

TABLE 1  
Risk of rupture associated with UCAs in Japan\*

Category	No. of Included Studies	No. of Cases	Patient-Years	No. of Ruptures	% Risk of Rupture per 100 Patient-Years (95% CI)	RR (95% CI)	OR of Rupture (95% CI)
overall	13	922	3801	104	2.7 (2.2–3.3)		
patient sex	10						
male†		294	1370	30	2.2 (1.5–3.1)		
female		435	1803	55	3.0 (2.3–4.0)	1.4 (0.9–2.2)	1.3 (0.85–2.22)
patient age (yrs)	11						
<60†		297	1543	35	2.3 (1.6–3.2)		
≥60		520	2077	62	3.0 (2.3–3.8)	1.2 (0.8–1.9)	1.17 (0.74–4.96)
history of SAH	13						
no†		709	2786	62	2.7 (2.1–3.4)		
yes		209	1010	28	2.8 (1.8–4.0)	1.3 (0.85–2.0)	1.06 (0.64–1.74)
symptoms present	13						
no†		876	3657	94	2.6 (2.1–3.1)		
yes		42	137	10	7.3 (3.5–13.4)	2.1 (1.1–3.9)‡	2.20 (0.97–4.96)
aneurysm site	10						
ant†		770	3370	59	1.8 (1.3–2.3)		
pst		127	618	20	3.2 (2.0–5.0)	2.3 (1.4–3.7)‡	2.18 (1.23–3.86)‡
aneurysm size (mm)	11						
<10†		585	2045	31	1.5 (1.0–2.2)		
≥10		100	344	32	9.3 (6.4–13.1)	6.4 (4.0–10.4)‡	8.68 (4.59–16.41)‡

\* Ant = aneurysm located in the anterior circle of Willis; pst = aneurysm located in the posterior circle of Willis.

† Referenced category.

‡ Statistically significant RR or rupture rate and OR of rupture were obtained according to the method described by Breslow and Day. In the table the OR represents (odds of rupture in the group)/(OR of rupture of the referenced group).

been obliterated or no other known source of hemorrhage was detected. 5) The UCA was diagnosed using either conventional or digital subtraction angiography, magnetic resonance angiography, three-dimensional computerized tomographic angiography, or a combination of these methods. 6) Documentation of the rupture of the aneurysm (that is, the SAH) could be confirmed with the aid of computerized tomography scanning, lumbar puncture, surgery, or a post-mortem examination.

If several publications originated at the same institution, the most recent series or the series including the largest number of cases was selected for review. In studies missing some inclusion criteria,<sup>17,19,20,27,28</sup> we sent requests to the authors and included the study if they could provide the missing data.

#### Data Extraction and Analysis

Once a study was deemed eligible for review, two of us independently extracted the following data: the total number of cases, the period of follow up, and the number of aneurysm ruptures. We also sent out requests to authors of the studies for additional detailed data not described in the papers. When data were available for stratification, we extracted information concerning patient age (11 studies) and sex (10 studies), the size of the aneurysm (11 studies), the site of the aneurysm (10 studies), any history of SAH (all 13 studies), symptoms caused by the aneurysm (all 13 studies), and incidences of death from SAH (11 studies). If data were available, we also extracted the number of surgeries performed on UCAs during the same period of study at the same institution, and the timing of lesion rupture. The influences of high blood pressure or smoking and their association with the rupture were discussed in very limited series<sup>2,7,17</sup> and were not assessed in this review.

To calculate the risk of aneurysm rupture, we multiplied

the total number of patients by the average period of follow up in each study to obtain the total number of patient-years of follow up. The number of patients with subsequent SAH was then divided by the number of patient-years to yield the risk of rupture per 100 patient-years.<sup>22</sup> We used this method to calculate the risk of rupture in all patients and in the various subgroups. We asked the authors of all 13 studies to provide follow-up periods for each subgroup, but only four authors could do so.<sup>17,26–28</sup> Therefore, the other subgroups were analyzed using the average follow-up period of the total number of cases in each study.<sup>3,22</sup>

#### Analysis of the Review

To identify any variations among the studies in this review, we compared data between factors in the following subgroups: 1) studies including more than and fewer than 50 patients; 2) studies published in English and those in Japanese; and 3) studies from four districts: Touhoku, Kanto, Kinki-Hokuriku, and Chyuugoku-Shikoku.

#### Comparison With Large-Scale Cohorts and Other Retrospective Reviews

We compared the findings of our review with those of two large-scale international cohort studies (the ISUIA retrospective and prospective cohorts)<sup>11,12</sup> and a systematic review by Rinkel, et al.<sup>22</sup> The review of Rinkel and colleagues incorporated three Japanese series, and we analyzed the data both to include and exclude Japanese cases. We also compared patient characteristics and the overall rupture rate obtained by calculating total patient-years and total cases of rupture.

#### Statistical Analysis

The chi-square and Fisher exact tests were used to com-

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TABLE 2  
Summary of included studies\*

Authors & Year	No. of Cases	Patient-Years	No. of Ruptures	% Rupture Rate (95% CI)	No. of Deaths	Location of Institution
Inagawa, et al., 1992	47	240	1	4.0 (0–1.5)	NA	CS
Asari & Ohmoto, 1993	54	197	11	5.6 (2.8–10.0)	10	CS
Mizoi, et al., 1995	49	211	4	1.9 (0.5–4.9)	3	TH
Yasui, et al., 1997	234	1463	34	2.3 (1.6–3.2)	18	TH
Ikawa, et al., 1998†	36	155	7	4.5 (1.8–9.3)	7	CS
Yasui, et al., 1998†	10	25	1	4.0 (0–14.8)	0	KH
Ikeda, et al., 2000†	33	158	11	6.9 (3.5–12.4)	NA	KH
Tsutsumi, et al., 2000	62	267	7	2.6 (1.1–5.4)	6	TH
Murata, et al., 2001†	48	121	4	3.3 (0.9–8.4)	1	KH
Suga, et al., 2002†	100	317	5	1.6 (0.5–3.7)	3	CS
Tsukahara, et al., 2002	110	217	7	3.2 (1.3–6.6)	2	NA
Matsumoto E, et al., 2003	48	158	7	4.4 (1.8–9.1)	7	KT
Matsumoto K, et al., 2003	91	273	5	1.8 (0.6–4.3)	4	KH

\* CS = Chyuugoku-Shikoku district; KH = Kinki-Hokuriku district; KT = Kanto district; NA = not available; TH = Touhoku district.  
† Published in Japanese.

pare differences between risk factors and the studies. The Mantel–Haenzel method was also used to compare differences in the included series and to obtain RRs and ORs between subgroups.<sup>3</sup> A probability value less than 0.05 was considered significant. Statistical analyses were performed with the aid of a commercially available software program (SAS, version 8; SAS Institute, Inc., Cary, NC).

### Results

#### Rupture Rate of Untreated UCAs in Japanese Institutions

Thirteen studies fulfilled our inclusion criteria.<sup>2,6–8,16,17,19,20,24–28</sup> Of these, eight were reported in English and the other five in Japanese. Twelve authors responded to our requests and nine provided additional information regarding subgroups.<sup>6,8,17,19,20,24,26–28</sup> Five studies included only asymptomatic cases,<sup>7,20,24,26,28</sup> and two studies included only cases without SAH.<sup>24,26</sup> One study included only aneurysms located in the anterior circulation,<sup>17</sup> and one included only patients older than 70 years.<sup>28</sup> We were able to incorporate these studies because the numbers of patients and ruptures were provided. A summary of the studies is shown in Table 1.

The 13 studies included 922 patients with UCAs who were followed up for a total of 3801 patient-years. There were 104 subsequent ruptures among the entire patient population (11%), constituting an annual rupture rate of 2.7% (95% CI 2.2–3.3%). The risk of rupture was significantly higher for large aneurysms (RR 6.4, 95% CI 4.0–10.4), aneurysms located in the posterior circulation (RR 2.3, 95% CI 1.4–3.7), and symptomatic aneurysms (RR 2.1, 95% CI 1.1–3.9) compared with each referenced category. Unruptured cerebral aneurysms tended to burst more often in patients presenting with SAH, in women, and in patients older than 60 years, but the differences between these groups were not significant. Because our rupture risks were calculated on the basis of an estimated average follow-up period in some series, we also obtained the OR for the net number of all cases of ruptured aneurysms/all cases of unruptured aneurysms in each subgroup, which would not be affected by the follow-up period. The OR of ruptured lesions in the

subgroups also showed a similar tendency for a high rupture risk in large aneurysms, posterior-circulation lesions, and aneurysms with symptomatic presentation (ORs 8.68, 2.18, and 2.20, respectively).

In eight reports the authors discussed the risk of rupture of small aneurysms (< 5 mm); seven of 40 ruptured aneurysms in these studies were smaller than 5 mm.<sup>6,8,17,19,20,24–26</sup> Among patients with aneurysms measuring 5 or 6 mm, an additional four of 40 lesions ruptured; hence, 27.5% (11 aneurysms) of the ruptured aneurysms were smaller than 7 mm. Because of the low number of small aneurysms and the lack of details about the follow-up period, we could not calculate the rupture rate for this group.

Data on this incidence of death caused by SAH from 11 studies showed that 61 (66%) of 92 patients died of SAH.

Eleven reports provided data about surgery during the same period as the studies.<sup>2,7,8,16,17,19,20,25–28</sup> In these 11 studies, surgery was performed in 1601 patients, compared with 787 who were only observed.

The timing of rupture was documented in 11 series, but the total number of ruptures in each year could be obtained from only eight series.<sup>2,6–8,20,24,26,28</sup> Among 47 cases of subsequent rupture, 19 lesions ruptured within 1 year, nine ruptured within the 2nd year, and the remaining 19 aneurysms ruptured after the 2nd year. When we included three additional series that provided Kaplan–Meier life tables showing the timing of rupture,<sup>16,17,27</sup> we noted no acute increase in the rupture rate in any specific year.

#### Variations Among the Included Studies

A summary of the included studies appears in Table 2 and Fig. 1. The series reported by Ikeda and colleagues<sup>7</sup> and Inagawa, et al.,<sup>8</sup> demonstrated statistically significant differences when they were compared with other studies regarding the rupture rate ( $p = 0.0068$ ). Excluding these two studies from the analysis did not alter the results of our review. Although the overall rupture rate was higher in studies written in Japanese and in those studies including fewer than 50 patients, no significant difference was noted between groups. Geographic location, which can influence the patient's living environment, did not influence the risk of rupture.

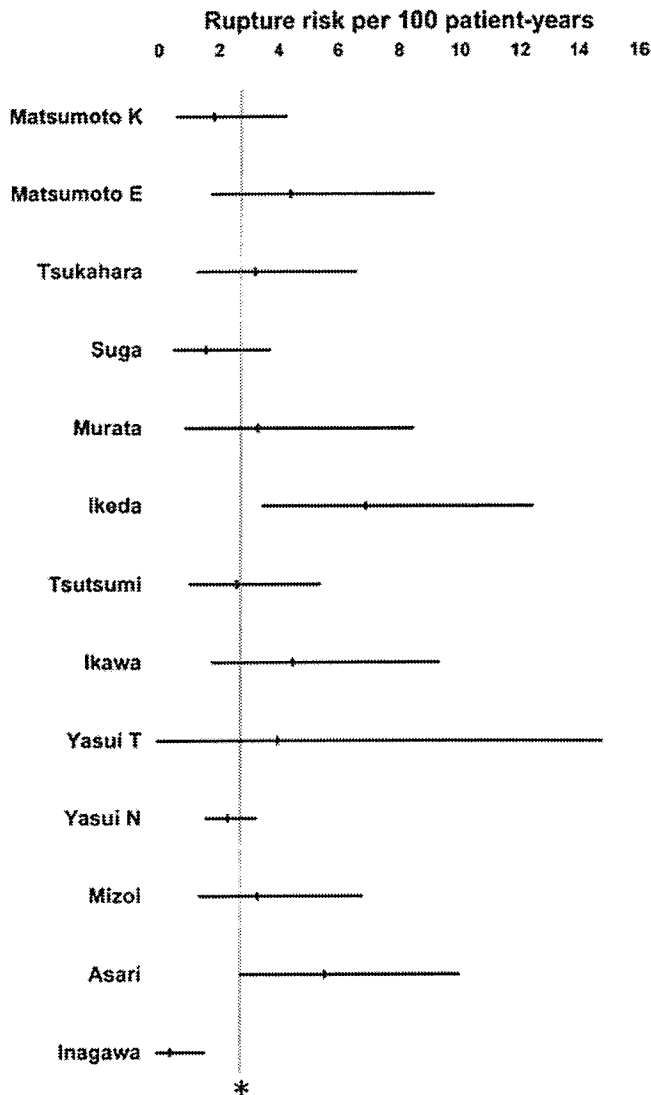


FIG. 1. Chart showing the risk of rupture associated with UCAs in the included studies. The asterisked line indicates the average rupture rate of all cases.

#### Comparison With International Studies

Table 3 offers a comparison of our findings and those of the ISUIA<sup>11,12</sup> and the systematic review from Europe by Rinkel and colleagues.<sup>22</sup> The ISUIA retrospective and prospective cohorts included significantly more female patients, symptomatic aneurysms, and lesions presenting with SAH than our review did. The retrospective cohort included significantly more cases of large aneurysms but fewer cases in which the lesion was located in the posterior circulation. Compared with our study, the prospective group did not show any differences in these subgroups. The ages of patients who were included could not be compared. The overall rupture rate in the 13 studies we reviewed was significantly higher compared with the rates of both the prospective and retrospective ISUIA cohorts. Our review also showed a higher risk of rupture compared with the review by Rinkel, et al.,<sup>22</sup> whereas the significance was less ( $p = 0.02$ ). Excluding Japanese series from the European study

did not change our findings. The European review included more cases of anteriorly located lesions, symptomatic aneurysms, and lesions with SAH.

#### Discussion

##### Rupture Rate Associated With UCAs

This systematic review shows that the rupture risk of UCAs observed over time in Japanese institutions is relatively high. Eleven percent of all patients who were included in the articles we reviewed experienced rupture of the aneurysm and the average annual rupture rate was 2.7% (95% CI 2.2–3.3%). An analysis of subgroups by RRs of rupture per 100 patient-years and the OR of cases of rupture versus those of nonrupture showed that significant factors influencing the rupture risk include the size, location, and symptomatic presentation of the aneurysm. Patient sex and age and a history of SAH also affected the risk, but these factors did not reach statistical significance. Papers published in Japanese and papers including fewer cases documented a slightly higher risk of rupture, but no significant difference was recognized. Multiple trials have failed to identify any influence of climate, physical stress, or emotional stress on the rate of SAH.<sup>23</sup> To determine whether there was any influence of climate or environmental stress on the rupture risk of UCAs among Japanese patients, in addition to the geographical location of the institution, we compared studies according to average yearly temperature, the largest difference in the average temperature within a year in the territory of each institution (data extracted from the database of the Japan Meteorological Society, [http://www.jma.go.jp/JMA\\_HP/jma/indexe.html](http://www.jma.go.jp/JMA_HP/jma/indexe.html)), and the population data (data obtained from the database of the Japan Statistics Bureau, <http://www.stat.go.jp/english/index.htm>). Japanese people usually seek medical care close to their homes, and thus we assumed that the statistics and climate information of a particular institute reflect the patient's living environment. Our comparison of 12 studies (the 13th was a multicenter study that could not be confined to a single area) revealed no apparent differences in climate or population subgroups. In this review, we could not assess other previously documented risk factors associated with the rupture rate, such as hypertension or smoking. Of note, the prevalence of hypertension or smoking in Japanese adults is not higher than that of the US population.<sup>5,18,21</sup>

By systematically collecting data from case series, we determined the number of patient-years and used that number in our analysis of the data. Although our review showed that there were fewer female patients and fewer patients with SAH or symptomatic aneurysms, overall these characteristics did not significantly differ from those of large cohort studies. The rupture rate documented in our review, however, was significantly higher.<sup>11,12</sup> A comparison with the systematic review by Rinkel, et al.,<sup>22</sup> also showed a difference, but this difference was smaller ( $p = 0.02$ ). Even in the patient group with the least risk (patients with aneurysms < 10 cm), the calculated rupture rate was 1.5% per year in our review (95% CI 1.0–2.15%). In addition, rupture was reported in 11 cases in which the aneurysm was smaller than 7 mm (27.5% of all ruptured cases in the pertinent series).

##### Race or Bias

To explain the high rupture rate documented in our re-

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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<sup>15</sup>—almost nine times higher than the incidence observed in Rochester, MN (11 per 100,000 annually<sup>10</sup>). Nevertheless, the prevalence of UCAs is not significantly different among the populations studied,<sup>22</sup> 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.,<sup>14</sup> 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.<sup>11</sup> Elderly people are also known to have a higher rate of SAH.<sup>15</sup> These factors elevate surgical risks,<sup>11</sup> 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<sup>11,12</sup> or the review written in Europe.<sup>22</sup> 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<sup>11</sup> 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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Nobuo Hashimoto and Akio Koizumi

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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.<sup>1,2</sup> 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.<sup>3-5</sup> 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.<sup>6,7</sup> 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.<sup>8-15</sup> Among these, elastin (*ELN*) and the LIM domain kinase 1 (*LIMK1*) genes in 7q11,<sup>8,16</sup> collagen alpha2(I) (*COL1A2*) in 7q22,<sup>17</sup> and tumor necrosis factor receptor, superfamily member 13B (*TNFRSF13B*) in 17cen have been

reported to be associated with IA.<sup>18</sup> 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,<sup>19-23</sup> 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.<sup>24</sup> 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.<sup>11</sup> 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.<sup>14,15,25</sup> 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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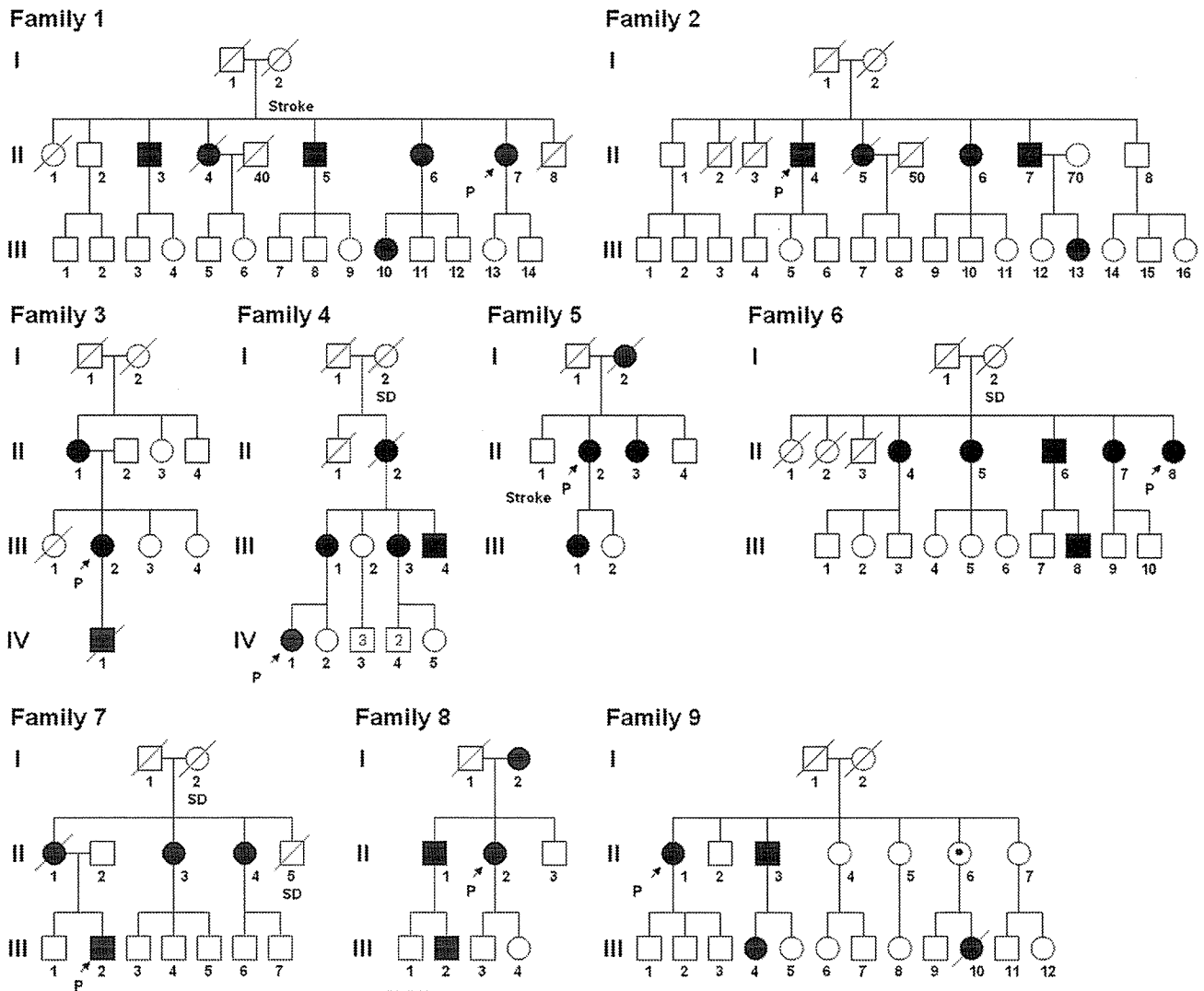
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**Figure 1.** Familial intracranial aneurysm pedigrees. Filled symbols represent affected individuals; open symbols, unaffected individuals; open symbols with black circles, obligate carriers; circles, women; squares, men; symbols with oblique bars, 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.<sup>13</sup> 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,<sup>13</sup> 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.<sup>13</sup>

### 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.marshfield>



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/](http://www.ncbi.nlm.nih.gov/genome/sts/)). Polymerase chain reactions were carried out in 7.5  $\mu$ L 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<sup>26</sup> and Merlin software<sup>27</sup> were used to detect genotyping errors and Mendelian inconsistency. When genotyping resulted in no call or an ambiguous one, the genotype was set to unknown.

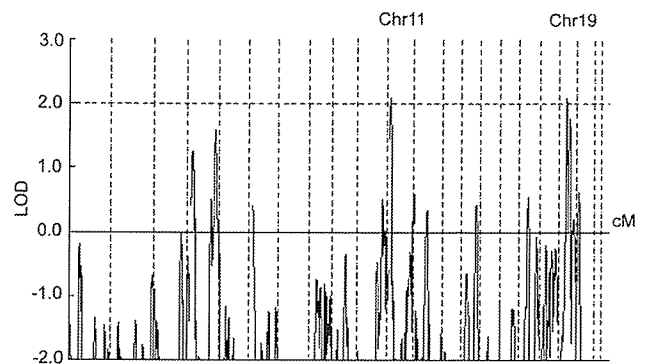
### Linkage Analysis

Because the estimated prevalence of IA is approximately 2%,<sup>28</sup> 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%<sup>29</sup> and that the percentage of families with an apparently AD mode of inheritance accounted for approximately 50%,<sup>34</sup> 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)<sup>30</sup> and Merlin software.<sup>27</sup> Because locus heterogeneity seems to be associated with IA, we obtained both LOD and HLOD scores.<sup>30</sup> 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.<sup>31</sup>

### Results

The results of the present study were obtained by reanalyzing the previously published data set.<sup>13</sup> 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.



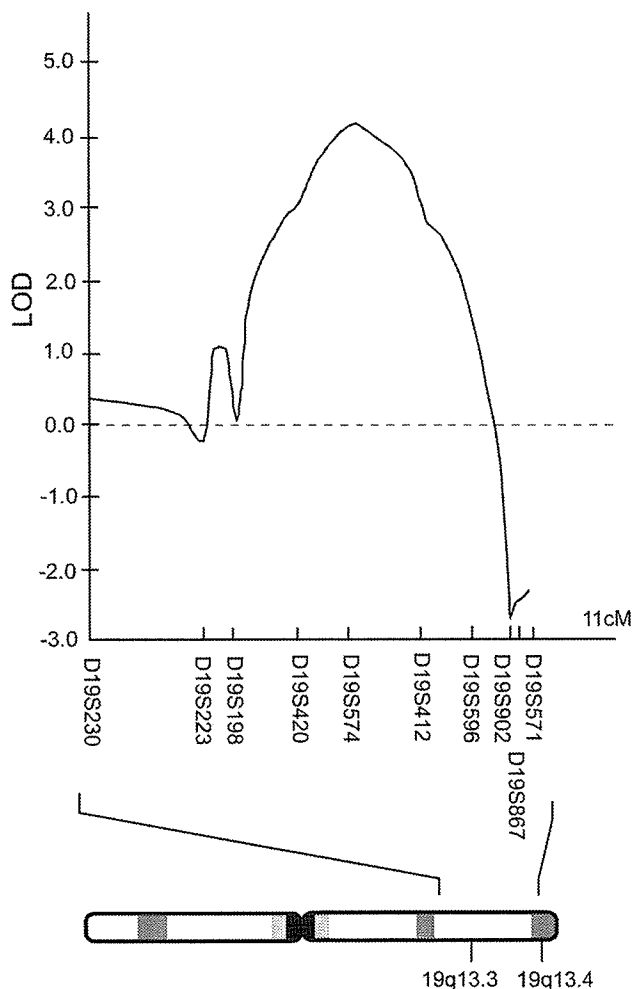
**Figure 2.** Genomewide screening of nine intracranial aneurysm families with dominant models. X-axis, genetic distance; Y-axis, logarithm of odds score. The vertical dotted lines show chromosomal boundaries, and the horizontal dotted line shows the cut-off for logarithm of odds score of  $>2.0$ . Chr 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.<sup>18</sup> 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  $P=0.019$ ).<sup>13</sup> 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.<sup>12</sup> 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.<sup>32</sup>



**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<sup>18</sup> 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.<sup>18</sup> 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.<sup>33</sup> *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.<sup>34</sup> Plasmin and pro-MMP-2 have been shown to be increased within intracranial aneurysms<sup>35,36</sup>; therefore, uPAR may be associated with aneurysmal formation through extracellular matrix remodeling. RAI inhibits the transcriptional activity of nuclear factor kappaB (NFkB),<sup>37</sup> which controls the expression level of MMPs, including MMP2.<sup>38</sup> PTGIR is a receptor of prostaglandin I<sub>2</sub> (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,<sup>39</sup> 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.<sup>1</sup> 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.<sup>2,3</sup> A positive family history is a risk factor as strong as smoking, hypertension, and heavy consumption of alcohol.<sup>4,5</sup>

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

Because disease and genetic heterogeneity are postulated for IA,<sup>11</sup> 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.<sup>12</sup> 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.<sup>9</sup>

### Methods

#### Study Design

Subjects from 3 groups participated. The first group comprised probands of 29 pedigrees with IA clustering.<sup>9</sup> 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
<i>TNFRSF13B</i>	Tumor necrosis factor receptor superfamily, member 13B	604907	16473152-16439349	NT_010718.15	33.804	879	4
<i>M-RIP</i>	Myosin phosphatase-Rho interacting protein	...	16543056-16686620	NT_010718.15	143.565	3114	29
<i>COPS3</i>	COP9 constitutive photomorphogenic homolog	604665	16782340-16747090	NT_010718.15	35.251	1269	12
<i>RAI1</i>	Retinoic acid induced 1	607642	17181736-17312516	NT_010718.15	130.781	5718	8
<i>SREBF1</i>	Sterol regulatory element binding transcription factor 1	184756	17338043-17312341	NT_010718.15	25.703	3441	21
<i>GRAP</i>	GRB2-related adaptor protein	604330	18548021-18522034	NT_010718.15	25.988	651	6
<i>MAPK7</i>	Mitogen-activated protein kinase 7	602521	18877883-18884469	NT_010718.15	6.587	2448	7
<i>MFAP4</i>	Microfibrillar-associated protein 4	600596	18888110-18883573	NT_010718.15	4.538	765	6
<i>AKAP10</i>	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.<sup>10</sup> 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.<sup>9</sup> 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>).<sup>13</sup> 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.<sup>10</sup>

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)**

	First Cohort (Pedigree and Nonpedigree): 29 Probands and 29 Unrelated Cases (n=58)	Nonpedigree Cohorts		P*
		Second Cohort: 304 Unrelated Cases (n=304)	Third Cohort: 332 Controls (n=332)	
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<sup>2</sup> 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)**

Region	Position	Contig Position	rs No.	Change	Minor Allele Frequencies in 3 Cohorts			GenBank Accession No. of mRNA and Effect on Products	Functional Polymorphisms Predicted by PolyPhen	Allele Frequency	
					First Cohort: 58 Cases	Second Cohort: 304 Cases	Third Cohort: 332 Controls			World Wide	Japanese
Promoter	c-247	16472985	rs4985754	G>T	0.362	0.339	0.315	NP_036584			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*, *COP3*, *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 Prologo (Prologo Primers & Probes, Kyoto, Japan; available at <http://www.prologo.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).<sup>9</sup>

### Association Study

SNPs of *TNFRSF13B* with allele frequency  $\geq 1\%$  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 *Aha*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  $\geq 1\%$  in the third cohort by THESIAS (Testing Haplotype Effects In Association Studies)<sup>14</sup> (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  $\geq 80\%$ <sup>15</sup> or a set of all haplotypes for which frequencies were  $\geq 5\%$ .<sup>16</sup> 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.

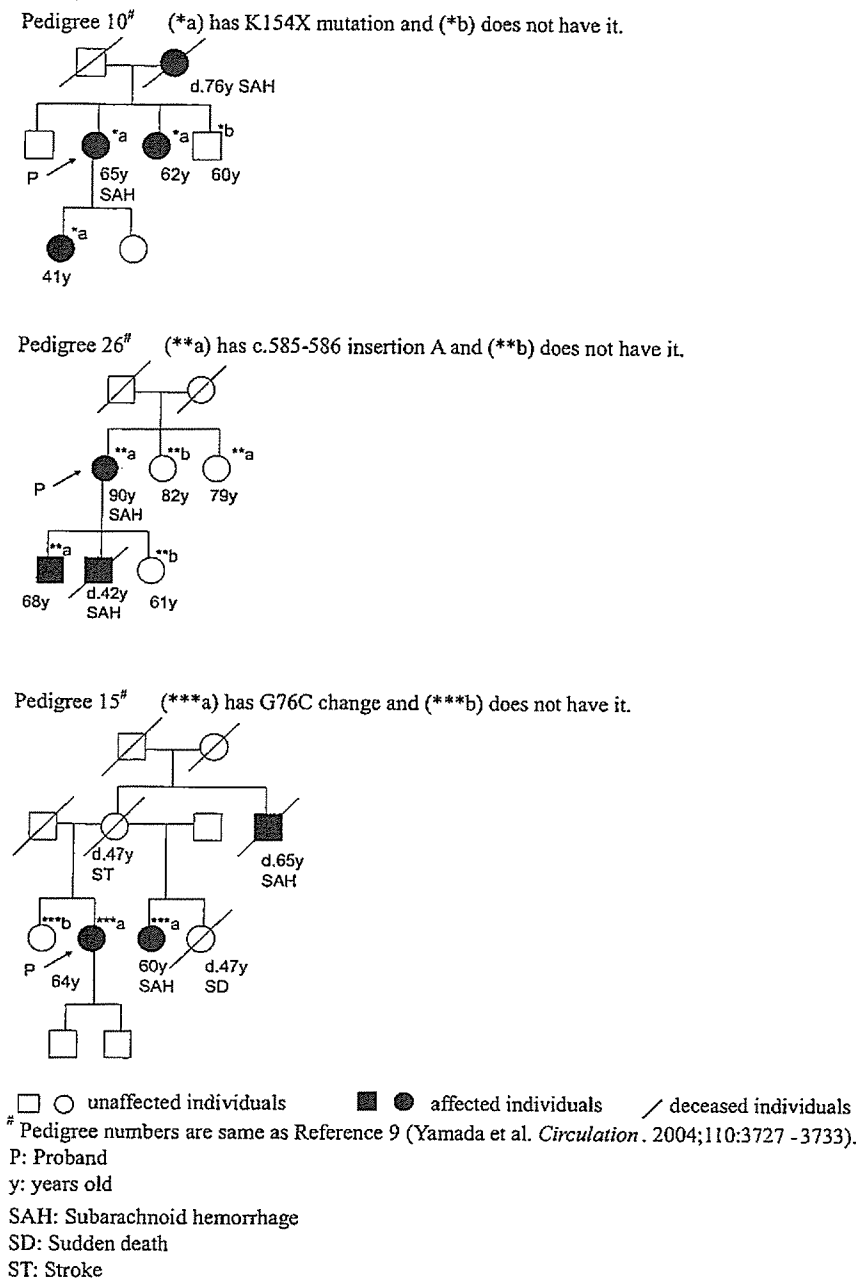


Figure 1. Segregation of the *TNFRSF13B* deleterious change with the IA phenotype in pedigrees.

## 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					
Intron5	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),<sup>9</sup> 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)<sup>9</sup>; 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

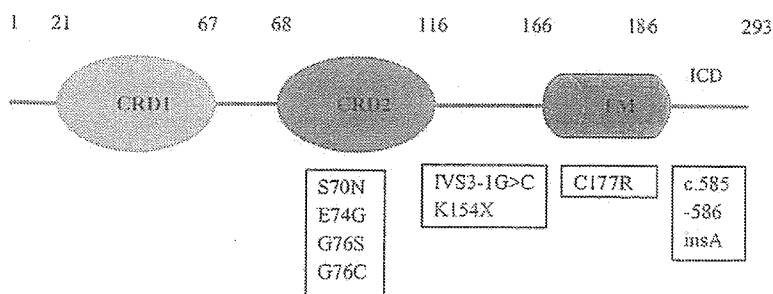
family (pedigree 15)<sup>9</sup>; 2 affected sisters had this variant, but an unaffected sister did not.

**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.<sup>17-19</sup> The 70th peptide S was conserved in

*TNFRSF13B*



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

CRD: cysteine-rich domain

TM: trans-membrane

ICD: intracellular regions



LD (Linkage Disequilibrium) structure of *TNFRSF13B*

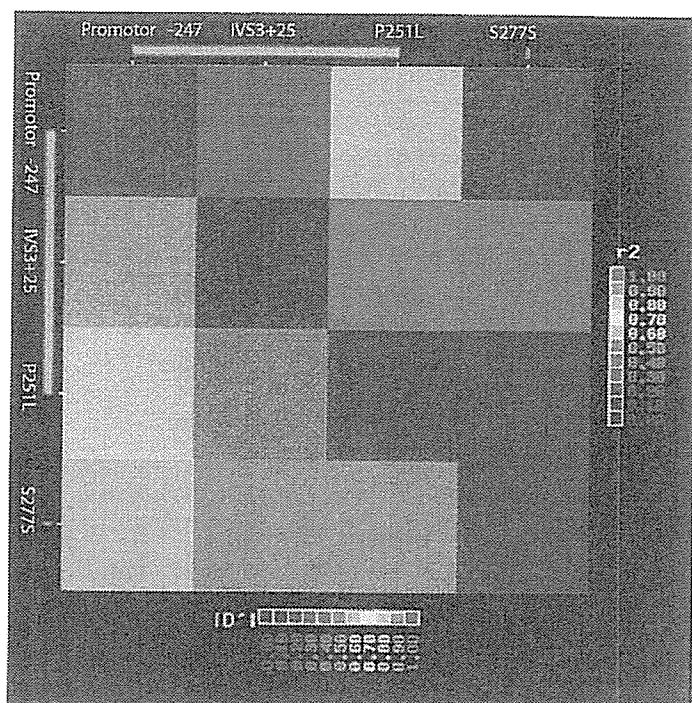


Figure 3. LD structure of *TNFRSF13B*.

*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  $\geq 1\%$  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 ( $P_{\text{corr}}=0.048$ ).

#### 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*,<sup>6</sup> *LOX*,<sup>20</sup> and *COL1A2*,<sup>21</sup> no gene has been claimed as a

candidate gene. There have been contradictions, however, in terms of involvement of *ELN* in IA.<sup>22,23</sup>

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,  $P_{\text{corr}}=0.048$ .

IA, which implies that the penetrance of IA is not as complete as was expected.<sup>24,25</sup> 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.<sup>26,27</sup> In 1 of these studies, 11 mutations (4.1%) were found in 270 chromosomes from 135 sporadic CVID cases.<sup>26</sup> 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.<sup>26,27</sup> 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  $\approx 24\%$ , 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 *TNFRSF13B*. 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.<sup>28</sup> 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.<sup>29,30</sup> 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*, *COP3*, *RAII*, *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.