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Abstract: Objective: Abnormalities in the coagulation pathway play an increasing role in the diagnostic work-up of stroke patients, especially in cases with stroke of undetermined cause. Methods: We investigated three common genetic variants within the coagulation cascade factor V Leiden, prothrombin mutation g.20210 G>A, factor XIII polymorphism Val34Leu in 167 patients with ischemic stroke defined by TOAST subclassification and 500 controls. Results: The factor V Leiden mutation was over-represented in patients with cardioembolic stroke for trend, whereas the prothrombin mutation g.20210G>A as well as the factor XIII polymorphism Val34Leu were not found to be associated with a stroke in general or any stroke subtype. The three polymorphisms showed no association with stroke in subgroups of patients defined by age (<40 y, 40-49 y, 50-59 y, ≥60 y). Discussion: This study suggests that the analysis of prothrombin mutation g.20210 G>A and factor XIII polymorphism Val34Leu is not a useful diagnostic procedure in the work-up of ischemic stroke, and the analysis of the factor V Leiden mutation might only be of some impact in patients with cardioembolic stroke.
Common genetic coagulation variants are not associated with ischemic stroke in a case-control study

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Column title: Genetic coagulation variants in ischemic stroke
Abstract

Objective: Abnormalities in the coagulation pathway are often included in the diagnostic work-up of stroke patients, especially in young adults with cryptogenic stroke.

Methods: Three common genetic variants within the coagulation cascade were investigated in 500 control subjects and in 167 patients with ischemic stroke defined by TOAST subclassification. Analyzed variants were factor V Leiden, prothrombin 20210 G>A, and factor XIII Val34Leu.

Results: The factor V Leiden mutation was over-represented in patients with cardioembolic stroke for trend, whereas the prothrombin 20210G>A variant and the factor XIII polymorphism Val34Leu were not associated with stroke of any subtype. The three polymorphisms showed no association with stroke in subgroups of patients defined by age (<40 y, 40-49 y, 50-59 y, ≥60 y).

Discussion: This study suggests that the analysis of prothrombin 20210 G>A and factor XIII Val34Leu is not a useful diagnostic procedure in the work-up of ischemic stroke.

Key words: factor V Leiden, prothrombin, factor XIII Val34Leu, ischemic stroke, hereditary thrombophilia
**Introduction**

The evaluation of coagulation abnormalities plays an important role in the diagnostic work-up of stroke patients, especially when no obvious cause such as a cardiac origin, severe atherosclerosis or any other vascular disease becomes apparent, and when stroke occurs in young patients.

The prothrombin (factor II) mutation g.20210 G>A was described by Poort et al. in 1996 and was found to confer a moderately increased risk of venous thrombosis. The factor V Leiden mutation (APC resistance) c.1691 G>A, first described by Bertina et al. in 1994, is associated with recurrent venous thrombosis and thromboembolism, especially in homozygous individuals or when combined with the prothrombin mutation. In a meta-analysis of genetic studies in ischemic stroke, both the prothrombin (19 studies on 3,028 cases and 7,131 controls) and factor V Leiden (26 studies on 4,588 cases and 13,798 controls) mutations were associated with ischemic stroke of any origin with an odds ratio of 1.44 and 1.33 respectively. The factor XIII polymorphism Val34Leu (c.103 G>T [NM_000129.3]) is associated with increased enzyme activity. In some studies, it was negatively associated with myocardial infarction, cerebrovascular disease, or venous thrombosis and positively associated with intracerebral hemorrhage. In other studies, however, no association with coronary heart disease including myocardial infarction, cerebrovascular disease, intracerebral hemorrhage, or deep venous thrombosis was detected.

These genetic variables have a considerable allele frequency within the Caucasian population with heterozygosity frequencies of 1-3% for the prothrombin mutation g.20210 G>A, 2-15% for factor V Leiden and 23-50% for factor XIII Val34Leu.

The present study aimed at investigating the effect of these three genetic polymorphisms on the risk of stroke in general and in stroke of different etiologies in a case-control set up.
Subjects and methods

Subjects

167 patients of German descent with ischemic stroke were recruited for this study between January 1999 and June 2000. Patients suffered from a neurological deficit with sudden onset. Diagnosis of ischemic stroke was confirmed by cerebral imaging (CCT or MRI). Additional examinations were also performed in order to determine the etiology of stroke: cardiac diagnostics (ECG, 24-hour-ECG, transthoracic or transesophageal echocardiography), ultrasound examination of the vessels of the neck and the brain and/or, if necessary, angiography/MR-angiography of these vessels, and laboratory analyses, e.g. to detect a state of hypercoagulopathy or vasculitis.

Etiology of stroke was classified according to the „Trial of Org 10172 in Acute Stroke Treatment“ (TOAST) classification as published by Adams et al. 18: (1) large vessel atherosclerosis, (2) cardioembolism, (3) small vessel occlusion, (4) stroke of other determined etiology including hypercoagulable states without obvious presence of criteria for (1) – (3) and artery dissection, and (5) stroke of undetermined etiology. Age subgroups were defined as <40 y, 40-49 y, 50-59 y, ≥60 y.

500 blood donors of German descent (29% female, mean age in years ± standard deviation 33 ± 11) of the University of Bonn were genotyped for the polymorphisms examined. All patients (or their legal trustees) gave informed and written consent; the study was approved by the local ethics committee.

Genetic analysis

In the genomic DNA samples, factor V Leiden mutation 19, prothrombin mutation g.20210 G>A 20, and factor XIII polymorphism Val34Leu 21 were analyzed by TaqMan technique as previously published.
Statistics

For statistics, we applied nominal regression analysis with stroke (respectively the different TOAST subgroups and age subgroups) as dependent variable and age and gender as covariables. Threshold for significance was defined as $p < 0.05$.

Results

A total of 167 patients were investigated in this study, 69 females and 113 males. Mean age at onset of stroke ($\pm$ standard deviation) was 55 $\pm$ 16 years. Of the 167 patients, 61 were classified into TOAST 1, 47 into TOAST 2, 28 into TOAST 3, 12 into TOAST 4, and in 29 patients the etiology of stroke could not be determined (TOAST 5).

None of the three polymorphisms investigated was associated with a significant risk of ischemic stroke (Table 1). Subgroup analysis for the different TOAST groups 1-5 (each in comparison to the healthy control individuals) revealed an over-representation of the factor V Leiden mutation in the group of patients with cardioembolic stroke (TOAST 2) for trend ($\chi^2=5.076; p=0.079$). In this subgroup, both other polymorphisms were not associated with the occurrence of stroke, neither was any of the three polymorphisms in all other stroke subgroups. There was no significant association of the polymorphisms with the occurrence of stroke in one of the subgroups defined by age (data not shown).

Discussion

Main findings:

The prothrombin mutation g.20210 G>A and factor V Leiden mutation are known to be associated with a higher risk of venous thrombosis. For the factor XIII polymorphism Val34Leu, an association with myocardial infarction and cerebrovascular disease has been discussed controversially. We analyzed these three genetic variants in a stroke case-control study. None of the analyzed variants was significantly associated with the risk of stroke. We
did not find a significant association of any of the genetic variants with stroke in any TOAST subgroup including class V (stroke of undetermined origin) in our study population. A subgroup analysis of young patients did not reveal a significant influence of any one of the genetic variants, either. The factor V Leiden mutation showed a tendency to predict cardioembolic stroke. This mutation is known to be associated with a risk of thrombosis, thus, a possible underlying mechanism could be paradoxical embolism in patients with patent foramen ovale or embolism in patients with atrial fibrillation.

Comparison with existing literature and limitations of the present study:

Studies concerning the association of ischemic stroke with genetic coagulation factor variants showed contradictory results. In an adult North Mediterranean population younger than 65 years, the prevalences of factor V Leiden and prothrombin 20210G>A mutations were higher in patients with ischemic stroke of any classification compared to controls matched for clinical risk factors\(^{22}\). In a recent study encompassing 367 patients with acute ischemic stroke and atrial fibrillation, both the factor V Leiden and the prothrombin g.20210G>A mutation were no risk factors for stroke associated with atrial fibrillation\(^ {23}\). Other studies investigating the role of the prothrombin mutation and factor V Leiden mutation in stroke associated with PFO by paradoxical embolism reported contradictory results: Lichy et al. found an association of the prothrombin mutation, but not factor V Leiden mutation, with stroke associated with PFO\(^ {24}\) whereas other studies described an association of both the factor V Leiden mutation and the prothrombin mutation with cryptogenic stroke and PFO\(^ {25,26}\). The large meta-analysis of genetic studies in ischemic stroke published by Casas et al. in 2004 encompasses data of 120 case-control studies with approximately 18,000 cases and 58,000 controls\(^7\). In accordance to our data, no significant association of factor XIII Val34Leu with ischemic stroke was found. For the factor V Leiden and the prothrombin mutations, however, a significant association with ischemic stroke was found with an odds ratio of 1.33 and 1.44 respectively.
In this meta-analysis, only white patients were included, but no other ethnic specification was performed, nor was any subgrouping for stroke etiologies done. Because of our much smaller sample size, minor effects of those two polymorphisms might have been missed in our study.

The implications for future research or clinical practice:

It would be of interest to investigate a larger cohort of patients with known cardioembolic stroke for the factor V Leiden mutation to confirm the association suggested by the present results. Possibly this could be done by retrospectively subgrouping patients according to stroke etiology from existing studies or meta-analyses. Such data could be helpful for strategies to define risk profiles for patients with patent foramen ovale or atrial fibrillation. In conclusion, our data suggest that these polymorphisms have no major influence on the risk of stroke of any TOAST class. For patients in whom any one of these genetic variants is diagnosed, consequences for treatment are controversial. Thus, genotyping of these variants in patients with ischemic stroke may be of questionable value for the clinical routine at present.

Competing Interests

The authors do not report competing interests.
Table 1. Influence of genetic variants of the coagulation factors factor V Leiden, prothrombin mutation 20210 G>A, and factor XIII Val34Leu on the occurrence of Stroke

<table>
<thead>
<tr>
<th></th>
<th>patients</th>
<th>controls</th>
<th>$\chi^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>55 ± 16</td>
<td>33 ± 11</td>
<td>260.205</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sex (F/M) (%)</td>
<td>37.9 / 62.1</td>
<td>29.2 / 70.8</td>
<td>7.476</td>
<td>0.006</td>
</tr>
<tr>
<td>prothrombin g.20210 G&gt;A (GG / GA / AA) (%)</td>
<td>95.8 / 4.2 / 0</td>
<td>98 / 2 / 0</td>
<td>0.23</td>
<td>0.878</td>
</tr>
<tr>
<td>factor V Leiden 1691 G&gt;A (GG / GA / AA) (%)</td>
<td>93.4 / 6 / 0.6</td>
<td>94 / 5.8 / 0.2</td>
<td>2.079</td>
<td>0.354</td>
</tr>
<tr>
<td>factor XIII Val34Leu G&gt;T (GG / GT / TT) (%)</td>
<td>49.1 / 44.3 / 6.6</td>
<td>56 / 38 / 6</td>
<td>2.399</td>
<td>0.301</td>
</tr>
</tbody>
</table>


Revision: Common genetic coagulation variants are not associated with ischemic stroke in a case–control study by S. Moskau et al.

Dear Prof. Dojovny,

thank you for the comments on our manuscript and the opportunity to resubmit after revision. We took care to revise the manuscript properly. We think that the paper has improved by the help of the comments and hope that you will find the revised version appropriate for publication the journal. Please find detailed explanations of the changes as follows:

This is an interesting paper which adds something to our knowledge. It needs minor revisions. The English language is not fluent and the paper should be re-written in English.

We have improved the English language.

Discussion section needs to be expanded with 2007–2008 references.
We have included 2007/2008 references in the discussion section.

A figure will help to show where this particular coagulation factor works. Otherwise it is fine
We prepared a scheme of the coagulation cascade including the coagulation factors examined in the actual study. We ask the editor to kindly decide, whether such figure (or a modified version of it) is necessary and should be published with the article.

With best regards
Alexander Semmler and Michael Linnebank for the authors