Matched Case-Control Study on Factor V Leiden and the Prothrombin G20210A Mutation in Patients With Ischemic Stroke/Transient Ischemic Attack Up to the Age of 60 Years

Wolfgang Lalouschek, MD; Martin Schillinger, MD; Kety Hsieh, MD; Georg Endler, MD; Susanne Tentschert; Wilfried Lang, MD; Suzanne Cheng, PhD; Christine Mannhalter, PhD

Background and Purpose—The role of the factor V Leiden mutation (FVL) and the G20210A mutation of the prothrombin (factor II [FII]) gene for arterial thrombosis is not clear.

Methods—We investigated the prevalence of these mutations in 468 patients with an acute stroke or transient ischemic attack (TIA) before the age of 60 years and in a healthy control population individually matched for age and gender. We also analyzed interactions between the mutations, gender, standard vascular risk factors, and stroke risk.

Results—The prevalence of the FVL did not differ significantly between patients and control subjects. However, we found a significant interaction between the FVL, smoking, and risk of stroke in women: female smokers without FVL had a somewhat increased risk of stroke of 2.6 (95% CI, 1.5 to 4.6; \( P = 0.001 \)) compared with nonsmoking noncarriers of the FVL. Stroke risk was markedly higher in female smokers who had the FVL (OR, 8.8; 95% CI, 2.0 to 38.0; \( P = 0.004 \)) after multivariate adjustment. No such interaction was observed in men. In contrast, the frequency of the FII G20210A mutation was significantly higher in male patients compared with controls (6% versus 1%; adjusted OR, 6.1; 95% CI, 1.3 to 28.3; \( P = 0.021 \)). In females, the prevalence of the mutation was 3% in both groups. We found no significant interactions of the FII G20210A mutation with other vascular risk factors and stroke risk.

Conclusions—Our data indicate a highly increased risk of ischemic cerebrovascular events in women up to 60 years who smoke and have FVL. We also found evidence for an increased risk of stroke/TIA in men who have the FII G20210A mutation but not in women in this age group. (Stroke. 2005;36:1405-1409.)

Key Words: factor V | mutation | prothrombin | stroke

The factor V Leiden mutation (FVL) (Arg506Gln) and the G20210A mutation of the prothrombin gene (factor II [FII] G20210A mutation) are the most common genetic risk factors of venous thrombosis.1 Their importance for arterial events, in particular myocardial infarction and ischemic stroke, is less clear. In unselected adult populations with a mean age of 65 years or older, no or only a modest association between these mutations and the risk of ischemic stroke has been found by recent meta-analyses.2-3 Some studies reported an association between one or both mutations and the risk of stroke in young patients (younger than 45 years in most studies), whereas others did not.4-15 Interactions of these mutations with exogenous vascular risk factors, particularly smoking, probably modulate the risk of arterial vascular events.16,17

In the present study, we investigated the prevalence of the FVL and the FII G20210A mutation in a large population of patients with ischemic cerebrovascular events (ischemic stroke or transient ischemic attack [TIA]) before the age of 60 years. We compared the occurrence of these variants in patients to healthy controls individually matched for age and gender. We also investigated interactions of these mutations with standard vascular risk factors and the effect on the risk of cerebrovascular events.

Materials and Methods

Patients and Control Subjects

468 consecutive patients with an acute ischemic stroke or TIA before age 60 years (177 female, 291 male; median age, 53 years; interquartile range [IQR], 45 to 57) who were documented in the Vienna Stroke Registry18 were included in the study. The diagnosis was established clinically by a neurologist and all patients underwent cranial CT or MR. The patients were documented according to a standardized protocol comprising the following information: vascular risk factors including hypertension, diabetes, cigarette smoking, body mass index, hyperlipidemia, oral contraception, or other hormone intake; medical history including general medical diseases (eg, previous cardiac, pulmonary, neurological, endocrine, gastrointestinal...
noral diseases) and previous medication with particular reference to vascular diseases (TIA/stroke, coronary artery disease, peripheral artery disease, venous thrombosis); laboratory and technical investigations including blood chemistry and blood count taken during hospitalization, cerebrovascular investigations (eg, carotid and vertebral ultrasound), cardiac investigations (eg, electrocardiography, echocardiography); and stroke cause and stroke severity as measured by validated scales.18,19

The patients included in the present study represent a subset of a larger cohort of patients documented in the Vienna Stroke Registry during the period from October 1998 until June 2001. During this period, 732 patients with an acute cerebrovascular event before age 60 years were admitted to one of the participating departments. From these, 69 patients with a hemorrhagic stroke or sinus thrombosis and 13 patients with a rare cause of stroke (confirmed arterial dissection or drug abuse) were excluded. Of 468 of the remaining 650 patients (72%), written informed consent and a blood sample could be obtained. The 468 control individuals matched for age (±2 years) and gender (median age, 52; IQR, 45 to 57 years) were participants in an official health care program.20 All were free of clinically manifest arterial vascular disease and reported no vascular diseases in first-degree relatives. Medical history, vascular risk factors, and results of laboratory investigations were documented according to a standardized protocol.

The study complied with the Declaration of Helsinki and was approved by the local ethics committee. All patients and control subjects gave their written informed consent for participation in the study.

Genotyping

The presence of FVL and FII G20210A were tested by one of two published methods: a mutagenic separated allele-specific polymerase chain reaction test21 or a multiplex polymerase chain reaction in combination with a hybridization to sequence-specific oligonucleotides immobilized on nylon membrane strips.22 The reagents for the latter test were kindly provided by Roche Molecular Systems, Alameda, Calif.

Statistical Methods

Continuous data are given as median and IQR (range from the 25th to the 75th percentile). Discrete data are given as counts and percentages. The McNemar test for dependent samples was used to compare groups of categorical data. Groups of continuous data were compared by the Wilcoxon Test.

Associations between the 2 polymorphisms and the risk of stroke were analyzed by means of conditional binary logistic regression models with adjustment for the conventional risk factors hypertension, diabetes, hyperlipidemia, current smoking, obesity, and hormone intake did not significantly affect these results. However, we found a significant interaction between FVL, current smoking, and risk of stroke in women. In nonsmoking women, the prevalence of the FVL was 5% in controls (6/117) and in patients (4/86). In women who smoked, the prevalence of FVL was 7% in controls (4/60) but 15% in patients (14/91). Clinical and demographic data of those female patients who smoked and had FVL are shown in Table 2. In a conditional logistic regression adjusted for other vascular risk factors, female smokers without the FVL had an increased risk of stroke of 2.6 (95% CI, 1.5 to 4.6; \( P=0.001 \)) compared with nonsmoking female noncarriers of the FVL. In women who had FVL and who smoked the risk of stroke was increased 8.8-fold (95% CI, 2.0 to 38.0; \( P=0.004 \)) after multivariate adjustment (Figure). In males, there was no relation between FVL, smoking, and stroke risk. We also found no other first-order interactions of the FVL with vascular risk factors (including age and hormone intake). We found no significant differences in the prevalence of the FVL in etiologically distinct subgroups (Table 3).

FII G20210A Mutation

The frequency of the FII G20210A mutation was significantly higher in patients than in control subjects (5% versus 2%; unadjusted OR, 2.3; 95% CI, 1.1 to 5.1; \( P=0.033 \); adjusted OR, 2.9; 95% CI, 1.1 to 7.9; \( P=0.032 \)). This difference was caused by a higher prevalence of the mutation in male patients compared with controls (6% versus 1%; unadjusted OR, 5.0; 95% CI, 1.4 to 17.2; \( P=0.011 \); adjusted OR, 6.1; 95% CI, 1.3 to 28.3; \( P=0.021 \)). In contrast, the prevalence of the mutation in female patients and controls was 3% in both groups (unadjusted OR, 1.0; 95% CI, 0.3 to 3.1; \( P=1.0 \); adjusted OR, 1.6; 95% CI, 0.4 to 5.8; \( P=0.502 \)). We did not observe significant interactions of the FII G20210A mutation with other vascular risk factors and stroke risk. We found no significant differences in the prevalence of the FII G20210A mutation in etiologically distinct subgroups (Table 3).

Two of 468 patients (0.4%) were combined carriers of FVL and the FII G20210A mutation but no combined mutation carrier was identified among the controls (\( P=0.157 \)).

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of Patients and Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n=468)</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Age median (IQR)</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
</tr>
<tr>
<td>BMI &gt;27 (%)</td>
</tr>
<tr>
<td>Current cigarette smoking (%)</td>
</tr>
<tr>
<td>Female hormone intake (women only)</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; IQR, interquartile range. *McNemar test. †Wilcoxon test.
Risk of stroke in women up to age 60 years in relation to FVL status and smoking (absolute numbers of individuals in each subgroup are given in the text).

### Discussion

Our results, obtained in a large sample of patients with ischemic cerebrovascular events with a median age of 53 years, indicate that the FVL mutation is not a general risk factor of stroke in this population. However, the significantly higher frequency of the mutation in female patients who smoke indicates a highly increased risk of stroke in these women. We also found evidence that the FII G20210A mutation is a significant risk factor of stroke in men in this age group, but not in women.

Recent meta-analyses suggest that the FVL and the FII G20210A mutation do not or only slightly contribute to the risk of stroke in older individuals.\(^2,^3\)

Some, but not all, investigations in young stroke patients (usually younger than 45 years) found a higher prevalence of these mutations in patients than in controls, particularly in patients with cryptogenic stroke.\(^4,^5,^7–^15\)

Most of these studies were small and/or did not test for interactions of these mutations with gender, other vascular risk factors, and stroke risk. Margaglioto et al studied 202 patients with stroke younger than 45 years and reported a markedly higher prevalence of the FVL mutation in female patients and a particularly increased stroke risk in smokers who had the FVL mutation.\(^1^1\) In contrast, Nabavi et al found no differences of the prevalence of the mutation in 225 young men and women with ischemic stroke or TIA. Carriers of the FVL were somewhat more frequently smokers (74% smokers among carriers versus 55% among noncarriers), but this difference did not reach significance (\(P=0.1\)). In this study, a potential interaction between the FVL mutation, smoking, and gender was not reported. Whereas these authors found a higher prevalence of the FVL mutation among patients with cryptogenic stroke, we could not detect an interaction between presumed etiology and FVL or the FII G20210A mutation.\(^1^0\) The FVL has previously been shown to play a role in arterial thrombosis in smokers. In a study by Rosendaal et al, a particularly high risk of myocardial infarction was described in young women who smoked and had the FVL mutation.\(^1^6,^1^7\)

A relationship between the FII G20210A mutation and stroke risk in younger individuals has also been reported. In some studies in which gender-specific calculations were performed, the prevalence of the mutation was similar in male and female patients.\(^1^1,^1^4\) and other studies did not separate the patient cohorts according to gender. Our observation of a markedly increased risk of stroke at age younger than 60 years in men but not in women constitutes a new finding and should be tested in independent populations.

There are several possible mechanisms that may underlie a different effect of prothrombotic genetic variations in men and women. Endogenous estrogens increase the resistance to activated protein C regardless of the presence of the FVL mutation.\(^2^1\) Also, the FVL has been shown to further increase the risk of venous thrombosis in oral contraceptive users.\(^2^4\) However, we did not find a significant interaction between hormone intake, the FVL, and stroke risk. Other possible explanations for gender differences are higher levels of factor VII:C and fibrinogen in women compared with men with

### Table 2. Clinical and Demographic Data of Female Patients Who Smoked and Had FVL

<table>
<thead>
<tr>
<th>N</th>
<th>Age</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>BMI &gt;27</th>
<th>Current Hormone Intake</th>
<th>TC, mmol/L</th>
<th>Type of Event</th>
<th>Presumed Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Major</td>
<td>CARD</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.73</td>
<td>Major</td>
<td>LVD</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.07</td>
<td>Major</td>
<td>LVD</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.83</td>
<td>TIA</td>
<td>SVD</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.37</td>
<td>TIA</td>
<td>UNDET</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.29</td>
<td>TIA</td>
<td>LVD</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.54</td>
<td>Major</td>
<td>LVD</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.23</td>
<td>Major</td>
<td>LVD</td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.74</td>
<td>Major</td>
<td>CARD</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.24</td>
<td>Major</td>
<td>UNDET</td>
</tr>
<tr>
<td>11</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.09</td>
<td>Major</td>
<td>SVD</td>
</tr>
<tr>
<td>12</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.72</td>
<td>Major</td>
<td>CARD</td>
</tr>
<tr>
<td>13</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.45</td>
<td>Minor</td>
<td>UNDET</td>
</tr>
<tr>
<td>14</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.02</td>
<td>Minor</td>
<td>SVD</td>
</tr>
</tbody>
</table>

FVL indicates factor V Leiden; CARD, cardioembolic; LVD, large-vessel disease; SVD, small-vessel disease; major, major stroke; minor, minor stroke; TC, total cholesterol; UNDET, undetermined.
coronary artery disease. Also, differences in body iron store have been considered to account for differences in stroke risk between men and women. In some studies, different effects of conventional vascular risk factors in men and women, and gender-specific changes in vascular function with age have been discussed.

We point out some potential limitations of our study. Our controls were not fully population-based. Therefore, we cannot exclude certain forms of bias, eg, regarding the distribution of vascular risk factors or the attitude toward a healthier living style in this population compared with our patients. However, our results remained stable after statistical adjustment for known vascular risk factors. Stratification into subgroups and testing for interactions may lead to relatively small sample sizes even in a relatively large study. Given these limitations and the case-control design of our study, the results, particularly the observed interactions between gender, smoking, mutations, and stroke risk, should be confirmed in other populations and/or in a prospective study.

In conclusion, our data indicate a highly increased risk of ischemic cerebrovascular events in women up to 60 years who smoke and have the FVL mutation. We also found evidence for an increased risk of stroke/TIA in men, but not women in this age group, who have the FII G20210A mutation. Because of the cross-sectional nature of our study, potential implications have to be regarded with care. Yet we believe that women who have been identified as carriers of the FVL mutation and who smoke should be informed about the significantly increased risk of cerebrovascular events in female mutation carriers who smoke.

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References

<table>
<thead>
<tr>
<th>TABLE 3. Prevalence of FVL and the Factor II G20210A Mutation According to Presumed Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large-Vessel Disease (n=72)</td>
</tr>
<tr>
<td>FVL* (%)</td>
</tr>
<tr>
<td>FII G20210A mutation* (%)</td>
</tr>
</tbody>
</table>

*Heterozygous and homozygous carriers combined (for each mutation there was one homozygous patient). FII indicates factor II.


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