The Thr715Pro Polymorphism of the P-Selectin Gene Is Not Associated With Ischemic Stroke Risk

Julia Ferrari, MD; Sandra Rieger, PhD; Georg Endler, MD; Stefan Greisenegger, MD; Marion Funk, MD; Thomas Scholze, MS; Wilfried Lang, MD; Wolfgang Lalouschek, MD; Christine Mannhalter, PhD

**Background and Purpose**—A Thr→Pro polymorphism at codon 715 in the coding region of the P-selectin gene has recently been described. Individuals carrying the Pro715 allele were reported to have a reduced risk of myocardial infarction. A possible association of this polymorphism with the risk of ischemic stroke is currently under discussion.

**Methods**—We investigated the prevalence of the 715 Thr→Pro polymorphism in 450 patients aged younger than 60 years with ischemic stroke or transient ischemic attack and in 450 controls without vascular disease matched for age and gender. We also investigated possible interactions of the polymorphism with other vascular risk factors, stroke severity and stroke etiology.

**Results**—The distribution of the two allelic variants of the 715Thr→Pro polymorphism did not differ significantly between patients and control subjects (78% versus 81% for Thr/Thr, 21% versus 18% for Thr/Pro and 1% versus 1% for Pro/Pro in patients and controls, respectively; adjusted odds ratio for carriers of the C allele: 1.0 [0.8 to 1.2; \( P=0.695 \)]. We found no significant interaction of this polymorphism with vascular risk factors, stroke severity, or stroke etiology.

**Conclusions**—Our study supports results from previous investigation showing that the 715Thr→Pro polymorphism of the P-selectin gene was not associated with a risk or clinical characteristics of ischemic stroke. *(Stroke. 2007;38:395-397.)*

**Key Words:** genetics ■ stroke

**Subjects and Methods**

The patients included in the present study represent a subset of a larger cohort of patients documented in the Vienna Stroke Registry during the period from October 1998 until June 2001. During this period 732 patients with an acute ischemic stroke or transient ischemic attack before the age of 60 years were admitted to one of the participating departments. From these, 69 patients with a hemorrhagic stroke or sinus thrombosis and 13 patients with a rare cause of stroke (8 patients with arterial dissection, 2 patients with cocaine abuse, and 3 patients with vasculitis) were excluded. Of 468 of the remaining 650 patients (72%) written informed consent and a blood sample could be obtained. Of 18 patients no DNA could be isolated. Thus, 450 (69%) patients (165 female, 285 male; median age 53 years; interquartile range, 45 to 57) could be included in the study. Of these, 97% of the patients were of white origin; only 3% were of nonwhite origin, ie, of Turkish, Arab, Asian, or African background. The diagnosis was established clinically and all patients underwent cranial CT or MRI. The patients were documented according to a...
standardized protocol with respect to risk factors, medical history, laboratory and technical investigations, stroke etiology, and severity as measured by validated scales.

The 450 controls (165 female, 285 male; median age 52 years; interquartile range 45 to 57) were mostly Austrian participants in an official health care program. All were free of clinically manifest arterial disease and did not report any arterial vascular diseases in first degree relatives. Medical history, vascular risk factors, and the results of laboratory investigations were documented according to a standardized protocol.

The study complied with the Declaration of Helsinki and was approved by the local Ethics Committee. All patients and control subjects gave their written informed consent.

**Genotyping**

For genetic analyses DNA was isolated from EDTA blood samples using standard procedures. The 715Thr->Pro polymorphism in the P-selectin gene was analyzed by mutagenically separated polymerase chain reaction (PCR) as previously described. The mutagenic separated PCR is a single-tube PCR technique with allele specific primers differing in length by 8 bp. Base mismatches in the allele specific primers introduce differences into the PCR products that minimize cross-reactions of the amplicons in subsequent cycles. Because of the 8-bp length difference, the alleles are easily discernible by high resolution electrophoresis. Mutagenic separated PCR were performed in a Perkin Elmer 9700 Cycler (Applied Biosystems) using 50 μL reaction volumes containing 5 μL 10× buffer, ~50 ng DNA, 2.0 mmol/L MgCl2, 200 μmol/L each dNTP (Amersham Pharmacia Biotech), 1.25 U AmpliTaq Gold (Applied Biosystems), and the following primers: 25 pmol P715-F common forward primer (5'-CTGTTGAAATGCTCAGAACTACATG-3'), 25 pmol P715-WTR reverse primer (5'-GCAGGTGCGACCGTGTTG-3'), and 7 pmol P715-MR reverse primer (5'-CCTGTCTTG-ATAGGGTGGC-CACGGAGG-3') (all Tib Molbiol, Berlin, Germany). A 10-minute denaturation at 95°C was followed by 35 cycles at 95°C for 60 seconds, 57°C for 45 seconds, and 72°C for 60 seconds, and a final extension step of 7 minutes at 72°C. The PCR products were analyzed by gel electrophoresis on Spreadex EL 400 gels (Elchrom) at 140 V for 3 hours and stained for 20 minutes with Sybr Green (1:10 000 dilution; Molecular Probes). The PCR product generated from the A-allele has a length of 182 bp; the C-allele gives a 190 bp product.

Control samples (heterozygous and homozygous controls) were included in each run. Assay results were only interpreted if all controls were correct. To exclude genotyping errors, we confirmed selected samples in a second analysis using the same method. The assay was generally performed by a different person who was unaware of the results. There was complete concordance between first and second assay in all samples.

**Statistical Methods**

Continuous data are given as median and interquartile range (range from the 25th to the 75th percentile). Discrete data are given as counts and percentages. The χ² tests or, if appropriate, exact tests were used to compare groups of categorical data. Groups of continuous data were compared by the Mann-Whitney U test.

Multivariate adjustments were performed by means of binary logistic regression models. The linearity of the logit assumption was checked for continuous predictor variables and an analysis of residuals was performed. Regression diagnostics and overall model fit were performed according to standard procedures.

Results of the logistic regression models are presented as an odds ratio and the 95% confidence interval. A 2-sided P≤0.05 was considered statistically significant. Calculations were performed using SPSS for Windows (Version 10.0; SPSS Inc). The power of our study was calculated as 0.815.

**Results**

We found an almost identical frequency of the variant allele of the 715 Thr->Pro polymorphism in patients and in controls.

**TABLE 1. Genotype Distribution in Patients with Stroke/Transient Ischemic Attack Compared With Healthy Controls**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n=352)</th>
<th>Control Subjects (n=98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr/Thr</td>
<td>53 (44–57)</td>
<td>52 (46–56)</td>
<td>0.824*</td>
</tr>
<tr>
<td>Thr/Pro</td>
<td>126 (36)</td>
<td>39 (40)</td>
<td>0.467†</td>
</tr>
<tr>
<td>Thr/Pro and Pro/Pro</td>
<td>209 (59)</td>
<td>59 (60)</td>
<td>0.882†</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>55 (16)</td>
<td>14 (14)</td>
<td>0.722†</td>
</tr>
<tr>
<td>Thr/Pro</td>
<td>192 (55)</td>
<td>54 (55)</td>
<td>0.812†</td>
</tr>
<tr>
<td>THR/Pro</td>
<td>252 (72)</td>
<td>73 (74)</td>
<td>0.571†</td>
</tr>
<tr>
<td>Male</td>
<td>56 (16)</td>
<td>14 (14)</td>
<td>0.694†</td>
</tr>
<tr>
<td>Female</td>
<td>78 (22)</td>
<td>21 (21)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>60 (17)</td>
<td>13 (13)</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>158 (45)</td>
<td>50 (51)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for hypertension, diabetes, smoking, hyperlipidemia, age and gender.

(Table 1). The adjusted odds ratio for carriers of the Pro allele to suffer from stroke was 1.0 (0.8 to 1.2; P = 0.695). The frequency of the Thr allele in male patients was equal 0.89 and in female patients was equal 0.88. The frequency of the Thr allele in male controls was equal 0.90 and in female controls was equal 0.89. As shown in Table 2, there were no

**TABLE 2. Clinical Characteristics in Patients Grouped According to Genotype**

<table>
<thead>
<tr>
<th>Age (median, interquartile range)</th>
<th>Patients (n=352)</th>
<th>Control Subjects (n=98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>53 (44–57)</td>
<td>52 (46–56)</td>
<td>0.824*</td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>126 (36)</td>
<td>39 (40)</td>
<td>0.467†</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>209 (59)</td>
<td>59 (60)</td>
<td>0.882†</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>55 (16)</td>
<td>14 (14)</td>
<td>0.722†</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>192 (55)</td>
<td>54 (55)</td>
<td>0.812†</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>252 (72)</td>
<td>73 (74)</td>
<td>0.571†</td>
</tr>
<tr>
<td>Stroke etiology, n (%)</td>
<td>56 (16)</td>
<td>14 (14)</td>
<td>0.694†</td>
</tr>
<tr>
<td>Small vessel</td>
<td>78 (22)</td>
<td>21 (21)</td>
<td></td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>60 (17)</td>
<td>13 (13)</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>158 (45)</td>
<td>50 (51)</td>
<td></td>
</tr>
</tbody>
</table>

*Mann-Whitney U test.
†χ² test.
associations of the P-selectin genotype with established vascular risk factors or clinical characteristics in the patients.

**Discussion**

We investigated the prevalence of the 715Thr->Pro variation in a large cohort of systematically documented consecutive patients with an acute ischemic cerebrovascular event before the age of 60 years and in individually matched control subjects without clinically manifest vascular disease. We did not find an association of this polymorphism with the risk of ischemic stroke/transient ischemic attack. This result is in agreement with data published by Volcik et al.,7 who found no correlation between this polymorphism and stroke risk in a large North American cohort containing both blacks and whites. We also analyzed for interaction with other vascular risk factors, stroke etiology, or stroke severity and found no association. Because of the relatively small number of patients in the stroke subgroups, we cannot draw any conclusions about the influence of this polymorphism for specific stroke etiologies.

The Pro715 allele has previously been reported to protect from myocardial infarction in a multicenter population-based case-control study (ECTIM-study); 574 male patients aged 25 to 64 were examined 3 to 9 months after myocardial infarction. The Pro715 variant allele of P-selectin was found to correlate with lower serum P-selectin levels in carriers of the Pro715 allele compared with homozygous carriers of the Thr715 allele.12 In the ECTIM extension study,13 a further population-based study performed in 2 regions of the UK in patients after a myocardial infarction, a protective effect of the Pro715 allele of similar magnitude was found in women. We therefore separated our results for men and women and tested for a gender-dependent association with stroke but did not find any gender-related effects. Our results indicate that the 715Thr->Pro polymorphism of P-selectin does not represent a risk factor for stroke in contrast to myocardial infarction. However, further studies are required to clarify the functional role of this genetic variant and elucidate the role of the P-selectin variant in vascular diseases (stroke and myocardial infarction).

**Limitations**

Assuming that genetic factors play a more important role in younger patients we tested patients who experienced the ischemic event when younger than 60 years. Our results are thus only valid for patients in this age group. We did not have access to serum or plasma samples and could not determine whether P-selectin levels correspond to the genotype in our population.

**Sources of Funding**

The VSR is supported by research grants from the Medizinisch Wissenschaftlicher Bund der Bürgermeisters der Bundeshauptstadt Wien (projects 1540 and 1829), Jubiläumsfond der Osterrichi-

**Disclosures**

None.

**References**

The Thr715Pro Polymorphism of the P-Selectin Gene Is Not Associated With Ischemic Stroke Risk
Julia Ferrari, Sandra Rieger, Georg Endler, Stefan Greisenegger, Marion Funk, Thomas Scholze, Wilfried Lang, Wolfgang Lalouschek and Christine Mannhalter

Stroke. 2007;38:395-397; originally published online January 4, 2007; doi: 10.1161/01.STR.0000254475.43533.dd
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 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/38/2/395

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/