Genetic Variation in the Lymphotoxin-Alpha Pathway and the Risk of Ischemic Stroke in European Populations

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Background and Purpose—Several genes involved in the lymphotoxin-α cascade (LTA, LGALS2, and PSMA6) have been linked with the risk of myocardial infarction. Here, we present a comprehensive analysis of these genes in patients with ischemic stroke (IS).

Methods—Twenty-three single nucleotide polymorphisms (SNPs) from LTA, LGALS2, and PSMA6 were genotyped in 601 German IS patients and 736 matched controls. SNPs and haplotypes were tested for association with overall IS, large vessel stroke, and cardioembolic stroke. Significant associations were replicated in an independent sample of 843 IS cases and 933 controls from the UK.

Results—Only one SNP (rs1048990 in PSMA6) showed association with overall IS, but this was not replicated in the UK sample. Three SNPs showed significant associations with stroke subtypes (P<0.05), but none of these associations could be replicated in the UK population.

Conclusions—Genetic variation in the lymphotoxin-α cascade (LTA, LGALS2, and PSMA6) is not a major risk factor for IS. (Stroke. 2009;40:970-972.)

Key Words: stroke ▪ genetics ▪ PSMA6 ▪ LTA ▪ LGALS2

Inflammatory pathways are important in the development of atherosclerosis.1–2 Recent work has implicated the inflammatory lymphotoxin-α cascade in the pathogenesis of myocardial infarction (MI): in a series of studies including a whole-genome association study and functional experiments, Ozaki and colleagues showed that variation in the genes for lymphotoxin-α (LTA), galectin-2 (LGALS2), and proteasome subunit α type 6 (PSMA6) is associated with MI in the Japanese population,3–5 although these results were not consistently replicated.6–10

MI and IS, particularly stroke attributable to large artery atherosclerotic disease, share common risk factors and mechanisms. However, aside from a small study on LTA,11 the role of the lymphotoxin-α cascade in IS remains unexplored. We therefore set out to perform a comprehensive analysis of LTA, LGALS2, and PSMA6 in a large German case–control sample with IS and an independent replication sample from the UK.

Subjects and Methods

601 European Caucasian patients (mean age 64 years, 377 men) with IS were recruited from a single dedicated stroke unit in Munich, Germany.15 IS was categorized using the TOAST classification system13 (large vessel: n=186; cardiac embolism: n=143; small vessel: n=86; other determined etiology: n=45; undetermined: n=141). Controls were recruited from the KORA study14 and comprised 736 unrelated age- and sex-matched individuals (mean age 62 years, 447 men) without prior stroke or TIA.

SNPs were selected with the tagger algorithm based on data of the International HapMap Project. All PSMA6 and LGALS2 SNPs from the initial Ozaki et al3,5 studies were included; for rs909253 from LTA,4 neighboring SNPs were used as surrogate markers because of almost complete LD observed in previous studies9 (for details on SNP selection cf. supplemental Table I, available online at http://stroke.ahajournals.org). Genotyping was performed by MALDI-TOF mass spectroscopy. Because of the reported role of the LTA pathway in atherosclerosis and MI, SNPs were tested for correlation with overall IS, large vessel stroke (LVS), and cardioembolic stroke (69 comparisons) using Chi-Sq-Tests (allelic model). The following software was used: WG-Permer Version 0.9.9, R Version 2.6.1 and UNPHASED Version 3.0.12. To correct for multiple testing, the Westfall and Young permutation method was applied.15 Haplotype analyses were performed with a sliding window approach (2, 3, and 4 markers).

SNPs with nominally significant associations were replicated in an independent sample from the UK which included 843 consecutive Caucasian IS patients (mean age 66.71 years, 491 men). All patients had brain imaging with CT or MRI and imaging of the carotid arteries with duplex or MRA. Echocardiography was performed when clinically indicated. IS was categorized as described above (large vessel: n=242;
cardiac embolism: n=133; small vessel: n=153; other determined etiology: n=5; more than one cause: n=86; undetermined: n=224). 933 white Caucasian community controls, age- and sex-matched (mean age 65.16 years, 538 men), free of symptomatic cerebrovascular disease were recruited by random sampling of family doctor lists. The study was approved by the local ethics committees.

Results
There were no deviations from Hardy-Weinberg equilibrium for any of the markers and study groups investigated. All markers were genotyped with a high call rate (average 97%); for rs2009658 analysis failed (call-rate of only 93%). Power calculations are presented in supplemental Table III.

All Ischemic Stroke
Only one SNP was associated with risk of IS as a whole; the minor allele of a single SNP from PSMA6 (rs1048990) was associated with a decreased risk of overall IS (Table 1; OR 0.795, 95% CI 0.640 to 0.987, \( P=0.037 \) uncorrected, \( P=0.477 \) corrected). This association could not be replicated in the UK population (OR 1.079, 95% CI 0.895 to 1.300, \( P=0.424 \) uncorrected).

Ischemic Stroke Subtypes
In subgroup analyses, the minor allele of rs1048990 was also associated with a decreased risk of LVS (supplemental Table II). This association was not replicated in the UK population.

In addition in the German population, the minor alleles of two LTA SNPs showed associations with an increased risk of LVS (rs2844484) and cardioembolic stroke (rs1800629) (supplemental Table II). Analysis of haplotypes constructed of a subset of SNPs did not increase the significance above the level observed with single-marker tests (data not shown). None of the single

Table 2. UK Replication Sample (Allelic Model)

<table>
<thead>
<tr>
<th>SNP No.</th>
<th>SNP-ID</th>
<th>Gene</th>
<th>TOAST Subtype</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P Value (Nominal)</th>
<th>P Value (WY-S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rs1048990</td>
<td>PSMA6</td>
<td>Overall IS</td>
<td>1.079</td>
<td>0.895–1.300</td>
<td>0.424</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>rs1048990</td>
<td>PSMA6</td>
<td>Large vessel</td>
<td>1.234</td>
<td>0.929–1.640</td>
<td>0.145</td>
<td>n.a.</td>
</tr>
<tr>
<td>17</td>
<td>rs2844484</td>
<td>LTA</td>
<td>Large vessel</td>
<td>1.177</td>
<td>0.950–1.459</td>
<td>0.136</td>
<td>n.a.</td>
</tr>
<tr>
<td>23</td>
<td>rs1800629</td>
<td>LTA</td>
<td>Cardioembolic</td>
<td>1.022</td>
<td>0.694–1.505</td>
<td>0.913</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; WY-S, corrected for testing multiple SNPs using the Westfall and Young method; MAF, minor allele frequency; n.a., not assessed. *Significant (\( P<0.05 \)); †SNPs from the original Ozaki et al.1–3 studies. For SNP No. 18 (rs2009658; cf. supplemental Table I), analysis failed because of a call-rate of only 93%.
marker associations could be replicated in the UK sample (Table 2). Genotypic ORs are presented in supplemental Table IV.

Discussion
This study suggests that genetic variation in LTA, LGALS2, and PSMA6 plays no major role in the pathogenesis of IS. No replicable association was found with overall IS or IS subtypes.

Strengths of our study include (1) adequate statistical power, (2) comprehensive analysis of all three genes (tag-SNP approach) in a large, carefully phenotyped sample and an independent replication sample, and (3) rigorous correction for multiple testing.

A limitation is the lack of control for vascular risk factors, which was impossible because of limited information in several German patients.

A previous study in a Hungarian population found significant associations between two SNPs (rs1041981 and rs909253) in LTA and risk of LVS.11 However, the sample was small (353 patients and 180 controls), and no replication was performed. rs1041981 showed no association with stroke in the current study. Taken together, these studies provide no convincing evidence for a role of the LTA gene in IS. They illustrate the need for immediate replication in stroke genetic studies.

Acknowledgments
The authors are grateful to all subjects who participated in this study.

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The KORA research platform was initiated and financed by the GSF–National Research Center for Environment and Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria.

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None.

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12. Lohmussaar E, Gschwendtner A, Mueller JC, Org T, Wichmann E, Hamann G, Meitinger T, Dichgans M. ALOX5AP gene and the risk of LVS.11 However, the sample was small (353 patients and 180 controls), and no replication was performed. rs1041981 showed no association with stroke in the current study. Taken together, these studies provide no convincing evidence for a role of the LTA gene in IS. They illustrate the need for immediate replication in stroke genetic studies.

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