Search on Chromosome 17 Centromere Reveals TNFRSF13B as a Susceptibility Gene for Intracranial Aneurysm
A Preliminary Study

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Background—Our previous studies have shown a significant linkage of intracranial aneurysms (IAs) to chromosome 17.

Methods and Results—Nine genes (TNFRSF13B, M-RIP, COPS3, RAI1, SREBF1, GRAP, MAPK7, MFAP4, and AKAP10) were selected from 108 genes that are located between D17S1857 and D17S1871 by excluding 99 genes that were pseudogenes, hypothetical genes, or well-characterized genes but not likely associated with IA. Direct sequencing of all coding and regulatory regions in 58 cases (29 pedigree probands and 29 unrelated nonpedigree cases) was performed. Deleterious changes were found only in TNFRSF13B, K154X, and c.585 to 586insA in exon4. The association of IA with TNFRSF13B was further studied in 304 unrelated cases and 332 control subjects. Rare nonsynonymous changes, a splicing acceptor site change and a frame shift, were found in unrelated cases (2.3%; 14 of 608) more frequently than in control subjects (0.8%; 5 of 664; P=0.035). The association study using single-nucleotide polymorphisms in an unrelated case-control cohort revealed a protective haplotype (odds ratio 0.69, 95% confidence interval 0.52 to 0.92, P=0.012) compared with the major haplotype after adjustment for covariates.

Conclusions—We propose that TNFRSF13B is one of the susceptibility genes for IA. (Circulation. 2006;113:2002-2010.)

Key Words: aneurysm ■ cerebrovascular disorders ■ genes ■ immune system

Intracranial aneurysms (IAs) are one of the major public health problems in Japan. The mortality rate from subarachnoid hemorrhages (SAHs), >90% of which are attributable to IA rupture, is estimated at 70 deaths per 10 000 person-years and accounts for 2% of annual total deaths.1 The consequences of SAH are catastrophic, with approximately half of IA ruptures resulting in immediate death.

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In familial IAs, there is a 3- to 5-fold increase in risk for first-degree relatives of affected individuals compared with the general population.2,3 A positive family history is a risk factor as strong as smoking, hypertension, and heavy consumption of alcohol.4,5

In an attempt to isolate susceptibility gene(s) for IA, 4 genome-wide linkage analyses have been reported.6–9 In a series of studies, we have failed to identify a positive association with reported candidate genes.9,10

Because disease and genetic heterogeneity are postulated for IA,11 extensive efforts are required to find the susceptibility gene(s) for IA, if the approach is limited to traditional positional cloning. On the other hand, the candidate gene approach relies on serendipity.

In the present study, we hypothesize that the many rare variants contribute to a common phenotype.12 We further assume that although deleterious changes are likely to be rare in the unaffected cohort, they may be more common in aggregate in the affected cohort. Consequently, we have assumed that variants associated with functional changes such as nonsense or nonsynonymous variants should be more abundant in candidate genes that determine susceptibility for IA. With this rationale, candidate genes were searched in a primary gene set in the 17-centromere region between D17S1857 and D17S1871, where we found the maximum nonparametric logarithm of the odds score peak (3.00) at D17S2196.9

Methods

Study Design
Subjects from 3 groups participated. The first group comprised probands of 29 pedigrees with IA clustering.9 The second group consisted of 333 unrelated nonpedigree cases with IA, and the
third group had 332 control subjects. Members of the first group and 29 unrelated cases, who were selected randomly from the second group, constituted the first cohort. The remaining 304 unrelated nonpedigree case subjects in the second group constituted the second cohort, and the third group constituted the third cohort. The response rates to our request of participation in the present study were 95.1% in the second group and 94.8% in the third group, respectively.

The target region was 4.3 Mb, which encompassed D17S1857, D17S1870, and D17S1871, where we found significant linkage in families with an IA cluster. A total of 108 genes are now assigned to this region (Data Supplement Table I). We set an exclusion principle to choose the primary candidate gene set: We excluded 99 genes; 26 pseudogenes; 29 hypothetical genes; 22 enzymes and transporters; 8 developmentally regulated genes; 4 genes associated with Smith-Magenis syndrome; 3 genes associated with neoplastic syndrome; 3 zinc finger proteins genes; 3 similar to keratin genes; and 1 open reading frame. Finally, 9 genes remained. These were TNFRSF13B, M-RIP, COPS3, RAI1, SREBF1, GRAP, MAPK7, MFAP4, and AKAP10 (Table 1). These 9 genes were directly sequenced in all subjects of the first cohort. Whether or not sequence variants were functional was predicted by PolyPhen (available at http://tux.embl-heidelberg.de/ramensky). Except for TNFRSF13B, none of the sequence variants in other genes were predicted to be deleterious. Further analysis was thus limited to TNFRSF13B. Using observed polymorphisms, an association study was conducted in the second cohort and the third cohort.

### Study Population

The probands of pedigrees and unrelated nonpedigree cases were diagnosed by digital subtraction angiography or in operations throughout collaborating hospitals in western Japan. We have excluded cases with IA affected with known heritable diseases or autoimmune diseases. Control subjects were screened at the brain checkup in the same hospitals as cases and met the following criteria: (1) confirmation of absence of IA by digital subtraction angiography, 3-dimensional computerized tomography, or magnetic resonance angiography; (2) an age at screening of ≥40 years old; (3) no medical history of any stroke, including IA or SAH; and (4) no family history of IA or SAH in first-degree relatives.

Individual and family history and lifestyle data were obtained by interviews. Past history and comorbidity were also examined by clinical charts at the hospitals or interview charts at the brain checkups. The study was approved by the Ethics Committee of

### Table 1: Nine Genes First Sequenced in Chromosome 17 Centromere in 58 Cases (First Cohort)

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>MIM No.</th>
<th>Position</th>
<th>GenBank Accession No. (2006/02/23)</th>
<th>Genomic Region, kb</th>
<th>mRNA Length, bp</th>
<th>No. of Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFRSF13B</td>
<td>Tumor necrosis factor receptor superfamily, member 13B</td>
<td>604907</td>
<td>16473152–16439349</td>
<td>NT_010718.15</td>
<td>33.804</td>
<td>879</td>
<td>4</td>
</tr>
<tr>
<td>M-RIP</td>
<td>Myosin phosphatase-Rho interacting protein</td>
<td>...</td>
<td>16543056–16686620</td>
<td>NT_010718.15</td>
<td>143.565</td>
<td>3114</td>
<td>29</td>
</tr>
<tr>
<td>COPS3</td>
<td>COP9 constitutive photomorphogenic homolog</td>
<td>604665</td>
<td>16782340–16747090</td>
<td>NT_010718.15</td>
<td>35.251</td>
<td>1269</td>
<td>12</td>
</tr>
<tr>
<td>RAI1</td>
<td>Retinoic acid induced 1</td>
<td>607642</td>
<td>17181736–17312516</td>
<td>NT_010718.15</td>
<td>130.781</td>
<td>5718</td>
<td>8</td>
</tr>
<tr>
<td>SREBF1</td>
<td>Sterol regulatory element binding transcription factor 1</td>
<td>184756</td>
<td>17338043–17312341</td>
<td>NT_010718.15</td>
<td>25.703</td>
<td>3441</td>
<td>21</td>
</tr>
<tr>
<td>GRAP</td>
<td>Grb2-related adaptor protein</td>
<td>604330</td>
<td>18548021–18522034</td>
<td>NT_010718.15</td>
<td>25.988</td>
<td>651</td>
<td>6</td>
</tr>
<tr>
<td>MAPK7</td>
<td>Mitogen-activated protein kinase 7</td>
<td>602521</td>
<td>18877883–18884469</td>
<td>NT_010718.15</td>
<td>6.587</td>
<td>2448</td>
<td>7</td>
</tr>
<tr>
<td>MFAP4</td>
<td>Microfibrillar-associated protein 4</td>
<td>600596</td>
<td>18888110–18883573</td>
<td>NT_010718.15</td>
<td>4.538</td>
<td>765</td>
<td>6</td>
</tr>
<tr>
<td>AKAP10</td>
<td>A kinase (PRKA) anchor protein 10</td>
<td>604694</td>
<td>19478475–19405569</td>
<td>NT_010718.15</td>
<td>73.177</td>
<td>1986</td>
<td>15</td>
</tr>
</tbody>
</table>

MIM indicates Mendelian Inheritance in Man; mRNA, messenger RNA.

### Table 2: Characteristics of the First Cohort (29 Pedigree Probands and 29 Unrelated Cases), the Second Cohort (304 Unrelated Cases), and the Third Cohort (332 Unrelated Controls)

<table>
<thead>
<tr>
<th>Nonpedigree Cohorts</th>
<th>First Cohort (Pedigree and Nonpedigree): 29 Probands and 29 Unrelated Cases (n=58)</th>
<th>Second Cohort: 304 Unrelated Cases (n=304)</th>
<th>Third Cohort: 332 Controls (n=332)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, %</td>
<td>70.7</td>
<td>66.8</td>
<td>54.5</td>
<td>0.0016†</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>Mean±SD 58.6±12.5</td>
<td>59.2±10.6</td>
<td>62.2±9.9</td>
<td>0.00017‡</td>
</tr>
<tr>
<td></td>
<td>Range 26–78</td>
<td>30–90</td>
<td>40–88</td>
<td></td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>55.2</td>
<td>56.3</td>
<td>42.5</td>
<td>0.0005‡</td>
</tr>
<tr>
<td>Ever-smoker, %</td>
<td>39.7</td>
<td>39.5</td>
<td>37.7</td>
<td>0.13†</td>
</tr>
<tr>
<td>Ever-drinker, %</td>
<td>43.1</td>
<td>38.5</td>
<td>43.7</td>
<td>0.18†</td>
</tr>
<tr>
<td>Family history of IA or SAH, %</td>
<td>58.6</td>
<td>17.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ruptured IA, %</td>
<td>62.1</td>
<td>47.0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Comparison between 304 unrelated cases (second cohort) vs 332 controls (third cohort).
†2x2 Test.
‡Student t test.
Kyoto University Institutional Review Board, and appropriate informed consent was obtained from all subjects.

**Direct Sequencing and Prediction of Functional Analysis for Detected Variants**

All exons, intron-exon boundaries, putative promoter sequences, and the 3′ untranslated region were analyzed by direct sequencing of 9 genes for 58 cases (the first cohort). For sequencing, we referred to TNFRSF13B, M-RIP, COP53, RAI1, SREBF1, GRAP, MAPK7, MFAP4, and AKAP10 on the NCBI Map Viewer (available at http://www.ncbi.nlm.nih.gov/mapview/maps.cgi/). Primers for coding exons were designed from an intron sequence to cover the entire regulatory region. After polymerase chain reaction amplification and purification, sequencing was done to cover the entire regulatory region. If the database suggested the existence of a regulatory region further away from the intron-exon boundary and commercially synthesized by Proligo (Proligo Primers & Probes, Kyoto, Japan; available at http://www.proligo.com). For regulatory regions, 500 bp upstream to the first exon was sequenced; however, if the database suggested the existence of a regulatory region further upstream, sequencing was done to cover the entire regulatory region. After polymerase chain reaction amplification and purification, sequencing was done on an ABI Prism 3100 Avant DNA sequencer (Applied Biosystems, Foster City, Calif.). We checked the single-nucleotide polymorphism (SNP) database as a reference (available at http://www.ncbi.nlm.nih.gov/SNP/index.html). Primers and polymerase chain reaction conditions for each gene are available from the Data Supplement Table II.

Among all the sequence changes identified by direct sequencing, we selected nonsense mutations and non synonymous variants as primary candidate variants. Then, we conducted functional analysis for each nonsynonymous variant by PolyPhen. The number of subjects who had rare nonsynonymous or deleterious changes was compared between the second cohort and the third cohort by the Fisher exact test with SAS software (version 8.2, SAS Institute Inc, Cary, NC).

**Testing Segregation in Pedigrees**

Three variants (K154X, c.585-586insA, and G76C) of TNFRSF13B found in 3 probands were investigated for concordance of segregation in these families (pedigree 10, pedigree 26, and pedigree 15).^{9}

**TABLE 3. Sequence Changes Detected in TNFRSF13B in the First Cohort (29 Pedigree Probands and 29 Unrelated Cases), the Second Cohort (304 Unrelated Cases) and the Third Cohort (332 Unrelated Controls)**

<table>
<thead>
<tr>
<th>Region</th>
<th>Position</th>
<th>Config Position</th>
<th>rs No.</th>
<th>Change</th>
<th>First Cohort: 58 Cases</th>
<th>Second Cohort: 304 Cases</th>
<th>Third Cohort: 332 Controls</th>
<th>GenBank Accession No. of mRNA and Effect on Products</th>
<th>Functional Polymorphisms Predicted by Polyphen</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter</td>
<td>c-247</td>
<td>16429865</td>
<td>rs4885754</td>
<td>G→T</td>
<td>0.362</td>
<td>0.339</td>
<td>0.315</td>
<td>NP_035584</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Exon3</td>
<td>16449689</td>
<td>A→G</td>
<td>0</td>
<td>0.002</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>S70N</td>
<td>Benign</td>
<td></td>
</tr>
<tr>
<td>Exon3</td>
<td>16449677</td>
<td>A→G</td>
<td>0</td>
<td>0.003</td>
<td>0</td>
<td>E74G</td>
<td>Possibly damaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon3</td>
<td>16449673</td>
<td>A→G</td>
<td>0</td>
<td>0.013</td>
<td>0.005</td>
<td>G76S</td>
<td>Possibly damaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron3</td>
<td>NS3+25</td>
<td>16449767</td>
<td>rs2274982</td>
<td>C→A</td>
<td>0.353</td>
<td>0.359</td>
<td>0.426</td>
<td></td>
<td>NCBII 0.49 ABII 0.36</td>
<td></td>
</tr>
<tr>
<td>Exon4</td>
<td>16447271</td>
<td>G→C</td>
<td>0</td>
<td>0.002</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon4</td>
<td>16441727</td>
<td>A→T</td>
<td>0.009*</td>
<td>0</td>
<td>0</td>
<td>K154X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon4</td>
<td>16441787</td>
<td>T→C</td>
<td>0</td>
<td>0.003</td>
<td>0.002</td>
<td>C177R</td>
<td>Possibly damaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon5</td>
<td>c.1385-596</td>
<td>insA</td>
<td></td>
<td></td>
<td>0.009*</td>
<td>0</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon5</td>
<td>16403830</td>
<td>C→T</td>
<td>0.345</td>
<td>0.360</td>
<td>0.342</td>
<td>P251L</td>
<td>Probably damaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon5</td>
<td>16402861</td>
<td>rs1078355</td>
<td></td>
<td></td>
<td>0.138</td>
<td>0.145</td>
<td>0.151</td>
<td></td>
<td>S277S</td>
<td></td>
</tr>
<tr>
<td>No. of subjects having rare variants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>14</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Variants found in only pedigree probands at the first cohort sequences.
†Change in intron sequence (splice acceptor site).
IVS indicates intervening sequence; UTR, untranslated region; ND, no data were available; NCBII, National Center for Biotechnology Information; and ABI, Applied Biosystems (http://www.appliedbiosystems.co.jp/website/jp/information/info.jsp).

**Association Study**

SNPs of TNFRSF13B with allele frequency ≥1% in 58 cases (the first cohort: 29 probands of the pedigrees and 29 unrelated cases) were all genotyped by direct sequencing (P251L and S277S) or by polymerase chain reaction–restriction fragment length polymorphism with Aliw for c-247G→TT and BfuAI for IVS3+25C→A in 304 unrelated cases (the second cohort) and 332 controls (the third cohort).

Haplotypes were constructed with sequence variants with allele frequency ≥1% in the third cohort by THESIAS (Testing Haplotype Effects In Association Studies)^1^ (available for download at http://genecanvas.ecgene.net/). We used the following criteria to choose a set of haplotypes for the association study: a set of the minimum number of haplotypes for which cumulative haplotype frequency was ≥80%15 or a set of all haplotypes for which frequencies were ≥5%.16 Associations were analyzed with adjustment for covariates including sex, hypertension, smoking, and drinking habit. Bonferroni correction was done for comparison of multiple haplotypes, not for experiment-wide multiple testing. Linkage disequilibrium (LD) was analyzed and visualized with the Genotype2LDBlock (available at http://cgen.uc.edu/cgi-bin/kzhang/genotype2LDBlock.cgi).

**Population-Attributable Risk**

The population-attributable risk for a given haplotype was calculated as follows:

Population-attributable risk = (OR − 1) × IE × P/IT

where IE is incidence of IA in the control cohort, IT is the incidence of IA in the general population, and P is the reference haplotype frequency in the general population. We assumed that IE was equal to IT and that P in the control cohort was equal to that in the general population. Thus, the population-attributable risk will be obtained as follows:

Population-attributable risk = (OR − 1) × P

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.
Demographic Features of the 3 Cohorts

As shown in Table 2, among unrelated subjects, the proportion of females or that of hypertension was higher in the second cohort than in the third cohort. Age at diagnosis was lower in the second cohort. No significant difference was found for either smoking or drinking habits.

Candidate Genes

The primary candidate gene set, after the exclusion of genes on the basis of defined criteria, was found to be related to immunity (TNFRSF13B), regulatory component (M-RIP), protein kinase (COPS3, MAPK7), transcriptional factor (RAI1, SREBF1), signaling protein (GRAP), cell adhesion (MFAP4), and signal transduction (AKAP10). Detected sequence changes in 58 cases (the first cohort) and their predicted effects on function are shown in Data Supplement Table III. We identified 7 sequence changes in TNFRSF13B, 20 sequence changes in M-RIP, 6 sequence changes in COPS3, 23 sequence changes in RAI1, 9 sequence changes in SREBF1, 9 sequence changes in GRAP, 5 sequence changes in MAPK7, 2 sequence changes in MFAP4, and 10 sequence changes in AKAP10.

TNFRSF13B had 2 nonsense mutations and 2 nonsynonymous variants predicted as “probably damaging” by Poly-Pedigree 10 has K154X mutation and (b) does not have it.

Pedigree 26 has c.585-586 insertion A and (b) does not have it.

Pedigree 15 has G76C change and (b) does not have it.

Figure 1. Segregation of the TNFRSF13B deleterious change with the IA phenotype in pedigrees.
Phen: K154X and frame shift (c.585-586insA) in exon4, G76C in exon3, and P251L in exon5. Apparent deleterious variants, including nonsense mutations and nonsynonymous variants, that were predicted to be “probably damaging” were identified only in **TNFRSF13B** (Table 3).

**Segregation of the TNFRSF13B Variants With the IA Phenotype in Pedigrees**

Two nonsense mutations and 1 nonsynonymous variant (“probably damaging” by PolyPhen) were found in probands in 3 pedigrees (Figure 1) among 29 families. In 1 family (pedigree 10), K154X was found in 2 affected siblings and 1 daughter, whereas it was not detected in an unaffected younger brother. Insertion A (c.585-586insA) was found in 1 family (pedigree 26); an affected mother and her son had this mutation. This mutation was also found in an unaffected sibling of the mother, who later developed stroke but was not investigated for pathogenesis. G76C was found in another family (pedigree 15); 2 affected sisters had this variant, but an unaffected sister did not.

**Direct Sequencing Exons 3 to 5 in 304 Unrelated Cases (Second Cohort) and 332 Controls (Third Cohort) in TNFRSF13B**

An extensive search was done in exons 3 to 5 because there are 2 deleterious variants and 2 “probably damaging” variants in these regions in **TNFRSF13B**. We further found additional sequence variants in the second and third cohorts (Table 3). The number of subjects having rare nonsynonymous changes, a splicing acceptor site change and a frame shift in **TNFRSF13B**, was significantly larger in the 304 unrelated cases than in the 332 controls (Fisher exact test, $P=0.035$; Table 4).

Locations of these variants are summarized in Figure 2. These nonsynonymous variants were located on the region critical for function. These 70th peptide S was conserved in **TNFRSF13B**.

![Figure 2. Schema of the domain structure of full-length TNFRSF13B.](image-url)
Xenopus laevis but not in mice, dogs, or rats. On the other hand, 74th E, 76th G, and 177th C were conserved in mice, dogs, and rats. Additionally, G76S and C177R were found in more than 1 unrelated subject; however, no one had more than 1 variant.

**Association Study**

Allele frequencies of 4 SNPs were found to be ≥1% in the third cohort (Table 3). We thus used 4 SNPs (c-247G>T, IVS3+25C>A, P251L, and S277S) of TNFRSF13B to construct haplotypes. LD structure was shown in Figure 3. Application of the selection criteria chose 4 haplotypes, which encompassed 87% of all haplotypes (Tables 5 and 6). Haplotype H1 (GACC) was found to be protective (OR 0.69, 95% CI 0.52 to 0.92, \( P = 0.012 \)) compared with the major haplotype H4 (TCTC). After Bonferroni correction for multiple comparisons, the probability value of H1 was still statistically significant (\( P_{corr} = 0.048 \)).

**Population-Attributable Risks**

The population-attributable risk was calculated to be approximately ~8% for the H1 haplotype versus H4, compared with 24% for smoking versus nonsmoking. Therefore, the attributable risk for the TNFRSF13B variants was approximately one third that of smoking.

**Discussion**

Extensive efforts have been made to search for susceptibility genes for IA. So far, 3 genome-wide linkage analyses have been done for the general population. With the exceptions of ELN, LOX, and COLIA2, no gene has been claimed as a candidate gene. There have been contradictions, however, in terms of involvement of ELN in IA.

In the present study, we have conducted a systematic approach targeting a linked region on chromosome 17. We selected 9 candidates from 108 genes and sequenced entire coding exons and regulatory regions in 58 cases (the first cohort). Because we found several variants that included obvious deleterious mutations in TNFRSF13B, we searched variants in 304 unrelated cases (the second cohort) and 332 control subjects (the third cohort), although searches were limited to those in exons 3 to 5, which covered the critical areas cysteine-rich domain 2 (CRD2), trans-membrane, and intracellular regions. The rare variants were significantly more frequent in IA unrelated cases than in control subjects. In addition, deleterious variants (K154X, frame shift [c.585-586insA], and G76C) were clearly segregated in the families, except in a family sibling who had c.585-586insA but did not have

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele Frequency</th>
<th>P (HWE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.67/0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.39/0.61</td>
<td>0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.65/0.35</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>0.85/0.15</td>
<td>0.43</td>
</tr>
</tbody>
</table>

HWE indicates Hardy-Weinberg equilibrium.

*Locus 1: rs4985754; locus 2: rs2274892; locus 3: SNP at 16440340; and locus 4: rs11078355."

Table 5. Allele Frequencies of TNFRSF13B Variants in 304 Unrelated Cases (the Second Cohort) and 332 Controls (the Third Cohort)
IA, which implies that the penetrance of IA is not as complete as was expected.24,25 Finally, case-control studies using sequence variants revealed a protective haplotype (GACC) against the most common haplotype (TCTC). With these lines of evidence, TNFRSF13B emerges as a candidate for susceptibility for IA.

Transmembrane activator and calcium modulator ligand interactor (TACI) encoded by TNFRSF13B mediates iso-type switching in B cells. The mutations in TNFRSF13B have recently been reported to be associated with common variable immunodeficiency (CVID) and immunoglobulin A (IgA) deficiency in humans.26,27 In 1 of these studies, 11 mutations (4.1%) were found in 270 chromosomes from 135 sporadic CVID cases.26 It is of particular interest that most sporadic cases with CVID had only 1 mutant allele, which suggests a mechanism of gain of function or haploinsufficiency.

Given that mutations of TNFRSF13B are associated with CVID or IgA deficiency, an unanswered question is why variants in TNFRSF13B are associated with IA. It is interesting that in the present study, 12 of 17 rare variants in IA cases and 3 of 5 rare variants in control subjects were found in the CRD2 domain, whereas the majority of mutations in cases with CVID or IgA deficiency were found at the C terminal side to the CRD2 region, which transfers signals from cell surface to intracellular domains. We postulate that variants at the ligand binding site may cause quantitative changes, whereas mutations in signal transduction result in qualitative changes. Different modes of functional impairments might be associated with different phenotypes. Studies are needed to investigate this further.

In the present study, we found 3 nonsense mutations (1 stop codon, 1 splicing acceptor site change, and 1 frame shift) and 5 rare nonsynonymous changes in 17 cases. Each case had a single variant. It is interesting that these variants are novel, and none were found in whites.26,27 The most common mutation among Japanese with IA is G76S (8/17), whereas in whites with CVID, it is A181E, which suggests founder mutations that are specific to ethnic groups. If this is true, genetic preposition to IA or CVID or IgA deficiency may be predicted by these founder mutations in the future.

The present study has several limitations. First, population-attributable risks of IA are calculated to be 7% to 10%, whereas that of smoking observed is 24%, which suggests that the risk attributable to TNFRSF13B is approximately one third that of smoking in the present cohort. However, further studies are needed, because only a small fraction of the risk is explained by THNFRS13B. Second, we have selected only 9 genes as the primary gene set from 108 genes. We excluded genes for which the functions are not well characterized or those with well-characterized functions that are not considered to be involved in IA. Although this is primary screening, this study cannot be free from selection bias. In the next study, we are expanding the gene set so that it includes some genes with unknown functions. Third, we tested with PolyPhen whether or not nonsynonymous variants were functional. Bioinformatics approaches may sometimes be misleading.28 In the future, we should explore other genes that had “possibly damaging” or “unknown” variants. Effects of variants on the function of TACI should also be confirmed experimentally in future. Fourth, there may be an argument for the hypothesis that rare variants contribute to common diseases. However, the hypothesis can provide criteria for positive selection of a susceptibility gene, which would have been overlooked by a haplotype-based association study. Fifth, in the present study, we did not determine CVID-related parameters such as B-cell expression of TACI and serum levels of immunoglobulin. Finally, we did not explore genes in LD with TNFRSF13B. The International HapMap Project (http://www.hapmap.org) suggests that there is LD between LOC96597 and TNFRSF13B. Further exploration of this will be needed in the future.

With positive findings, the above rationale, and reasonable background, we proposed that TNFRSF13B is one of the candidate genes for susceptibility for IA, notwithstanding several limitations. This in turn proposes that immu-
nologic mechanisms may play a role in IA development to a discernible extent. Our hypothesis is in accordance with clinical experiences in which IA is often found in subjects with autoimmune diseases. Further studies are needed to strengthen our hypothesis. In addition, the present results might pave the way for an investigation of a link between immunologic events and IA development.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

A number of genetic studies conducted on familial intracranial aneurysms (IA) have reported positive findings for various chromosomal regions, including 1p, 2p, 7cent, 17cent, 19q and Xp. In the present study, we have extensively searched for genes on chromosome 17cent. A total of 9 candidate genes (TNFRSF13B, M-RIP, COPS3, RAI1, SREBF1, GRAP, MAPK7, MFAP4, and AKAP10) were selected from 108 genes within this linked region. TNFRSF13B was the only gene tested that was associated with intracranial aneurysms in the 58 cases (29 pedigree probands and 29 unrelated non-pedigree cases). The association of IA with TNFRSF13B was further studied in 304 unrelated cases and 332 control subjects. In unrelated cases, deleterious or nonsynonymous variants were found at a higher frequency (2.3%) than in control subjects (0.8%) ($P=0.035$). The association study using single nucleotide polymorphisms in an unrelated case-control cohort revealed a protective haplotype (odds ratio=0.69, 95% confidence interval, 0.52 to 0.92; $K_p=0.012$) to the major haplotype. We propose that TNFRSF13B is one of the genes which determine susceptibility for IAs. Other genes are also involved in IAs, as the population attributable risk of TNFRSF13B is small (7% to 10%). Interestingly, TNFRSF13B, one of the members that transduces key signals in the regulation for the survival and the apoptosis of immune cells, has recently been reported to be associated with common variable immunodeficiency and IgA deficiency. The present finding provides support for the hypothesis that immunological mechanisms play a role in the development of IA.
Search on Chromosome 17 Centromere Reveals *TNFRSF13B* as a Susceptibility Gene for Intracranial Aneurysm: A Preliminary Study

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