第八章　予後（自然歴）

1.　小児もやもや病

一過性脳虚血が最も多く発生するのは発症後の数年間でありその後、減少はするが、知能障害と機能障害を有する患者は発症から時間が経過するほど増加し、その程度も増悪する[1]。年齢が低い乳幼児ほど脳梗塞、特に皮質梗塞の発生が多く、脳梗塞の存在が機能予後に最も大きく関与すると考えられている[2-4]。小児例では多くの例で病期が進行するが、思春期になると進行は緩徐となる[5,6]。長期的に経過観察すると、片側病変が両側病変に移行したり、当初、無症候だった大脳半球の 65%でも TIA が出現するとの報告がある[7]。成人に移行した場合、ADL が良好な例は少数で[8]、少数ながら頭蓋内出血が死因となりうる[5,9]。

脳血行再建術の効果を検証した RCT は存在しないが、脳血行再建術を実施した場合、その術式に関わらず TIA は消失あるいは減少し脳梗塞の再発はきわめて稀で、自然歴と比較すると機能予後は良好であると考えられている[4,10-19]。頭痛は脳血行再建術により減少するが、脳循環動態の改善に関係なく、術後も頭痛が遷延したり、稀に術後に新たに頭痛が出現することも報告されている[20,21]。大脳高次機能も予後を左右する重要な因子であり、発症から 5 年以上経過すると IQ の低下が明らかとなってくる[22]。脳血行再建術は知能予後を改善させると考えられている[23]。

2.　成人もやもや病

発症病型に関わらず、成人もやもや病の未治療例は外科治療例よりも脳血管イベントの再発率は高く予後も不良との報告が多く[24,25]、小児と同様、脳血行再建術を考慮すべきである。

最近では、以前考えられていたよりも病期の進行が高頻度であることが判明している[26-29]。症候例・無症候例、確診例・疑診例に関わらず、非手術半球の約 20%で病期が進行し、その半数は TIA/脳梗塞あるいは頭蓋内出血がおきる。女性で病期の進行が生じやすいことが知られている[30]。もやもや病罹患女性の妊娠・分娩に関しては、ときに頭蓋内出血など重篤な脳卒中が生じ得ることが知られている。エビデンスに基づく管理指針は未だ確立されていないが、産科医と脳神経外科医が緊密に連携できる環境の下で妊娠継続期・分娩・産褥期の綿密な管理を行うことが推奨される[31,32]。

1)　成人虚血型もやもや病

小児と同様、脳血行再建術の効果を検証した RCT は存在しないが、脳血行再建術後に TIA や脳梗塞の発生は著明に減少する。しかし少数ながら経過観察期間中に頭蓋内出血や非手術半球における病期の進行に起因する脳梗塞が生じることがあり術後も長期間の経過観察が予後を良好に維持する上で重要と考えられている[17,33-36]。

2)　成人出血型もやもや病

初回の頭蓋内出血による死亡率は 6.8～20%である。再出血は機能予後を悪化させ死亡率を上昇させる[37,38]。再出血は初回と同一または異なる部位から生じる[39]。

保存的治療をした場合、再出血は 30～65%の症例において初回の出血から 2～20 年後に

生じ、観察期間が長いほど高率となる傾向がある[37,38,40-42]。前脈絡叢動脈や後交通動脈の分枝の異常な拡張を有する例で再出血のリスクが高いとの報告がある[42,43]。もやもや血管に生じた動脈瘤が脳血行再建術により消失することも報告されている[44]。

現時点では血行再建術の再出血予防効果については不明だが、脳血行再建術実施の有無にかかわらず、長期にわたる経過観察が必須と考えられている。

3. 無症候性もやもや病について

近年、非侵襲的画像診断法の進歩と普及によって、発症以前にもやもや病と診断される症例が増加している。最近、本研究班が実施した追跡調査では、加齢に伴って病期が進行していること、脳梗塞、脳循環障害を有していた症例はそれぞれ 20%、40%と少なからず潜在的な脳虚血を有していることが判明した[45]。

無症候性もやもや病の予後には不明な部分が多い。これまでの報告によれば、33 例のうち 4 例が TIA をきたし、2 例の死因が頭蓋内出血と考えられ[46]、10 例のうち 1 例で病期の進行に伴って脳梗塞をきたしている[47]。最近の追跡調査では、未治療の 34 例のうち 5 例で病期が進行し、脳梗塞・頭蓋内出血が発生するリスクは年間 3.2%と報告されている。診断時に脳虚血を有している例で脳梗塞をきたしやすかったのに対して、脳血行再建術を実施した 6 例では脳血管イベントは生じなかったことが報告されている[45]。したがって、無症候性もやもや病は脳血管イベントをきたす可能性を潜在的に有していると考えられ、保存的に経過観察する場合も MRI/MRA を用いた注意深い経過観察が長期にわたって必要と考えられている。

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平成 20-22 年度もやもや病（ウイリス動脈輪閉塞症）
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ウイリス動脈輪閉塞症における病態・治療に関する研究班について

橋本 信夫（国立循環器病センター 総長）

もやもや病の概説 ガイドラインの作成について

冨永 悌二（東北大学脳神経外科 教授）

もやもや病の疫学（無症候性を含めて）における最近の特徴

寶金 清博（札幌医科大学脳神経外科 教授）

もやもや病における脳出血（妊娠関連も含めて）

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厚生労働省難治性疾患克服研究事業
「ウイリス動脈輪閉塞症における病態・治療に関する研究班」主催

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もやもや病

研究と臨床の最前線

参加費 無料
（定員200名）

平成20年 **11月23日**（日）13:00-15:00（開場12時30分）

会場：千里ライフサイエンスセンター（ライフホール）

地下鉄（北大阪急行電鉄）　御堂筋線 千里中央行　終点・千里中央駅下車（北出口すぐ）
大阪モノレール　千里中央駅下車（徒歩約5分）

【申込み方法】往復ハガキに参加ご希望の方のお名前・連絡先を記入の上、お申し込みください。返信用ハガキにもお名前、連絡先をご記入ください。

【申込み締切】平成20年11月10日(月)

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Novel epidemiological features of moyamoya disease

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Novel epidemiological features of moyamoya disease

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ABSTRACT

Background: Many clinical features that are specific to moyamoya disease have been reported and cited in textbooks based on previous data. The purpose of this study is to investigate the present epidemiological features of moyamoya disease based on recently obtained regional all-inclusive data.

Methods: The authors performed an all-inclusive survey of moyamoya disease in Hokkaido, one of the major islands in Japan that has a population of 5.63 million. The epidemiological features were analysed based on the data from 267 newly registered patients with moyamoya disease in Hokkaido from 2002 to 2006. These analysed data were adjusted to the whole Japanese population at 2005.

Results: The detection rate of the disease per year was 0.94 patients per 100 000 people, and prevalence was 10.5 patients per 100 000 people. The incidence of ischaemia concerned with the disease was 0.53 patients per 100 000 people-years and haemorrhage was 0.2 patients per 100 000 people-years. The ratio of female to male patients was 2.18. The ratio of patients aged 10 years and above to under 10 years of age at onset was 6.18. Two peaks for age of onset were seen: the highest was observed between 45 and 49 years, and the second between 5 and 9 years. Asymptomatic patients comprised 17.8% of the total number of patients.

Conclusion: The epidemiological features of moyamoya disease determined by this survey varied considerably from previous data. The detection rate and prevalence of the disease were higher than those reported previously. The highest peak of onset age was older than those reported previously. In addition, it was revealed that asymptomatic moyamoya patients are not always rare in Japan.

It is not always easy to determine the true epidemiological features of a specific disease that has a low incidence. Most published studies depend on patient data obtained from selected large hospitals or compiled from numerous previously reported literature. Consequently, these studies have many inherent selection biases. A nationwide all-inclusive study, which appears to be an ideal approach to exclude bias, is not always feasible as the registration of patients suffering from diseases with a low incidence takes several years; furthermore, many omissions in registration can take place because of the weak incentives provided to doctors and patients. Indeed, the epidemiological data of many neurological diseases that appear even in textbooks may suffer from serious selection biases. Moyamoya disease is one such typical neurological disease that has a low incidence. The present epidemiological data for this disease has been obtained mainly from select community and university hospitals and may suffer from a serious selection bias.[1–4]

In addition, the development of a brain check-up system in Japan revealed many asymptomatic cerebrovascular diseases,[5 6] including cases of familial occurrence of moyamoya disease. This means that the previous epidemiological data may have underestimated the number of cases.

However, moyamoya disease has been designated as an intractable disease by the Japanese Ministry of Health, Labour and Welfare, which is fortunate from the point of view of disease tracking. Patients who are officially registered with moyamoya disease are qualified to receive a medical allowance from the government.[7] Therefore, nearly all patients are considered to have a strong incentive to seek registration. Hokkaido is the second largest island in Japan, and the data of patients with moyamoya disease have been well documented for the past several years, including demographic and radiological data. Therefore, analysis of patient registry data in Hokkaido may help to reveal the true features of moyamoya disease. In this study, all-inclusive continuous data from a well-limited area in Japan are analysed to reveal the contemporary epidemiological features of moyamoya disease.

PATIENTS AND METHODS

Geographical facts

Hokkaido is the second largest island in Japan, with an area of 78 000 km^2; it accounts for 22% of the area of Japan. This prefecture has a population of 5.63 million, which is 4.4% of the total Japanese population. In addition, although Hokkaido is a large island, it has only one prefectural government; Japan has 47 prefectures in total (fig 1). The other major islands are divided into many prefectures. Thus, Hokkaido presents us with an ideal opportunity to obtain sufficient data from a relatively isolated area that represents a single prefecture.

Patient registration

This survey was based on the certification system of registered intractable diseases of the Ministry of Health, Labour and Welfare in Japan from 1979. Each prefectural government is required to certify, register and provide financial support to patients with moyamoya disease as it has been officially designated an intractable disease by the Japanese government.[7] Patients who were suspected to have moyamoya disease in hospital could make an application to the prefectural government to be granted radiological assessment, including magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and/or conventional cerebral angiography data, in order to confirm the

Figure 1 Geographical location of Hokkaido (grey) in Japan.

particular vascular changes associated with moyamoya disease, including basal moyamoya vessels. These applications were evaluated by the Commission of Certification by specialists in each prefecture. The applications were sorted into the following classifications: definite moyamoya disease, probable moyamoya disease (unilateral moyamoya disease) or improbable cases (eg, atherosclerosis without moyamoya vessels or quasi-moyamoya disease related to other diseases) based on the criteria prepared by the Research Committee on Spontaneous Occlusion of the Circle of Willis (moyamoya disease) in Japan.[8]

The subjects surveyed in this study were patients with definite and probable moyamoya disease, as certified by the Japanese Ministry of Health, Labour and Welfare in Hokkaido from 2002 to 2006. We analysed newly certificated data: onset age for symptomatic cases and detected age for asymptomatic cases, gender, clinical presentation at onset, familial disease from 2002 to 2006, and the number of already registered patients in 2005.

Analysis

In this study, the types of clinical findings were divided into four subgroups: (a) ischaemia, including infarction and transient ischaemic attacks; (b) haemorrhage; (c) no symptoms—asymptomatic patients were identified by the brain check-up system or by administration of screening examinations to individuals with familial history; and (d) other symptoms, including headaches, seizures and involuntary movements. Furthermore, the following were investigated: (1) detection rate, prevalence and incidence of ischaemia and haemorrhage; (2) gender differences; (3) age distribution at onset or detection; (4) type of clinical findings at onset; and (5) proportion of family history. As far as age and gender were concerned, the data in this study obtained from the Hokkaido area were standardised based on data of the whole Japanese population at 2005. This figure was the most accurate data based on the national census in past years. Prevalence was calculated using all of the registered patients at 2005. The method of statistical analysis used was Chi-square test.

Protection of privacy act

In this study, all data were obtained, under agreement, from data from the Department of Health and Welfare of the Hokkaido Prefecture Government. All data used in this study were irretrievably unlinked to personal information.

RESULTS

Detection rate, incidence and prevalence

A total of 283 new applications for disease certification were submitted during the 5 years from 2002 to 2006. There were 592 cases from individuals who had already been diagnosed with the disease in 2005. The new applications comprised 233 definite cases, 34 probable cases and 16 improbable cases that did not meet the criteria for valid diagnosis. Therefore, a total of 267 newly enrolled cases were investigated in this study. The 60 definite cases (22.5%) were assessed by MR examination without cerebral angiography. The prevalence of risk factor within these applications was as follows: hypertension, 0.3%;

Table 1 Epidemiological features of moyamoya disease

	Present study	Previous study—1997[2]
Number of cases	267	1176
Detection rate (per 100 000)	0.94	0.35
Prevalence (per 100 000)	10.5	3.16
Sex ratio (male:female)	1:2.2	1:1.8
Patients younger than 10 years old at onset (%)	15.1%	47.8%
Pattern of age distribution	2 peaks	2 peaks
Highest peak in age distribution (in years)	45–49	10–14
Second peak in age distribution (in years)	5–9	40–49
Patients with family history	15.4%	10.0%

diabetes, 0.3%; hyperlipidaemia, 0%; and systemic atherosclerotic disease; 0.7%. The detection rate of the disease per year was 0.94 patients per 100 000 people (95% confidence interval (CI): 0.71 to 1.24), and the prevalence was 10.5 patients per 100 000 people (95% CI: 9.44 to 11.7). These figures are higher than those previously reported (table 1). The incidence of ischaemia concerned with the disease was 0.53 patients per 100 000 people-years, and the incidence of haemorrhage was 0.2 patients per 100 000 people-years.

Gender differences

The ratio of female to male patients was 2.18 (table 1). However, no significant gender differences were observed, although this figure appears to be slightly higher than that of 1.8, as reported previously.[2]

Age distribution

The percentage of patients under 10 years of age at onset was 15.1% (table 1). This percentage was significantly lower than that of 47.8%, as reported previously (table 1).[2] Figure 2 shows the age distribution of the new patients. The highest peak of detection rate was observed at 45–49 years and a smaller peak was observed at 5–9 years. In female patients, two definite peaks in age distribution were observed—a higher peak at 45–49 years and a smaller peak at 5–9 years; this was similar to the pattern of total age distribution. In male patients, this two-peak pattern of age distribution was not clear. It appears that the age distribution pattern was mainly influenced by the female patients. This two-peak pattern was also observed in the previous report.[2] However, the main peak was shifted towards the adult age group.

Types of clinical findings

The percentage of haemorrhage cases was 21.0% and these cases showed only one peak at 35–39 years (figs 3, 4). The percentage of cases with ischaemia was 57.4%; these cases showed two peaks—one at 5–9 years and the other at 45–49 years. The percentage of asymptomatic cases was 17.8% and had two small peaks at 5–9 years and adult age.

In the disease pattern by gender, the ratio of ischaemia in females was significantly smaller than that in males (53.0% vs 65.9%; $p<0.05$). Female patients showed a higher incidence of haemorrhage (22.2%) and asymptomatic patterns (20.5%) than male patients (19.5%, 12.2%). These data were not significant ($p = 0.62$ and $p = 0.10$, respectively). In the disease pattern by age, 78.4% of the patients below 10 years of age had ischaemia compared with 53.5% of the patients of 10 years and older ($p<0.01$). For haemorrhage, 2.7% of the younger group versus 24.3% of the older group ($p<0.01$) were affected.

Familial occurrence

Familial history was observed in 15.4% of patients. This figure was higher than that of 10.0%, as reported previously (table 1).[2] In the younger age group, 37.8% patients had familial moyamoya disease, which was significantly higher than that in the older age group (12.2%) ($p<0.01$).

DISCUSSION

The epidemiological features of moyamoya disease have been reported several times in Japanese literature.[1–4] However, as shown in table 1, there are several differences in the epidemiological features of moyamoya disease between the data from previously and our study. The results produced from our study might not exclude latent regional bias, although this bias seems to be small enough to neglect. However, in order to minimise this regional bias, the age and gender of this data obtained from Hokkaido were adjusted to those of the whole Japanese population. As far as race/ethnicity is concerned, all data in this study were obtained from Japanese individuals. The results of this study revealed higher detection rates and prevalence compared with previous studies, as well as a peak shift in detection rate from children to adults, and a change in the type of clinical findings and higher familial occurrence.

Higher detection rate and prevalence

The higher detection rates and prevalence of moyamoya disease disclosed in this study do not necessarily indicate that the values of these two important epidemiological parameters have actually increased. Indeed, the increase in the registered number of adult patients with moyamoya disease has resulted in an increase in the values of detection rate and prevalence. These higher figures than previous reports[2 9] probably reflect the availability of appropriate diagnostic tools and the brain check-up system that has been extensively developed in Japan. On the contrary, this figure could be lower than the actual figure as this system can register fatal cases due to moyamoya disease. In any case, the higher detection rate and prevalence reflects the actual features of moyamoya disease.

Peak shift from children to adults

One of the well-known specific features of moyamoya disease is its two-peak pattern of age distribution and its higher incidence in childhood in Japan. This study also revealed a two-peak pattern similar to that reported in previous papers.[1–4 10] This study, however, revealed that the higher peak observed in adults, particularly in female patients, is more prominent than in children. This result is similar to the data of Uchino *et al.*, which is a multi-race/ethnicity study in non-Asian countries.[9] It is conceivable that this difference is caused by the method of data sampling. Most of the previous surveys were conducted by

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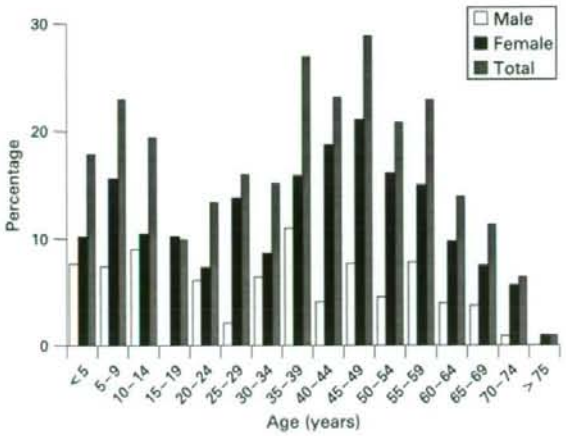

Figure 2 Onset age distribution of moyamoya disease.

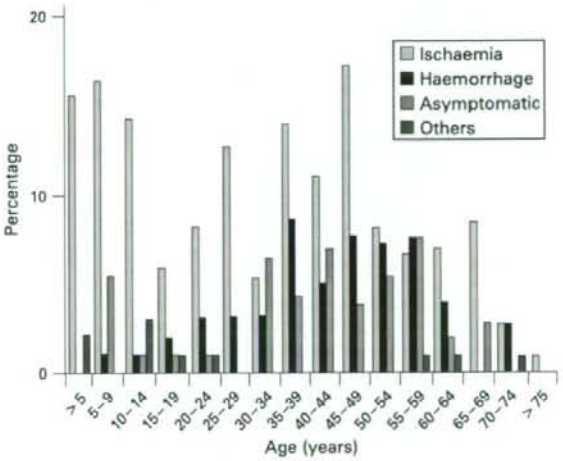

Figure 3 Age distribution of disease pattern at onset.

using questionnaires on the epidemiological features of moyamoya disease. This method was affected by sampling hospitals. Previous surveys had an inclination to use mainly university hospitals and public general hospitals. In this study, however, there were 33.3% patients from large hospitals and 15.4% from university hospitals. The other 51.3% patients were from numerous neurosurgical, neurological or paediatric specialised small hospitals or clinics. The selection of hospitals might lead to an overestimation of the number of psediatric patients and an underestimation of the number of adult cases in the past literature.

Changes in types of clinical findings and familial occurrence

Minor changes in the types of clinical findings, including an increase in haemorrhage onset, is not always remarkable as it simply reflects an increased number of adult patients with moyamoya disease in this study. However, with regards to clinical findings, the most remarkable difference observed in this study was an increase in the number of patients with asymptomatic moyamoya disease. These patients accounted for 18.0% of the total number of patients. The driving force for the elevated number of asymptomatic cases is believed to be the brain check-up system in Japan[5 6] and the knowledge of the familial occurrence of moyamoya disease. These factors were responsible for the identification of asymptomatic patients and indicate that many patients with asymptomatic moyamoya disease have still not been identified. The familial occurrence rate is 15.4% in this study and was higher than that reported previously.[10–12] This increase is also a reflection of the screening of the families with a history of moyamoya disease.

CONCLUSION

The detection rate, incidence and prevalence of moyamoya disease are higher than previously reported. The two-peak pattern was recognised as previously reported. However, the peak of detection rate in adult patients was higher than that in paediatric patients. In addition, it was revealed that patients with asymptomatic moyamoya disease are not always rare in Japan.

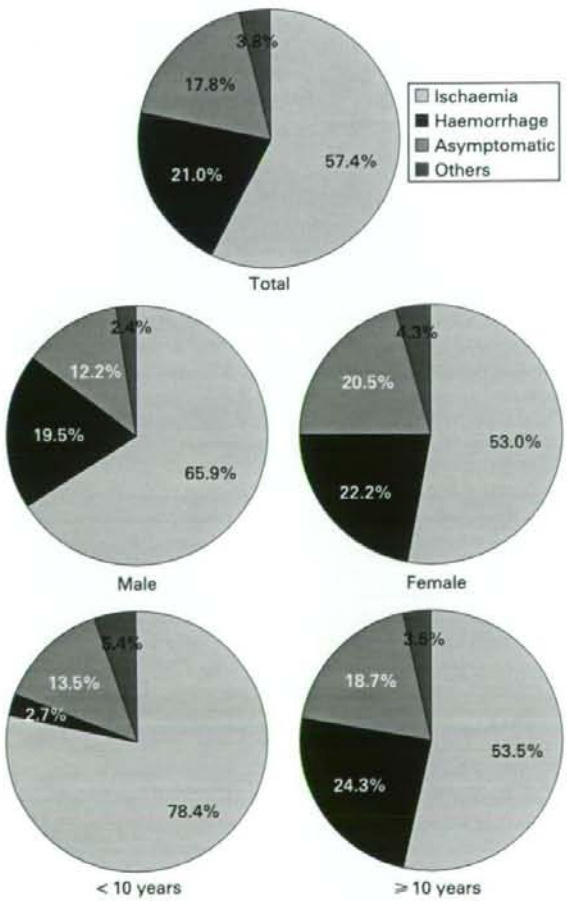

Figure 4 Disease patterns of moyamoya disease at onset.

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Autosomal dominant moyamoya disease maps to chromosome 17q25.3

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Supplemental data at www.neurology.org

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ABSTRACT

Background: Moyamoya disease (MMD) is an idiopathic steno-occlusive cerebrovascular disease that represents an important cause of stroke. However, etiology of the disease has remained largely unknown.

Methods: We previously showed that the inheritance pattern of MMD is autosomal dominant with incomplete penetrance. Here, we report the genome-wide parametric linkage analysis for MMD in 15 extended Japanese families. We conducted linkage analyses under two diagnostic classifications: narrow and broad. Affected member-only analysis was applied due to incomplete and age-dependent penetrance of the disease.

Results: Under both classifications, significant evidence of linkage was only observed on chromosome 17q25.3, with maximum multipoint logarithm of odds (lod) scores of 6.57 (under the narrow classification) and 8.07 (under the broad classification) at D17S704. Haplotype analysis revealed segregation of a disease haplotype in all families but one, and informative crossovers enabled mapping of the MMD locus to a 3.5-Mb region between D17S1806 and the telomere of 17q, encompassing 94 annotated genes.

Conclusions: Our data suggest that there is a major gene locus for autosomal dominant moyamoya disease on chromosome 17q25.3. ***Neurology*® 2008;70:2357-2363**

GLOSSARY

ACAs = anterior cerebral arteries; **HLOD** = heterogeneity-adjusted logarithm of odds; **ICAs** = internal carotid arteries; **MCAs** = middle cerebral arteries; **MMD** = moyamoya disease; **MRA** = MR angiography; **NF1** = neurofibromatosis type 1; **NPL** = nonparametric linkage; **RCMJ** = Research Committee on the Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and Welfare, Japan; **RFLP** = restriction fragment length polymorphism; **SNP** = single nucleotide polymorphism.

Moyamoya disease (MMD) is an idiopathic cerebrovascular disorder characterized by steno-occlusive lesions around the terminal portions of the bilateral internal carotid arteries (ICAs) accompanied by collateral vessels (moyamoya vessels) at the base of the brain.[1] The disease extends over all age groups, but is predominant in the age group <10 years.[2] Most juvenile patients develop transient ischemic attacks or cerebral infarctions, which cause severe disabilities including motor weakness or mental retardation.[3] On the other hand, adult patients are more prone to have hemorrhagic stroke. Although there are few epidemiologic studies for stroke in children in Asian countries, an annual incidence of stroke in Hong Kong Chinese children was estimated to be 1.9 to 2.1 per 100,000 children-years, and 6.0% of the patients had MMD (23.1% for stroke caused by vascular disease in children).[4] In addition, moyamoya angiopathy has been shown to be an inde-

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pendent risk factor for recurrence of arterial ischemic stroke in children.[5] Thus, MMD is an important cause of stroke especially for children. Moreover, MMD is a progressive disease, and there are no effective treatments for preventing the narrowing of the intracranial arteries. Surgery using an extracranial-intracranial bypass can be used as a treatment option,[6,7] but it is a heavy burden to carry, particularly for children.

Two previous genome-wide linkage studies that mainly involved affected siblings or relative pairs identified two loci for MMD at 3p24.2-p26[8] and 8q23.[9] Another candidate locus at 17q25 was proposed from chromosome-wide linkage analyses.[10] However, no disease-causing gene has yet been identified. The different loci identified by these linkage studies most likely represent locus heterogeneity. In the context of genetic heterogeneity, linkage analyses using affected siblings or relative pairs have only limited statistical power and are often confounded by false-negative results.[11] Alternatively, parametric analyses using extended pedigrees of Mendelian traits provide us more chances to identify a disease-causing gene. In the present study, we selectively recruited families of ≥3 generations without consanguinity, in which an autosomal dominant mode of inheritance can be rationally assumed. Genome-wide parametric analyses with the affected member-only method provided significant evidence of a major gene locus for autosomal dominant MMD in the telomeric region of chromosome 17q. Identification of a gene responsible for MMD would lead to understanding of the disease pathogenesis, thereby enabling the development of new therapeutic options to prevent disease progression.

METHODS Clinical evaluation. According to the official diagnostic criteria proposed in 1997 by the Research Committee on the Spontaneous Occlusion of the Circle of Willis of the Ministry of Health and Welfare, Japan (RCMJ),[12] MMD should be defined as follows: 1) steno-occlusive lesions around the terminal portions of the ICAs including the proximal portions of the anterior cerebral arteries (ACAs) and middle cerebral arteries (MCAs); 2) moyamoya vessels at the base of the brain illustrated by abnormal vascular networks on conventional angiography or more than two flow voids in the basal ganglia on MRI; and 3) these findings are present bilaterally. Patients with unilateral involvement of these findings were diagnosed as probable MMD. However, MMD is a progressive disease and its phenotype changes dynamically according to the disease stage. For example, collateral vessels cannot be detected in the early and end stages,[1,11] and unilateral MMD can progress to bilateral involvement during its follow-up.[14,15] Therefore, the core spectrum of the diagnostic criteria for MMD is the steno-occlusive lesions around the terminal portion of the ICAs. On the basis of these observations, we analyzed our data under two diagnostic classifications: narrow and broad (table 1). The narrow classification only included patients with definite MMD according to the RCMJ criteria. For the broad diagnostic classification, we employed modified diagnostic criteria, in which any steno-occlusive lesions around the terminal portions of the ICAs, either unilaterally or bilaterally, were considered to be in the continuum of the MMD phenotype even without findings of moyamoya vessels. The modified diagnostic criteria may be applicable only to those who have a family history of definite MMD. Accordingly, the broad classification contained the following phenotypes: 1) definite MMD; 2) probable MMD (unilateral MMD); and 3) either bilateral or unilateral steno-occlusive lesions around the terminal portion of the ICAs without findings of moyamoya vessels.

The medical records of participants who had already been diagnosed were checked to confirm the diagnosis of MMD. A clinical interview and examinations by MRI and magnetic resonance angiography (MRA) were carried out for all available relatives, and conventional angiography was conducted when necessary. We ascertained the medical his-

Table 1 Definition of the narrow and broad diagnostic classifications of familial moyamoya disease (MMD)

Narrow classification
Definite MMD that fulfills all of the following findings in the RCMJ criteria in 1997[12]:
1) Steno-occlusive lesions around the terminal portions of the internal carotid arteries (including the proximal portions of the anterior cerebral arteries and middle cerebral arteries)
2) Moyamoya vessels at the base of the brain illustrated by abnormal vascular networks on conventional angiography or more than two flow voids in the basal ganglia on MRI
3) The findings 1 and 2 are present bilaterally
4) Known diseases with similar angiographic findings (i.e., arteriosclerosis, autoimmune disease, meningitis, brain neoplasm, Down syndrome, neurofibromatosis type 1, head trauma, and irradiation to the head) should be ruled out
Broad classification
Any steno-occlusive lesions that fulfill the following findings:
1) Steno-occlusive lesions around the terminal portions of the internal carotid arteries
2) Findings of moyamoya vessels are not essential
3) Bilateral involvement is not essential
4) Known diseases with similar angiographic findings should be ruled out

RCMJ = Research Committee on Moyamoya Disease of the Ministry of Health and Welfare, Japan.

tory, age, age at onset, age at diagnosis, first sign at onset, course of the disease, treatment option, and associated diseases. Families with moyamoya syndrome, that is, MMD with known causes such as neurofibromatosis type 1 (NF1), protein C deficiency, Down syndrome, or past radiation therapy in the head, were excluded from this study. The present study was approved by the Ethics Committee of the Kyoto University Institutional Review Board and approved written informed consent was obtained from all subjects or their parents if the subjects were children younger than 16 years. An autopsy brain was obtained from II-9 in Family 6 (figure e-1, on the *Neurology*® Web site at www.neurology.org), with written informed consent from a family member.

Family pedigrees. As shown in figure e-1 we recruited 15 pedigrees of familial MMD for whom DNA samples were available from ≥3 affected and unaffected individuals. These families had never participated in previous linkage studies. Twelve families (families 1 through 6, 8 through 10, and 13 through 15) were reported in our earlier report,[16] and the family IDs in the present study correspond to those in the previous report (DNA samples were not available from Families 7, 11, and 12). We expanded Family 2, in which Individuals I-2, II-1, II-3, II-6, and III-1 were newly recruited and individual IV-4 had received an initial screening examination by MRI and MRA. The remaining three families were assigned as Families 16 through 18. Under the broad classification, all the families ranged for ≥3 generations from the founders (we assumed the nearest common ancestor of the affected individuals to be the founder), justifying a parametric model as an autosomal dominant transmission.[16] We consistently used a parametric analysis with a dominant model even under the narrow classification, since 12 of the 15 families ranged for ≥3 generations and satisfied a dominant model (Families 8, 17, and 18 would be one or two generational families). We defined an obligatory carrier as a non-founder with affected offspring, if one's phenotype had been confirmed to be normal by MRI and MRA or one was deceased.

The demographic features of the participating pedigrees and pedigree members are shown in table 2. We observed a total of 55 patients (38 women and 17 men) with MMD phenotype under the broad diagnostic classification, of which 53 patients (the other two had been deceased) were finally enrolled in the study. Twenty-five patients showed childhood onset (<15 years of age). The female and childhood predominances were compatible with the characteristics of MMD in a previous report.[3] Among the 55 patients, 43 (28 women and 15 men) were patients with definite MMD and five were patients with probable MMD (II-2 in Family 1, II-2 in Family 8, II-2 in Family 9, I-2 in Family 10, and II-4 in Family 17). Stenoses around the terminal portion of the ICAs without collateral vessels were found bilaterally in one patient (II-3 in Family 1) and unilaterally in six patients (II-2 in Family 5, II-3 in Family 9, II-2 in Family 10, IV-4 in Family 14, I-2 in Family 17, and III-3 in Family 18). Observations of patients with various phenotypes (unilateral or bilateral, or with or without collateral vessels) in a single family justified our assumption that these phenotypes were within the continuum of MMD phenotypes. This assumption is also supported by the findings that two pairs of identical twins (IV-3 and IV-4 in Family 14 and II-3 and II-4 in Family 17) showed qualitative differences in their disease phenotypes. We observed the skipping of a generation phenomenon and assumed 13 obligatory carriers, of whom five (II-3 in Family 3, II-2 in Family 13, III-9 in Family 14, and III-3 and III-7 in Family 15) were confirmed not to have any abnormal findings on MRI and MRA, indicating incomplete penetrance of MMD. In addition, we observed rapid disease emergence in Individual III-3 in family 18, a 37-year-old woman without any vascular risk factors, who had no occlusive lesions around the terminal portions of the ICAs in 2005 at the start of her participation in the study but had developed lesions 1 year later in 2006, suggesting age-dependent penetrance of the disease.

Table 2 Characteristics of the pedigrees of familial moyamoya disease

Item	No.
No. of patients	
Definite moyamoya disease	43 (15 men, 28 women)
Bilateral steno-occlusive lesions without collaterals	1 (1 man, 0 women)
Probable moyamoya disease	5 (0 men, 5 women)
Unilateral steno-occlusive lesions without collaterals	6 (1 man, 5 women)
Total	55 (17 men, 38 women)
No. of obligatory carriers	13 (4 men, 9 women)
Pedigree size under the broad classification	
3 Generations	11
4 Generations	3
5 Generations	1
All	15
Type of onset	
Childhood onset (<15 y)	25
Adult onset (≥15 y)	15
Asymptomatic	13
Unknown	2
Symptom at onset	
Ischemic stroke	29
Hemorrhagic stroke	6
Epilepsy	4
Headache	1
Asymptomatic	13
Unknown	2

Genotyping. Genomic DNA was extracted from blood samples from living patients and the autopsy brain from a deceased participant (II-9 in Family 6) using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). For genotyping in the genome-wide linkage study, an ABI Prism Linkage Mapping Set (Version 2; Applied Biosystems, Foster City, CA) with 382 markers for 22 autosomes and 18 markers for the X chromosome with more than 75% heterozygosity was used at about 10-cM resolution as described previously.[17] Briefly, genomic DNA extracted from peripheral blood was amplified by PCR with fluorescently labeled markers, and the PCR products were analyzed by an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems) using the Genescan program. Fine mapping markers were designed according to information from the Marshfield genetic map (http://research.marshfieldclinic.org/genetics/). We used

single nucleotide polymorphism (SNP) markers with minor allele frequencies of >10% if we could not obtain microsatellite markers. Genotyping of SNP markers was performed using a PCR-restriction fragment length polymorphism (RFLP) method as previously described.[18] A total of 17 markers were genotyped at 12.5-Mb intervals at the 17q25-qter linkage region. The marker locations were obtained from the uniSTS reference physical map (http://www.ncbi.nlm.nih.gov/genome/sts/). Merlin software[19] (http://www.sph.umich.edu/csg/abecasis/Merlin) was used to detect genotyping errors and Mendelian inconsistency. When genotyping resulted in no calls or an ambiguous call, the genotype was set to "unknown." Overall successful call rate was 99.8%.

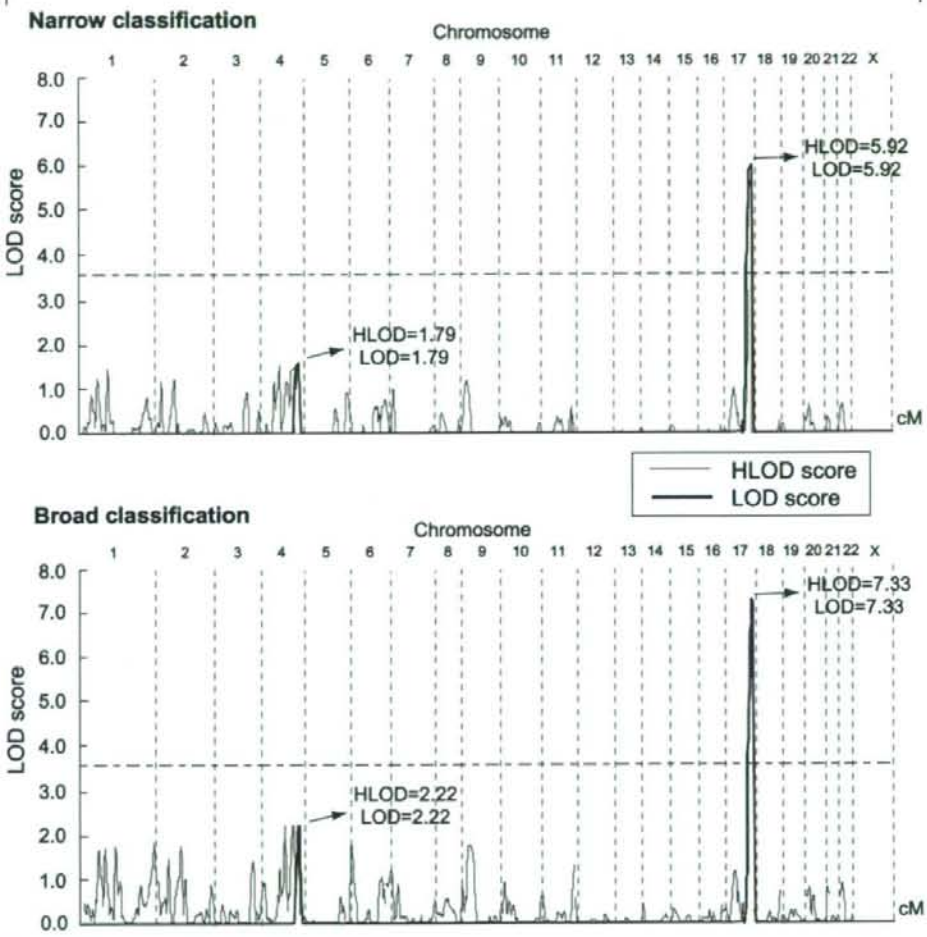
Linkage and haplotype analyses. We conducted a parametric linkage analysis assuming a dominant model. An affected member-only analysis was employed on the basis of observations of incomplete and age-dependent penetrance of MMD. In the analysis, we assigned an affected individual as either one whose phenotype was clinically diagnosed by either the narrow or broad classification or an obligatory carrier. The rationale for the assumption that obligatory carriers should be treated as affected was the low penetrance and low disease allele frequency. To increase the accuracy of haplotype estimation in affected individuals, we included unaffected individuals and non-founder spouses. The phenotype of unaffected related individuals in the pedigrees was assigned as unknown, while non-founder spouses were assigned as unaffected. On the basis of the observed prevalence of 3.16 per 100,000 persons,[2] the disease allele frequency should be set at 0.0000158, but we set it more conservatively at 0.0001 due to the increasing number of asymptomatic patients who have recently been diagnosed by MRI and MRA.[20] We assumed a phenocopy frequency of 0.00001. Population allele frequencies for each microsatellite marker were estimated from all the unrelated founders using the Merlin software,[19] with the exception of the X chromosome. For the X chromosome, we assigned equal portions for individual alleles. A two-point linkage analysis was performed using the MLINK program of LINKAGE version 5.2 (http://linkage.rockefeller.edu/soft/linkage).[21] Multipoint analyses for autosomes and X chromosome were run with a one-tailed probability value using GENEHUNTER version 2.0 (http://www.broad.mit.edu/ftp/distribution/software/genehunter/).[22] Since locus heterogeneity could be associated with MMD, we obtained both logarithm of odds (lod) and heterogeneity-adjusted logarithm of odds (HLOD) scores.[22] We employed a multipoint lod score of >3.6 as the threshold for genome-wide significance, and a lod score of >3.3 for the two-point analysis.[22] Haplotypes were constructed with the GENEHUNTER program. To avoid mis-specification of the inheritance pattern, we confirmed our results by nonparametric analysis. Nonparametric linkage (NPL) score was obtained using the GENEHUNTER program.

Mutation analysis of candidate genes. The 3.5-Mb candidate interval on chromosome 17q25-qter contains 94 annotated genes, of which 28 are predicted genes of unknown function (NCBI Human Genome Resources). There appear to be several putative candidate genes around the interval, which included BAI1-associated protein 2 (*BAIAP2*), tissue inhibitor metalloproteinase-2 (*TIMP2*), ras-related C3 botulinum toxin substrate 3 (*RAC3*), an important regulator of cell growth and cytoskeletal reorganization, or RAB40B, member of ras oncogene family (*RAB40B*). These four candidate genes were directly sequenced for a proband of five families (Families 1, 2, 5, 14, and 15) including coding exons, exon-intron boundaries, and 3′ untranslated regions in the same way as described previously.[23] Any polymorphic sites identified through our sequencing were searched for SNP database (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/index.html). When a minor allele frequency of a variant has not been determined yet in the database, genotyping was performed in 32 control subjects using PCR-RFLP method as described previously.[18] Variants with a minor allele frequency of >1% were considered not to be associated with autosomal dominant MMD because it is a relatively rare disease and is likely to be caused by a mutation. As −418G>C in *TIMP2* gene on chromosome 17q25 has been shown to be associated with familial MMD in a recent report,[24] we genotyped −418G>C for the probands of families using PCR-RFLP method.

RESULTS The results of genome-wide screening are shown in figure 1. Significant evidence of linkage was only observed on chromosome 17q25-qter, with maximum multipoint lod scores of 5.92 (HLOD score = 5.92) under the narrow classification and 7.33 (HLOD score = 7.33) under the broad classification at D17S928. No other regions showed genome-wide lod scores of >3.6. Two-point lod scores of 4.23 and 4.45 under the narrow classification (5.25 and 5.48 under the broad classification) with a recombination fraction of 0.05 were obtained at two nearby genome-wide screening markers, D17S784 and D17S928 (table e-1). Both the two-point and multipoint lod scores were linked to the locus in all families, with the exception of Family 14 (data not shown). Results of non-parametric analysis showed practically same result, with only a prominent peak at chromosome 17q25 with a maximum NPL score of 4.51 under the narrow classification (5.51 under the broad classification) at D17S928 (data not shown). Taken together, we considered 17q25-qter as a major genetic locus for MMD with an autosomal dominant mode of inheritance.

To narrow down the genetic locus, we conducted fine mapping of the 17q25-qter linkage region at an average resolution of 0.8 Mb, which gave a maximum multipoint lod score of 6.57 ($p = 0.000021$) under the narrow classification and 8.07 ($p = 0.0000034$) under the broad classification at D17S704 (figure 2). A maximum HLOD score of 6.81 under the narrow classification and 8.11 under the broad classification was observed at the same marker. Both the lod and HLOD scores decreased sharply at D17S1806. Sensitivity analyses using various sets of parameter combinations, i.e., the disease allele frequencies (0.0001 or 0.00001) and phenocopy frequencies (0.001, 0.0001, or 0.00001) for either narrow classification or broad classification did not alter either lod or HLOD scores more than 1% (data not shown). As shown in figure e-1,

Figure 1 Multipoint parametric lod and heterogeneity-adjusted logarithm of odds scores in the genome-wide screening for familial moyamoya disease (MMD) under the narrow diagnostic classification, in which only patients with definite MMD are assigned as affected, and under the broad diagnostic classification, in which patients with unilateral or bilateral steno-occlusive lesions around the terminal portion of internal carotid arteries including definite MMD and unilateral MMD are designated as affected



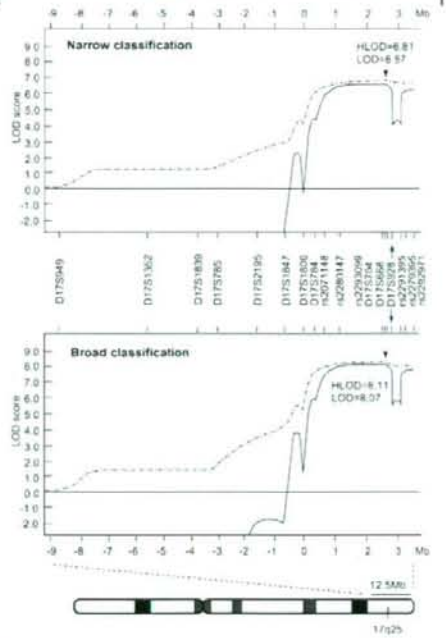
The dotted line shows the cutoff for a lod score of >3.6.

all affected individuals in each family shared a disease haplotype, with the exception of Family 14. Family 14 failed to show a common haplotype in the affected family members, suggesting the possible involvement of locus heterogeneity. Haplotype analysis with fine mapping revealed an obligate recombination between markers D17S1806 and D17S784 in Individual III-2 of Family 2 (figure e-1), which defined the critical MMD gene location within a 3.5-Mb region from D17S1806 to the end of chromosome 17q (uniSTS reference physical map).

The MMD locus at 17q25 reported by Yamauchi et al. spans from D17S785 to D17S836,[10] which lies 1.5-Mb centromeric to D17S1806 of the present locus, suggesting the possibility that their locus may not be identical to our locus. On the other hand, maximum lod and HLOD scores within MMD locus at 3p24-p26.1[8] were −8.95 and 0.24 under the narrow classification, and −9.04 and 0.30 under the broad classification. As for MMD locus at 8q23,[9] maximum lod and HLOD scores were −4.18 and 0.15 under the narrow classification, and −2.99 and 0.42 under the broad classification. These two loci satisfied the linkage exclusion criteria (lod < −2.0).

We sequenced four candidate genes (*BAIAP2*, *TIMP2*, *RAC3*, and *RAB40B*) and identified a total of 37 variants, of which four cause amino acid substitution (data not shown). However, all the nonsynonymous variants were found in the database and their minor allele frequencies were above 1%. A

Figure 2 Multipoint lod and heterogeneity-adjusted logarithm of odds (HLOD) scores in fine mapping of chromosome 17q25-qter under the narrow and broad classifications



The definition of the classifications is described in table 1. The location of marker D17S1806 is arbitrarily set at 0 Mb, and the other markers are scaled on the basis of their distances (in Mb) from D17S1806. The lod scores are shown by the continuous line and the HLOD scores are shown by the broken line. The lower figure shows the chromosomal positions of the fine mapping markers.

promoter polymorphism −418G>C in *TIMP2* gene was not polymorphic in our studied population.

DISCUSSION This study presents compelling evidence that a major locus for autosomal dominant MMD lies in the telomeric region of 17q25. The restriction of employment of pedigrees to multigenerational families, which is compatible with autosomal dominant transmission, justified a genome-wide parametric analysis. To circumvent uncertainty in phenotyping arising through low and age-dependent penetrance, we applied an affected member-only analysis after MRI and MRA screening examinations. We analyzed the data under both a narrow classification, in which only patients with definite MMD were assigned as affected, and a broad classification, in which patients with any steno-occlusive lesions around the terminal portions of the ICAs were designated as affected. With such a conservative approach, the present study succeeded in robustly revealing significant evidence of linkage to chromosome 17q25.3 under both classifications, with maximum multipoint lod scores of 6.57 (HLOD score of 6.81, $p = 0.000021$) under the narrow classification and 8.07 (HLOD score of 8.11, $p = 0.0000034$) under the broad classification. Segregation of a disease haplotype in all affected individuals was observed in 14 of the 15 families. An informative crossover defined the candidate interval between D17S1806 and the telomere of 17q, corresponding to a distance of about 3.5 Mb. We selected four candidate genes around the disease locus; however, mutation analysis could not identify any deleterious mutations.

The robust results of our linkage analyses under both the narrow and broad diagnostic classifications in turn suggest that our modified diagnostic criteria in the broad classification can be safely applied for the diagnosis of patients with MMD at least in familial cases. If so, the present study, in turn, indicates that MCA stenosis or MCA occlusion of uncertain causes as well as unilateral MMD may also be considered to be in the spectrum of MMD.

One ambiguity in the present study would be the assignment of the obligatory carriers, since we could not detect any lesions in the spectrum of MMD even with MRI and MRA examinations. One explanation for the absence of stenotic lesions in the obligatory carriers would be age-dependent progression of MMD, as observed in III-3 of Family 18.

Our locus is close to the previous reported MMD locus at 17q25,[10] but the two loci might not overlap with each other. At present, further evidence is required to confirm whether these two loci are identical. On the other hand, we failed to replicate the other MMD loci at 3p24-p26.1[8] and 8q23.[9] This failure of replication may be attributable to genetic heterogeneity, since the present study restricted the studied pedigrees to multigenerational families, while the other studies mainly used affected siblings or relative pairs. Isolation of a gene responsible for autosomal dominant MMD in our locus will provide evidence for or against genetic heterogeneity.

We selected four candidate genes on the basis of their biologic functions: *BAIAP2*, *TIMP2*, *RAC3*, and *RAB40B*. *BAIAP2* interacts with brain-specific angiogenesis inhibitor-1 (*BAI1*), which is an inhibitor of basic fibroblast growth factor (*bFGF*)-induced angiogenesis.[25] A promoter polymorphism of *TIMP2* gene was found to be associated with familial MMD in the Korean population.[24] However, it was not polymorphic in our studied population, although we genotyped only a limited number of

population. *RAC3* and *RAB40B* are members of ras oncogene family and important regulators of cell growth and cytoskeletal reorganization. No mutation was identified through the mutation analysis of these candidate genes. Further analyses will be needed.

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特 集：脳卒中と遺伝子

もやもや病の遺伝要因の探索の現状

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特 集 Ⅷ 脳卒中と遺伝子

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Current Knowledge on the Genetic Factors Involved in Moyamoya Disease

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Abstract

Moyamoya disease (MMD) is characterized by progressive stenosis and occlusion of the terminal portion of the bilateral carotid arteries as well as arterial collateral vessels. The etiology of MMD, however, remains unknown.

Several pieces of evidence suggest the involvement of genetic factors in MMD: over 10% of MMD patients have affected blood relatives; concordance in the affection status has been proven in 80% of identical twins; and there is an ethnic predisposition to MMD, the incidence of the disease being the highest in the Asian population. With regard to genetic factor (s), transmission of MMD does not follow the classic Mendelian law, i. e., skipping of a generation and discordance in identical twins, thereby indicating that genetic influence is likely to determine the susceptibility to MMD.

This study aimed to overview the recent findings related to the genetic determinants in MMD and to provide research perspectives for the next decade.

Pathophysiological investigations at molecular levels have uncovered the upregulation of various growth and stress response factors that are associated with angiogenesis in occlusive cerebral arteries.

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