and 3 relatively common mutations
within the LPL gene. We hypothesized that women carrying ≥1 of these mutations would have an increased incidence of preeclampsia.

**Methods**

**Patients and Control Subjects**

Whole blood or cheek swab samples were obtained from 250 preeclamptic patients and 265 control subjects (all white) at the University of Iowa Hospital and Wake Forest University. Control subjects were women who had undergone ≥2 term pregnancies unaffected by preeclampsia, did not have a family history of preeclampsia, and did not have chronic hypertension. Mild preeclampsia was defined as mild hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg) plus proteinuria (1+ on dipstick or 300 mg/24 h urine). Severe preeclampsia was defined as the presence of ≥1 of the following: (1) high blood pressure, ≥160/110 mm Hg on 2 occasions ≥4 hours apart; (2) proteinuria, ≥5 g/24 h or 4+ on dipstick; (3) thrombocytopenia, <100,000 platelets per milliliter; (4) oliguria, <400 mL/24 h; (5) pulmonary edema; or (6) elevated liver enzymes (SGOT/PT). A severe variant of preeclampsia called HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) was defined by thrombocytopenia (<100,000 platelets per milliliter), SGOT/PT (>70), and hemolysis (as demonstrated by any of the following: total bilirubin >1.2 mg/dL, serum lactate dehydrogenase ≥600 U/L, and hemolytic anemia on smear). Nulliparous preeclampsia was defined as preeclampsia in the first pregnancy progressing beyond 20 weeks. Multiparous preeclampsia was defined as the absence of preeclampsia in the first pregnancy beyond 20 weeks but preeclampsia diagnosed in a subsequent pregnancy. The study was approved by the institutional review committees at both Wake Forest University and the University of Iowa.

**TABLE 1. Primer Sequences Used in Genotyping**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker Location</th>
<th>Primer (5’ to 3’)</th>
</tr>
</thead>
</table>
| LPL Asp9Asn| Exon 2                           | Forward, CTCGAGTCACCTTACATCC
                     | Reverse, CACCACCCCATGACTC     |
| LPL−93G promoter| −93 Position of promoter region | Forward, GGGAAGTCCTTACATCTGT
                     | Reverse, GACACTTTCGGCAGAGCTGC |
| LPL Asn291Ser| Exon 6                           | Forward, TGGACGAGCAGTGAAAGGT
                     | Reverse, TGGCTGCTTTGTGCTCTC  |

**Mutation Detection Enhancement Analysis**

DNA fragments were amplified from the genomic DNA via polymerase chain reaction (PCR). Amplification was carried out in a total volume of 10 µL containing 50 ng genomic DNA, 200 mmol/L dNTPs, and 0.3 µmol/mL each primer in Bioline USA Inc PCR buffer with 0.5 U Taq DNA polymerase. All reactions were at 94°C for 30 seconds, the appropriate annealing temperature for 30 seconds, and 72°C for 30 seconds, for 35 cycles. Primer sequences were all previously published for the LPL Asp9Asn mutation\(^1\) and the LPL −93T/G promoter mutation.\(^2\) The primer for the LPL Asn291Ser mutation was newly developed. Primer sequences are listed in Table 1. The amplified DNA were loaded onto 0.5× mutation detection enhancement (FMC) gels containing 2.5% glycerol and electrophoresed for 6 hours at 20 W and 51°C. After electrophoresis, the gels were silver stained and scored.

**Statistical Analysis**

Genotype results were analyzed by χ² analysis or Fisher’s exact test, and allele frequency results were analyzed by a χ² contingency table. Linkage disequilibrium between the 3 variant sites (LPL Asp9Asn versus LPL −93T/G promoter) was estimated by use of the 2-way program developed by the University of Iowa. Statistical significance was accepted for \(P<0.05\).

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Results**

The results showing the distribution of genotypes and alleles for the Asp9Asn mutation are presented in Table 2. The incidence of the Asp9Asn mutation among preeclamptic, severe preeclamptic, and HELLP syndrome patients was not significantly different from that of the control subjects. In

**TABLE 2. Distribution of Genotypes and Alleles for LPL Asp9Asn Mutation in Control Subjects and Preeclampsia Patients**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotypes (%)</th>
<th>Alleles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Control</td>
<td>248</td>
<td>238 (96.0)</td>
</tr>
<tr>
<td>PRE</td>
<td>250</td>
<td>243 (97.2)</td>
</tr>
<tr>
<td>Nullip PRE</td>
<td>203</td>
<td>198 (97.5)</td>
</tr>
<tr>
<td>Parous PRE</td>
<td>47</td>
<td>45 (95.7)</td>
</tr>
<tr>
<td>Severe</td>
<td>166</td>
<td>163 (98.2)</td>
</tr>
<tr>
<td>Nullip severe</td>
<td>126</td>
<td>124 (98.4)</td>
</tr>
<tr>
<td>Parous severe</td>
<td>33</td>
<td>32 (97.0)</td>
</tr>
<tr>
<td>HELLP</td>
<td>18</td>
<td>17 (94.4)</td>
</tr>
<tr>
<td>Nullip HELLP</td>
<td>12</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td>Parous HELLP</td>
<td>5</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>Offspring</td>
<td>102</td>
<td>98 (96.1)</td>
</tr>
</tbody>
</table>

PRE indicates preeclampsia; nullip, nulliparous; and severe, severe preeclampsia. Offspring are of preeclamptic pregnancies.
addition, we subclassified preeclampsia, severe preeclampsia, and HELLP syndrome by parity. There was no significance difference between nulliparous or parous patients with preeclampsia, severe preeclampsia, and HELLP syndrome and control subjects. In addition, offspring of preeclamptic mothers were no more likely to be carriers of the Asp9Asn mutation than control subjects.

Genotype and allele frequencies for the \(-93T/G\) promotor mutation are presented in Table 3. The incidence of the \(-93T/G\) promotor mutation among preeclamptic, severe preeclamptic, and HELLP syndrome patients was not significantly different from that of control subjects. Among 17 cases of HELLP syndrome, we found no carriers of the LPL \(-93T/G\) promotor mutation. There was also no significance difference between nulliparous or parous patients with preeclampsia and severe preeclampsia and control subjects. Offspring of preeclamptic mothers were carriers of the LPL \(-93T/G\) promotor mutation with a frequency similar to that of control subjects.

The distributions of genotypes and alleles for the Asn291Ser mutation are presented in Table 4. The incidence of the Asn291Ser mutation among preeclamptic and severe preeclamptic patients was also not significantly different from that of control subjects. There was no significant difference between nulliparous or parous patients with preeclampsia and severe preeclampsia and control subjects. Between nulliparous HELLP syndrome patients and control subjects, there was a significant difference in the occurrence of the Asn291Ser mutation (16.7% versus 3.0%, \(P=0.01\)) although none of the 5 parous HELLP syndrome patients were carriers of the Asn291Ser mutation.

As noted in Table 5, we found a strong linkage disequilibrium between the Asp9Asn allele and the LPL \(-93T/G\) promotor allele \((P<0.001, \chi^2=86.3)\).

### Discussion

Our data from a large cohort of white subjects indicate that the LPL Asp9Asn mutation, LPL \(-93T/G\) promotor muta-
tion, and LPL Asn291Ser mutation are not associated with an increased risk for preeclampsia. In this study, we did observe that the Asn291Ser mutation shows a significant association with an increased risk for nulliparous HELLP syndrome in a small subgroup of patients. We caution, however, that this finding is based on a population of only 12 nulliparous patients diagnosed with HELLP syndrome.

Although the pathogenesis of preeclampsia is unclear, there is increasing recognition that marked perturbations in lipid and lipoprotein metabolism may be of fundamental importance in the pathogenesis of preeclampsia. Preeclamptic women have marked hyperlipidemia as reflected in higher serum levels of triglycerides and free fatty acids and exhibit markedly elevated concentrations of triglyceride-rich lipoproteins in the circulation. Lipid and lipoprotein metabolism may be of fundamental importance in the pathogenesis of preeclampsia. Among the described LPL mutations, the Asp9Asn missense mutation 11 is reportedly involved in hypertriglyceridemia. In our data, between nulliparous preeclampsia patients and control subjects, there was a significant difference in the prevalence of the Asn291Ser substitution was likely to be a significant predisposing factor contributing to the expression of different forms of hyperlipidemia. In our data, between nulliparous HELLP syndrome patients and control subjects, there was a significant difference in the prevalence of the Asn291Ser mutation (16.7% versus 3.0%, P=0.01). Although we found this positive association, we did not observe a significant association between this mutation and the development of preeclampsia when the entire group of preeclamptics (n=224) was analyzed. This finding again is different from that previously reported. The discrepancy between our findings and the data from the Pittsburgh group may be explained 3 possible ways. The first possibility is gene-gene interaction. The presence of these mutations alone may not be sufficient for the development of preeclampsia. Other gene variants (ie, LPL receptor gene or apolipoprotein E2 gene) may be necessary for the expression of preeclampsia. The second possibility is gene-environment interaction. Dietary differences may be important for the expression of preeclampsia. The effect of the LPL Asp9Asn mutation and the LPL −93T/G promoter mutation for dyslipidemia could be masked by altered dietary intake. Third, the present study involved a much larger cohort of preeclamptic subjects (n=250 compared with n=100 in the previous report by Hubel et al). It has been our experience that positive associations observed in relatively small populations may not persist when larger populations of subjects are genotyped.

In summary, results from this study suggest that the Asp9Asn mutation, −93T/G promoter mutation, and Asn291Ser mutation are not significantly associated with an increased risk for preeclampsia. The Asn291Ser mutation was found to be more prevalent in a small cohort of nulliparous patients with HELLP syndrome. This finding will need to be confirmed, however, in a larger cohort of patients with HELLP syndrome.

Acknowledgments

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References


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