TABLE 3
Comparison of the current review with international studies

Authors & Year	Total No. of Cases	Patient-Years	No. of Ruptures	% Rupture Rate (95% CI)	p Value*
ISUIA, 1998	1449	12023	32	0.3 (0.2–0.4)	<0.0001
Rinkel, et al., 1998	NA	3907	75	1.9 (1.5–2.4)	0.02
ISUIA, 2003	1692	6544	51	0.8 (0.6–1.0)	<0.0001
present study	922	3801	104	2.7 (2.2–3.3)	

* Fisher exact test.

view, we considered at least two factors. First, UCAs in Japanese patients may have a higher risk of rupture due to a genetic or habitual background. The rate of SAH in the Japanese population is reported to be very high, as high as 96 per 100,000 annually[15]—almost nine times higher than the incidence observed in Rochester, MN (11 per 100,000 annually[10]). Nevertheless, the prevalence of UCAs is not significantly different among the populations studied,[22] which might indicate that UCAs in Japanese patients may rupture more frequently than they do in the Caucasian population. An SAH, however, is not necessarily a consequence of the rupture of a UCA that has already formed. Kataoka, et al.,[14] noted that the pathological analysis of ruptured aneurysms showed evidence of acute formation of the lesion's wall, and some SAHs may be caused by acutely formed aneurysms, which would not be detected as UCAs during routine physical or imaging examinations. Furthermore, the prevalence of SAH may differ according to the study design and how the rate was determined. Nevertheless, the high rupture risk of documented UCAs may explain the high rate of SAH in Japan, and this high risk may relate to the genetic or habitual backgrounds of the Japanese patients.

Secondly, we considered that our review might simply incorporate series that included highly biased cases. Two possible steps could create such a bias in patient selection. Large aneurysms and those located in the posterior circulation are known to be associated with a high risk of rupture.[11] Elderly people are also known to have a higher rate of SAH.[15] These factors elevate surgical risks,[11] which may make surgeons reluctant to perform surgery in patients with these factors. Hence, these patients may be left untreated. Except for the inclusion of more cases with aneurysms of the posterior circulation, however, the characteristics of patients in this review included more patients with lower risk than those found in either the ISUIA cohorts[11,12] or the review written in Europe.[22] Therefore, this bias does not appear to be the main reason for the difference in rupture risk.

As a second bias, we may need to consider intrinsic variations in the design of the studies. Large-scale cohorts, especially in prospective studies, may include all patients who have been encountered, even those seen only in outpatient clinics. On the other hand, retrospective series tend to include patients who were admitted to the hospital for some reason and then evaluated for intervention. These nuances in the selection criteria for patients might produce a bias in the studies. In part of this study (11 series), the number of patients who underwent surgery for UCAs was twice that of patients who were merely observed. Hence, our study does not represent all UCAs that we encountered in our daily practice and should always take into account the selection bias.

Based on these findings, patients with UCAs that were not treated but observed in Japanese institutions may indeed carry a relatively high risk of lesion rupture. These patients, however, might also have high medical risks, surgical risks, or both, and treatment should be chosen according to a detailed risk–benefit analysis for each scenario. Although we cannot currently define the genetic factors influencing rupture, we would like to warn physicians that UCAs might have a different natural course in different races, and the data reported by the ISUIA[11] may not apply to all cases.

To understand the real risks of rupture in all patients with UCAs encountered in daily practice, we should rely on prospective cohort studies conducted in individual countries or on those with an international basis. In Japan, there are two ongoing prospective studies. When these studies are completed, we should be better equipped to determine the natural history of UCAs, whether that history differs among countries or races, and what treatment should be used for patients with UCAs in Japan.

Conclusions

A systemic review of untreated UCAs in Japan showed that the risk of rupture is significantly higher than that reported by international large-scale cohort studies. This difference in the rupture risk might be induced by differences in racial or genetic backgrounds or by differences in study designs or patient backgrounds. Based on these findings, we can state that untreated UCAs in Japan, once excluded from surgical indications, have a significantly high rate of rupture, but this rate may not apply to all patients treated in outpatient clinics.

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Model-Based Linkage Analyses Confirm Chromosome 19q13.3 as a Susceptibility Locus for Intracranial Aneurysm

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Model-Based Linkage Analyses Confirm Chromosome 19q13.3 as a Susceptibility Locus for Intracranial Aneurysm

Youhei Mineharu, MD; Kayoko Inoue, MD, MPH, PhD; Sumiko Inoue, PhD; Shigeki Yamada, MD, PhD; Kazuhiko Nozaki, MD, PhD; Nobuo Hashimoto, MD, PhD; Akio Koizumi, MD, PhD

Background and Purpose—In previous studies of familial intracranial aneurysm (IA), parametric linkage analyses have been undertaken for five unrelated families, four providing maximum logarithm of odds (LOD) scores with dominant models and one with a recessive model. Each family was linked to a distinct locus, indicating locus heterogeneity. This study aimed to examine whether Japanese IA families consistent with autosomal-dominant mode of inheritance support linkage to these loci.

Methods—We performed genomewide linkage analysis using the GENEHUNTER program. Affected-only parametric linkage analysis was used for 41 affected members in nine unrelated IA families with dominant models, which were selected from 29 families used for a nonparametric (model-free) linkage analysis in our previous study.

Results—We failed to support the linkage to previously reported autosomal-dominant loci. Instead, we found linkage to chromosome 19q13.3 with a maximum multipoint LOD score of 4.10. The LOD-1 interval (regions with LOD scores of >1) was 8.0 cM between D19S198 and D19S902.

Conclusions—A genomewide scan for IA families with dominant models in Japan confirmed the locus at chromosome 19q13.3, which has also been reported as a candidate locus in a Finnish population. (***Stroke*. 2007;38:000-000.**)

Key Words: autosomal-dominant ■ familial ■ genetics ■ intracranial aneurysm ■ linkage

Intracranial aneurysm (IA) is of great concern in public health fields because of its high morbidity and mortality rate in ruptured cases. Rupture of an IA causes subarachnoid hemorrhage (SAH), and results in immediate death in 50% of cases with an additional 30% of cases left with a severe disability.[1,2] However, despite advances in diagnostic and treatment techniques, the mortality and morbidity rates associated with SAH remain unimproved.

Several factors such as age, hypertension, smoking, heavy alcohol intake, perceived mental stress, lean body mass index, and blood transfusion have been reported to be associated with SAH.[3–5] Evidence suggests that genetic factors also contribute to the pathogenesis of IA. The risk of SAH is four times higher in first-degree relatives and six times higher in siblings of patients with SAH than in the general population.[6,7] To clarify the genetic background of IA formation, several linkage analyses have been conducted. A total of 10 suggestive linkage regions have been identified to date: chromosomes 1p34.3-p36.13, 2p13, 5p15.2-p14.3, 5q22-q31, 7q11.2-q22.1, 11q24-q25, 14q23-q31, 17cen, 19q12-q13, and Xp22.[8–15] Among these, elastin (*ELN*) and the LIM domain kinase 1 (*LIMK1*) genes in 7q11,[8,16] collagen alpha2(I) (*COL1A2*) in 7q22,[17] and tumor necrosis factor receptor, superfamily member 13B (*TNFRSF13B*) in 17cen have been reported to be associated with IA.[18] It is noteworthy that mutations were detected in *TNFRSF13B*. However, a linkage to 7q11 or the association of *ELN* polymorphisms with IA has failed to be replicated in several studies,[19–23] and the association of *COL1A2* or *TNFRSF13B* with IA has not been replicated in an independent study.

Most of the linkage studies applied nonparametric (model-free) linkage analyses because the mode of inheritance of familial IA has not been determined, although autosomal-dominant (AD), autosomal-recessive, and undetermined modes have been reported.[24] The first parametric linkage analysis was done by Roos et al, who analyzed a large consanguineous family with a recessive model and showed linkage to 2p13.[11] On the other hand, genomewide scans of four unrelated families with dominant models revealed linkage to four distinct loci at 1p34.3-p36.13, 5p15.2-p14.3, 11q24-q25, and 14q23-q31.[14,15,25] These findings suggest that locus heterogeneity (different responsible locus for each family) may be associated with the disease. To examine whether Japanese IA families share common linkage regions, we undertook parametric linkage analysis using large unrelated families consistent with an AD mode of inheritance. These families were selected from 29 families previously analyzed in a model-free linkage analysis.

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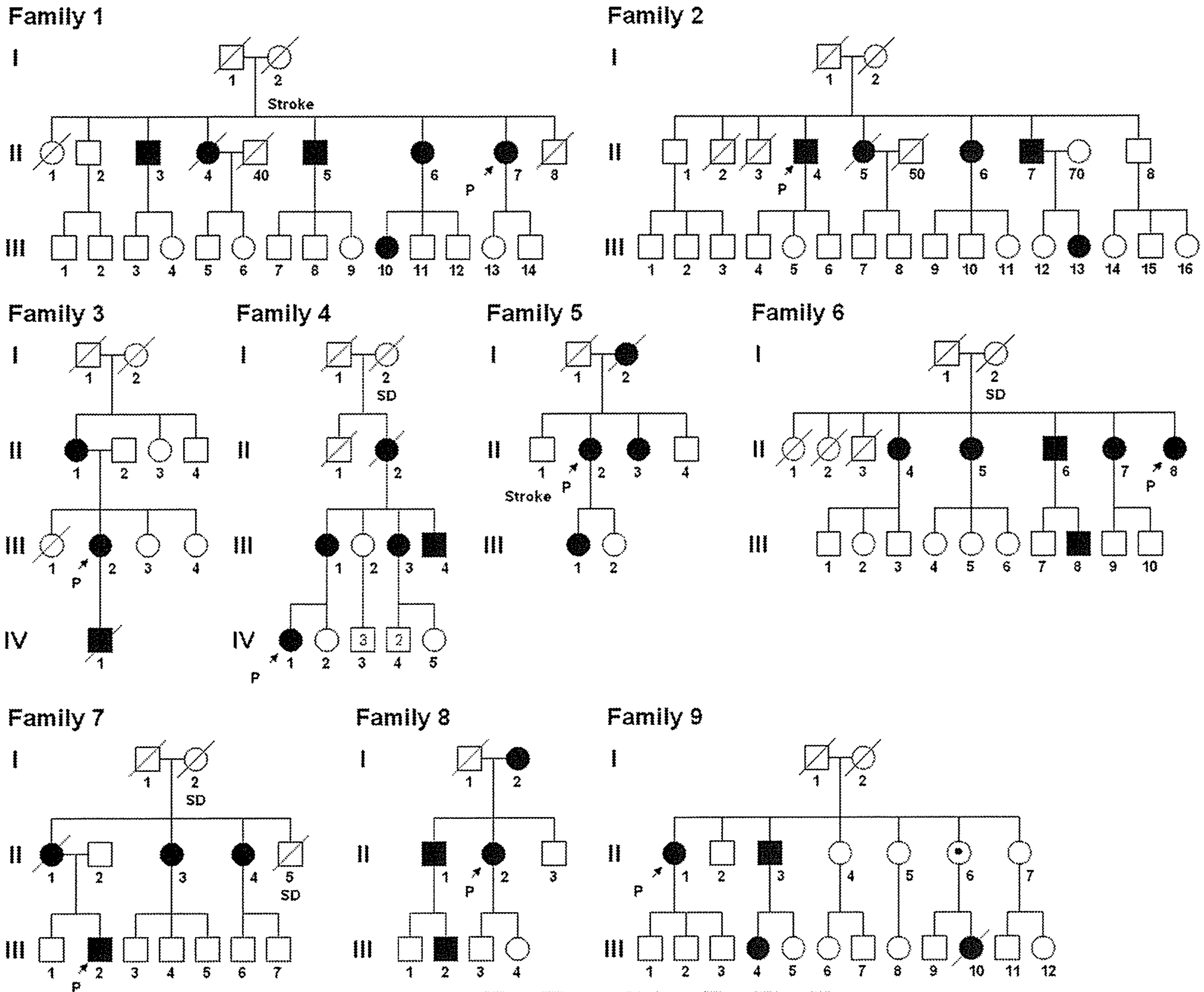

Figure 1. Familial intracranial aneurysm pedigrees. Filled symbols represent affected individuals; open symbols, unaffected individuals; open symbols with black circles, obligatory carriers; circles, women; squares, men; symbols with oblique bars, dead individuals. *Individuals whose DNA was available; P, probands; SD, sudden death by unknown causes; numbers in symbols, number of offspring; numbers below symbols, individual ID; Roman number, number of generation.

Materials and Methods

Study Population

The study was approved by the Ethics Committee of the Kyoto University Institutional Review Board and appropriate informed consent was obtained from all subjects. We used the same study population and fully detailed how we recruited the IA families in an earlier report.[13] Briefly, we recruited patients with IA with a family history of IA or aneurysmal SAH from collaborating hospitals in western Japan. Probands were confirmed to have saccular aneurysms from medical records. Clinical interviews and magnetic resonance angiographies were carried out for all available relatives aged 30 years or older. In those suspected to have aneurysms, an additional examination (digital subtraction angiography or three-dimensional computed tomography) was conducted. All images for diagnosis were examined by at least three neurosurgeons and neuroradiologists. History, family history, and comorbidity were examined using the clinical charts of individual participants. We excluded families with known heritable diseases or autoimmune diseases.

From among the 29 Japanese IA families in our previous report,[13] we selected nine large families of two or more generations without consanguinity and for which the AD mode can be reasonably assumed. The inclusion criteria was as follows: (1) families with affected individuals in three consecutive generations or families with three or more affected individuals in the parental generation and one or more affected individuals in the offspring generation, and (2) no consanguinity. The pedigree charts of IA families analyzed in this study are shown in Figure 1. Families 1 to 9 in this study represent pedigrees 1, 2, 5, 6, 10, 13 14, 16, and 29 in our previous report, respectively.[13]

Genotyping

Genomic DNA was extracted from blood samples from live participants or preserved umbilical cords from deceased participants (IV-1 in family 3 and III-10 in family 9) using a QIAamp DNA Blood Mini Kit (Qiagen Inc). The genotypes of some deceased affecteds (II-4 in family 1, II-5 in family 2, II-2 in family 4, I-2 in family 5, and II-2 in family 7) were reconstructed from the genotypes of offspring and spouses. Polymerase chain reaction amplification from genomic DNA was performed with fluorescence-labeled (6-FAM, HEX, NED) and tailed primers. Polymerase chain reaction primers to analyze microsatellite markers comprised an approximate 10 cM human index map (ABI Prism Linkage Mapping Set Version 2: 382 markers for 22 autosomes and 18 markers for the X chromosome; Applied Biosystems), and fine mapping markers were designed according to information from the Marshfield genetic map (http://research.marsh-

fieldclinic.org/genetics). We used single nucleotide polymorphism markers for fine mapping if we could not obtain a suitable microsatellite marker. Marker location was obtained from the uniSTS reference physical map (www.ncbi.nlm.nih.gov/genome/sts/). Polymerase chain reactions were carried out in 7.5 μL with 50 ng genomic DNA using AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a two-step amplification program. DNA fragments were analyzed on an ABI Prism 3100 Avant Genetic Analyzer. SimWalk2[26] and Merlin software[27] were used to detect genotyping errors and Mendelian inconsistence. When genotyping resulted in no call or an ambiguous one, the genotype was set to unknown.

Linkage Analysis

Because the estimated prevalence of IA is approximately 2%,[28] we used parametric affected member-only analysis assuming an AD mode of inheritance with a disease allele frequency of 0.01 and a nongenetic phenocopy frequency of 0.01. We set a penetrance at 70%, although penetrance value is inapplicable in an affected-only analysis. Given the different genetic backgrounds between sporadic and familial cases (ie, familial cases are likely to be caused by a mutation, whereas sporadic cases are likely to be a phenocopy or have risk alleles of susceptibility genes), a mutated allele frequency should be much smaller than 2%. Based on the previous findings that familial occurrence of IA is approximately 10%[29] and that the percentage of families with an apparently AD mode of inheritance accounted for approximately 50%,[24] the number of patients with IA in apparent AD families is relatively small at approximately 0.1%. Given a low mutation frequency and high prevalence, phenocopy frequency is likely to be high. Thus, we also calculated logarithm of odds (LOD) and heterogeneity (HLOD) scores with a disease frequency of 0.001, a phenocopy frequency of 0.02, and a penetrance of 0.70. Population allele frequencies for each microsatellite marker were estimated from all of the unrelated founders using Merlin software. To increase the accuracy of haplotype estimation in affected individuals, we included unaffected individuals and obligatory carriers in the analysis. The phenotype of these individuals was assigned as "unknown" so as not to influence the statistical power. Multipoint analyses for autosomes were run with a one-tailed probability value using GENEHUNTER (Version 2.0 and 1.3)[30] and Merlin software.[27] Because locus heterogeneity seems to be associated with IA, we obtained both LOD and HLOD scores.[30] We used a two-stage design; first, all chromosomal regions were screened by genotyping at approximately 10 cM density (screening), and second, the regions with multipoint LOD >2.0 in the screening analysis were further finely mapped at approximately 1 to 2 cM densities (fine mapping). Regions with LOD >3.6 were considered to be probable linkage regions.[31]

Results

The results of the present study were obtained by reanalyzing the previously published data set.[13] In the previous report, 29 families were analyzed in a model-free linkage analysis, whereas in the present study, we reanalyzed nine of 29 families in a model-based approach (Figure 1) assuming an AD mode of inheritance. These nine families had a total of 41 affected individuals and one obligatory carrier. DNA samples were available from 53 individuals, including 36 affected, one obligatory carrier, and 16 unaffected individuals.

In genomewide screening, an LOD score of >2.0 was found around marker D11S1338 at chromosome 11p15 and D19S420 at chromosome 19q13 (Figure 2). A HLOD score of >2.0 was also found around the same regions. No other region filled the criteria of LOD or HLOD scores of >2.0. As for the previously reported autosomal-dominant IA loci, 1p34.3-p36.13, 5p15.2-p14.3, 11q24-q25, and 14q23-q31, the maximum LOD scores in these loci were −0.18, −1.2, 0.6, and 0.31, respectively.

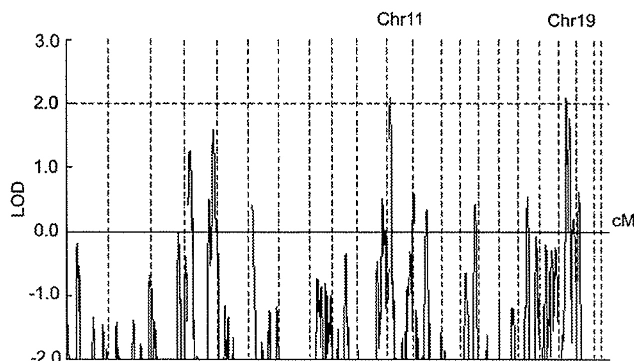

Figure 2. Genomewide screening of nine intracranial aneurysm families with dominant models. X-axis, genetic distance; Y-axis, logarithm of odds score. The vertical dotted lines show chromosomal boundaries, and the horizontal dotted line shows the cutoff for logarithm of odds score of >2.0. Chr indicates chromosome. Logarithm of odds scores of >2.0 were observed at chromosomes 11p15 and 19q13.

Fine mapping was done for the regions at chromosomes 11p15 and 19q13. The maximum multipoint LOD and HLOD scores at chromosome 11p15 were 0.75 and 1.40, respectively, whereas at chromosome 19q13, they were both 4.10 (Figure 3). The maximum score was obtained at D19S574 and the LOD-1 interval (regions with LOD score of >1) was 8.0 cM (6.2 Mbp) between D19S198 and D19S902 at chromosome 19q13.3, harboring 197 genes. Changing the disease allele frequency and phenocopy frequency did not appreciably alter the results (the maximum LOD score at D19S574 was 4.12 with a disease frequency of 0.001, a phenocopy frequency of 0.02, and a penetrance of 0.70).

In family 5, a nonsense mutation (K154X) in *TNFRSF13B* on chromosome 17cen was shown to be segregated in our previous study.[18] This family showed a maximum LOD score of 0.04 at D19S574.

Discussion

In an earlier report, we analyzed 29 IA families in a model-free linkage analysis, which gave a maximum nonparametric LOD score of 3.00 at chromosome 17cen (nominal P=0.001), 2.15 at chromosome 19q13 (nominal P=0.020), and 2.16 at chromosome Xp22 (nominal P=0.019).[13] In the present study, we reanalyzed nine of 29 families in a model-based approach assuming AD mode of inheritance. We failed to show any linkage to previously reported autosomal-dominant IA loci; 1p34.3-p36.13, 5p15.2-p14.3, 11q24-q25, and 14q23-q31. Instead, we found linkage to 19q13.3 with a maximum multipoint LOD score of 4.10, and the LOD-1 interval ranged over 8.0 cM between D19S198 and D19S902. Thus, we confirmed that Japanese families with autosomal-dominant IA linked to chromosome 19q13.3. Chromosome 19q13.3 has already been shown to be linked to IA in a Finnish population.[12] The loci in the two studies overlap by 1.3 cM between markers D19S545 and D19S902. Thus, chromosome 19q13.3 was found to be linked to IA in different ethnicities, indicating that this is a reliable locus for IA. Additionally, it is interesting that the region is also proposed as a candidate locus for abdominal aortic aneurysm.[32]

FINAL PROOF

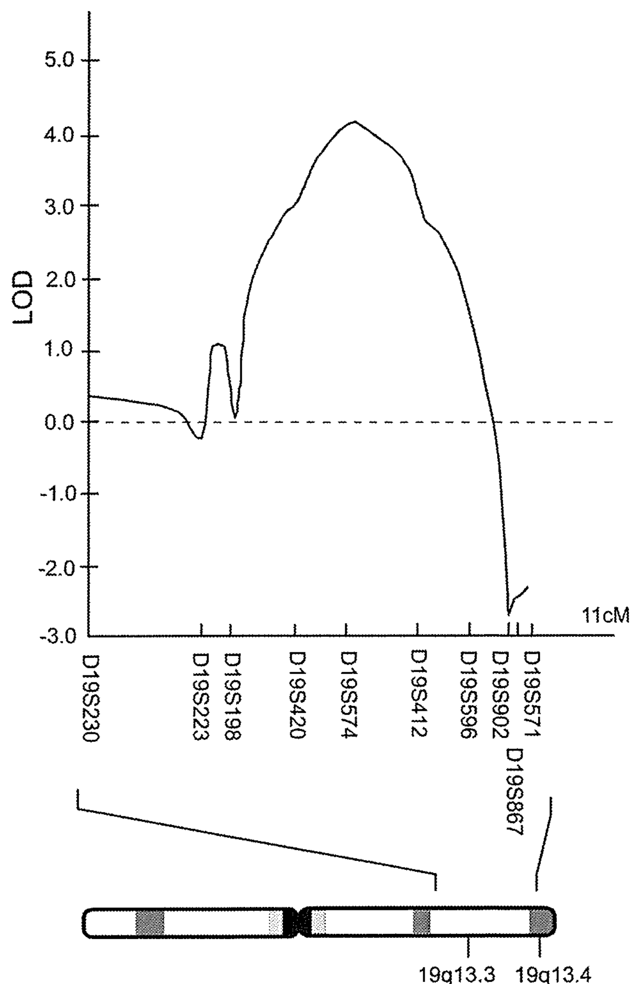

Figure 3. Logarithm of odds scores resulting from fine mapping for chromosome 19q13. X-axis, genetic distance; Y-axis, logarithm of odds score. The relative position of fine markers was indicated on X-axis. The maximum logarithm of odds score of 4.10 was observed at D19S574. The logarithm of odds-1 interval ranged between D19S198 and D19S902.

In this study, we included a family that in our previous study[18] showed a segregation of mutation in *TNFRSF13B* on chromosome 17cen because the exclusion of such a family would have led to a selection bias. A nonsense mutation in exon 4 (K154X) in *TNFRSF13B* was shown to be segregated in family 5.[18] Family 5 showed a maximum LOD score of 0.04 at D19S574, which had little influence on the linkage signal at chromosome 19q13.3.

The LOD-1 interval at chromosome 19q13.3, encompassing 197 genes, contains several interesting candidate genes. These include Ets2 repressor factor (ERF), urokinase-type plasminogen activator receptor (PLAUR), RelA-associated inhibitor (RAI), and prostacyclin receptor (PTGIR). ERF is a repressor of Ets2, a member of the Ets family of transcription factors. The Ets family plays an important role in angiogenesis through the activation of matrix metalloproteinases (MMPs). Inhibition of Ets has been shown to prevent the development of abdominal aortic aneurysm in a rat model by downregulating MMPs.[33] *PLAUR* is a key molecule in the regulation of plasmin activity on cell surfaces. PLAUR and its ligand, together with prometalloproteinase-2 (pro-MMP-2), promote angiogenic processes through their proteolytic properties.[34] Plasmin and pro-MMP-2 have been shown to be increased within intracranial aneurysms[35,36]; therefore, uPAR may be associated with aneurysmal formation through extracellular matrix remodeling. RAI inhibits the transcriptional activity of nuclear factor kappaB (NFkB),[37] which controls the expression level of MMPs, including MMP2.[38] PTGIR is a receptor of prostaglandin I2 (prostacyclin), which plays a major role as a potent mediator of vasodilatation and is an inhibitor of platelet aggregation. Both RAI and prostacyclin stimulating factor have been shown to be significantly less expressed in ruptured intracranial aneurysms compared with the superficial temporal artery in patients with IA,[39] indicating RAI and PTGIR may be associated with IA.

In conclusion, a genomewide scan in a Japanese population with a dominant model of familial IA revealed linkage to chromosome 19q13.3, which is also a linkage region found in a Finnish population. Further efforts to identify susceptible genes in this locus should be worthwhile.

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Disclosures

None.

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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

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TABLE 1. Nine Genes First Sequenced in Chromosome 17 Centromere in 58 Cases (First Cohort)

Gene Symbol	Gene Name	MIM No.	Position	GenBank Accession No. (2006/02/23)	Genomic Region, kb	mRNA Length, bp	No. of Exons
TNFRSF13B	Tumor necrosis factor receptor superfamily, member 13B	604907	16473152–16439349	NT_010718.15	33.804	879	4
M-RIP	Myosin phosphatase-Rho interacting protein	...	16543056–16686620	NT_010718.15	143.565	3114	29
COPS3	COP9 constitutive photomorphogenic homolog	604665	16782340–16747090	NT_010718.15	35.251	1269	12
RAI1	Retinoic acid induced 1	607642	17181736–17312516	NT_010718.15	130.781	5718	8
SREBF1	Sterol regulatory element binding transcription factor 1	184756	17338043–17312341	NT_010718.15	25.703	3441	21
GRAP	GRB2-related adaptor protein	604330	18548021–18522034	NT_010718.15	25.988	651	6
MAPK7	Mitogen-activated protein kinase 7	602521	18877883–18884469	NT_010718.15	6.587	2448	7
MFAP4	Microfibrillar-associated protein 4	600596	18888110–18883573	NT_010718.15	4.538	765	6
AKAP10	A kinase (PRKA) anchor protein 10	604694	19478745–19405569	NT_010718.15	73.177	1986	15

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

third group had 332 control subjects.[10] 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 and D17S1871, where we found significant linkage in families with an IA cluster.[9] 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).[13] 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.[10]

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 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)

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

*Comparison between 304 unrelated cases (second cohort) vs 332 controls (third cohort).
†χ^2 Test.
‡Student t test.

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)

					Minor Allele Frequencies in 3 Cohorts			GenBank Accession No. of mRNA and Effect on Products	Functional Polymorphisms Predicted by Polyphen	Allele Frequency	
Region	Position	Contig Position	rs No.	Change	First Cohort: 58 Cases	Second Cohort: 304 Cases	Third Cohort: 332 Controls			World Wide	Japanese
								NP_036584			
Promoter	c-247	16472985	rs4985754	G>T	0.362	0.339	0.315				ND
Exon3		16449689		G>A	0	0.002	0	S70N	Benign		
Exon3		16449677		A>G	0	0.003	0	E74G	Possibly damaging		
Exon3		16449672		G>A	0	0.013	0.005	G76S	Possibly damaging		
Exon3		16449672		G>T	0.009*	0	0	G76C	Probably damaging		
Intron3	IVS3+25	16449376	rs2274892	C>A	0.353	0.358	0.426			NCBI 0.49	ABI 0.36
Intron3	IVS3-1†	16441271		G>C	0	0.002	0				
Exon4		16441210		A>T	0.009*	0	0	K154X			
Exon4		16441187		T>C	0	0.003	0.002	C177R	Possibly damaging		
Exon4	c0.585-586	16441143		insA	0.009*	0	0.002				
Exon5		16440340		C>T	0.345	0.360	0.342	P251L	Probably damaging		
Exon5		16440261	rs11078355	C>T	0.138	0.145	0.151	S277S			ABI 0.14
No. of subjects having rare variants					3	14	5				

*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; NCBI, National Center for Biotechnology Information; and ABI: Applied Biosystems (http://www.appliedbiosystems.co.jp/website/jp/information/info.jsp?).

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*, *COPS3*, *RAI1*, *SREBF1*, *GRAP*, *MAPK7*, *MFAP4*, and *AKAP10* on the NCBI Map Viewer (available at http://www.ncbi.nih.gov/mapview/maps.cgi?). Primers for coding exons were designed from an intron sequence >50 bp 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 nonsynonymous 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]

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 *Alw*I for c-247G>TT and *Bfu*AI 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)[14] (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://cgi.uc.edu/cgi-bin/kzhang/genotype2LDBlock.cgi).

Population-Attributable Risk

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

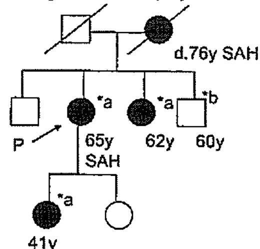
$$\text{Population-attributable risk} = (OR - 1) \times IE \times P / IT$$

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:

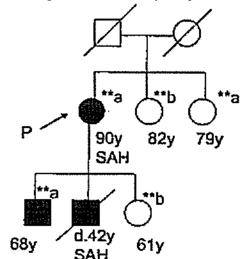
$$\text{Population-attributable risk} = (OR - 1) \times P$$

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.

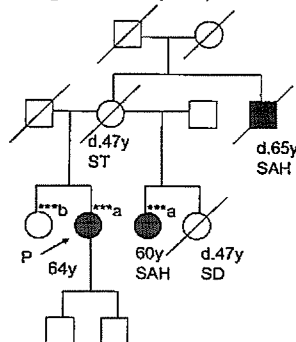
Pedigree 10# (*a) has K154X mutation and (*b) does not have it.



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



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



□ ○ unaffected individuals ■ ● affected individuals / deceased individuals
Pedigree numbers are same as Reference 9 (Yamada et al. *Circulation*. 2004;110:3727-3733).
P: Proband
y: years old
SAH: Subarachnoid hemorrhage
SD: Sudden death
ST: Stroke

Figure 1. Segregation of the *TNFRSF13B* deleterious change with the IA phenotype in pedigrees.

Results

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-

TABLE 4. Four Rare Nonsynonymous Changes, a Splicing Acceptor Site Change, and a Frame Shift in *TNFRSF13B* and the Detected No. of Subjects in 304 Unrelated Cases (the Second Cohort) and 332 Controls (the Third Cohort)

Position	Nucleotide Change	Amino Acid Change	Detected No. of Subjects		P
			Unrelated Case	Control	
Rare nonsynonymous changes					
Exon3	c. 222G>A	S70N	1	0	
Exon3	c. 234A>G	E74G	2	0	
Exon3	c. 239G>A	G76S	8	3	
Exon4	c. 542T>C	C177R	2	1	
Splicing acceptor site change					
Intron3	IVS3-1		1	0	
Frame shift					
Exon4	c.585-586 Insertion A		0	1	
Total			14	5	0.035*

GenBank Accession No. NM_012452.
*Fisher exact test.

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),[9] 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)[9]; 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)[9]; 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.[17–19] The 70th peptide S was conserved in

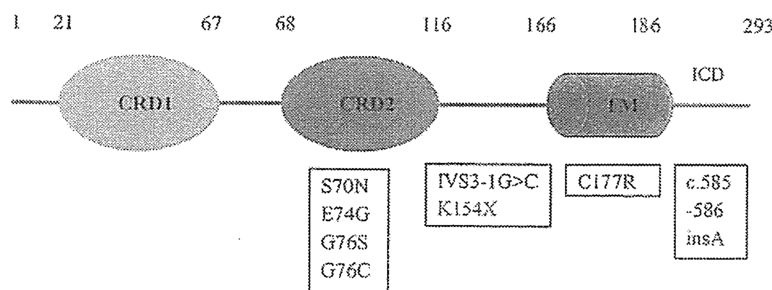


Figure 2. Schema of the domain structure of full-length *TNFRSF13B*.

LD (Linkage Disequilibrium) structure of *TNFRSF13B*

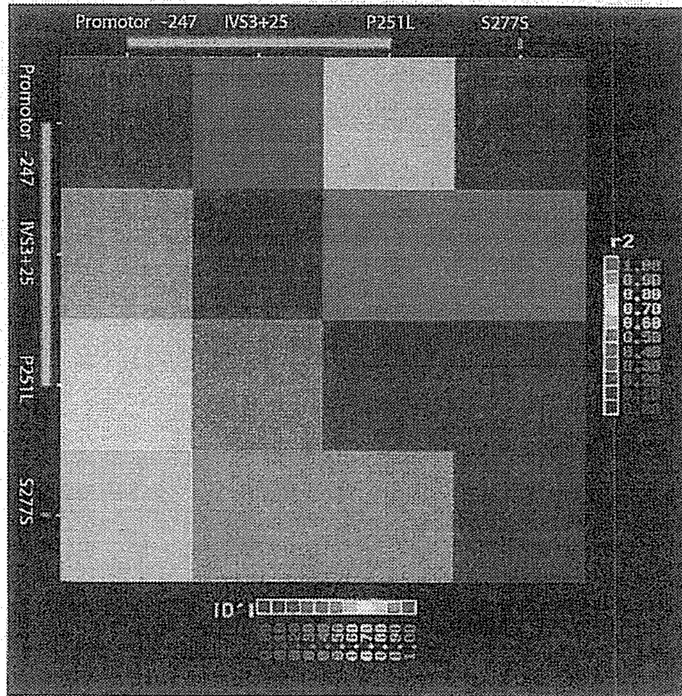


Figure 3. LD structure of *TNFRSF13B*.

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*,[6] *LOX*,[20] and *COL1A2*,[21] no gene has been claimed as a candidate gene. There have been contradictions, however, in terms of involvement of *ELN* in IA.[22,23]

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 5. Allele Frequencies of *TNFRSF13B* Variants in 304 Unrelated Cases (the Second Cohort) and 332 Controls (the Third Cohort)

	Allele Frequency	*P* (HWE)
Locus 1 (G/T)*	0.67/0.33	0.21
Locus 2 (A/C)*	0.39/0.61	0.14
Locus 3 (C/T)*	0.65/0.35	0.23
Locus 4 (C/T)*	0.85/0.15	0.43

HWE indicates Hardy-Weinberg equilibrium.

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

TABLE 6. Haplotype Association Study With Adjustment for Covariates by THESIAS (the Third Cohort)

Haplotype Identification Code	Haplotype Sequence	Frequency of Haplotype		OR (95% CI)	*P*
		Unrelated Case	Control		
H1	GACC	0.211	0.268	0.69 (0.52–0.92)	0.012*
H2	GACT	0.117	0.131	0.82 (0.57–1.18)	0.29
H3	GCCC	0.251	0.215	1.11 (0.79–1.42)	0.70
H4	TCTC	0.289	0.271	Intercept	
Covariate					
Sex (female vs male)				2.26 (1.55–3.30)	0.000024
Hypertension				1.97 (1.44–2.70)	0.000027
Smoking (ever-smoker vs nonsmoker)				1.64 (1.12–2.42)	0.011864
Alcohol (ever-drinker vs nondrinker)				0.91 (0.63–1.33)	0.63

*After Bonferroni correction, P_{corr}=0.048.

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 isotype 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.[29,30] 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.

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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.

Optic Strut as a Radiographic Landmark in Evaluating Neck Location of a Paraclinoid Aneurysm

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OBJECTIVE: The optic strut (OS) is a candidate landmark in computed tomographic (CT) angiographic scans for the discrimination of intradural and extradural/intracavernous aneurysms involving the paraclinoid segment of the internal carotid artery. The goal of this study is to examine and confirm the qualifications of the OS as a landmark in CT angiographic scans for the preoperative evaluation of aneurysms in this region.

METHODS: Seventeen consecutive patients with 18 unruptured paraclinoid aneurysms who underwent preoperative CT angiography scans and direct surgery between 1998 and 2005 were evaluated retrospectively. We focused on the relationships of the necks of aneurysms to the OS in CT angiographic scans and that of the necks to proximal dural rings during intraoperative examinations.

RESULTS: Direct surgery revealed that 14 aneurysms, the necks of which were distal to the OS on CT angiographic scans, arose distal to the proximal dural rings. All aneurysms were clipped, except one exhibiting calcification of the neck. Three aneurysms, for which the neck was proximal to the OS on CT angiographic scans, revealed only a portion or nothing of their domes instead of their necks through the proximal dural rings after dissection of the distal dural rings. Dome coating with fibrin glue and a piece of muscle tissue or mere exploration was performed. Another aneurysm, of which the neck straddled the OS on CT angiographic scans, was found to arise across the proximal dural ring. Clipping of the neck was performed after dissection of the proximal dural ring. Of the source images of CT angiographic scans, the axial images were the most useful in evaluating the relationship of the neck of an aneurysm to the OS.

CONCLUSION: On CT angiographic scans, the OS is a precise identification of the proximal dural ring that forms the superior border of the cavernous sinus. The aneurysms whose necks arise obviously distal to the OS on CT angiographic scans are able to be clipped without dissection of the proximal dural ring.

KEY WORDS: Computed tomographic angiography, Optic strut, Paraclinoid aneurysm, Proximal dural ring

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Discriminating between intradural and extradural/intracavernous aneurysms of the internal carotid artery (ICA) is critical when considering treatment options. It remains difficult to make this distinction, however, because of the lack of reliable landmarks in image examinations (8). We focused our attention on the optic strut (OS), which is the small (3–5 mm) and obliquely oriented osseous bridge superiorly connecting the anterior clinoid process and medially connecting the sphenoid bone. The OS is an attachment site for the proximal dural ring (i.e., the anterior part of the proximal ring attaches to the inferior surface of the OS). The inferior border of the paraclinoid region is formed by the proximal dural ring, which is the anterior roof of the cavernous sinus (17, 18). Gonzalez et al. (4) considered the OS to be a candidate for the identification of the proximal dural ring during computed tomographic (CT) angiographic scans. This small osseous bridge is also visible or easily detectable in axial and coronal source images of CT angiographic scans. We demonstrate here the accuracy of the OS as a landmark for image examination during preoperative evaluation of a paraclinoid aneurysm.

PATIENTS AND METHODS

Between 1998 and 2005, 55 patients with 58 paraclinoid aneurysms were admitted to our hospital. Eight of these patients who

were observed, but did not undergo surgery, were excluded from our study. Six patients with ruptured aneurysms were also excluded because it would have been inappropriate to examine the precise relationship of their necks to the proximal dural rings during surgery. The remaining 44 unruptured paraclinoid aneurysms in 41 patients were treated by direct surgery or endovascular treatment (EVT) (*Table 1*). Of the 21 lesions treated by direct surgery, 20 underwent the ipsilateral pterional approach and one underwent the contralateral pterional approach. In all surgical cases, the carotid bifurcation was exposed at the neck, the anterior clinoid process was drilled out, and the distal dural ring was dissected. Twenty-three lesions received either EVT only or extracranial-intracranial bypass followed by EVT. The method of treatment was chosen by each surgeon based primarily on the findings of digital subtraction angiographic scans. The lesions treated by direct surgery were categorized into four groups, including two subgroups according to al-Rodhan et al.'s (1) classification: one anterior wall lesion (Group Ia), four ventral paraclinoid lesions (Group Ib), nine true ophthalmic artery lesions (Group II), three carotid cave lesions (Group III), three transitional lesions (Group IV), and one cavernous lesion (Group V) (*Table 1*). Three lesions lacking preoperative CT angiographic scans were not included in our study. Eighteen lesions in 17 consecutive patients who underwent preoperative high-resolution CT angiographic scans and direct surgery were retrospectively evaluated using CT angiographic scans (i.e., using the axial, coronal, and sagittal source images) (*Table 2*). We focused on the relationship of the necks of aneurysms to the OS in CT angiographic scans and that of the necks to the proximal dural rings in the intraoperative findings. A neurosurgeon and a neuroradiologist blinded to the intraoperative findings identified the neck of aneurysms and the OS and judged the relationship between the location of the aneurysmal neck and that of the OS (i.e., whether the aneurysm is located distally or proximally to the OS) on the basis of CT angiographic scans.

TABLE 1. Number of aneurysms in each group of al-Rodhan classification

al-Rodhan's classification	Surgical group	Endovascular group
Ia	1	2
Ib	4	1
II	9	8
III	3	7
IV	3	0
V	1	5
Total	21	23

TABLE 2. Summary of 21 patients in the surgical group[a]

Patient no.	Age (yr)/sex	Side	Group	Operating method	Site of neck to optic strut
1	38/F	L	Ia	Neck clipping	Distal
2	56/F	R	Ib	Neck clipping	X[c]
3	57/F	R	Ib	Neck clipping	Distal
4	55/F	L	Ib	Neck clipping	Distal
5	61/F	R	Ib	Neck clipping	Distal
6	69/F	L	II	RA + trapping (calcification)[b]	Distal
7	54/F	L	II	Neck clipping	Distal
8	68/F	L	II	Neck clipping	Distal
9	66/F	R	II	Neck clipping	X[c]
10	56/F	R	II	Neck clipping	Distal
11	19/M	L	II	Neck clipping	Distal
12	22/F	L	II	Neck clipping	Distal
13	50/F	L	II	Neck clipping	Distal
14	59/F	R	II	Neck clipping	Distal
15	41/M	R	III	Neck clipping	Distal
16	54/F	R	III	Neck clipping	Distal
17	61/F	L	III	Neck clipping	Both
18	69/F	R	IV	Coating only	Proximal
19	59/F	R	IV	Coating only	Proximal
20	64/F	R	IV	Exploration only	X[c]
21	69/M	L	V	Exploration only	Proximal

[a] L, left; R, right; RA, radial artery graft.
[b] Impossible to clip because of neck calcification.
[c] Patients who did not undergo preoperative computed tomographic angiography.

CT angiographic scans were performed on an eight-detector-row multidetector helical scanner (Aquilion, Toshiba Medical Systems, Tokyo, Japan). CT scans were obtained using 1.0-mm section collimation during a bolus intravenous injection of 50 ml of contrast medium. Multiplanar reformats were created using a 1.0-mm section thickness in the axial, coronal, and sagittal planes. We selected images that best profiled the OS and its relationship to the necks of paraclinoid aneurysms. Evaluations by CT angiographic scans were compared with the intraoperative findings.

RESULTS

Between 1998 and 2005, 17 consecutive patients with 18 unruptured paraclinoid aneurysms (three men; 14 women; mean age, 54.7 yr; age range, 19–69 yr) who underwent preoperative CT angiographic scans and subsequent direct

surgery were evaluated in regard to their CT angiographic scanning data and operative findings. During surgery, 14 out of 18 lesions distal to the OS in CT angiographic scans were found arising distal to the proximal dural ring. All lesions were clipped, except one exhibiting neck calcification. It was impossible to clip the lesion with neck calcification, so we performed an extracranial-intracranial bypass with a radial artery graft and trapping of the ICA. In Patient 10 (*Table 2*), the aneurysm was clearly distal to the OS in the axial and coronal source images by CT angiographic scans (*Fig. 1*, Group II). During surgery, the neck and the dome were found distal to the proximal dural ring, and the neck was clipped. In Patient 18, the neck was proximal to the OS, and only part of the dome (instead of the neck) was visible distal to the proximal dural ring (*Fig. 2*, Group IV). Dome coating was performed with fibrin glue and a section of muscle. In Patient 17, the neck straddled the OS from proximal to distal on CT angiographic scans, and direct surgery demonstrated that a portion of the neck was proximal to the proximal dural ring (*Fig. 3*, Group III). The proximal end of the neck was not visible even after the optic nerve was mobilized by canal unroofing and the distal dural ring was dissected, whereas the majority of the neck was distal to the proximal ring. Premature rupture occurred when neck clipping was attempted without sufficient dissection of the proximal dural ring. Finally, neck clipping was performed after sufficient dissection of the proximal dural ring, division of the ipsilateral ophthalmic artery, retrograde suction decompression (2), and exposure of the proximal end of the neck.

The coronal and axial source images of CT angiographic scans demonstrated in exquisite detail the necks of the paraclinoid aneurysms and the OS, whereas the sagittal source images often failed to demonstrate these features. The axial images, especially, precisely showed the relationship of the paraclinoid aneurysm necks to the OS.

DISCUSSION

It is extremely important to identify the locations of dural rings by radiographic examination because, if aneurysms in

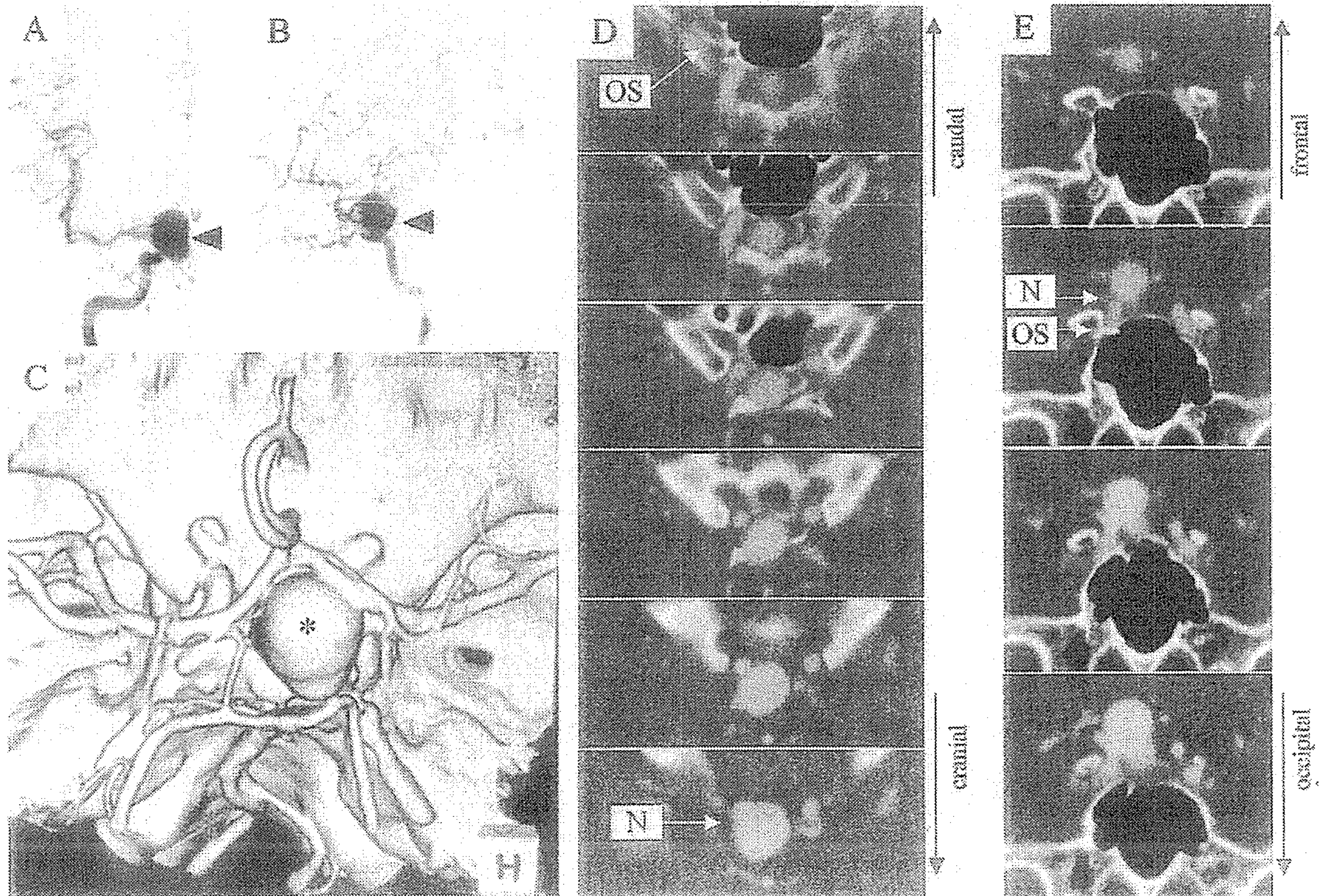

FIGURE 1. *Patient 10. Preoperative anteroposterior (A) and lateral (B) digital subtraction angiographic scans showing IC-ophthalmic aneurysm (arrowheads) (al-Rodhan Group II). C, preoperative three-dimensional reformatted CT angiographic scanning study, with an asterisk denoting the aneurysm. Preoperative axial (D) and coronal (E) reformatted images. Aneurysm arises distal to the OS. Upper images in D are more caudal, and upper images in E are more frontal. This aneurysm was clipped. N, neck of aneurysm.*