Polymorphisms in Platelet Glycoprotein 1bα and Factor VII and Risk of Ischemic Stroke
A Meta-Analysis

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Background and Purpose—Platelets and components of the coagulation cascade are known to be instrumental in the pathogenesis of arterial occlusive disorders. The aim of this meta-analysis is to test the hypothesis that genetic variation in the platelet glycoprotein 1bα and Factor VII genes influence the occurrence of ischemic stroke. All genetic association studies that examined the R353Q (rs6046) polymorphism of the Factor VII gene and 2 polymorphisms of the platelet glycoprotein (1bα) gene (Thr/Met rs9251 and Kozak sequence –5 C/T rs2243093) in relation to ischemic stroke were examined.

Methods—Electronic databases Embase, Medline, and HuGEnet were searched for all years up until June 2006 for all studies that evaluated any of these candidate genes and stroke.

Results—Pooled ORs were calculated with 95% CIs using both fixed and random effects models. Meta-analysis for Factor VII (R353Q) did not detect any effect on ischemic stroke risk. Further estimation resulted in pooled OR1 QQ versus RR 0.9 (95% CI: 0.4 to 1.9) and pooled OR2 for RQ versus RR 0.9 (95% CI: 0.6 to 1.4). These results were robust and homogeneous. Pooling ORs for the platelet glycoprotein 1bα Kozak variant –5 T/C polymorphism showed extreme heterogeneity with differing effect directions across studies. Fisher’s method of pooling was therefore used to calculate a combined probability value, which was highly significant (P<0.001). The pooled OR for platelet glycoprotein 1bα Met/Met v Thr/Thr was 1.0 to 2.0, depending on the sensitivity analyses, and for Thr/Met versus Thr/Thr, the pooled OR was between 1.3 and 1.4. These results were consistent, reasonably robust, and implied a dominant genetic effect.

Conclusion—This analysis provides strong evidence that the Factor VII R353Q gene polymorphism is not associated with ischemic stroke, that the Thr/Met polymorphism of GP1bα is associated with ischemic stroke in a dominant genetic model, and that the Kozak sequence polymorphism of GP1bα may be close to another causative locus that is associated with ischemic stroke. (Stroke. 2008;39:000-000.)

Key Words: Factor VII ■ meta-analysis ■ platelet glycoprotein ■ polymorphism ■ stroke

Stroke is a complex multifactorial disease and is the third leading cause of death in Australia, accounting for 7.1% and 11.3% of all deaths in men and women, respectively.1 The occurrence of stroke in Australia has been estimated at up to 48 000 events each year2 and has been ranked as the fourth leading cause of burden and disease injury.1 Traditional risk factors for stroke are well described and include smoking, hypertension, diabetes, and high cholesterol; however, the underlying etiology of ischemic stroke cannot be determined in over 40% of patients.2 Evidence of genetic susceptibility for ischemic stroke is demonstrated in family studies,3 twin studies,4 animal models,5 and from epidemiological data derived from large prospective investigations such as the Framingham Study.6 Despite these indications, precise genetic risk factors and their impact on cerebrovascular disease remain largely unknown.

Polymorphisms in genes encoding for proteins involved in the thrombotic and thrombolytic cascades are plausible candidates for potential influence on stroke occurrence. This meta-analysis focuses on 2 particular components of these
cascades that have received particular attention, platelet glycoprotein1bα and Factor VII.

Platelet glycoprotein1bα is a major receptor for von Willebrand factor and thrombin and plays an important role in the initial development of thrombi. It is a transmembrane platelet glycoprotein with 3 adhesive ligands; von Willebrand factor, thrombin, and P-selectin. The platelet glycoprotein 1bα gene has several variants, including a −5 T/C dimorphism in the untranslated Kozak sequence, which has been shown to influence antigen expression levels, as well as 2 changes in the coding region, Thr/Met (alloantigen HPA-2) and a variable number tandem repeat sequence, which affect the structure of the protein. These polymorphisms have been targeted for novel antithrombotic strategies for ischemic stroke. Platelet glycoproteins play a crucial role in the initial stage of thrombus formation and without them, the interaction between von Willebrand factor and the glycoprotein complex would not be initiated. Alteration in the function of these integrins due to polymorphic variations may affect thrombus formation and it is therefore biologically plausible that platelet glycoprotein polymorphisms in 1bα such as Thr/Met and Kozak −5 T/C may play a role in the development of ischemic stroke.

Factor VII is a serine protease single chain glycoprotein and is an essential component in the initiation stage of coagulation. It contributes to the prothrombotic state and is the initial enzyme in this process. Circulating Factor VII binds to tissue factor and is activated to Factor VIIa. High plasma levels of Factor VII have been associated with increased risk for coronary heart disease. Many polymorphisms have been described for the Factor VII gene, including A1/A2 and R353Q (Arg353Gln or G10976A). The R353Q polymorphism is found in exon 8 of the Factor VII gene and results from a single base substitution of alanine by guanine in the second position of the codon for residue 353. This substitution leads to a change in the protein from arginine to glutamine. The A1/A2 polymorphism is a decanucleotide insertion/deletion mutation that occurs in the promoter region of the gene. Current evidence that supports the influence of polymorphisms in Factor VII on ischemic stroke risk is equivocal. In a meta-analysis of 3 studies, Casas et al examined the A1/A2 polymorphism and found no association for an increased risk of ischemic stroke. The R353Q polymorphism was examined in a prospective cohort study of initially healthy American men, the R353Q Factor VII polymorphism was of marginal significance and was not found to be an independent risk factor.

The publication of a number of new association studies investigating Factor VII and GPIbα in stroke led us to undertake meta-analyses of all published case–control association studies examining these possible risk factors.

Method

Electronic databases Embase, Medline, and HuGeNet were searched for all years up until June 2006 for all studies that evaluated any of these candidate genes and stroke. Review articles and meta-analysis were also included. Search terms included the following key words/MeSH headings: cerebrovascular accident, stroke, cerebrovascular infarction, brain infarction, and cerebral ischemia, glycoproteins, Factor VII, and polymorphism (s). The search was limited to human studies. For non-English articles, English abstracts were obtained and full papers translated to determine if the study should be included in the review process. Secondary references of review articles and meta-analyses were examined and any papers of interest by title or author were retrieved for possible inclusion. Studies were considered for inclusion if they examined ischemic stroke risk and platelet glycoprotein or Factor VII polymorphisms. Stroke subtyping was preferred, but studies were not excluded on that basis. Studies were excluded if they examined hemorrhagic stroke, but included if they examined both ischemic and hemorrhagic stroke and the data were presented separately for each classification. Only genetic association studies that used a case–control or cohort design and were published in peer-reviewed journals were reviewed. Studies were not excluded on the basis of the ethnicity of study participants.

Data Extraction

Genotype frequencies were extracted from each study by 2 reviewers (J.M. and J.A.) to determine cases and controls and confirmed by a third researcher (A.T.). Any disagreements were adjudicated by a third author (A.T.).

Quality Assessment of Studies

Studies were critiqued to assess for quality independently and in duplicate by 2 reviewers (J.M. and S.W.) using a standardized extraction form. Covariables such as mean age, gender, and ethnicity were also extracted for each study. Quality assessment included review for selection bias, case and control ascertainment methods, information bias, laboratory methods that were clearly described and quality measures addressed, calculation of Hardy-Weinberg equilibrium (HWE), and representativeness of cases and controls. Type of genetic statistical model used was noted if reported. Quality assessment scores were then calculated on each study; the total scores are reported in Table 1. Agreement was met between both reviewers (SW, JM). The studies included in the meta-analysis were sufficiently similar in regard to case–control design, ascertainment of cases and controls, and representativeness of cases. There was minor variation in control representativeness because controls were derived from both population and hospital sources (see Table 1).

Statistical Analysis

Allele frequency was described for each study. HWE was assessed using the exact test. Where studies were not in HWE, degree of disequilibrium was calculated and adjusted for using methods previously described. Continuity correction was performed by adding 0.5 for those studies that had cells with zero counts. Pooling was performed according to methods previously described. In brief, heterogeneity was assessed for OR9 (AA versus aa) and OR10 (Aa versus aa) using a Q test where A and a are the 2 alleles at a polymorphic site. Overall gene effect across all 3 genotype groups (eg, AA, Aa, aa) was then assessed using logistic regression. If this was positive, we went on to estimate pooled ORs. Where the degree of heterogeneity across studies was high (ie, I²>75%), we used Fisher’s method for pooling probability values as described previously. All analyses were performed using STATA 9.0 (STATA Corp, College Station, Texas). A probability value <0.05 was considered statistically significant, except for heterogeneity where P<0.1 was used.

Results

Seventeen papers were identified that examined platelet glycoprotein 1bα polymorphisms. Four of these did not examine the polymorphism of interest. One study in Japanese and another in Chinese were eligible for inclusion based on the abstract and English translations were sought. One study examined the Kozak sequence polymorphism, but genotype counts could not be obtained despite attempts to contact the authors. This study was therefore excluded from the analysis. Among the remaining 12 studies, 7 studies
focused on Thr/Met (rs6065) polymorphism, 7, 20, 21, 23–26, 3 studies examined Kozak sequence /H11002 5 T/C (rs2243093) polymorphism, 27–29 and 2 studies examined both polymorphisms 30, 31 (see Table 1). The more recent (2001) Sonoda paper 31 used the same data as their earlier paper (2000) with slightly more cases23 to conduct a haplotype analysis on Thr/Met and Kozak sequence /H11002 5 T/C polymorphism; hence, our meta-analysis used the 2001 study for both Kozak sequence /H11002 5 T/C and Thr/Met polymorphisms. Therefore, 8 studies were included in the meta-analysis for Thr/Met.

**Table 1. Characteristics of Studies Included in Meta-Analyses That Examine Platelet Glycoprotein Polymorphisms Thr-Met (rs6065) and/or Kozak Sequence – 5 T/C (rs2243093) and Factor VII Polymorphism R353Q (rs6046) and Ischemic Stroke Risk**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Polymorphism</th>
<th>Study Design</th>
<th>Percent Males, Case</th>
<th>Percent Males, Control</th>
<th>Age, Years (mean)</th>
<th>Ethnicity</th>
<th>Source of Controls</th>
<th>Stroke Subtype</th>
<th>Analysis QA</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlsson</td>
<td>1997</td>
<td>Thr-Met</td>
<td>CC</td>
<td>47</td>
<td>54</td>
<td>60.3</td>
<td>German</td>
<td>Hospital</td>
<td>No</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Gonzalez-Conejero</td>
<td>1998</td>
<td>Thr-Met</td>
<td>CC</td>
<td>52</td>
<td>52</td>
<td>65.8</td>
<td>Mediterranean White</td>
<td>Hospital</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Reiner</td>
<td>2000</td>
<td>Thr-Met</td>
<td>CC</td>
<td>0</td>
<td>0</td>
<td>37.8</td>
<td>White</td>
<td>Population</td>
<td>No</td>
<td>7</td>
<td>Yes</td>
</tr>
<tr>
<td>Chen</td>
<td>2000</td>
<td>Thr-Met</td>
<td>CC</td>
<td>78</td>
<td>50</td>
<td>18–86†</td>
<td>Chinese</td>
<td>Population</td>
<td>No</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Baker</td>
<td>2001</td>
<td>Kozak Thr-Met</td>
<td>CC</td>
<td>64</td>
<td>64</td>
<td>66.6</td>
<td>White</td>
<td>Population</td>
<td>Yes</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>Sonoda</td>
<td>2001</td>
<td>Kozak Thr-Met</td>
<td>CC</td>
<td>79*</td>
<td>71</td>
<td>62.5</td>
<td>Japanese</td>
<td>Hospital staff</td>
<td>Yes</td>
<td>7</td>
<td>Yes</td>
</tr>
<tr>
<td>Ishii</td>
<td>2004</td>
<td>Thr-Met</td>
<td>CC</td>
<td>76</td>
<td>76</td>
<td>58.5</td>
<td>Japanese</td>
<td>Blood donors</td>
<td>Yes</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Gao</td>
<td>2005</td>
<td>Kozak Thr-Met</td>
<td>CC</td>
<td>71</td>
<td>71</td>
<td>60.9</td>
<td>Chinese Han</td>
<td>Hospital</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Corral</td>
<td>2000</td>
<td>Kozak</td>
<td>CC</td>
<td>52</td>
<td>52</td>
<td>65.7</td>
<td>Mediterranean White</td>
<td>Hospital</td>
<td>No</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Frank</td>
<td>2001</td>
<td>Kozak</td>
<td>CC</td>
<td>0</td>
<td>0</td>
<td>39.0</td>
<td>White, African American, Other</td>
<td>Population</td>
<td>No</td>
<td>8</td>
<td>No</td>
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<tr>
<td>Hsieh</td>
<td>2004</td>
<td>Kozak</td>
<td>CC</td>
<td>53</td>
<td>52</td>
<td>60.1</td>
<td>White</td>
<td>Population</td>
<td>No</td>
<td>9</td>
<td>Yes</td>
</tr>
<tr>
<td>Heywood</td>
<td>1997</td>
<td>R353Q</td>
<td>CC</td>
<td>53</td>
<td>60</td>
<td>73.2</td>
<td>White</td>
<td>Population</td>
<td>Yes</td>
<td>9</td>
<td>No</td>
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<tr>
<td>Nishiuma</td>
<td>1997</td>
<td>R353Q</td>
<td>CC</td>
<td>45</td>
<td>52</td>
<td>68.2</td>
<td>Japanese</td>
<td>Population</td>
<td>Yes</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Kain</td>
<td>2002</td>
<td>R353Q</td>
<td>CC</td>
<td>64</td>
<td>51</td>
<td>52.7</td>
<td>South Asian</td>
<td>Population</td>
<td>No</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Yeh</td>
<td>2004</td>
<td>R353Q</td>
<td>CC</td>
<td>75</td>
<td>84</td>
<td>44.6</td>
<td>Taiwanese</td>
<td>Hospital</td>
<td>Yes</td>
<td>6</td>
<td>No</td>
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<tr>
<td>Zee</td>
<td>2004</td>
<td>R353Q</td>
<td>Nested CC</td>
<td>100</td>
<td>100</td>
<td>58.7</td>
<td>White</td>
<td>Population</td>
<td>Yes</td>
<td>9</td>
<td>Yes</td>
</tr>
<tr>
<td>Rubattu</td>
<td>2005</td>
<td>R353Q</td>
<td>CC</td>
<td>60</td>
<td>57</td>
<td>74.0</td>
<td>Mediterranean White</td>
<td>Hospital</td>
<td>Yes</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

*Gender data reported in earlier paper.  
†Age range.  
QA indicates total quality score; CC, case–control.

Platelet Glycoprotein 1bα Polymorphisms

**Thr/Met Variant**

Genotype distributions are shown in Table 2 for the 8 studies that were used in the meta-analysis for the Thr/Met variant. In total, there were 1287 cases and 1774 controls. Two studies 21, 30 were not in HWE and thus were not included in final pooling. No heterogeneity was observed for OR1 (Met/Met versus Thr/Thr, $\chi^2=4.53$, df=5, $P=0.475$), but there was moderate heterogeneity with degree of heterogeneity (I²) of 58% for OR2 (Thr/Met versus Thr/Thr, $\chi^2=11.84$, df=5, $P=0.001$).

**Table 2. Genotype Frequencies for Genetic Association Studies That Examine the Glycoprotein 1bα Polymorphism Thr/Met Included in the Meta-Analysis**

<table>
<thead>
<tr>
<th>Lead Author</th>
<th>Year</th>
<th>No. of Cases/Controls</th>
<th>Stroke TT</th>
<th>Stroke TM</th>
<th>Stroke MM</th>
<th>Controls TT</th>
<th>Controls TM</th>
<th>Controls MM</th>
<th>HWE*§</th>
<th>OR1 (95% CI)</th>
<th>OR2 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlsson</td>
<td>1997</td>
<td>218/165</td>
<td>181</td>
<td>34</td>
<td>3</td>
<td>134</td>
<td>31</td>
<td>0</td>
<td>0.365</td>
<td>5.2 (0.3–101.2)</td>
<td>0.8 (0.1–5.8)</td>
</tr>
<tr>
<td>Gonzalez-Conejero</td>
<td>1998</td>
<td>104/104</td>
<td>81</td>
<td>22</td>
<td>1</td>
<td>93</td>
<td>10</td>
<td>1</td>
<td>0.285</td>
<td>1.1 (0.1–18.6)</td>
<td>2.5 (0.4–17.9)</td>
</tr>
<tr>
<td>Sonoda</td>
<td>2001</td>
<td>235/317</td>
<td>177</td>
<td>56</td>
<td>2</td>
<td>272</td>
<td>43</td>
<td>2</td>
<td>0.684</td>
<td>1.5 (0.2–11.0)</td>
<td>2.0 (0.3–14.2)</td>
</tr>
<tr>
<td>Reiner</td>
<td>2000</td>
<td>36/346</td>
<td>28</td>
<td>6</td>
<td>2</td>
<td>290</td>
<td>54</td>
<td>2</td>
<td>1.000</td>
<td>10.4 (1.4–76.3)</td>
<td>1.2 (0.2–8.1)</td>
</tr>
<tr>
<td>Chen</td>
<td>2000</td>
<td>188/270</td>
<td>184</td>
<td>3</td>
<td>1</td>
<td>240</td>
<td>26</td>
<td>4</td>
<td>0.014</td>
<td>0.3 (0.0–2.9)</td>
<td>0.2 (0.1–0.5)</td>
</tr>
<tr>
<td>Baker</td>
<td>2001</td>
<td>206/191</td>
<td>175</td>
<td>31</td>
<td>0</td>
<td>169</td>
<td>19</td>
<td>3</td>
<td>0.034</td>
<td>0.1 (0.0–2.7)</td>
<td>1.6 (0.2–11.1)</td>
</tr>
<tr>
<td>Ishii</td>
<td>2004</td>
<td>200/281</td>
<td>147</td>
<td>51</td>
<td>2</td>
<td>228</td>
<td>49</td>
<td>4</td>
<td>0.505</td>
<td>0.8 (0.1–4.3)</td>
<td>1.6 (1.0–2.5)</td>
</tr>
<tr>
<td>Gao</td>
<td>2005</td>
<td>100/100</td>
<td>88</td>
<td>12</td>
<td>0</td>
<td>84</td>
<td>16</td>
<td>0</td>
<td>1.000</td>
<td>1.0 (0.0–48.6)</td>
<td>0.7 (0.1–5.1)</td>
</tr>
</tbody>
</table>

*HWE exact test assessed in controls.
Logistic regression analysis with the random effect model was therefore applied and found a significant overall gene effect (likelihood ratio $= 10.89$, $P = 0.004$). The pooled OR$_1$ and OR$_2$ were 2.08 (95% CI: 0.78 to 5.54) and 1.43 (95% CI: 1.13 to 1.81), respectively (see Figures 1 and 2), ie, patients who had the Met/Met and Thr/Met genotypes demonstrated approximately 2 and 1.4 times higher risk of stroke than patients with genotype Thr/Thr. Sensitivity analysis was performed by including the Chen$^{21}$ and Baker$^{30}$ studies; this resulted in pooled OR$_1$ and OR$_2$ of 1.05 (95% CI: 0.47 to 2.36) and 1.27 (95% CI: 1.03 to 1.56), respectively. Adjusting all studies for HWE yielded pooled OR$_1$ and OR$_2$ estimates of 1.26 (95% CI: 0.55 to 2.88) and 1.34 (95% CI: 1.08 to 1.66), respectively. Although there was moderate heterogeneity in OR$_2$, pooling using fixed and random effects models yielded similar results. These results are consistent and reasonably robust in indicating a genetic effect and possibly point to a dominant genetic model, although the magnitude of the homozygous Met/Met genetic effect varies considerably in the sensitivity analysis and is also consistent with an additive genetic model.

**Kozak Variant**

Overall, there were 1984 cases and 1932 controls included in the analysis for this variant. Three studies$^{29–31}$ were not in HWE; given that this was too high a number to do sensitivity analysis, we included all studies and adjusted for degree of disequilibrium (see Table 3). Heterogeneity was high ($\chi^2 = 27.9$, df$=4$, $P<0.001$) with the direction of the genetic effect going from risk-increasing to protective. Although variability may have been due to methodological differences between individual studies (see Table 1), we interpreted it as an indication of varying linkage disequilibrium (LD) structure across the populations in the studies. In this case, Fisher’s method can combine the probability values across studies without reference to the direction of the effect; results of Fisher’s method in this case were highly statistically significant ($\chi^2 = 45.2$, $P = 4.536 \times 10^{-8}$).

**Factor VII Variant Arg353Gln**

Nine studies were identified that looked at the Factor VII R353Q (rs6046) polymorphism. Three of these did not publish sufficient data for analysis.$^{32–34}$ This left 6 studies to be included in the meta-analysis.$^{11,16,35–38}$ (see Table 1). Genotype frequencies between cases and controls are described in Table 4 and used 1537 cases and 3133 controls. One study$^{38}$ was not in HWE and thus 5 studies were pooled. There was no evidence of heterogeneity for both OR$_1$ (QQ versus RR, $\chi^2 = 1.45$, df$=4$, $P = 0.836$) and OR$_2$ (RQ versus QQ versus RR, $\chi^2 = 1.45$, df$=4$, $P = 0.836$) and OR$_2$ (RQ versus QQ versus RR, $\chi^2 = 1.45$, df$=4$, $P = 0.836$).
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RR, $\chi^2=2.31$, df=4, $P=0.678$). The fixed effect model was applied for pooling and the pooled OR, and OR$_1$ were further estimated, ie, 0.9 (95% CI: 0.4 to 1.9) and 0.9 (95% CI: 0.6 to 1.4), respectively. Including the study not in HWE yielded very similar results with OR$_1$ and OR$_2$ of 0.9 (95% CI: 0.8 to 1.1) and 0.9 (95% CI: 0.6 to 1.2), respectively; adjusting for HWDE also gave similar results with OR$_1$ and OR$_2$ of 0.9 (95% CI: 0.6 to 1.3) and 1.0 (95% CI: 0.7 to 1.3), respectively. These results consistently indicate no effect of this polymorphism on occurrence of ischemic stroke.

### Discussion

The previous inconsistent results of studies that examine platelet glycoprotein 1bα and Factor VII polymorphisms and risk of ischemic stroke have been attributed to methodological factors such as small samples sizes, poorly matched cases and controls, or variation between ethnic populations. The aim of this meta-analysis was to calculate a pooled OR from identified studies and largely address the issue of small sample size.

#### Platelet Glycoprotein 1bα Polymorphisms

To date, results for the Kozak sequence polymorphism were conflicting. For example, Hsieh and colleagues using data from the Vienna Stroke Registry found that patients who were homozygous for the CC genotype had a 3.5-fold increased risk for ischemic cerebrovascular events (95% CI: 1.5 to 7.9, $P=0.0003$) compared with the TT or TC genotype carriers. Streifler investigated whether the development of stroke or transient ischemic attack in patients with significant carotid stenosis (>50% occlusion) was associated with several platelet glycoprotein polymorphisms. They compared symptomatic and asymptomatic patients and found no significant differences in the frequency of the Kozak sequence polymorphism between the 2 groups. In another study, there was no increase in risk of nonfatal stroke and myocardial infarction in carriers of the Kozak sequence C-allele.

This meta-analysis indicates that the Kozak sequence −5T/C polymorphism demonstrates a strong association with risk of ischemic stroke but that the direction of the association is highly variable. Population variation across ethnicities is one possible interpretation of variability; however, Lohmueller et al showed that stratifying for ethnicity does not necessarily remove or diminish heterogeneity and Ioannides et al showed that although allele frequencies varied, the vast majority of genetic associations were consistent across ethnic groups in terms of magnitude. Another interpretation of this variability of direction of association is variation in the LD structure across the populations in the studies, ie, the Kozak locus is close to another, presumably causative, locus, and in some populations, one Kozak allele is in LD with the causative allele and in other populations, the other Kozak allele is in LD with the causative allele. In this case, Fisher’s method can combine the probability values across studies without reference to the direction of the effect. In essence, this answers the question “Is there an association signal for this locus?” without reference to which allele is linked. The results from this meta-analysis indicate that there is an association signal present that is worth pursuing, but that the true causative locus may be nearby in LD with the Kozak sequence −5 T/C polymorphism.

The second polymorphism examined in glycoprotein 1bα is the C/T transition, which results in an amino acid change at

### Table 3. Allele Frequencies of Kozak Variant (rs2243093) and Association Between Cases and Controls

<table>
<thead>
<tr>
<th>Lead Author</th>
<th>Year</th>
<th>T</th>
<th>C</th>
<th>OR1 (95% CI)</th>
<th>OR2 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corral</td>
<td>2000</td>
<td>184</td>
<td>24</td>
<td>1.0 (0.2–5.6)</td>
<td>1.0 (0.2–5.9)</td>
</tr>
<tr>
<td>Baker</td>
<td>2001</td>
<td>336</td>
<td>74</td>
<td>1.4 (0.2–10.1)</td>
<td>0.8 (0.1–5.7)</td>
</tr>
<tr>
<td>Frank</td>
<td>2001</td>
<td>70</td>
<td>12</td>
<td>0.3 (0–2.1)</td>
<td>0.6 (0.1–4.3)</td>
</tr>
<tr>
<td>Sonoda</td>
<td>2001</td>
<td>349</td>
<td>121</td>
<td>1.3 (0.2–8.9)</td>
<td>1.5 (0.2–10.3)</td>
</tr>
<tr>
<td>Hsieh</td>
<td>2004</td>
<td>2415</td>
<td>383</td>
<td>0.3 (0–2.1)</td>
<td>0.6 (0.1–4.3)</td>
</tr>
</tbody>
</table>

*HWE exact test assessed in controls.
†P value for assessing association.

### Table 4. Genotype Frequencies for Genetic Association Studies That Examine the Factor VII Polymorphism R353Q (RS6046) Included in the Meta-Analysis

<table>
<thead>
<tr>
<th>Lead Author</th>
<th>Year</th>
<th>No. of Cases/Controls</th>
<th>RR</th>
<th>RQ</th>
<th>QQ</th>
<th>OR1 (95% CI)</th>
<th>OR2 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zee</td>
<td>2004</td>
<td>319/2902</td>
<td>256</td>
<td>59</td>
<td>4</td>
<td>1.5 (0.2–11.1)</td>
<td>1.4 (0.2–9.9)</td>
</tr>
<tr>
<td>Nishiuma</td>
<td>1997</td>
<td>118/97</td>
<td>106</td>
<td>12</td>
<td>0</td>
<td>0.8 (0.1–6.0)</td>
<td>0.9 (0.1–6.0)</td>
</tr>
<tr>
<td>Rubattu</td>
<td>2005</td>
<td>294/286</td>
<td>182</td>
<td>104</td>
<td>8</td>
<td>1.0 (0.2–8.9)</td>
<td>1.5 (0.2–10.3)</td>
</tr>
<tr>
<td>Yeh</td>
<td>2004</td>
<td>213/200</td>
<td>197</td>
<td>16</td>
<td>0</td>
<td>0.3 (0–2.1)</td>
<td>0.6 (0.1–4.3)</td>
</tr>
<tr>
<td>Heywood</td>
<td>1997</td>
<td>317/198</td>
<td>249</td>
<td>63</td>
<td>5</td>
<td>0.749</td>
<td>0.8 (0.1–5.7)</td>
</tr>
<tr>
<td>Kain</td>
<td>2002</td>
<td>294/280</td>
<td>114</td>
<td>105</td>
<td>75</td>
<td>0.000</td>
<td>1.0 (0.2–5.9)</td>
</tr>
</tbody>
</table>

*HWE exact significance test.
position 145 (threonine/methionine). Ethnic variation of this polymorphism frequency has been reported. For example, the Met/Met genotype is absent in the Chinese Han ethnic group, whereas the Thr/Met heterozygote has a low population frequency of 6.2%. The platelet alloantigen system has several antigens and the Thr/Met polymorphism is the molecular basis for HPA2; 2a/2b. The HPA-2a C and D allele associated with Thr/Met are present in 80% (C allele) and 10% (D allele) of the white population. It is apparent that the presence of this variant may vary widely depending on the population being investigated.

Several modestly sized studies have examined the platelet glycoprotein polymorphism Thr/Met and possible associations with ischemic stroke. Our pooled results indicate that the presence of one or more Met alleles may in fact confer some increase in risk for ischemic stroke. This finding is likely due to the increased power that results from pooling. As reviewed previously, individual studies tended to find ORs that were suggestive of association but did not reach statistical significance, although cases did appear to have the Met allele at higher frequencies.

The main results and the sensitivity analyses tend to suggest a dominant genetic model, although the CIs are wide and are also consistent with an additive genetic model, also called codominant, which means that there is a gene–dose effect.

**Factor VII Polymorphisms**

Two polymorphisms have been identified in the Factor VII gene, A1A2 and R353Q, and both have been investigated in terms of their contribution to ischemic stroke risk. It is known that these 2 polymorphisms, A1/A2 and R353Q, are in LD ($\Delta>0.7$). The implications of this LD are that the 2 risk alleles are associated in some way to linked loci or are both located close to another’s causative locus. It has been suggested that investigation of adjacent markers on the same gene may confirm the nature of an association being either causal or due to LD. An association detected on one allele may be interpreted to a degree by associations seen in the other allele. In the case of Factor VII A1A2, repeated studies across multiple different ethnic groups have shown no association with ischemic stroke risk, although it was not clear whether this was a true-negative or a finding driven by lack of power due to small sample sizes. Indeed, Casas et al examined the A1/A2 polymorphism in a meta-analysis of 3 studies and found no association with ischemic stroke in a combined sample ($n=545$) acknowledged by the authors as being limited. The robust and homogeneous nature of our results combined with the presence of LD between A1A2 and R353Q strengthen our interpretation of the findings of the present meta-analysis of 5 studies on the Factor VII gene R353Q polymorphism as a true-negative.

**Methodological Issues**

It is worth noting that we have chosen to pool studies across ethnic groups. For example, 2 of the studies that looked at the Kozak $-5$ T/C sequence polymorphism were taken from a Japanese study population, whereas the remaining studies consisted of white participants. The population frequency for the Kozak $-5$ T/C sequence polymorphism has been described as 17% in Japanese and 8% in whites, although an Australian study found the white frequency to be somewhat higher at 22.8%. Although ethnicity can obviously influence allele frequency, current evidence indicates that if the allele is present, the magnitude of the effect is likely to be similar across ethnicities because the biological mechanism is likely to be the same.

One study in the meta-analysis for Kozak sequence $-5$ T/C polymorphism used only female participants. To date, evidence suggests that most claims of gender-related differences in genetic association studies are either spurious or insufficiently documented; therefore, we did not consider gender to be a strong contributing factor to heterogeneity.

It is also worth noting that this meta-analysis also nicely encapsulates the range of answers that meta-analysis of genetic association studies can provide, robust negative results (Factor VII), robust positive results (GP1bα Thr/Met), or heterogeneity and remaining uncertainty. In the latter case, the use of Fisher’s method can indicate that there is an association signal somewhere in a genomic area, but that the causative allele is perhaps in LD with the polymorphism that is genotyped. Fisher’s method pools probability values rather than ORs; this can be useful where the ORs vary from protective to risk increasing and where usual methods might falsely indicate a pooled OR around 1. However, the caveat is that Fisher’s method is particularly susceptible to publication bias; a positive signal may simply reflect strongly significant studies published regardless of whether they indicated a risk or a protective effect.

In conclusion, we find that the Thr/Met polymorphism of GP1bα is strongly associated with ischemic stroke, likely in a dominant or additive manner, that the Factor VII gene polymorphism is not associated with ischemic stroke, and that the Kozak sequence polymorphism of GP1bα may be close to another causative locus that is associated with ischemic stroke.

**Source of Funding**

J.M.M.’s PhD candidature is supported by an Australian Postgraduate Award from The University of Newcastle, Newcastle, Australia.

**Disclosures**

None.

**References**

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A Meta-Analysis

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Stroke, published online April 10, 2008;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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