

Mapping of a Familial Moyamoya Disease Gene to Chromosome 3p24.2-p26

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Summary

Moyamoya disease is characterized by bilateral stenosis and/or occlusion of the terminal portion of the internal carotid artery. Moyamoya disease is prevalent among patients <10 years of age. Although most cases appear to be sporadic, ~10% occur as familial cases. The incidence of familial cases has been increasing because noninvasive diagnostic equipment, such as magnetic-resonance imaging and magnetic-resonance angiography, can detect the disease in almost all affected patients, including asymptomatic patients, during screening studies. In this study, we performed a total genome search to identify the location of a familial moyamoya disease gene in 16 families, assuming an unknown mode of inheritance. A linkage was found between the disease and markers located at 3p24.2-26. A maximum NPL score of 3.46 was obtained with marker D3S3050. This is the first genetic locus found to be involved in the molecular pathogenesis of familial moyamoya disease.

Introduction

Spontaneous occlusion of the circle of Willis, first described as “moyamoya disease” (MIM 252350) in Japan (Suzuki and Takaku 1969), is characterized by bilateral stenosis and/or occlusion of the terminal portion of the internal carotid artery and by the development of abnormal netlike vessels at the base of the brain. A high incidence of moyamoya disease occurs in Asia, especially in Japan (Ikezaki et al. 1997). Moyamoya disease is not associated with any location of unusually high incidence

in Japan (Goto and Yonekawa 1992). Moyamoya disease occurs more frequently in females (male-to-female ratio of 2:3) and is prevalent among patients <10 years of age (Suzuki 1983). Juvenile patients with moyamoya disease initially present with transient motor disturbances—that is, symptoms of transient brain ischemia—whereas adults present with intracranial hemorrhage. The symptoms in juvenile patients are due to the narrowing or occlusion of the circle of Willis, and those in adults are due to a collapse of collateral circulation, which gradually develops as a result of the occlusion of the carotid fork at a younger age.

Most cases of moyamoya disease appear to be sporadic, but ~10% are familial cases (Fukuyama et al. 1991; Fukui 1997). Approximately 70% of cases of moyamoya disease among family members occur in siblings, and 24% occur in a parent and offspring (Fukuyama et al. 1991). The incidence of such familial cases has been increasing because noninvasive diagnostic equipment, such as magnetic-resonance imaging (MRI) and magnetic-resonance angiography (MRA), can detect the disease in almost 100% of affected patients, including asymptomatic patients, during screening studies (Ikezaki et al. 1997). Therefore, extensive genetic study of moyamoya disease has become urgent and important, although moyamoya disease has long been thought to be caused mainly by environmental factors (Suzuki 1983). In this study, we tried to identify the locus associated with moyamoya disease, by performing a genomewide search with genotypes of microsatellite polymorphic markers.

Subjects and Methods

Family Pedigree and DNA Extraction

We studied 77 Japanese individuals (28 males and 49 females) in 16 families with 3–13 members (fig. 1). No history of intermarriage was evident. Moyamoya disease was detected in 37 of the 77 individuals, on the basis of the guidelines for the diagnosis of moyamoya disease set by the Research Committee on Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and

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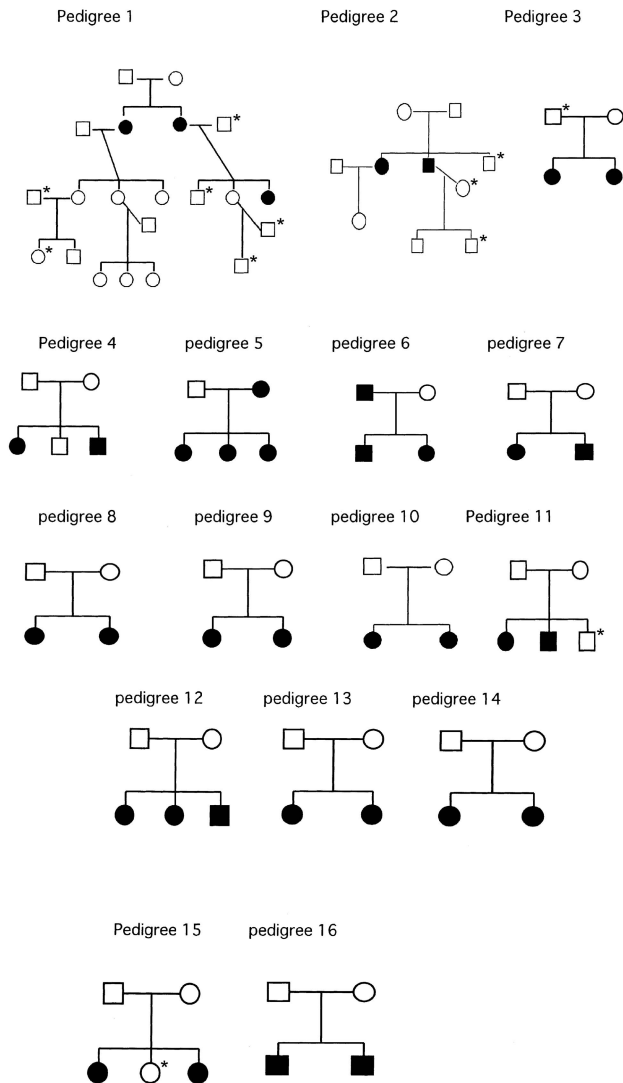


Figure 1 Pedigrees of the families with moyamoya disease. All individuals shown were genotyped, except those who are deceased and those marked with an asterisk (*). Blackened squares and circles indicate individuals with moyamoya disease, and unblackened symbols indicate normal individuals. The mean age at diagnosis was 12 years.

Welfare of Japan (Ikezaki et al. 1997). These guidelines require diagnosis, by MRA and MRI, of both of the following specific findings: (1) bilateral narrowing or stenosis of the carotid fork, detected by MRA, and (2) abnormal vascular networks in the bilateral basal ganglia, detected by MRI as more than two flow voids in each brain hemisphere. Two of the 37 patients were asymptomatic and were identified by chance during MRA screening.

Genomic DNA was extracted from the peripheral blood of 22 individuals in 3 families (both affected and unaffected individuals were analyzed) and also from 55

blood samples (from 13 families) generously supplied by the blood bank holding blood samples from individuals with moyamoya disease. The DNA was prepared from the blood samples by proteinase K digestion and the phenol-chloroform-extraction method.

Genotyping

Genotyping for 371 highly polymorphic microsatellite markers spanning the 22 autosomes was performed by use of the Weber human linkage screening set, version 6 (Research Genetics). One marker on chromosome 3p (3D1560; The Genome Database [GDB] accession number 238670) was added. The spacing between adjacent markers was 7–20 cM, and the mean heterozygosity of the markers was 76%. PCR was performed with 50- μ l mixtures (pH 8.4 or 8.6) of 100 ng genomic DNA, 200 μ M dATP, 200 μ M dTTP, 200 μ M dGTP, 20 μ M dCTP, 20 pmol of each primer, 0.5 U *Taq* polymerase, 1.0–2.5 mM MgCl₂, 50 mM KCl, and 0.1 μ Ci α [³²P]-dCTP. PCR amplifications were performed in a PC-800 (ASTEC) by use of the following parameters: initial denaturation at 94°C for 5 min, followed by 35 cycles (94°C for 1 min 30 s, 52°C–60°C for 1 min 30 s, and 71°C for 2 min) and a final elongation at 71°C for 5 min. The annealing temperature was optimized for each set of primers. The PCR-amplified product solution was used for electrophoresis loading (80 W for 2–3 h at constant power) on a 6% polyacrylamide gel. Autoradiography was performed by exposure of the film, at 80°C, for 2–72 h.

Initially, eight families (pedigrees 1–8) were used to screen for any locus suggestive of linkage. Then, eight more families (pedigrees 9–16) were included, to analyze the region of interest.

Linkage Analysis

To screen the loci suspected to be responsible for moyamoya disease, two-point LOD scores were calculated, under the assumption of either autosomal recessive inheritance or autosomal dominant inheritance (incomplete penetrance), by use of the MLINK program as implemented in the LINKAGE package, version 5.20 (Terwilliger and Ott 1994). Full multipoint calculations for nonparametric analysis were determined, by use of the GENEHUNTER program (Lander and Kruglyak 1995), because nonparametric analysis provides a robust significance of linkage irrespective of the mode of inheritance. The penetrance of the familial disease was estimated to be nearly 100%. The disease-allele frequency chosen was .000035, on the basis of the 1994 annual report of the Research Committee on Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and Welfare of Japan (Fukui 1996, p. 36). Allele frequencies of the markers were obtained from the

GDB, and markers on chromosome 3p24.2-p26 were determined on the basis of samples of DNA from 50 normal Japanese subjects.

Results

For the initial screening of eight families, we used all the markers on chromosomes 1–22. Linkage analysis under the assumption of autosomal recessive inheritance revealed that the D3S2387 (The Cooperative Human Linkage Center [CHLC] accession number 31795) and D3S3050 (CHLC accession number 41102) loci on chromosome 3 showed LOD scores >1.0 , whereas LOD scores were <1.0 for the rest of the markers. The LOD score for D3S2387 was 1.493576 at a recombination fraction (θ) of .1 (nonparametric LOD [NPL] score 2.31, information content .848), and that for D3S3050 was 3.184154 at $\theta = .01$ (NPL score 1.80, information content .892). Nonparametric linkage analysis showed an NPL score >1.96 at loci between D3S2387 and D3S3050 and an information content of .785–.892. These two loci are both on chromosome 3p24.2-p26.

For the study of the eight additional families, we used four markers (D3S2387, D3S3050, D3S1560, and D3S1304 [GDB 62981]) at 3p24.2-p26. Nonparametric analysis revealed a significant linkage at marker D3S3050 (NPL score 3.46, information content .851; table 1).

Discussion

Moyamoya disease is a rare cerebrovascular disorder of unknown etiology. There are two major hypotheses concerning the etiology of moyamoya disease—namely, that it is a genetically inherited disease or that it is an acquired disease. The latter etiology includes infection, such as laryngitis, and has been thought to induce an autoimmune response against some components of the circle of Willis, resulting in specific angiitis (Suzuki 1983). However, there is no definite evidence that infections can actually induce moyamoya disease. Certain environmental agents are also suspected to be the cause of acquired disease. However, a massive case-control study has revealed no positive linkage with environmental agents (Fukui 1997). An epidemiological study (Graham and Matoba 1997) showed that moyamoya disease in Hawaii has a higher incidence and prevalence than in the rest of the United States, mostly owing to the larger percentage of Asians, particularly Japanese, living in Hawaii, which suggests that moyamoya disease is caused by genetic, rather than environmental, factors. In Japan, no special incidence of moyamoya disease has been linked with occupation, lifestyle, or site of residence. Such evidence strongly suggests that environ-

Table 1

NPL Scores Obtained by Nonparametric Linkage Analysis at 3p24.2-p25

Locus and Distance (in cM) ^a	NPL Score	P Value	Information Content
D3S2387:			
.00	2.79470	.002527	.887136
1.67	2.89951	.001819	.758705
3.34	3.01797	.001225	.708900
5.02	3.15065	.000745	.702517
6.69	3.29816	.000459	.739142
D3S3050:			
8.36	3.46119	.000238	.850934
9.48	3.35205	.000360	.752518
10.60	3.24986	.000527	.717286
11.72	3.15447	.000738	.716760
12.84	3.06571	.001049	.750260
D3S1304:			
13.96	2.98345	.001345	.842207
14.71	2.77165	.002805	.745647
15.46	2.57414	.004968	.701351
16.21	2.39063	.008511	.686057
D3S1304:			
16.95	2.22085	.013161	.699786
17.70	2.06458	.019914	.765910

^a Distance is from top of chromosome 3.

mental factors are not important in the development of moyamoya disease.

The prominent histological change of the bilateral carotid forks that is associated with moyamoya disease is an eccentric fibrosis, or cellular-fibrous thickening, of the intima. The internal elastic lamina shows tortuosity and duplication, whereas the media shows atrophy and thinning. The adventitia does not show marked change (Oka 1981; Suzuki 1983; Ikeda 1991). In general, these affected vessels do not show arteriosclerotic or inflammatory changes. The most severely affected site is confined to the carotid fork, and the lesion usually is bilateral. Histologically, the vessel wall is narrowed or obstructed by eccentric proliferation of the endothelium, which is composed of several laminated elastic lamina intermingled with fibrosis. A whole-body-autopsy study of patients with moyamoya disease, aged 6–58 years (mean 37 years), showed that 32 of 36 patients had several abnormal changes in arteries other than those in the brain that were similar to changes found in the affected cerebral vessels. Such systemic vascular changes included intimal hypertrophy of the renal, coronary, pancreatic, and pulmonary arteries (Suzuki 1983; Ikeda 1991). Morphometric analysis (Ikeda 1991) revealed significant intimal thickening of the pulmonary arteries, renal arteries, and pancreatic arteries in patients with moyamoya disease, compared with age- and sex-matched controls. Thus, the pathological lesion of this disease is not localized but is systemic, and it is not

formed by the inflammatory or aging process (Ikeda 1991). These histopathological findings strongly suggest that the lesion is formed by a progressive and continuous process and that the most susceptible site, which may be genetically determined, is the bilateral carotid fork. Therefore, moyamoya disease can be regarded as a severe intracranial manifestation of systemic arterial disease, and it is triggered by a noninflammatory, nonarteriosclerotic, eccentric thickening of the intima of the bilateral carotid fork.

These pathologic and epidemiological facts suggest that genetic factors play a more important role in the pathogenesis of moyamoya disease than do acquired factors. Recently, a hypothesis accounting for the intimal thickening proposed that abnormal regulation of extracellular-matrix (ECM) metabolism may lead to increased steady-state levels of elastin mRNA and elastin accumulation in arterial smooth-muscle cells (SMCs) in patients with moyamoya disease (Yamamoto et al. 1997). Such intrinsic abnormalities of SMCs in patients with moyamoya disease also suggest that the pathogenesis of moyamoya disease is genetically determined. The gene for a second form of Marfan syndrome (MIM 154700), a connective-tissue disorder characterized by skeletal and cardiovascular anomalies, also may be located on chromosome 3p25-24.2 (Collod et al. 1994). Elastin and collagen are the major components of the ECM, and congenital vascular defects or abnormalities tend to result from defects in these structural proteins. The fact that both moyamoya disease, which is presumably caused by disturbance of the ECM, and the fibrous-connective-tissue disorder Marfan syndrome have been mapped to the same region of the chromosome is noteworthy.

Investigation of the mode of inheritance of moyamoya disease is complicated by the variety of symptoms caused by this disease. The diagnosis of moyamoya disease depends on only the finding of bilateral carotid angiography. The initial symptoms of moyamoya disease are transient brain ischemia, caused by the narrowing and/or obstruction of the carotid fork, before 10 years of age. Progressive narrowing of the carotid fork results in gradual development of collateral circulation, which is detected, by carotid angiography, as an abnormal vascular network. Eventual rupture of the abnormal vessels results in symptoms of intracranial hemorrhage, after 30 years of age. Therefore, although the symptoms are different in the adult and juvenile types of the disease, both types are considered to be different aspects of a single process at different times in the course of the disease. In particular, the severity of the clinical symptoms has no significant correlation with the degree of angiographical stenosis of the carotid fork. Thus, genetic heterogeneity in moyamoya disease is difficult to identify from the symptomatology. Also, cases of moyamoya disease prob-

ably exist among the healthy population, owing to delayed consultation of a physician because the symptoms in childhood are mild and transient. Even if juvenile patients mature to adulthood without a diagnosis of moyamoya disease, identification remains difficult unless the fragile collateral vessels are ruptured, especially since the asymptomatic period of affected patients can be long. Moyamoya disease cannot be diagnosed without invasive examination by bilateral carotid angiography, but adult patients with moyamoya disease often are comatose at the onset of intracranial hemorrhage, so invasive examination is impossible. These various factors suggest that the actual frequency of the disease allele is much higher than the reported value of .000035, which is based on the statistical research of the Research Committee on Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and Welfare of Japan.

We analyzed three families with affected parents and children (pedigrees 1, 5, and 6). The initial symptoms were intracerebral hemorrhage in all affected parents. The age at onset in the parents was 53 and 51 years in pedigree 1, 45 years in pedigree 5, and 39 years in pedigree 6. The age at initial onset in the children was 8 years in pedigree 1; 16 and 18 years in pedigree 5, with an incidental asymptomatic identification; and 14 years in pedigree 6, with an asymptomatic identification. The symptoms included ischemic brain manifestations in each of these children. Symptomatic heterogeneity was observed in these families, and clinical anticipation—that is, earlier age at onset in younger patients—also was observed. Whether clinical anticipation was due to delayed diagnosis because of underdeveloped diagnostic tools or a poor standard of medicine or because the same molecular phenomenon seen in CAG-repeat disease (Takiyama et al. 1995) may occur in moyamoya disease is not certain. The observation of both juvenile and adult types in the same families strongly supports the hypothesis that both types of moyamoya disease are caused by the same genetic disorder.

The results of an extensive genetic study are not available, but the most likely pathogenesis of moyamoya disease is thought to be polygenic, on the basis of estimates (Fukuyama et al. 1991) calculated by means of Edwards's (1960) hypothesis. However, the microsatellite polymorphism D3S3050, mapped to chromosome 3p24.2-p26, showed strong evidence of linkage by non-parametric analysis, with an NPL score of 3.46. Other genes close to the linked region on chromosome 3p include the genes responsible for Marfan syndrome and the von Hippel-Lindau disease gene (MIM 193300; Latif et al. 1993) responsible for hemangioblastoma, which is a vascular tumor. Since the moyamoya disease gene maps to this region of chromosome 3p and may encode a gene product that is fundamentally important for the formation and maintenance of vascular-wall homeosta-

sis, this region of the genome is of great interest in the study of inherited vascular disease. Eventual molecular characterization of this disease may provide valuable insights into the processes of progressive stenosis and occlusion of the terminal portion of the internal carotid artery and possibly may suggest a new therapeutic method, such as gene therapy.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, The, <http://www.chlc.org> (for marker D3S3050 [CHLC.GATA88H04])
 Genome Database, The, <http://www.gdb.org/> (for markers 3D1560 [238670] and D3S2387, D3S3050, D3S1560, and D3S1304 [62981])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for moyamoya disease [MIM 252350], Marfan syndrome [MIM 154700], and von Hippel-Lindau disease [MIM 193300])

References

- Collod G, Babron MC, Jondeau G, Coulon M, Weissenbach J, Dubourg O, Bourdarias JP, et al (1994) A second locus for Marfan syndrome maps to chromosome 3p24.2-p25. *Nat Genet* 8:264-268
- Edwards JH (1960) The simulation of Mendelism. *Acta Genet Stat Med* 10:63-70
- Fukui M (ed) (1996) Annual report 1995. The Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Fukuoka, Japan
- Fukui M (1997) Current state of study on moyamoya disease in Japan. *Surg Neurol* 47:138-143
- Fukuyama S, Kanai M, Osawa M (1991) Clinical genetic analysis on the moyamoya disease. In: Fukui M (ed) Annual report 1990. The Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Fukuoka, Japan, pp 53-59, 141-146
- Goto Y, Yonekawa Y (1992) Worldwide distribution of moyamoya disease. *Neurol Med Chir (Tokyo)* 32:883-886
- Graham JF, Matoba A (1997) A survey of moyamoya disease in Hawaii. *Clin Neurol Neurosurg* 99 Suppl 2:S31-S35
- Ikeda E (1991) Systemic vascular changes in spontaneous occlusion of the circle of Willis. *Stroke* 22:1358-1362
- Ikezaki K, Han DH, Kawano T, Kinukawa N, Fukui M (1997) A clinical comparison of definite moyamoya disease between South Korea and Japan. *Stroke* 28:2513-2517
- Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241-247
- Latif F, Tory K, Gnarr J, Masahiro Y, Duh FM, Orcutt ML, Stackhouse T, et al (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 260:1317-1320
- Oka K, Yamashita M, Sadoshima S, Yanaka K (1981) Cerebral haemorrhage in moyamoya disease at autopsy. *Virchows Arch* 392:247-261
- Suzuki J (ed) (1983) Moyamoya disease. Springer-Verlag, Berlin
- Suzuki J, Takaku A (1969) Cerebrovascular "moya-moya" disease. *Arch Neurol* 20:288-299
- Takiyama Y, Igarashi S, Rogaeva EA, Endo K, Rogaev EI, Tanaka H, Sherrington R, et al (1995) Evidence for intergenerational instability in the CAG repeat in the MJD1 gene and for conserved haplotypes at flanking markers amongst Japanese and Caucasian subjects with Machado-Joseph disease. *Hum Mol Genet* 4:1137-1146
- Terwilliger JD, Ott J (1994) Two-point linkage analysis. In: Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore, pp 13-104
- Yamamoto M, Aoyagi M, Tajima S, Wachi H, Fukai N, Matsushima Y, Yamamoto K (1997) Increase in elastin gene expression and protein synthesis in arterial smooth muscle cells derived from patients with moyamoya disease. *Stroke* 28:1733-1738