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

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

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

Results—Fibrinogen $\gamma'/total$ 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 $\gamma'/total$ fibrinogen ratio. In contrast, $FGG$-H2 was associated with a decreased fibrinogen $\gamma'/total$ fibrinogen ratio, but not with risk of stroke.

Conclusions—Fibrinogen $\gamma'/total$ 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 $\gamma'/total$ 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 $\gamma'/total$ 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 with 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 anti-human 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 8486C>T (rs2066865, 10034C>T by SeattleSNPs) tagged $FGG$ haplotype 2 (H2), SNP 7792T>C (rs1049636, 9340T>C by SeattleSNPs) tagged $FGG$-H3 and SNP 4288G>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 $\gamma'/total$ fibrinogen ratio were determined using Haplo.Stats version 1.2.2 (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html).

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Results

In the acute phase of stroke, the mean plasma fibrinogen $\gamma'$ 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 $\gamma'$/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 $\gamma'$ level and fibrinogen $\gamma'$/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 $\gamma'$/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).

No association between FGG haplotypes and total fibrinogen levels was present. Adjustment for cardiovascular risk factors and CRP levels did not affect these relationships.

Discussion

Fibrinogen $\gamma'$ 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 $\gamma'$ are more important than its prothrombotic properties. This seems to be in contrast with previous studies where increased fibrinogen $\gamma'$ levels or fibrinogen $\gamma'$/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 $\gamma'$/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 $\gamma'$ levels or fibrinogen $\gamma'$/total fibrinogen ratios. In patients with ischemic stroke, the decrease in the convalescent phase may more reflect the prestroke levels, which would suggest that the antithrombotic properties of fibrinogen $\gamma'$ are more important than its prothrombotic properties. This seems to be in contrast with previous studies where increased fibrinogen $\gamma'$ levels or fibrinogen $\gamma'$/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 ratios. As expected, the fibrinogen \( \gamma' / \text{total fibrinogen ratio} \) 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 \( \gamma A \) specific polyadenylation site in the FGG-H2 allele, which may inhibit the formation of fibrinogen \( \gamma' \) specific mRNA.\(^8\) No relationship between FGG-H2 and risk of stroke was observed, which is consistent with previous studies.\(^4,8\)

In conclusion, this study shows that the fibrinogen \( \gamma' / \text{total fibrinogen ratio} \) 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.

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**Disclosures**
None.

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