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

Youhei Minehara, MD; Kayoko Inoue, MD, MPH, PhD; Sumiko Inoue, PhD; Shigeki Yamada, MD, PhD; Kazuhiro 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;38:1174-1178.)

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, 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, LIMK1 and 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 COLIA2 or TNFRSF13B with IA has not been replicated in an independent study.24

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.
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. 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, 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.

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-

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.
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 and Merlin software 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%, 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% and that the percentage of families with an apparently AD mode of inheritance accounted for approximately 50%, 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 obligatorily affected 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) and Merlin software. Because locus heterogeneity seems to be associated with IA, we obtained both LOD and HLOD scores. 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.

**Results**

The results of the present study were obtained by reanalyzing the previously published data. 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 D11S538 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.

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. 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). 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. The loci in the two studies overlap by 1.3 cM between markers D19S45 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.
In this study, we included a family that in our previous study\textsuperscript{18} showed a segregation of mutation in \textit{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 \textit{TNFRSF13B} was shown to be segregated in family 5.\textsuperscript{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.\textsuperscript{33} PLAUR is a key molecule in the regulation of plasmin activity on cell surfaces. PLAUR and its ligand, together with prometallproteinase-2 (pro-MMP-2), promote angiogenic processes through their proteolytic properties.\textsuperscript{34} Plasmin and pro-MMP-2 have been shown to be increased within intracranial aneurysms\textsuperscript{35,36}; therefore, uPAR may be associated with aneurysmal formation through extracellular matrix remodeling. RAI inhibits the transcriptional activity of nuclear factor kappaB (NFkB),\textsuperscript{37} which controls the expression level of MMPs, including MMP2.\textsuperscript{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,\textsuperscript{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.

**Acknowledgments**

We thank Norio Matsuura and Eriko Mori for technical assistance and the following doctors for patient recruitment and help in ascertaining MRA examinations: Shinsuke Tominaga, Hiroshi Hasegawa, and Yoshihiko Inui (Tominaga Hospital); Shyunichi Yoneda and Yoshiito Nareu (Nihonbashi Hospital); Yoo Kang (Ohno Kinen Hospital); Hirooyasu Yamakawa (Gero-spring Hospital); Atsushi Kawarazaki (Kawarazaki Hospital); Masayuki Matsuda (Shiga University of Medical Science); Michiyasu Suzuki and Sadahiro Nomura (Yamaguchi University School of Medicine); Takaaki Kaneko and Takuya Moriwaki (Hikone Municipal Hospital); Nozomu Murai (Otowa Hospital); Tomoo Tokime, Shoichi Tani, and Susumu Kanemoto (Tenri Hospital); Tatsumiho Yamagami (Kyoto Kizugawa Hospital); Motoharu Fujii (International University of Health and Welfare); Hitaru Ohishi, Kiminari Ohtaka, Junko Sasaki, Koki Iwaya, and Masaya Iwakawa (Senboku Kumiai Sougou Hospital); Kenji Kikuchi and Yutaka Yamazaki (Yuri Kumia General Hospital); Kiyohiro Houkin and Osamu Mabuchi (Sasekai General Hospital); Ichiro Nakahara and Toshiyuki Higashi (Kokura Memorial Hospital); Takashi Yoshizawa and Kenjiro Ito (Yokohama Shintoshi Neurosurgical Hospital); and Jun Takahashi, Nobuhiro Mikuni, Ken-ichiro Kikuta, Yasushi Takagi, and Yo Kishi (Kyoto University Graduate School of Medicine).

**Sources of Funding**

This work was supported by a grant from the Ministry of Education, Science, Sports, and Culture of Japan to A.K. (Tokutei Kenkyu: 15012231, 16012232, 17019034, and 18018022) and a grant from the Japan Society for the Promotion of Science (JSPS) to A.K. (Kiban Kenkyu A: 14207016 and S: 17109007). Y.M. is a member of the JSPS research fellows.

**Disclosures**

None.

**References**


Model-Based Linkage Analyses Confirm Chromosome 19q13.3 as a Susceptibility Locus for Intracranial Aneurysm
Youhei Mineharu, Kayoko Inoue, Sumiko Inoue, Shigeki Yamada, Kazuhiko Nozaki, Nobuo Hashimoto and Akio Koizumi

Stroke. 2007;38:1174-1178; originally published online February 22, 2007;
doi: 10.1161/01.STR.0000259657.73682.03
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/4/1174

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/