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 rs6065 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
increased risk for coronary heart disease. Many plasma levels of Factor VII have been associated with binds to tissue factor and is activated to Factor VIIa. High significance and was not found to be an independent risk factor.16 The R353Q Factor VII polymorphism was of marginal significance and was not found to be an independent risk factor.16 Although the sample size was limited to 545 cases. In a case–control study, no association for an increased risk of ischemic stroke, although the sample size was limited to 545 cases. In a meta-analysis of 3 studies,11,13,14 a third author (A.T.). Any disagreements were adjudicated by a third researcher (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. Covariates 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 (S.W., JM). The studies included in the meta-analysis were sufficiently similar in regard to case–control designs, 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.20 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.3 In brief, heterogeneity was assessed for ORs (AA versus aa) and ORs (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.21 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.20,21 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.22 Among the remaining 12 studies, 7 studies
focused on Thr/Met (rs6065) polymorphism, 7,20,21,23–26, 3 studies examined Kozak sequence/H110025 T/C (rs2243093) polymorphism,27–29 and 2 studies examined both polymorphisms30,31 (see Table 1). The more recent (2001) Sonoda paper31 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 polymorphism; hence, our meta-analysis used the 2001 study for both Kozak sequence/H11002 and Thr/Met polymorphisms. Therefore, 8 studies were included in the meta-analysis for Thr/Met.

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 studies21,30 were not in HWE and thus were not included in final pooling. No heterogeneity was observed for OR1 (Met/Met versus Thr/Thr, \( \chi^2 = 5.2, df = 5, P = 0.475 \)), but there was moderate heterogeneity with degree of heterogeneity (I\(^2\)) of 58% for OR2 (Thr/Met versus Thr/Thr, \( \chi^2 = 11.84, df = 5, P = 0.001 \)).
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 OR1 and OR2 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 Chen21 and Baker30 studies; this resulted in pooled OR1 and OR2 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 OR1 and OR2 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 OR2, 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 studies29–31 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 (\( \chi^2 = 45.2, P = 4.536 \times 10^{-08} \)).

**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 study38 was not in HWE and thus 5 studies were pooled. There was no evidence of heterogeneity for both OR1 (QQ versus RR, \( \chi^2 = 1.45, \) df = 4, \( P = 0.836 \)) and OR2 (RQ versus
carotid stenosis (50% occlusion) was associated with severe stroke or transient ischemic attack in patients with significant sample size.

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 sample sizes, poorly matched cases and controls, or variation between ethnic populations. The aim of this meta-analysis was to calculate a pooled OR from cases and controls, or variation between ethnic populations. The analysis also gave similar results with OR1 and OR2 of 0.9 (95% CI: 0.6 to 1.2), respectively. These results consistently indicate no effect of this polymorphism on occurrence of ischemic stroke.

**Discussion**

The fixed effect model was applied for pooling and the pooled OR, and OR2 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 OR1 and OR2 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 OR1 and OR2 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.

**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. Streiffer 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 5’T/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 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>
<th>HWE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corral</td>
<td>2000</td>
<td>184</td>
<td>24</td>
<td>1.5 (0.2–11.1)</td>
<td>1.4 (0.2–9.9)</td>
<td>0.047</td>
</tr>
<tr>
<td>Baker</td>
<td>2001</td>
<td>336</td>
<td>74</td>
<td>1.0 (0.2–5.9)</td>
<td>1.0 (0.2–5.9)</td>
<td>0.271</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>
<td>0.550</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>
<td>0.001</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>
<td>0.070</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>QO</th>
<th>QQ</th>
<th>OR1 (95% CI)</th>
<th>OR2 (95% CI)</th>
<th>HWE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zee</td>
<td>2004</td>
<td>319/2992</td>
<td>256</td>
<td>59</td>
<td>4</td>
<td>1554</td>
<td>500</td>
<td>0.854</td>
</tr>
<tr>
<td>Nishiuma</td>
<td>1997</td>
<td>118/97</td>
<td>106</td>
<td>12</td>
<td>0</td>
<td>86</td>
<td>11</td>
<td>1.000</td>
</tr>
<tr>
<td>Rubattu</td>
<td>2005</td>
<td>294/286</td>
<td>182</td>
<td>104</td>
<td>8</td>
<td>203</td>
<td>76</td>
<td>1.000</td>
</tr>
<tr>
<td>Yeh</td>
<td>2004</td>
<td>213/200</td>
<td>197</td>
<td>16</td>
<td>0</td>
<td>176</td>
<td>23</td>
<td>0.550</td>
</tr>
<tr>
<td>Heywood</td>
<td>1997</td>
<td>317/198</td>
<td>249</td>
<td>63</td>
<td>5</td>
<td>149</td>
<td>47</td>
<td>0.749</td>
</tr>
<tr>
<td>Kain</td>
<td>2002</td>
<td>294/280</td>
<td>114</td>
<td>105</td>
<td>75</td>
<td>107</td>
<td>98</td>
<td>0.000</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; a2a/b2b. 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, A1A2 and R353Q, are in LD (Δ>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 A1A2 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 −5T/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.

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**Disclosures**

None.

**References**

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