Factor V Leiden and Thermolabile Methylenetetrahydrofolate Reductase Gene Variants in an East Anglian Preeclampsia Cohort

Kevin M. O’Shaughnessy, Beiyuan Fu, Franco Ferraro, Ian Lewis, Sarah Downing, Nick H. Morris

Abstract—Preeclampsia is a heritable condition that develops as a result of widespread vascular endothelial dysfunction. The thrombotic tendency in this condition has suggested a number of candidate genes, and there have been recent reports of positive association with the Leiden variant of factor V and the thermolabile variant of methylenetetrahydrofolate reductase. We attempted to reproduce these results in a large cohort of well-characterized women with preeclampsia, recruited prospectively within the East Anglian region of the United Kingdom. Women in the preeclampsia cohort (n=283) were genotyped for both the Leiden variant (G1691A) of factor V and the thermolabile variant (C677T) of methylenetetrahydrofolate reductase. Genotype and allele frequencies were compared with those of 2 control groups, one consisting of women recruited prospectively (n=100) from the same maternity hospital as the subjects and another consisting of normotensive women (n=100) from East Anglia. No significant differences were detected. Specifically, the carrier rate for the Leiden variant was 5.3% in the preeclampsia group and 5.5% in the combined control group. T677 homozygotes for methylenetetrahydrofolate reductase were 11% and 11.5% in the 2 groups, respectively. We conclude that there is no evidence of association of preeclampsia with either of these 2 polymorphisms in our study population. (Hypertension. 1999;33:1338-1341.)

Key Words: preeclampsia • thrombosis • polymorphisms • factor V • methylenetetrahydrofolate reductase • association study

Preeclampsia is a common pregnancy-related hypertension syndrome that poses a major risk of mortality and morbidity on both the woman and fetus.1 It has been appreciated for almost 4 decades that it has a familial basis, although the exact mode of inheritance remains unclear.2 The pathophysiology of preeclampsia reflects widespread dysfunction of the maternal vascular endothelium,3 and vascular diseases such as diabetes, essential hypertension, and antiphospholipid syndrome predispose pregnant women to preeclampsia. This, together with the marked thrombotic tendency of some women with preeclampsia,4 has suggested a number of candidate genes that may be involved in the pathophysiology of preeclampsia.5 Reports published to date suggest that functional variants of 2 of these genes, the blood clotting factor V (Leiden variant)6,7 and methylenetetrahydrofolate reductase (MTHFR, thermolabile variant),7,8 are more frequent in women with preeclampsia.

The Leiden variant of factor V causes resistance to activated protein C by substituting the Glu506 residue with arginine at the cleavage site for activated protein C.9 Activated protein C resistance is an important cause of dominantly inherited familial thrombophilia and venous thrombosis10 and has been implicated more recently in excessive early fetal loss.11 In contrast, the thermolabile variant of MTHFR causes modest elevations in plasma homocyst(e)ine levels under conditions of folate depletion.12 The level of this amino acid is an important vascular risk factor for patients with established coronary artery disease,13 but case-control studies with the thermolabile variant have yielded conflicting results.

Hyperhomocyst(e)inemia is, however, more frequent in preeclampsia, and the effects of folate depletion in pregnancy would certainly be exaggerated in mothers who carry the thermolabile variant of MTHFR.4,14 However, association studies using these polymorphisms have used relatively small samples. In this study, therefore, we sought to replicate these findings in a large and well-characterized population of Northern European women with preeclampsia from the East Anglian region of the United Kingdom.

Methods

Subjects
The study was approved by the local ethical committee of the Cambridge Health Authority. A total of 283 women (230 primigravida) with preeclampsia were recruited prospectively (at diag-
necrosis) from the Rosie Maternity Hospital at Addenbrooke’s Hospital, Cambridge, over the preceding 24 months. All gave written informed consent to participate and met the following criteria of preeclampsia: they were proteinuric (>300 mg/24 hours or at least ++ of proteinuria on a dip stick) and hypertensive with a blood pressure of >140/90 mm Hg occurring after 20 weeks of gestation with at least a 25-mm Hg rise in diastolic blood pressure. All these features were resolved by 3 months postpartum, and no participants had a multiple-birth pregnancy, concurrent diabetes, renal disease, or essential hypertension. Within the cohort, 149 had severe disease (as determined on the basis of deliveries before 37 weeks of gestation) and 25 developed the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).

An aged-matched control group of 100 pregnant women was recruited over the same period from the Rosie Maternity Hospital. All women in this group had pregnancies uncomplicated by preeclampsia. Another control sample of normotensive women was selected from our CLEAREST database, which contains data collected from a large community survey of cardiovascular risk factors conducted in the East Anglian region between 1990 and 1994. One hundred normotensive (seated blood pressure, 140/90 mm Hg) occurring after 20 weeks of gestation with at least a 25-mm Hg rise in diastolic blood pressure. All these features were resolved by 3 months postpartum, and no participants had a multiple-birth pregnancy, concurrent diabetes, renal disease, or essential hypertension. Within the cohort, 149 had severe disease (as determined on the basis of deliveries before 37 weeks of gestation) and 25 developed the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).

An aged-matched control group of 100 pregnant women was recruited over the same period from the Rosie Maternity Hospital. All women in this group had pregnancies uncomplicated by preeclampsia. Another control sample of normotensive women was selected from our CLEAREST database, which contains data collected from a large community survey of cardiovascular risk factors conducted in the East Anglian region between 1990 and 1994. One hundred normotensive (seated blood pressure, 140/90 mm Hg) women with no history of preeclampsia and <50 years old at screening were randomly selected from the CLEAREST cohort. All subjects in this study were white Northern Europeans.

Genotyping
Genomic DNA was isolated from a venous blood sample and then genotyped by a polymerase chain reaction/restriction fragment length polymorphism method with the use of previously published methods. In brief, the affected exon in each gene considered was amplified by polymerase chain reaction, and products were digested with an appropriate restriction enzyme. Fragments were finally size-fractionated on a 2% agarose gel to allow allele assignment. The polymerase chain reaction primers and restriction enzymes used are shown in Table 1.

Statistical Analysis
Statistical differences between allele and genotype frequencies were determined with the chi² statistic with significance set at <0.05. Relative risk was expressed as the odds ratio, with 95% confidence intervals computed with the logit method. Power estimations were carried out with the StatGraphics software package (version 2.6).

Table 1: Primer Sequences and Restriction Enzymes Used in Genotyping

<table>
<thead>
<tr>
<th>Gene Variant</th>
<th>Forward Primer (5′→3′)</th>
<th>Reverse Primer (5′→3′)</th>
<th>Restriction Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR C677T</td>
<td>TGAGGAGAAGGGTGTCGCGGGA</td>
<td>AGGACGCTCGGGTGAGAGTG</td>
<td>HincII</td>
</tr>
<tr>
<td>Factor V G1691A</td>
<td>CATGAGAGACATCGCCCTCG</td>
<td>GACCTAACATGTTCTAGCCAGAG</td>
<td>MnlI</td>
</tr>
</tbody>
</table>

Table 2: Distribution of Genotypes and Alleles Among Women With Preeclampsia and Those in Control Groups

<table>
<thead>
<tr>
<th>Gene Variant</th>
<th>Individual Genotypes*</th>
<th>Alleles*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia cases</td>
<td>138 (0.488)</td>
<td>114 (0.403)</td>
</tr>
<tr>
<td>Pregnant controls</td>
<td>51 (0.510)</td>
<td>37 (0.370)</td>
</tr>
<tr>
<td>Population controls</td>
<td>48 (0.480)</td>
<td>41 (0.410)</td>
</tr>
<tr>
<td>Factor V G1691A</td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Preeclampsia cases</td>
<td>268 (0.947)</td>
<td>15 (0.053)</td>
</tr>
<tr>
<td>Pregnant controls</td>
<td>94 (0.940)</td>
<td>6 (0.060)</td>
</tr>
<tr>
<td>Population controls</td>
<td>95 (0.950)</td>
<td>5 (0.050)</td>
</tr>
</tbody>
</table>

*Number and frequency (in parentheses) are given.

Results
Genotype and allele frequencies for factor V G1691A and MTHFR C677T polymorphisms are shown in Table 2. All polymorphisms were in Hardy-Weinberg equilibrium. The frequencies were not significantly different between the control group of pregnant women and the population control groups of normotensive women. Therefore, the 2 control groups were combined for further comparison with the preeclamptic women.

For the MTHFR polymorphism, there was no evidence of a significant excess of homozygotes for the T677 variant, with frequencies of 11% and 11.5% in the preeclampsia and pooled control group, respectively (odds ratio, 1.05; 95% confidence interval, 0.74 to 1.51). The observed frequency of T677 homozygotes in our control subjects is the same as that reported in the study by Sohda et al but somewhat lower than reported for a Southern European population. In the preeclampsia group, the T677 frequency was not affected by disease severity; the genotype was present in 15 of 149 (10.1%) subjects who delivered before 37 weeks and in 2 of 25 (8%) subjects who developed the HELLP syndrome.

The carrier rate for the factor V Leiden variant was 5.5% in the pooled controls, with no individuals homozygous for the A1691 variant. This frequency is similar to the frequency previously reported in Northern European and American populations. The carrier rate among the preeclampsia group was not significantly different at 5.3%, and no A1691 homozygotes were detected (odds ratio, 0.96; 95% confidence interval, 0.43 to 2.14). Disease severity in the preeclampsia group did not significantly affect carrier rate for the Leiden variant; it was present in 5 of 149 (3.4%) subjects who delivered before 37 weeks and in 2 of 25 (8%) subjects who developed the HELLP syndrome.

Discussion
We did not find either an excess of the T677 homozygotes for MTHFR or carriers for the Leiden variant of factor V in our
well-characterized preeclamptic population. On the basis of delivery before 37 weeks of gestation, more than half of our subjects with preeclampsia had severe disease, yet separate analysis of this subgroup did not significantly alter the study findings. This is again at variance with suggestions that these polymorphisms are markers of disease severity in women with preeclampsia, and, in any case, results of the study by Grandone et al.17 were positive despite the predominance of subjects with very mild disease. It is also unlikely that our negative result reflects a simple type II error due to an inadequate sample size. Previous studies reporting a significant excess of these genotypes were substantially smaller than ours; eg, the 2 studies on MTHFR genotypes reported on only 96 and 67 preeclamptic women, respectively.7,8 Our sample size of 283 would be expected to have at least 92% power to detect a doubling of the Leiden variant frequency and >99% power to detect a doubling of the TT MTHFR genotype frequency.

Association studies are susceptible to the definition of both patient and control groups. Stratification may not be appreciated but may confound the matching of the 2 groups. The preeclamptic women in this study were all recruited from a regional teaching center within the East Anglian region, and the pregnant control subjects were recruited from the same center at the same time. Subjects with preeclampsia were carefully phenotyped with the use of a stringent definition of preeclampsia that requires a minimum elevation of diastolic blood pressure.13 We are confident that all of our subjects had definite preeclampsia as opposed to isolated pregnancy-induced hypertension. Association studies are also frequently confounded by the definition of the control population. Because the East Anglian population is largely rural with little geographic movement until recent times, we assumed that our gene pool is relatively homogenous. This is supported by the finding that our pregnancy control group had frequencies for both polymorphisms that mirrored the more general East Anglian population as sampled in our normotensive controls.

Several other polymorphisms have been reported to be positively associated with preeclampsia in case-control studies. The best known is the angiotensinogen M235T variant, which was actually the first positive association to be described with data also suggesting linkage of the angiotensinogen gene with preeclampsia.18 Nevertheless, at least 1 recent study failed to replicate the association with sample populations from Australia and mainland China.19 It is not clear whether the lack of association found in the present study can also be simply attributed to population differences.

Despite the reported associations of the MTHFR and factor V polymorphisms with preeclampsia, their interpretation is debatable. They may, eg, represent susceptibility genes themselves or, as seems more likely, reflect linkage disequilibrium between these polymorphisms and the real susceptibility loci. Both scenarios have important consequences for the mode of inheritance of preeclampsia; the simple mendelian model usually suggested giving way to a complex model with 2 (or more) susceptibility loci for the disorder. This being the case, it seems imperative that in populations that show positive associations, methods such as transmission disequilibrium testing20 are used to establish whether linkage disequilibrium exists. The attraction of the transmission disequilibrium testing approach is the relative ease with which the necessary nuclear pedigrees can be recruited; the relative youth of preeclamptic subjects also ensures a high probability that probands will have living parents.

From a pathophysiological standpoint, it is conceivable that the thermolabile variant of MTHFR may contribute to the 2-fold elevation of plasma homocysteine recently reported in a small sample of preeclamptic women.21 In pregnancy uncomplicated by preeclampsia, homocysteine levels are almost 50% lower than in nonpregnant women despite the increased folate demand in pregnancy.22 Hence, it seems relatively unlikely that the homocysteine levels in preeclampsia reflect simple folate depletion and hence expression of the biochemical phenotype of the T677 homozygotes. Wide-spread prescription of folate supplements to pregnant women will also ensure that folate depletion to levels sufficient to express the homozygous T677 phenotype will become increasingly uncommon. Data on plasma homocysteine levels in pregnant women with the various C677T genotypes are clearly needed to further address this issue.

In summary, we did not find any evidence that either factor V Leiden or the thermolabile variant of MTHFR are associated with preeclampsia in our study population from the East Anglian region of the United Kingdom. Unless the associations can be confirmed independently in all populations, suggestions that they might be used in genetic risk prediction seem premature.9

Acknowledgments

This work was supported by a grant from the British Heart Foundation (to Drs O'Shaughnessy and Morris). Ian Lewis is a PhD student and is funded by the British Heart Foundation.

References


Factor V Leiden and Thermolabile Methylenetetrahydrofolate Reductase Gene Variants in an East Anglian Preeclampsia Cohort

Kevin M. O'Shaughnessy, Beiyuan Fu, Franco Ferraro, Ian Lewis, Sarah Downing and Nick H. Morris

Hypertension. 1999;33:1338-1341
doi: 10.1161/01.HYP.33.6.1338

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/33/6/1338