Linkage of Familial Moyamoya Disease (Spontaneous Occlusion of the Circle of Willis) to Chromosome 17q25

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**Background and Purpose**—Moyamoya disease is a cerebrovascular disease of unknown cause that mainly affects Japanese children. The incidence of familial occurrence accounts for 9% of cases. The characteristic lesions of moyamoya disease are occasionally seen in neurofibromatosis type 1, of which the causative gene (NF1) has been assigned to chromosome 17q11.2.

**Methods**—To determine whether a gene related to moyamoya disease is located on chromosome 17, we conducted microsatellite linkage analyses on 24 families containing 56 patients with moyamoya disease. Leukocyte DNA extracted from the family members was subjected to polymerase chain reaction for a total of 22 microsatellite markers on chromosome 17. The amplified polymerase chain reaction fragments were analyzed with GeneScan on an automated sequencer.

**Results**—Two-point linkage analysis gave a maximum log10 odds (LOD) score of 3.11 at the recombination fraction of 0.00 for the marker at locus D17S939. The affected pedigree member method also showed a significantly low P value (<1.0 × 10−3) for the 5 adjacent markers at 17q25. Multipoint linkage analysis also indicated that the disease gene is contained within the 9-cM region of D17S785 to D17S836, with a maximum LOD score of 4.58.

**Conclusions**—A gene for familial moyamoya disease is located on chromosome 17q25. (Stroke. 2000;31:930-935.)

**Key Words:** child • genetics • moyamoya disease

Spontaneous occlusion of the circle of Willis (moyamoya disease) is a clinical entity of unknown cause that is characterized primarily by angiographic findings of bilateral occlusion at the terminal portion of the internal carotid artery with a characteristic telangiectasic vascular network (“moya-moya” vessels) at the base of the brain. Although the disease is extremely uncommon in non-Asian populations,1 its estimated prevalence in the entire Japanese population is ≥ 3 per 100 000 persons.2 Together with the elevated incidence in the Japanese population, there are several lines of evidence that indicate moyamoya disease is related to genetic factors3–5: (1) familial occurrence is seen in ~9% of cases, (2) the incidence of the disease in both monozygotic twins is 80%, and (3) the sib recurrence rate and relative incidence rate in offspring are 42 and 34 times higher than the incidence rate in the general population. On the basis of the statistical data, Osawa et al4 concluded that moyamoya disease is inherited most probably in a polygenic mode or an autosomal dominant fashion with a low penetrance.

In this regard, it is quite interesting that the characteristic lesions of moyamoya disease are occasionally associated with neurofibromatosis type 1 (NF1; von Recklinghausen’s disease), of which the responsible gene, NF1, is located on chromosome 17q11.2.6 More than 50 cases of such an association have been reported so far.4,7–9 In the present study, we conducted linkage analyses on pedigrees of familial moyamoya disease to test the hypothesis that moyamoya disease is linked to a particular chromosomal region.

**Subjects and Methods**

**Ascertainment**

Each member of 24 families who agreed to participate in the present study was examined by 1 of the authors (K.H., S.K., T.M., or M.F.). All of the participants underwent magnetic resonance (MR) imaging at least once and MR angiography (MRA), conventional angiography, or both when indicated. The presence of moyamoya disease was diagnosed according to the criteria shown in Table 1.10 All of the individuals without symptoms were screened with MRA or angiography. An asymptomatic patient (individual 6) was diagnosed as having definite moyamoya disease on MRA or angiography (criterion II) in addition to criterion III. All of the affected patients were diagnosed as having definite moyamoya disease (Figure 1), and no “probable” cases were included. The analyzed pedigrees are shown
TABLE 1. Diagnostic Guidelines for Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease)

I. A. 1. Age of onset varies, but the young and females are more frequently affected. Solitary occurrence is common, but familial occurrence is occasionally seen.
2. Symptoms and course vary, presenting no symptoms (incidental finding), transient disorder, or fixed neurological deficits of a slight or severe degree.
3. Cerebral ischemia is predominant in children, whereas intracranial hemorrhage is more common in adults.

B. In children, hemiparesis, monoparesis, sensory impairment, involuntary movement, headache, or convulsive seizure often appears repeatedly and occasionally on alternating sides. Mental retardation or fixed neurological deficits may be observed. Unlike in adults, intracranial hemorrhage is rare.

C. In adults, symptoms similar to those in children may appear, but intraventricular, subarachnoid, or intracerebral hemorrhage of sudden onset occurs more frequent. Recovery from such hemorrhage with or without neurological sequelae is seen in most of the patients except for those with a severe course and lethal outcome.

II. Cerebral angiography is indispensable for the diagnosis and presents with at least the following findings:
A. Stenosis or occlusion at the terminal portion of the internal carotid artery and at the proximal portion of the anterior and middle cerebral arteries on the opposite side is also diagnosed as a definite case.
B. Abnormal vascular network seen in the vicinity of the arterial occlusion in arterial phase
C. Findings that are bilateral.

When magnetic resonance imaging and magnetic resonance angiography fulfill these criteria, cerebral angiography is not mandatory.

III. Cause is unknown. There are no basic diseases as arteriosclerosis, meningitis, neoplasia, Down’s syndrome, von Recklinghausen’s disease; trauma; or irradiation. Quasi- or Akin-Moyamoya disease includes fibromuscular dysplasia, vascular malformations (angioma), Sturge-Weber disease, Tolosa-Hunt syndrome, circulating lupus anticoagulants.

IV. Pathological findings helpful for the diagnosis:
A. Intimal thickening that causes stenosis or occlusion of the lumen is observed in and around the terminal portion of the internal carotid artery, usually on both sides. A deposit of lipids is occasionally seen in the proliferating intima.
B. Arteries constituting the circle of Willis, such as the anterior and middle cerebral and posterior communicating arteries, often show stenosis of varying degrees or occlusion associated with fibrocellular thickening of the intima, waving of the internal elastic lamina, and attenuation of the media.
C. Numerous small arteries (perforating and anastomotic branches) are observed around the circle of Willis.
D. Reticular conglomerates of small vessels are often seen in the pia mater.

Diagnostic Criteria

Referring to I, the diagnostic criteria are classified as follows. Autopsy cases without cerebral angiography should be investigated separately referring to IV.

A definite case is one that fulfills all of I and II.

In children, however, a case that fulfills II, A and B, on one side and clearly presents with narrowing at the terminal portion of the internal carotid artery on the opposite side is also diagnosed as a definite case.

A probable case (unilateral) is one that fulfills II III II, C.

Modified from Fukui.10

in Figure 2. Among them, 5 families have been reported elsewhere. The mean ages (range, median) of the affected patients and unaffected individuals were 19.5 (3 to 56, median 14) years and 44.0 (18 to 65, median 47) years, respectively.

DNA Typing and Search Strategy

Genomic DNA was extracted from peripheral blood with DNAzol (Life Technologies). For an initial screening of chromosome 17 to determine the approximate locus, the ABI PRISM linkage mapping set of fluorescence-labeled markers (D17S849, D17S938, D17S945, D17S799, D17S925, D17S784, and D17S928; Perkin-Elmer Applied Biosystems) with the Gold polymerase (Perkin-Elmer Applied Biosystems) was used to generate linkage maps.

Two Internet databases [GeneMap98 (http://www.ncbi.nlm.nih.gov/genemap98/) and NCBI Entrez Chromosomal Map (http://www.ncbi.nlm.nih.gov/Entrez/Genome/org.html)] were used to determine the approximate locus, the ABI PRISM linkage mapping set of fluorescence-labeled markers (D17S849, D17S938, D17S945, D17S799, D17S925, D17S784, and D17S928; Perkin-Elmer Applied Biosystems) with the Gold polymerase (Perkin-Elmer Applied Biosystems) was used to generate linkage maps.
Linkage Analysis

Two-point LOD scores between the disease locus and each individual marker were calculated with the MLINK program of the LINKAGE 5.2 package.\textsuperscript{12} Osawa et al\textsuperscript{4} assumed on the basis of their statistical study that moyamoya disease was inherited in a polygenic mode or an autosomal dominant manner with a low penetrance. Because of the actual inheritance observed in the present pedigrees (Figure 2), in which 1 of the parents in several families had moyamoya disease as well as the children, we considered that there was a certain major gene that determined the disease occurrence at least apparently in a dominant fashion. We thus used a dominant transmission model as the most appropriate model for the 2-point linkage analysis. Based on the incidences of the disease in the parents and the offspring, penetrances of 0.2, 0.5, and 0.67 were used. The frequency of the abnormal allele was set at 0.00001 on the basis of the prevalence of 3.16 per 100 000 persons and the familial aggregation.\textsuperscript{5} Recombination frequency ($\theta$) was assumed to be equal between males and females. LOD scores were also calculated with the use of a recessive model with an allele frequency of 0.006 and penetrance of 0.2, 0.5, 0.67, 0.8, and 1.0 for a reference. Unified parametric and nonparametric multipoint analyses were performed with GENEHUNTER,\textsuperscript{13} and the results were plotted contiguous to each other.\textsuperscript{14} Marker order and intermarker distances were based on the linkage maps available on Internet databases. The studied markers and the distances between them are shown in Figure 3.

To further confirm the results, the affected pedigree member (APM) method\textsuperscript{15} was performed with the APM package. As weighing functions, $f(p)=1/\sqrt{p}$ and $1/p$ were applied. Empirical $P$ values were calculated with SIM and HIST in the APM package.

Results

The screening of 4 families (individuals 1 to 5, 6 to 9, 12 to 22, and 23 to 28 in Figure 2) with 10 microsatellite markers showed a positive LOD score at the D17S784 marker on chromosome 17q25 (maximum LOD score $[Z_{\text{max}}] 0.32$ at $\theta =0.00$). With a dominant transmission model, detailed analysis for all of the 24 families (individuals 1 to 103) with the 9 additional markers around D17S784 yielded a cumulative

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Figure 1. Cerebral carotid angiogram of a representative case of moyamoya disease (individual 6 in Figure 2).

Figure 2. Pedigrees of the families studied indicating male (■) and female (○) individuals. Filled squares and circles indicate individuals with moyamoya disease. All of the numbered individuals were genotyped. Individuals 62 to 65, 70 to 73, 78 to 81, 33 to 44, 45 to 52, and 82 to 90 were also analyzed by Ikeda et al.\textsuperscript{16}
maximum LOD score of 3.11 at $\theta=0.00$ for the marker D17S939. Different estimates of disease penetrance did not significantly alter the LOD scores. With an autosomal recessive model of inheritance, the maximum LOD scores were 2.02 (penetrance 0.2), 2.26 (0.5), 2.45 (0.67), 2.56 (0.8), and 2.82 (1.0) at the recombination fraction of 0.10 for the marker D17S939. The cumulative LOD scores obtained are shown in Table 2. Multipoint linkage analysis in the 24 families according to the method of Kruglyak et al\textsuperscript{13} revealed that the moyamoya disease gene was most likely to lie in the 9-cM interval between D17S785 and D17S836, with a maximum LOD score of 4.58 (Figure 4).

The application of the APM method to all of the affected individuals showed high statistical values and significantly low empirical $P$ values ($P<0.00001$) for the 5 markers surrounding D17S939 with a weighting function $f(p)=1/\sqrt{p}$ (Table 3). With $f(p)=1/p$, the empirical $P$ value was the lowest for the marker D17S939.

### Discussion

We performed linkage analysis to map the locus of familial moyamoya disease on chromosome 17. Despite the small pedigree and the limited number of microsatellite markers on chromosome 17, we successfully identified the disease locus in the telomeric region of the long arm of chromosome 17. With the APM method, we confirmed the linkage at a significantly low $P$ value. During the initial period of this study, we expected moyamoya disease to be related to the $NFI$ gene located on chromosome 17q11.2. The NFI protein is a GTPase-activating protein that regulates cellular growth through control of the Ras oncogene. We hypothesized that aberration of this regulation might give rise to the proliferation of endothelial and smooth muscle cells in the internal carotid arteries and their branches. The present data, however, did not support the direct participation of the $NFI$ gene in the occurrence of moyamoya disease and instead indicated the importance of a distinct gene located closer to the telomere of chromosome 17 (17q25). At the present moment, the region 17q 25 between the markers D17S785 and D17S836 includes no likely candidate genes that may be relevant to angiogenesis or cellular proliferation, according to the GeneMap'98 available on the Internet (http://www.ncbi.nlm.nih.gov/genemap98/). However, adjacent regions contain some possibly relevant genes, including epidermal growth factor receptor-binding protein 2 (GenBank accession number M96995), integrin-$\beta_4$ subunit (X51841), and tissue inhibitor of metalloproteinase 2 (J05593).

Ikeda et al\textsuperscript{16} recently demonstrated that a gene of familial moyamoya disease is mapped to chromosome 3p24.2-p26 region. Their result, however, is not necessarily contradictory...
Figure 4. Unified multipoint linkage analysis between familial moyamoya disease and markers on chromosome 17q24-25, determined with the use of genotypes from all the pedigrees. Marker D17S794 was set at map position zero. The LOD score at each point was computed according to the method of Kruglyak et al.13

Our result, because the previously conducted genetic studies predicted that the disease is most probably polygenic (i.e., >1 gene can be responsible for the disease).3 For instance, linkage of several independent loci, including chromosomes 5q, 6, 11q, 12q, 13, and 14q, has been found to show linkage to asthma.17 Because the maximum LOD score of 3.11 (OR >1200) in the 2-point linkage analysis was considered marginal to indicate the presence of a linkage and because this type of analysis is susceptible to the assumed model of inheritance, we conducted 2 further nonparametric analyses. With the unified parametric and nonparametric methods of Kruglyak et al.,13 the maximum LOD score of 4.58 (OR >38 000) was observed at a locus between D17S785 and D17S836. This was confirmed with the APM method, which showed significantly low P values (P<0.00001) in the same region. The amply significant statistical LOD scores and P values according to different analytical methods in the present study definitely demonstrate the presence of a relevant gene on chromosome 17q25, although further studies should be warranted to determine the relationship between the genes at the different loci.

Moyamoya disease is the most critical cause for childhood stroke in the Japanese population. The disease is considered most frequent in northern Asian countries, and it is distributed worldwide at a lower frequency. Because this study dealt with only familial cases of moyamoya disease, the results may not be directly applicable to the majority of sporadic cases. However, the elucidation of a genetic cause for familial moyamoya disease should help us to better understand sporadic moyamoya disease as well. Based on this point of view, we are conducting a project to clone the causative gene in the chromosome 17q25 region.

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