Fibrinogen $\gamma'$ in Ischemic Stroke
A Case-Control Study

Elim Y.L. Cheung, MSc; Shirley Uitte de Willige, PhD; Hans L. Vos, PhD; Frank W.G. Leebeek, MD, PhD; Diederik W.J. Dippel, MD, PhD; Rogier M. Bertina, PhD; Moniek P.M. de Maat, PhD

**Background and Purpose**—To determine the contribution of fibrinogen $\gamma'$ levels and FGG haplotypes to ischemic stroke.

**Methods**—Associations between fibrinogen $\gamma'$ levels, fibrinogen $\gamma'/\text{total fibrinogen ratio}$, and FGG haplotypes with the risk of ischemic stroke were determined in 124 cases and 125 controls.

**Results**—Fibrinogen $\gamma'/\text{total fibrinogen ratio}$ was higher in patients than in controls during the acute phase of the stroke and lower in the convalescent phase 3 months after the stroke. FGG haplotype 3 (H3) was associated with a reduced risk of ischemic stroke (odds ratio 0.60; 95% CI, 0.38 to 0.94), but not with the fibrinogen $\gamma'/\text{total fibrinogen ratio}$. In contrast, FGG-H2 was associated with a decreased fibrinogen $\gamma'/\text{total fibrinogen ratio}$, but not with risk of stroke.

**Conclusions**—Fibrinogen $\gamma'/\text{total fibrinogen ratio}$ is associated with ischemic stroke, especially in the acute phase of the disease. In addition, FGG-H3 haplotype appears to be protective against ischemic stroke. (*Stroke*. 2008;39:000-000.)

**Key Words:** FGG haplotypes ■ fibrinogen $\gamma'/\text{total fibrinogen ratios}$ ■ ischemic stroke

Fibrinogen, a central protein in the hemostatic system, has a high degree of heterogeneity in healthy individuals. One variant, fibrinogen $\gamma'$, carries an extended $\gamma$ chain formed by alternative processing of the fibrinogen $\gamma$ pre-mRNA and comprises 7% to 15% of the fibrinogen molecules. Fibrinogen $\gamma'$ has both antithrombotic (binding sites for thrombin and disruption of platelet binding to fibrinogen) and prothrombotic properties (binding site for the factor XIII B subunit). Associations with deep venous thrombosis (DVT) and coronary artery disease indicate that plasma fibrinogen $\gamma'$ levels may contribute to the pathology of thrombotic disease. Fibrinogen $\gamma$ gene (FGG) variation is associated with fibrinogen $\gamma'$ levels, fibrinogen $\gamma'/\text{total fibrinogen ratio}$, risk of DVT and myocardial infarction (MI). The aim of this study was to determine the role of fibrinogen $\gamma'$ levels and common FGG gene variations in ischemic stroke.

**Patients and Methods**
We performed a case-control study of 124 first-ever ischemic stroke or transient ischemic attack (TIA) patients, and 125 population controls, aged 18 to 75 years old. The study design has been described previously. Baseline characteristics are given in Table 1. Blood was collected from an unselected subgroup of 47 patients 7 to 14 days (acute phase) and 3 months after the stroke in tubes containing 1/10 volume of 0.129 mol/L sodium citrate.

Fibrinogen $\gamma'$ antigen levels were measured by ELISA as described previously. Pooled normal plasma calibrated against purified human $\gamma'$ fibrinogen (a gift from Dr M. Mosesson, Blood Center of Wisconsin, Milwaukee, Wis) was used as calibrator. Total fibrinogen levels were measured according to von Clauss, and C-reactive protein (CRP) was measured using an in-house ELISA with polyclonal rabbit antihuman CRP antibodies (DAKO).

**Genetic Analyses**
We genotyped 3 haplotype-tagging single nucleotide polymorphisms (SNPs) that tag the total common genetic variation in FGG in whites (http://pga.gs.washington.edu). SNP 8486G>T (rs2066865, 10034C>T by SeattleSNPs) tagged FGG haplotype 2 (H2), SNP 7927T>C (rs1049636, 9340T>C by SeattleSNPs) tagged FGG-H3 and SNP 4228G>A (rs2066860, 5836G>A by SeattleSNPs) tagged FGG-H4. FGG-H1 was assigned to subjects who possessed the common alleles of the 3 SNPs. Annotation of the SNPs uses AF350254 as reference sequence with nucleotide +1 being the translation initiation nucleotide. We genotyped the 3 SNPs using 5’ nuclease/TaqMan assays (primer sequences available on request).

**Statistical Analyses**
Differences between groups were examined by analysis of variance (ANOVA) or by paired-samples $t$ test for acute phase versus convalescent phase in patients. We adjusted for risk factors for vascular diseases (smoking, hypertension, diabetes mellitus, and hyperlipidemia). The association between haplotypes, taking the haplotype ambiguity into account, and risk of stroke, fibrinogen $\gamma'$ level and fibrinogen $\gamma'/\text{total fibrinogen ratio}$ were determined using Haplo.stats version 1.2.2 (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html).
Table 1. Baseline Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients (n=124)</th>
<th>Controls (n=125)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>56 (±12)</td>
<td>56 (±12)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>58 (47%)</td>
<td>59 (47%)</td>
<td>NS</td>
</tr>
<tr>
<td>Index event</td>
<td>Stroke: TIA</td>
<td>115:9</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>61 (49%)</td>
<td>37 (30%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60 (48%)</td>
<td>24 (19%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18 (14%)</td>
<td>5 (4%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>78 (63%)</td>
<td>84 (67%)</td>
<td>0.700</td>
</tr>
<tr>
<td>Positive family history for cardiovascular disease</td>
<td>75 (61%)</td>
<td>56 (45%)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Data for age are presented as mean±SD. Other data are counts and (percentages). TIA indicates transient ischemic attack; NS, not significant.

Results

In the acute phase of stroke, the mean plasma fibrinogen γ’ level was higher in patients (0.42±0.17 g/L) than in controls (0.34±0.10 g/L; P<0.001; Table 2). The fibrinogen γ’/total fibrinogen ratio was calculated to adjust for the acute phase increase of fibrinogen, and this ratio was also significantly higher in patients (0.113±0.034) than in controls (0.100±0.029; P=0.002; Table 2). Adjustment for CRP and common cardiovascular risk factors did not influence these relationships.

In the samples collected 3 months after the event, the mean fibrinogen γ’ level and fibrinogen γ’/total fibrinogen ratio (0.29±0.09 g/L and 0.088±0.02 g/L, respectively) were significantly lower than during the acute phase of stroke, and also significantly lower than in controls (Table 2).

Carriers of the FGG-H3 allele had a significantly reduced risk of ischemic stroke (OR 0.60, 95% CI, 0.38 to 0.94). No clear association with stroke was observed for the other haplotypes (FGG-H1: reference; FGG-H2: OR 0.91; 95% CI, 0.58 to 1.43; FGG-H4: OR 0.75; 95% CI, 0.30 to 1.88).

Only FGG-H2 was associated with a significantly decreased fibrinogen γ’/total fibrinogen ratio in patients during the acute phase (decrease of 0.093±0.013 and 0.085±0.020, respectively, both P<0.001), in the convalescent phase (decrease of 0.060±0.015, P<0.001) and in controls (Figure).

Table 2. Fibrinogen γ’ Levels, γ’/Total Fibrinogen Ratio and Fibrinogen Levels in Patients and Controls

| | Patients | | Controls | | | |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Acute Phase N=114 | Convalescent Phase N=47 | | | | | | |
| Fibrinogen γ’ (g/L) | 0.42±0.17 | 0.29±0.09 | 0.34±0.10 | <0.001 | 0.01 | <0.001 |
| Fibrinogen γ’/total fibrinogen ratio | 0.113±0.034 | 0.088±0.020 | 0.100±0.029 | 0.002 | 0.01 | <0.001 |
| Fibrinogen (g/L) | 3.69±1.06 | 3.34±0.76 | 3.41±0.61 | 0.006 | 0.54 | 0.003 |
| CRP (mg/L) | 2.33 (0.49–8.18) | 1.15 (0.62–5.17) | 1.30 (0.61–3.18) | 0.007 | 0.11 | 0.85 |

Presented are means±SD or §: median and interquartile range (IQR); P*: P value between controls and patients in acute phase, P†: P value between controls and patients in convalescent phase, P‡: P value between patients in acute phase and in convalescent phase.

Discussion

Fibrinogen γ’ levels were elevated in the acute phase of ischemic stroke and decreased in the convalescent phase. The increase in the acute phase is probably a result of the event. The decrease in the convalescent phase may more reflect the prestroke levels, which would suggest that the antithrombotic properties of fibrinogen γ’ are more important than its prothrombotic properties. This seems to be in contrast with previous studies where increased fibrinogen γ’ levels or fibrinogen γ’/total fibrinogen ratios were associated with increased risk of arterial thrombotic disease,3,7 but these latter results may have been influenced by the acute phase. It is a limitation of our study that it has a relatively small sample size, especially for the unselected group of patients that has been followed-up in the convalescent phase.

Fibrinogen γ’/total fibrinogen ratio is only elevated in the acute phase of stroke and not in the convalescent phase suggesting that the acute phase affects alternative splicing as already suggested for other genes.

We observed that carriers of FGG-H3 have a reduced risk of ischemic stroke, but this is not consistent with previous studies.2,4,5,8 It is unclear what the underlying mechanism might be because FGG-H3 gave only a slight, nonsignificant increase of fibrinogen γ’ levels or fibrinogen γ’/total fibrinogen ratio was calculated to adjust for the acute phase increase of fibrinogen, and this ratio was also significantly higher in patients (0.113±0.034) than in controls (0.100±0.029; P=0.002; Table 2). Adjustment for CRP and common cardiovascular risk factors did not influence these relationships.
fibrinogen ratios. As expected, the fibrinogen γ'/total fibrinogen ratio was strongly reduced in FGG-H2, both in cases (acute phase and convalescent phase) and in controls. This observation may be explained by the improved cleavage stimulatory factor binding site near the γA specific polyadenylation site in the FGG-H2 allele, which may inhibit the formation of fibrinogen γ' specific mRNA. No relationship between FGG-H2 and risk of stroke was observed, which is consistent with previous studies.

In conclusion, this study shows that the fibrinogen γ'/total fibrinogen ratio is increased in the acute phase of stroke, which may reflect an antithrombotic defense mechanism of the human body. Additionally, carriers of the FGG-H3 haplotype appear to be protected against ischemic stroke.

**Sources of Funding**

This study was supported by grants from the Dutch program for Tissue Engineering, revolving fund from the Erasmus University Medical Center and grant 912-02-036 from the Netherlands Organization for Scientific Research (NWO).

**Disclosures**

None.

**References**

Fibrinogen γ in Ischemic Stroke. A Case-Control Study
Elim Y.L. Cheung, Shirley Uitte de Willige, Hans L. Vos, Frank W.G. Leebeek, Diederik W.J. Dippel, Rogier M. Bertina and Moniek P.M. de Maat

Stroke. published online January 31, 2008;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2008/01/31/STROKEAHA.107.495499.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/