Fibrinogen Gene Promoter −455 A Allele as a Risk Factor for Lacunar Stroke

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Background and Purpose—Elevated fibrinogen levels are suggested to increase the risk of myocardial infarction and stroke. Carriers of the A allele of the fibrinogen −455G/A polymorphism have increased plasma fibrinogen levels. We studied the association of this polymorphism with stroke subtype in the Stroke Aging Memory (SAM) cohort.

Methods—The SAM cohort comprises 486 consecutive patients 55 to 85 years of age who, 3 months after ischemic stroke, completed a detailed stroke assessment. Stroke subtypes were examined with MRI. −455G/A genotype was determined by polymerase chain reaction. MRI and genotype data were available for the 299 patients who constitute the present study population.

Results—Genotype distributions were 64.9% (GG), 31.8% (GA), and 3.3% (AA). In a logistic regression model with age, sex, hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, myocardial infarction, arhythmia, atrial fibrillation, peripheral arterial disease, and smoking as possible confounders, there was a significant association between A+ genotype and ≥3 lacunar infarcts (odds ratio [OR], 2.57; 95% CI, 1.23 to 5.36; P = 0.01). Hypertensive patients carrying the A allele had increased risk (OR, 4.24; 95% CI, 1.29 to 13.99; P = 0.02) for ≥3 lacunar infarcts. A similar increase in risk was observed among smokers with the A+ genotype (OR, 2.67; 95% CI, 0.92 to 7.77; P = 0.07).

Conclusions—Stroke patients carrying the A allele of the Bβ-fibrinogen −455G/A polymorphism frequently presented with multiple lacunar infarcts. This association was stronger among hypertensives and smokers. These associations suggest that the A allele may predispose to atherothrombotic events in cerebrovascular circulation. (Stroke. 2003;34:01–07.)

Key Words: fibrinogen n genetics n infarcts n lacunar infarction n stroke

Stroke is a leading cause of death in Western societies, resulting in disability and socioeconomic burden. There are several known risk factors for stroke and cerebral atherosclerosis, including dyslipidemia, hypertension, diabetes, smoking, heart failure, atrial fibrillation (AF), and increasing age.1,2 Little is known about inherited factors that could predispose or modify the type and consequences of stroke. Some studies have reported that family history of stroke is an independent risk factor for all stroke types, whereas others have failed to find such an association.3,4 Several studies indicate the role of fibrinogen as a risk factor for ischemic heart disease, myocardial infarction (MI), stroke, venous thrombosis, and peripheral arterial disease (PAD).5–14 Blood viscosity and fibrin formation are affected by circulating fibrinogen concentration.15 Immobilized fibrinogen on endothelial cells acts as a substrate for platelet aggregation by binding to αIIb/β3 integrins on the adjacent platelet surfaces and adhering to the vessel wall/subendothelial collagen.16–19 Fibrinogen levels rise transiently as a result of inflammation, smoking, and cold.20–23 Age, sex, and genetic and hormonal factors also contribute to fibrinogen levels.21–23,25 G/A variability in the −455 locus of the Bβ-fibrinogen promoter region, especially the carrier status of the A allele, has previously been shown to be associated with elevated fibrinogen levels and to increase the risk of cardiovascular diseases and ischemic stroke.24,26,27 In this study, we evaluated the association of the Bβ-fibrinogen −455G/A promoter polymorphism with the type and number of strokes in a population of 299 stroke patients.

Subjects and Methods

Patients

Procedures of the Helsinki Stroke Aging Memory (SAM) stroke cohort were detailed in a report on methods and baseline findings.34

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Briefly, 486 consecutive patients 55 to 85 years of age were evaluated 3 months after ischemic stroke (the index stroke). Subjects underwent a structured medical and neurological history based on review of all available hospital charts, interview of the subject and a knowledgeable informant, and a structured clinical and neurological examination performed by a board-certified neurologist (T.P.). In addition, all cases were reviewed by a senior neurologist (T.E.). History of hypertension was defined as blood pressure ≥160/95 mm Hg. The neurological examination focused on factors and features related to dementia and stroke similar to the method of the Memory Research Unit, Department of Neurology, University of Helsinki, and the National Stroke Data Bank.35,36 Laboratory evaluations included total and high-density lipoprotein cholesterol, triglycerides, and fasting blood glucose. Total cholesterol was considered high at ≥6.5 mmol/L. History of main vascular risk factors was obtained as described earlier.34,35 The study was approved by the ethics committee of the Department of Clinical Neurosciences, Helsinki University Central Hospital (Helsinki, Finland). The study design was fully explained, and written information was offered to the patients; if they agreed to participate, they signed a written consent form.

**General Clinical Assessment**

A total of 396 patients (81.5%) in the SAM cohort underwent MRI. From the MRI data, stroke subtypes were then examined. Inclusion and exclusion criteria are detailed elsewhere.38-40 Fibrinogen -455G/A genotype was successfully determined for 371 subjects (76.3%). Information on both genotype and MRI was available on 299 subjects (61.5%), who formed the final study population. The study population did not differ from the remaining 187 patients (-455G/A genotype or MRI data were not available) in terms of demographic and clinical characteristics (age, sex, history of hypertension, smoking, and diabetes with lacunar and large-vessel infarcts as confounders).38-40 Logistic regression analysis (enter and forward stepwise models) with age, sex, hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, MI, AF, PAD, and smoking as confounders was used to further study the association of -455G/A genotype with lacunar and large-vessel infarcts, as well as the dependence between genotype and conventional risk factors.

**Infarct Subtypes**

Different infarct subtypes were determined by MRI findings. Infarction was defined as lacunar if it was situated in deep white or gray matter and its diameter was 3 to 9 mm. By definition, a large-vessel infarct was located in the corticosubcortical layers of cerebral hemispheres in the territories of superficial branches of anterior, middle, or posterior cerebral artery, and its diameter was ≥10 mm.39,40

**DNA Procedures**

DNA was separated from frozen blood samples through standard procedures. Polymerase chain reaction (PCR) for DNA amplification was carried out as previously described.34 PCR reactions were performed with a PTC 100 (Perkin-Elmer) in a 50-μL reaction with 50 ng of genomic DNA, 200 ng of each appropriate primer (5’-CTCCTCATTGTGCTGACACCCTGGGC-3’ and 5’-GAATTGGGAAATCGAATCTCTGCTACCTT-3’), 200 μmol/L of each deoxynucleotide triphosphate, and 1 U of Dynazyme II DNA polymerase in 1× reaction buffer (Finnzymes OY). Samples were incubated for 5 minutes at 95°C, followed by 34 cycles of 1 minute at 95°C, 1 minute at 55°C, and 1 minute at 72°C. PCR products (20 μL) were digested with 10 U of the HaeIII restriction enzyme (Promega Corp) and resolved in 2% agarose gel for determination of -455G/A genotype.

**Statistical Analysis**

SPSS/Win (version 10.0, SPSS Inc) software was used to carry out statistical analyses. The association of age with the lacunar and large-vessel infarcts was calculated by Student’s t test. The associations of age, hypertension, hypercholesterolemia, hypertriglyceridemia, smoking, and diabetes with lacunar and large-vessel infarcts were examined by Pearson’s χ² test. Logistic regression analysis (enter and forward stepwise models) with age, sex, hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, MI, arrhythmia, AF, PAD, and smoking as confounders was used to further study the association of -455G/A genotype with lacunar and large-vessel infarcts, as well as the dependence between genotype and conventional risk factors.

**Results**

**Fibrinogen -455G/A Polymorphism and Lacunar Infarcts in Stroke Patients**

The allele distribution was in Hardy-Weinberg equilibrium. Genotype distributions of the -455G/A locus were 64.9% for GG, 31.8% for G/A, and 3.3% for A/A. The frequency of the A allele was 19.2%. These frequencies closely correspond to the population frequencies among whites; in the Etude Cas-Temoins sur l’Infarctus du Myocarde (ECTIM) study, the allele frequencies were 65.9%, 29.1%, and 4.9%, respectively.26 No relation between known stroke risk factors and -455G/A genotypes was observed (Table 1). Of the 299 patients, 61.9% (n = 185) had lacunar and 58.2% (n = 174) had large-vessel infarcts. Thus, 20.1% of the patients (n = 60) had both types of stroke.

Patients with the A+ genotype were overrepresented (P = 0.01) among individuals with ≥3 lacunar infarcts (49.1% versus 27.3%), whereas patients who had 1 lacunar infarct were more often G/G homozygotes. In a forced logistic regression analysis, there was an association between the A+ genotype (odds ratio [OR], 2.72; 95% CI, 1.18 to 6.27; P = 0.02) and ≥3 lacunar infarcts (Table 2). Age was significantly associated with 2 (OR, 1.06; 95% CI, 1.00 to 1.13; P = 0.04) and ≥3 (OR, 1.06; 95% CI, 1.00 to 1.13; P = 0.04) lacunar infarcts. The association between A+ genotype and ≥3 lacunar infarcts persisted in a forward stepwise logistic analysis.
regression model (OR, 2.57; 95% CI, 1.23 to 5.36; \( P = 0.01 \)). Multiple lacunar infarcts showed no association with other known risk factors.

### Dependence Between −455G/A Genotype and Risk Factors for Lacunar Infarcts

In a logistic regression model, the −455G/A genotype showed a significant interaction with hypertension (\( P = 0.004 \)) and smoking (\( P = 0.03 \)) on the occurrence of multiple lacunar infarcts, whereas there was no significant dependence between genotype and other stroke risk factors. The significant interactions between genotype and hypertension or smoking were further studied by forming a new variable with different risk factor combinations. In these variables, categories were generated in ascending order of possible risk for stroke: (1) GG homozygotes without risk factor, (2) GG homozygotes with risk factor, (3) carriers of the A allele (GA + AA) without other risk factor, and (4) individuals with both risk factors (A allele and other). When studying the dependence between age and genotype, we used mean age (71.1 years) as a cutoff point to form a categorical variable, which was then combined with genotype.

Forced logistic regression analysis of the genotype-hypertension variable, including sex, age, diabetes, hypertriglyceridemia, hypercholesterolemia, MI, arrhythmia, AF, PAD, and smoking as confounders, revealed an association in which individuals with both risk factors (genotype and hypertension) had a significantly higher risk (OR, 4.24; 95% CI, 1.29 to 13.99; \( P = 0.02 \)) for multiple lacunar infarcts compared with individuals who were GG homozygotes without hypertension (Table 3). In addition, smokers carrying the A allele had increased risk (OR, 2.67; 95% CI, 0.92 to 7.77; \( P = 0.07 \)) for developing multiple lacunar infarcts, although the association was marginal. Similar results were observed in forward stepwise logistic regression analysis.

### Large-Vessel Infarcts and the −455G/A Polymorphism

In a forced logistic regression model, male sex (\( P = 0.03 \)) was associated with 2 large-vessel infarcts compared with 1 large-vessel infarct (Table 4). In addition, age (\( P = 0.01 \)) was associated with ≥3 large-vessel infarcts (Table 4). In a

### TABLE 2. Comparisons of Characteristics Between 1 and 2 Lacunar Infarcts and Between 1 and Multiple (≥3) Lacunar Infarcts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lacunar Infarcts</th>
<th>Significance</th>
<th>≥3 Lacunar Infarcts</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=77)</td>
<td>2 (n=55)</td>
<td>OR (95% CI)*</td>
<td>P*</td>
</tr>
<tr>
<td>Mean age (SD), y</td>
<td>69.6 (7.76)</td>
<td>72.3 (6.55)</td>
<td>1.06 (1.00–1.13)</td>
<td>0.04</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>44.2</td>
<td>45.5</td>
<td>1.67 (0.73–3.86)</td>
<td>0.23</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>51.9</td>
<td>54.5</td>
<td>1.34 (0.61–2.93)</td>
<td>0.47</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>19.5</td>
<td>16.4</td>
<td>0.77 (0.28–2.08)</td>
<td>0.60</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>15.6</td>
<td>16.4</td>
<td>2.47 (0.75–8.10)</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertriglyceridemia, %</td>
<td>3.9</td>
<td>0</td>
<td>NA†</td>
<td>0.71</td>
</tr>
<tr>
<td>MI, %</td>
<td>13.0</td>
<td>20.0</td>
<td>1.28 (0.44–3.72)</td>
<td>0.66</td>
</tr>
<tr>
<td>Arrhythmia, %</td>
<td>22.1</td>
<td>27.3</td>
<td>0.70 (0.17–2.88)</td>
<td>0.62</td>
</tr>
<tr>
<td>AF, %</td>
<td>14.3</td>
<td>18.5</td>
<td>1.76 (0.33–9.50)</td>
<td>0.51</td>
</tr>
<tr>
<td>PAD, %</td>
<td>10.4</td>
<td>18.2</td>
<td>2.32 (0.75–7.18)</td>
<td>0.14</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>53.2</td>
<td>47.3</td>
<td>0.57 (0.24–1.35)</td>
<td>0.20</td>
</tr>
<tr>
<td>−455G/A A+, %</td>
<td>27.3</td>
<td>34.5</td>
<td>1.51 (0.68–3.35)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Forced logistic regression model.
†Not available because of 0 frequencies.

### TABLE 3. Dependence Between −455G/A Genotypes and Risk Factors (Hypertension and Smoking) and Their Associations With Number of Lacunar Infarcts

<table>
<thead>
<tr>
<th>Interaction Variables</th>
<th>Lacunar Infarcts</th>
<th>Significance</th>
<th>≥3 Lacunar Infarcts</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=77)</td>
<td>2 (n=55)</td>
<td>OR (95% CI)*</td>
<td>P*</td>
</tr>
<tr>
<td>Hypertension and GG-genotype, %</td>
<td>32.5</td>
<td>29.1</td>
<td>Reference</td>
<td>28.3</td>
</tr>
<tr>
<td>Hypertension and GG-genotype, %</td>
<td>40.3</td>
<td>36.4</td>
<td>1.16 (0.45–2.97)</td>
<td>0.75</td>
</tr>
<tr>
<td>Hypertension and A+ genotype, %</td>
<td>15.6</td>
<td>16.4</td>
<td>1.21 (0.39–3.80)</td>
<td>0.74</td>
</tr>
<tr>
<td>Hypertension and A+ genotype, %</td>
<td>11.7</td>
<td>18.2</td>
<td>2.18 (0.67–7.05)</td>
<td>0.19</td>
</tr>
<tr>
<td>Smoking and GG-genotype, %</td>
<td>37.7</td>
<td>30.9</td>
<td>Reference</td>
<td>22.6</td>
</tr>
<tr>
<td>Smoking and GG-genotype, %</td>
<td>35.1</td>
<td>34.5</td>
<td>0.92 (0.34–2.51)</td>
<td>0.88</td>
</tr>
<tr>
<td>Smoking and A+ genotype, %</td>
<td>9.1</td>
<td>21.8</td>
<td>3.42 (1.03–11.34)</td>
<td>0.05</td>
</tr>
<tr>
<td>Smoking and A+ genotype, %</td>
<td>18.2</td>
<td>12.7</td>
<td>0.65 (0.19–2.17)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

*Forced logistic regression model.
The fibrinogen −455G/A polymorphism has been associated with stroke in 3 previous studies. Kessler et al found an association between the AA genotype and large-vessel infarcts (P=0.045), and Liu et al associated the A+ genotype with ischemic stroke (P<0.02). The significant association (OR, 2.05; P=0.05) between fibrinogen genotype and ischemic stroke has been reported among hypertensive patients by Nishiuma et al. They also found that the A+ genotype was more common in the atherothrombotic (P=0.009) and lacunar (P=0.063) stroke groups than in controls.

The limitations of our study include the fact that plasma fibrinogen measurements were not available. However, several studies have shown that the A allele of the −455G/A polymorphism is associated with elevated plasma fibrinogen concentration and that A instead of G in the −455 position produces a 1.2- to 1.5-fold increase in fibrinogen Bβ chain transcription. The Bβ chain transcription is a rate-limiting step in the synthesis of the total functional fibrinogen molecule. Fibrinogen is synthesized in hepatocytes, and cytokines and growth factors participate in the process. Increased viscosity and higher available substrate quantity resulting from elevated plasma fibrinogen concentration may promote coagulation and act as a risk for small-vessel thrombotic occlusion, thus affecting the phenotype of the cerebral infarction.

Smoking is a risk factor for atherosclerosis, and it increases fibrinogen levels by increasing Bβ chain transcription similar to an ongoing low-grade acute-phase reaction. Along that line, we found an association between the A+ genotype and smoking on the occurrence of multiple lacunar infarcts, although the statistical significance was marginal (P=0.07). We believe that the A+ genotype and smoking, both of which increase fibrinogen levels, act synergistically to increase the risk of stroke and that hypertension participates in the atherosclerotic process by injuring vascular endothelial cells. In our study, hypertension showed a significant association with the A+ genotype as a predisposing factor for multiple lacunar infarcts. It has been shown that in hypertensive patients higher fibrinogen levels are associated with target-organ damage.

Our results may be explained by assuming that the A+ genotype and resulting increased fibrinogen concentration in circulation may contribute to the progression of atherosclerosis primarily in smaller cerebral arteries with slower blood flow rather than in large vessel. In this way, it may predispose to the development of occlusions in small cerebral arteries and finally to multiple lacunar infarcts. The present results also suggest that along with known risk factors,
genetic variation in fibrinogen synthesis seems to play a strong role both as a risk factor and as a modifying factor affecting stroke phenotype.

It is also known that cardiovascular disease phenotypes are complex and polygenic in nature and that disease phenotype is under the negative or positive influence of gene-environment interactions. Our study thus suggests that combining genetics with the traditional risk factors may increase diagnostic accuracy and provide possibilities for more targeted preventive interventions, progression indicators, and possibly effective treatments. These findings also suggest that high-risk groups could be screened for a prothrombotic variant associated with multiple lacunar infarcts. More research is needed to reveal the complex picture of gene-environment interactions.

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