Intronic c.573+79G>A polymorphism of protein Z gene in haemorrhagic and ischaemic stroke

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Dear Sir,

Protein Z (PZ) down-regulates coagulation by enhancing the inhibition of activated factor X by PZ-dependent protease inhibitor (1). Theoretically, increased blood levels of PZ should predispose to bleeding, although conflicting association between blood PZ concentration, thrombotic phenotype and bleeding tendency has been reported in clinical studies (2–8).

The A allele at the PZ gene (PROZ) polymorphism c.573+79G>A (IVS6+79G/A or intron F G79A) is consistently associated with lower PZ concentration and activity in plasma (9–11). Lichy et al. have reported that the A allele was more prevalent in controls than in young patients with ischaemic stroke, suggesting a protective effect for cerebral ischaemia of this allele (9). More recently, Staton et al. showed no significant association with this polymorphism in an unselected population of ischaemic stroke patients (10). Here, we assess whether the distribution of c.573+79G>A genotypes differs in haemorrhagic compared with ischaemic stroke patients or asymptomatic controls in a Spanish population. We studied 156 consecutive patients presenting with haemorrhagic stroke associated with ischaemic stroke patients or asymptomatic controls in a Spanish population. We studied 156 consecutive patients with acute primary haemorrhagic stroke (HS) admitted to our Stroke Unit. Also, 390 patients with acute ischaemic stroke (IS) and 147 subjects without prior history of cerebrovascular symptoms (asymptomatic controls) were assessed as control groups. All patients had at least a brain CT scan at hospital admission. A brain-MRI was also performed in 273 patients. Haemorrhagic stroke was defined on CT scan as a homogenous and well defined area of increased density in the brain parenchyma. Patients with mixed areas of hypodense and hyperdense signals consistent with haemorrhagic transformation of an ischaemic infarction were not included in the study, because this condition is on occasion difficult to differentiate from HS. Additionally, patients presenting with a haemorrhagic stroke associated to atrial fibrillation were excluded in order to avoid the possibility of an early haemorrhagic transformation of an ischaemic stroke. Haemorrhagic stroke due to anticoagulants was also excluded from the study. Asymptomatic controls were recruited among patients’ partners or by random-digit dialing of the same geographic area.

Age, gender and vascular risk factors (current smoking, arterial hypertension, diabetes, and hypercholesterolemia) were recorded as previously described (12). All patients received a clinical workup in order to classify stroke subtypes. Haemorrhagic stroke was further dichotomized as lobar (frontal, parietal, temporal occipital or cerebellar cortex, n=43) or deep (brainstem, basal ganglia, internal capsule, or dentatus nucleus, n=105). In eight patients with HS both deep and lobar territories were involved. Ischaemic strokes were classified according to TOAST criteria (12). Informed consent was obtained from patients and controls and the study was approved by the local Ethics Committee. Demographic data are shown in Table 1A. Patients with HS were significantly older than IS or asymptomatic controls. Moreover, the prevalence of males was higher in patients with ischaemic stroke compared to controls or HS patients. As expected, stroke patients have a higher prevalence of vascular risk factors than controls.

DNA was isolated from venous blood. The c.573+79G>A polymorphism of the PROZ gene was analyzed by polymerase chain reaction (PCR) amplification as described (9). The two alleles PZ*A and PZ*G were identified after digestion of the PCR product with Hpa I (MBI Fermentas). Categorical variables were compared using the χ2-test or Fisher’s exact test. Logistic regression models were used to determine the independent association of the PROZ c.573+79 G>A polymorphism and stroke subtype. All statistical analysis was performed with SPSS v12.

Table 1B shows the allele and genotype frequencies, the later being consistent with Hardy-Weinberg equilibrium. The prevalence of the A allele in our control population was slightly lower (22 vs. 23 to 24%) than in other studies (9–11). Among patients, the prevalence of the A allele was lower in HS patients compared with asymptomatic controls (14.7% vs. 22.1%; P=0.02), or with IS patients (14.7% vs. 20.3%; P=0.03), while it was similar in IS and asymptomatic controls (20.3% vs. 22.1%; P=0.17). The frequency of the A allele did not differ in lobar or deep HS. As also shown in Table 1B, logistic regression models indicated homozygosity for the A allele as a protective factor for HS (OR 0.20, 95% CI: 0.04–0.97), although it did not reach statistical signifi-
cance when adjusted for age, current smoking, history of hypertension, and diabetes (OR 0.21, 95% CI: 0.04–1.10). No overall difference in the distribution of c.573+79G>A PROZ genotypes in patients with IS and controls was found, even when stroke subtypes were analyzed (not shown).

In summary, the c.573+79G>A polymorphism of the PROZ gene has been assessed in HS for the first time, as well as in a large population of ischaemic stroke patients. We found a lower prevalence of the A allele in patients with HS compared with ischaemic stroke or asymptomatic controls. After adjustment for age and vascular risk factors, the association between this polymorphism and HS did not reach statistical significance, thereby excluding it as an independent protection factor. This could be due to a necessary association of the effect of the A allele with other risk factors for HS, as previously described for the association between low PZ levels and venous thromboembolism (13). Alternatively, the number of individuals studied could be too small for measuring an independent protective effect of the A allele. One limitation of our study is that we lack the appropriate samples to measure PZ plasma levels. Nevertheless, the c.573+79A allele has been repeatedly associated with lower PZ levels in other studies (9–11). Therefore, we hypothesise that the different allelic distribution observed in HS patients would result on different plasma PZ concentration. The possible effect of this polymorphism and its relation to PZ blood levels in haemorrhagic stroke deserves further studies (14).

Table 1: A) Main characteristics of the study population. B) PROZ c.573+79G>A genotype and A allele distribution.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=147)</th>
<th>Ischaemic stroke (n=390)</th>
<th>Haemorrhagic stroke (n=156)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean ± SD</strong></td>
<td>65 ± 9.6</td>
<td>67 ± 11</td>
<td>72 ± 11*</td>
</tr>
<tr>
<td><strong>Male gender (%)</strong></td>
<td>73 (50)</td>
<td>266 (68)*</td>
<td>90 (59)*</td>
</tr>
<tr>
<td><strong>Current smoking (%)</strong></td>
<td>28 (19)</td>
<td>156 (40)*</td>
<td>36 (23)</td>
</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>36 (24)</td>
<td>244 (63)*</td>
<td>109 (68)*</td>
</tr>
<tr>
<td><strong>Diabetes (%)</strong></td>
<td>13 (9)</td>
<td>119 (31)*</td>
<td>30 (19)</td>
</tr>
<tr>
<td><strong>Hypercholesterolaemia (%)</strong></td>
<td>24 (16)</td>
<td>110 (28)*</td>
<td>18 (12)</td>
</tr>
</tbody>
</table>

**Data are: number (%), *p<0.01 compared to controls, †p<0.01 compared to ischaemic stroke.**

References