The Versican Gene and the Risk of Intracranial Aneurysms

Ynte M. Ruigrok, MD; Gabriël J.E. Rinkel, MD, FAHA; Cisca Wijmenga, PhD

Background and Purpose—The proteoglycan versican is an excellent candidate gene for intracranial aneurysms (IAs) because it plays an important role in extracellular matrix assembly and is localized in a previously implicated locus for IAs on chromosome 5q.

Methods—We analyzed all the common variations using 16-tag single nucleotide polymorphisms (SNPs) and haplotypes in the versican gene using a 2-stage genotyping approach. For stage 1, 16 SNPs were genotyped in 307 cases and 639 controls. For stage 2, the two SNPs yielding the most significant associations (P<0.01) were genotyped in a second independent cohort of 310 cases for confirmation of the associations.

Results—In stage 1, we found several SNPs in strong linkage disequilibrium and haplotypes constituting these SNPs associated with IAs in the Dutch population (strongest SNP association for rs173686 with odds ratio=1.34, 95% CI 1.09 to 1.65, P=0.004). In stage 2, we confirmed association for the 2 SNPs with the most significant associations (strongest SNP association for rs173686 with odds ratio=1.36, 95% CI 1.11 to 1.67, P=0.003).

Conclusion—SNPs in strong linkage disequilibrium and haplotypes constituting these SNPs in the versican gene are associated with IAs suggesting that variation in or near the versican gene plays a role in susceptibility to IAs. (Stroke. 2006;37:2372-2374.)

Key Words: genetics ■ intracranial aneurysm ■ subarachnoid hemorrhage

We hypothesized that disruption of the extracellular matrix (ECM) of the arterial wall is a likely factor in the pathogenesis of intracranial aneurysms (IAs).1 Interesting candidate genes are structural genes of the ECM and genes involved in ECM assembly. The proteoglycan versican plays an important role in the ECM assembly process and is localized close to a previously implicated locus for IAs on chromosome 5q in a Japanese cohort.2

We performed a comprehensive genetic association study analyzing all the common variants using tag single nucleotide polymorphisms (SNPs) and haplotypes between these SNPs in the versican gene in a Dutch case–control population.

Materials and Methods

We used a 2-stage genotyping approach. For stage 1, 16 SNPs were genotyped in 307 cases and 639 controls. For stage 2, the two SNPs yielding the most significant associations (P<0.01) were genotyped in a second cohort of 310 cases for confirmation of the associations.

For stage 1, we included 307 prospectively collected Dutch white patients with ruptured and unruptured IAs admitted to the University Medical Center (UMC) Utrecht and 639 ethnically matched Dutch controls. For stage 2, DNA was available from a second independent cohort of 310 Dutch patients with aneurysmal subarachnoid hemorrhage also from the UMC Utrecht. Patients in stage 2 were retrospectively collected because these patients, who have been treated for aneurysmal subarachnoid hemorrhage in the past by surgical clipping of the aneurysms, participated in a screening study for the formation of new aneurysms by means of CT angiography.3 During this screening, these patients were asked to give blood for DNA analysis. The UMC Utrecht ethical review board approved our study protocol. All cases and controls were from The Netherlands, of European descent and with at least three of the four grandparents also born in The Netherlands.

Sixteen tag SNPs in the versican gene were selected from the International HapMap Project (www.hapmap.org) using Tagger (Paul de Bakker, http://www.broad.mit.edu/mpg/tagger/) (Figure). The pairwise linkage disequilibrium (LD) between the SNPs in the gene is shown in the Figure. Genotyping was performed using Taqman assays on coded genomic DNA samples (ie, blind to the diagnostic group).

Differences in allele and haplotype SNP frequencies between patients and controls were assessed as odds ratios (ORs) using the χ² test with corresponding 95% CIs and probability values. Haplotype frequencies were analyzed using Haplovie.4 Assuming an autosomal-recessive model2 and a 5% high-risk allele frequency, our results had 80% power for detecting a susceptibility locus with a relative risk ≥1.7 at a significance level of 0.05 (genetic power calculator, SGDP Statistical Genetics Group, http://statgen.iop.kcl.ac.uk).

Received November 1, 2005; final revision received March 28, 2006; accepted May 3, 2006.

From the Department of Neurology, Rudolf Magnus Institute of Neuroscience (Y.M.R., G.J.E.R.) and the Complex Genetics Section, Department of Biomedical Genetics (C.W.), University Medical Center Utrecht, Utrecht, The Netherlands.

Correspondence to Ynte M. Ruigrok, MD, Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. E-mail y.m.ruigrok@neuro.azu.nl

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Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000236499.55301.09
The characteristics of patients analyzed in stage 1 and 2 and of controls are shown in Table 1. The distribution of the SNP genotypes was consistent with Hardy-Weinberg equilibrium. We had 0.2% missing genotypes. Analyzing patients of stage 1 versus controls, the rs251124, rs173686, rs308362, rs309557, rs308371, rs309593, and rs2290673 SNPs were associated with IAs (P < 0.05), with rs251124 (OR = 1.43, 95% CI = 1.09 to 1.88, P = 0.008) and rs173686 (OR = 1.34; 95% CI = 1.09 to 1.65, P = 0.004) having the strongest association (Figure). The SNPs rs308362, rs309557, rs308371, rs309593, and rs2290673 are all on the same strong LD block with high D' values (D' > 0.8). The SNPs rs251124 and rs173686 are in high LD with each other with a high D' value of 1.0 and are in high LD with most of the SNPs in the block, including the previous five SNPs (D' values > 0.7). We

The pairwise LD between all SNPs in the versican gene and comparison of the allelic frequencies of the 16 tagged SNPs between 307 patients with IA patients of stage 1 and 639 healthy controls. 95% CI lower indicates 95% CI lower limit; 95% CI upper, 95% CI upper limit. The exons of the gene are shown as black squares.

**Results**

The characteristics of patients analyzed in stage 1 and 2 and of controls are shown in Table 1. The distribution of the SNP genotypes was consistent with Hardy-Weinberg equilibrium. We had 0.2% missing genotypes. Analyzing patients of stage 1 versus controls, the rs251124, rs173686, rs308362, rs309557, rs308371, rs309593, and rs2290673 SNPs were associated with IAs (P < 0.05), with rs251124 (OR = 1.43, 95% CI = 1.09 to 1.88, P = 0.008) and rs173686 (OR = 1.34; 95% CI = 1.09 to 1.65, P = 0.004) having the strongest association (Figure). The SNPs rs308362, rs309557, rs308371, rs309593, and rs2290673 are all on the same strong LD block with high D' values (D' > 0.8). The SNPs rs251124 and rs173686 are in high LD with each other with a high D' value of 1.0 and are in high LD with most of the SNPs in the block, including the previous five SNPs (D' values > 0.7). We

**TABLE 1. Characteristics of the Analyzed Patients With Stage 1 and 2 Intracranial Aneurysms and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Patients Stage 1 (n=307)</th>
<th>Patients Stage 2 (n=310)</th>
<th>Controls (n=639)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>58.0</td>
<td>55.1</td>
<td>51.6</td>
</tr>
<tr>
<td>(range, 20 to 93)</td>
<td>(range, 26 to 72)</td>
<td>(range, 18 to 91)</td>
<td></td>
</tr>
<tr>
<td><strong>Familial aneurysms</strong></td>
<td>52.3</td>
<td>44.6</td>
<td></td>
</tr>
<tr>
<td>(range, 12 to 65)</td>
<td>(range, 11 to 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unruptured aneurysms</strong> (no SAH)</td>
<td>36 (11.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple aneurysms</strong></td>
<td>76 (24.8%)</td>
<td>50 (16.2%)</td>
<td></td>
</tr>
<tr>
<td><strong>MCA aneurysms</strong></td>
<td>72 (23.4%)</td>
<td>70 (22.5%)</td>
<td></td>
</tr>
</tbody>
</table>

SAH indicates subarachnoid hemorrhage; MCA, middle cerebral artery complex.
constructed haplotypes using the SNPs (rs251124 and rs173686) most strongly associated with IAs and then using all the SNPs associated with IAs (Table 2). The strongest haplotype association with IAs was found for the rs251124 and rs173686 SNPs (C, A) (ie, C allele SNP rs251124, A allele for SNP rs173686; OR = 0.74, 95% CI = 0.60 to 0.91, \( P = 0.003 \)) and (T, G) (OR = 1.40, 95% CI = 1.06 to 1.84, \( P = 0.02 \)).

In stage 2, the SNPs rs251124 and rs173686 yielding the most significant associations were genotyped in the second cohort of patients. Comparing the allele frequency of these SNPs in this patient group with the allele frequency of the control group again, significant associations with IAs are found for both SNPs rs251124 (T allele 18.1% in patients versus 12.8% in controls; OR = 1.35; 95% CI = 1.14 to 1.60, \( P = 0.003 \)) and rs173686 (G allele 39.3% in patients versus 32.2% in controls; OR = 1.36; 95% CI = 1.11 to 1.67, \( P = 0.003 \)). When combining the patients of stage 1 and 2 the associations with IAs become stronger for both SNPs (rs251124 OR = 1.47; 95% CI = 1.17 to 1.84, \( P = 0.0006 \); rs173686 OR = 1.35; 95% CI = 1.14 to 1.60, \( P = 0.0003 \)). Adjustment for age and sex using logistic regression did not change our conclusions.

### Discussion

In conclusion, we found that SNPs in strong LD and haplotypes constituting these SNPs in the versican gene are associated with IAs. We replicated our findings in a second independent cohort of patients with IA. Our findings suggest that variation in or near the versican gene plays a role in susceptibility to IAs, which would then confirm our hypothesis that diminished maintenance of the ECM is important in the development of IA.

Our results are consistent with the linkage findings of a study in a Japanese cohort showing linkage to 5q22–31 (this locus lies in the vicinity of the versican gene), because we also observed increased allele sharing in the versican gene. However, our sample of affected sib pairs with IAs was very small (14 pairs), resulting in probability values below the threshold levels for linkage in genomewide screens (data not shown). Interestingly, this IA locus on chromosome 5q lies close to a locus identified in thoracic aortic aneurysms on 5q13–14, which may suggest a common genetic factor for the two types of aneurysms. Moreover, because versican has already been suggested as a positional candidate gene in subarachnoid hemorrhage, variation in the versican gene may be a common genetic risk factor for both these diseases.

The SNPs showing association with IAs lie in a region of the versican gene that includes the two largest exons (6 and 7) in which alternative RNA splicing occurs. These alternatively spliced exons encode glycosaminoglycan attachment sites, which can bind chondroitin sulfate chains that are believed to have an antiadhesive function. The largest spliced variant (V0) includes the two largest exons, whereas the smallest variant (V3) lacks these exons. Consequently, V3 has no glycosaminoglycan attachment sites and therefore a lower number of chondroitin sulfate chains attached. Overexpression of V3 in arterial smooth muscle cells enhances cell adhesion, reduces growth and migration, and induces tropoelastin synthesis. In this way, V3 may influence the accumulation of ECM components. In patients with IA, the splicing process of versican may be altered resulting in a higher proportion of larger isoforms and thus in diminished ECM assembly.

The demonstrated association of versican with IAs should be replicated by studies in other populations. These should include large numbers of patients because IA is a complex disease and most of the genetic factors will therefore have only a small effect. Indeed, the odds ratios of the associated SNPs and haplotypes that we found are relatively low. The same holds true for the previously reported linkage data because the 5q linkage peak appeared moderately high with a maximum LOD score of 2.24. After the association has been confirmed, the causal variant in the versican gene should be identified by sequencing and functional analysis.

### Acknowledgments

We thank Z. Urban for his comments on a previous version of the manuscript and J. L. Senior for copy editing the article and for help improving the English.

### Sources of Funding

Y. M. Ruigrok was supported by The Netherlands Organization for Scientific Research (NWO), project no. 940-37 to 023.

### Disclosures

None.

### References

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Stroke. 2006;37:2372-2374; originally published online August 17, 2006;
doi: 10.1161/01.STR.0000236499.55301.09
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

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World Wide Web at:
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