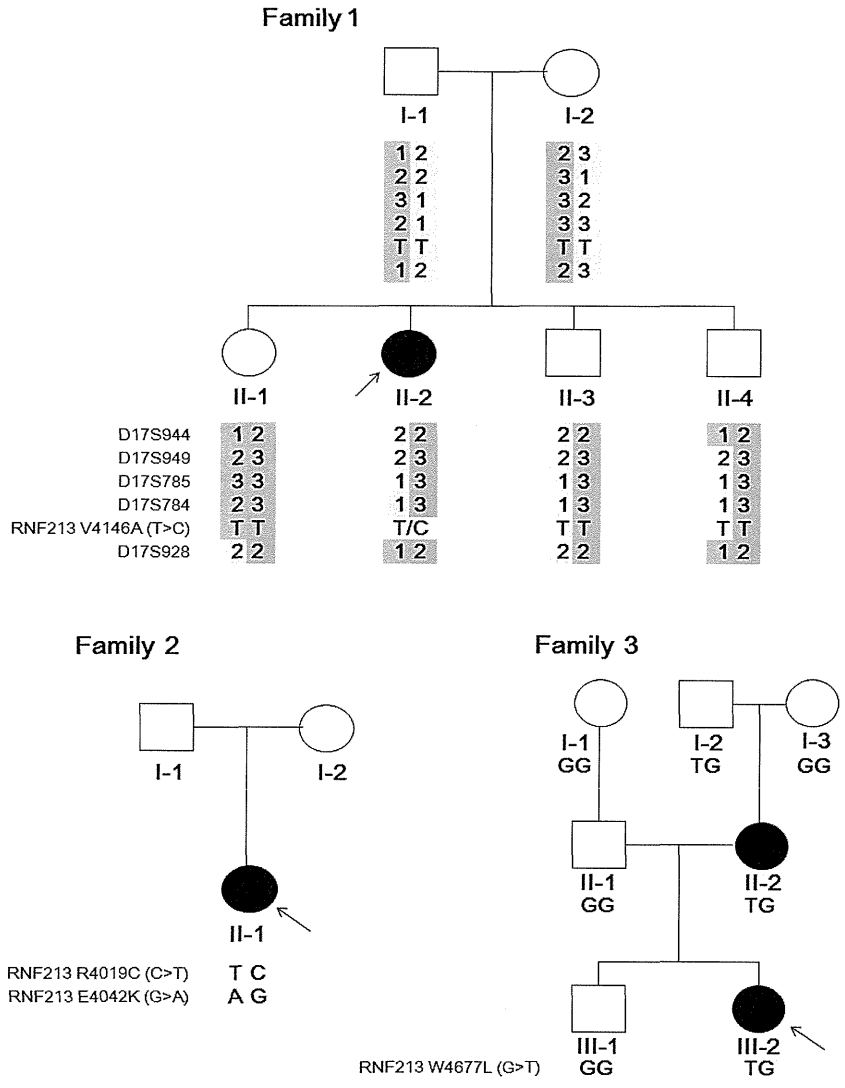

図1.チェコスロバキア人もやもや病患者に見出されたRNF213 rare variant。矢印はprobandを示す。

E. 文献

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F. 特許

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平成27年度厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

もやもや病成人出血発症例の治療方針に関する研究

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研究要旨

JAM Trial は出血発症もやもや病に対する直接バイパス手術の再出血予防効果を明らかにするための無作為割り付け試験である。2014 年の主要結果報告では、primary endpoint、secondary endpoint のいずれの発生率も手術群で有意に抑えられ、直接バイパスの再出血予防効果が証明された。さらに二次解析結果が 2016 年に報告され、後方出血群は前方出血群に比べ自然予後不良で、手術効果が高いサブグループであることが明らかとなった。

現在 JAM Trial Group では、前・後方出血群で予後に差異が生じる機序を明らかにするため、脳血管撮影所見の解析を行っている。これによると、後方出血群は前方出血群と比べ脈絡叢動脈からの異常側副路や後大脳動脈病変を有する割合が多い、という特徴が明らかとなった。

A. 研究目的

(1) 出血発症もやもや病に対するバイパス手術の再出血予防効果を明らかにすることを目的とする。
(2) 出血部位による出血性もやもや病の自然歴や手術効果の違いを明らかにする。（サブ解析 1）
(3) 出血部位による脳血管撮影上の特徴を明らかにする。（サブ解析 2）

B. 研究方法

多施設間共同臨床試験として登録５年・追跡５年の prospective randomized trial を行う。［倫理面への配慮］参加各施設の医の倫理委員会の審議と登録前の informed consent を必須とする。

頭蓋内出血発作を 1 年以内に認めたモヤモヤ病確定診断例で、ADL が modified Rankin disability scale 0～2 のものを対象とし、事務局による登録条件のチェックの後、保存的治療のみの「非手術群」と STA-MCA anastomosis を実施する「手術群」への randomization を行う。出血部位により前方循環出血群（A 群）、後方循環出血群（P 群）に分類、層別割り付けを行うことで手術群・非手術群間の出血部位の偏りを排除する。

登録時、登録６ヶ月後、１年後、その後１年毎に規定の諸検査を行いながら臨床経過を観察する。「再出血発作」、「ADL を悪化させる虚血発作」、「その他の死亡ならびに重篤な ADL

悪化」、「内科医の判断による手術への移行（虚血発作頻発等）」が研究の primary end point、再出血発作単独が secondary endpoint である。目標症例数は 80 例（平成 18 年 1 月症例数見直し：手術群、非手術群各 40 例）とする。

C. 研究結果

1. 主要結果

平成 13 年 1 月より症例登録を開始し、本症の呼称として Japan Adult Moyamoya (JAM) trial を採択した。登録施設数は 22 施設。平成 20 年 6 月にこの症例数に到達し新規登録を終了した。80 症例の内訳は手術群 42 例、非手術群 38 例である。

平成 25 年 6 月に最終症例登録から 5 年が経過、全症例の観察期間が終了した。手術群 6 例 (3.2%/年)、非手術群 13 例 (8.2%/年) に primary end point に該当するイベントが発生した。再出血の発生 (secondary endpoint) は手術群 5 例 (2.7%/年)、非手術群 12 例 (7.6%/年) であった。

登録状況を表 1 に、また end point 到達症例の詳細を表 2 に示す。

表 1. JAM trial 登録状況

	A 群	P 群	計
手術群	24	18	42
非手術群	21	17	38
計	45	35	80

表 2. Primary end point 到達症例

(1) 手術群

性別	出血部位	登録からの期間	原因
F	A	3 ヶ月	再出血
M	P	8 ヶ月	脳幹梗塞死
M	A	9 ヶ月	再出血
F	A	1.4 年	再出血
F	A	2.3 年	再出血
F	A	4.8 年	再出血

(2) 非手術群

性別	出血部位	登録からの期間	原因
F	P	7 ヶ月	再出血
F	P	7 ヶ月	再出血
M	P	8 ヶ月	再出血
F	P	1.2 年	再出血
F	P	1.7 年	再出血
M	A	2.0 年	再出血
F	P	2.4 年	再出血
F	P	3.3 年	再出血
F	A	3.5 年	再出血
F	P	4.0 年	再出血
F	A	4.5 年	虚血発作増強
M	P	4.98 年	再出血
F	P	3.9 年	再出血

手術群、非手術群で患者の年齢、性別、併存全身合併症、過去の神経学的イベント、出血様式や部位に有意差はなかった。

Primary endpoint

手術群 : 0.032/patient-year

非手術群 : 0.082/ patient-year

(a) Log rank 検定 p=0.048

(b) Cox regression analysis

手術群の Hazard ratio (HR)

0.391 (95%CI: 0.148-1.029, p=0.057)

Secondary endpoint (再出血)

手術群 : 0.027/patient-year

非手術群 : 0.076/patient-year

(a) Log rank 検定 p=0.048

(b) Cox regression analysis

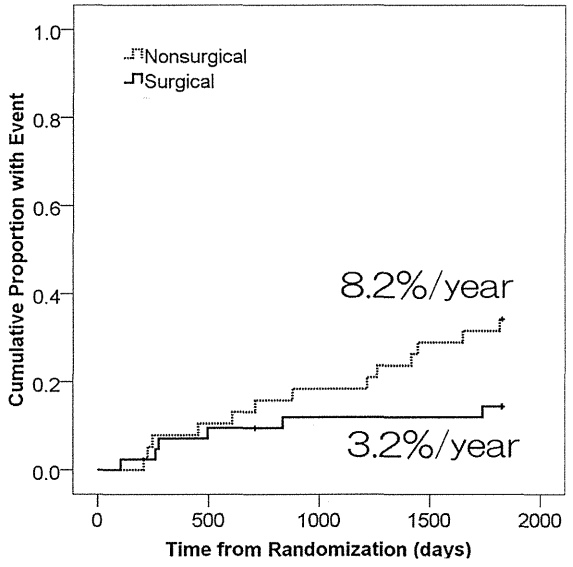
手術群の HR

0.355 (95%CI: 0.125-1.009, p=0.052)

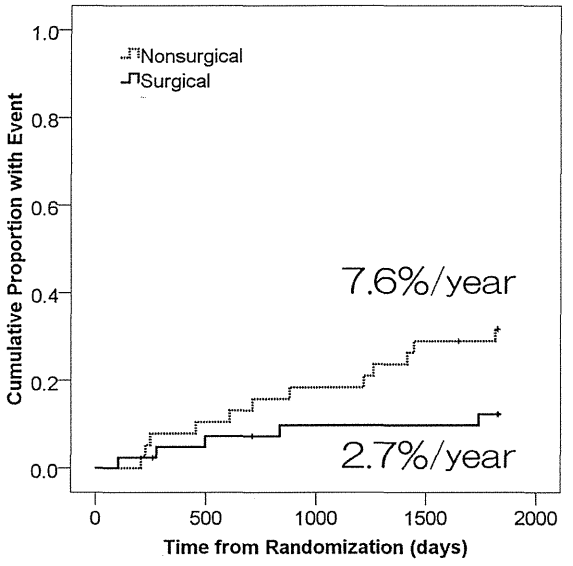
Primary endpoint, secondary endpoint に関する Kaplan-Meier 曲線を図 1 に示す。

図1　Kaplan-Meier 曲線

Primary endpoint



Secondary endpoint

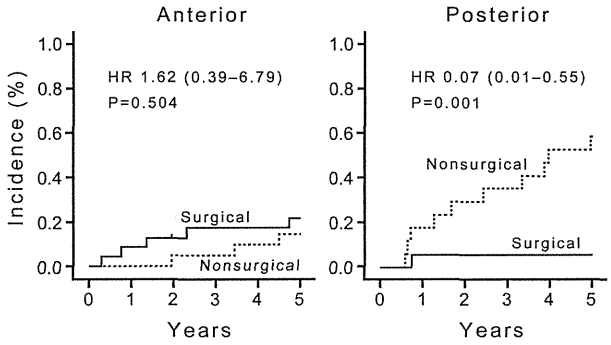


2. サブ解析 1

(1) 出血部位による手術効果の差異

P 群における HR(primary endpoint)は 0.07 (95%CI:0.01-0.55)であり、手術群で有意に予後が良好であるのに対し、A 群における HR は 1.62(95%CI:0.39-6.79)であり、有意な手術効果は認められなかった（図 2)。交互作用検定では A・P 群間で手術効果が有意に異なることが示された（P=0.013)。

図2　サブ解析 1 (primary endpoint)



(2) 出血部位による非手術群予後の差異

非手術群 38 例のみを対象として A・P 群間での予後の違いを検討した。

Primary endpoint:

Log rank P=0.003

P 群の HR: 5.83 (95%CI 1.60-21.27)

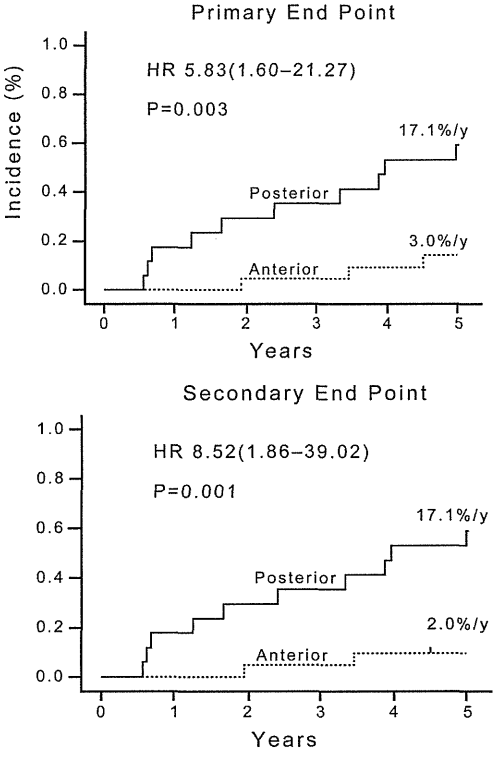
Secondary endpoint:

Log rank P=0.001

P 群の HR: 8.52 (95%CI 1.89-39.02)

であり、両 end point とも P 群で発生率が有意に高かった（図 3)。以上の結果は、2016 年に Stroke 誌に発表された。

図 3. 非手術群における Kaplan-Meier 曲線



3. サブ解析 2

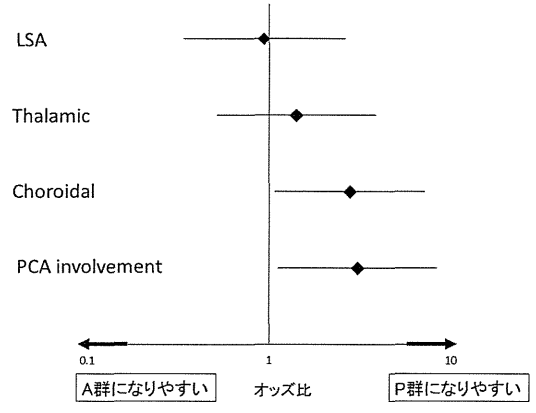
(1) 出血部位と異常側副路の関係

前・後方出血群間で予後に差異が生じる機序を明らかにするため、脳血管撮影所見の解析を行った。全 80 例中、出血側の判定が困難であった等の 5 例を除く 75 例において、出血部位（A・P 群）と、出血側半球における異常側副路の発達や後大脳動脈狭窄性病変との関係を解析した。

異常側副路を、lenticulostriate type、thalamic type、choroidal type の 3 タイプに分類し、それぞれの発達の程度をスコア化して評価した。

P 群出血となるオッズ比は lenticulostriate type: 0.94、thalamic type: 1.41、choroidal type: 2.77、後大脳動脈病変: 3.06 であり、後方出血には脈絡叢動脈からの異常側副路と後大脳動脈病変が関連する可能性が示唆された（図 4）。これら 2 要因は、前述の 4 要因を投入したロジスティック回帰モデルでも有意に後方出血に関連した。

図 4. 各異常側副路と出血部位との関係

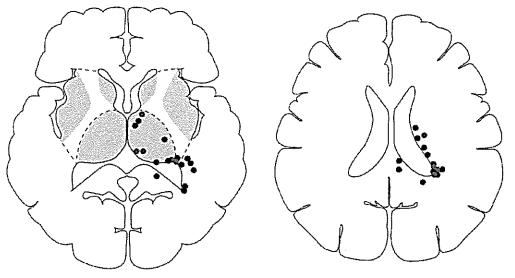


(2) 出血点の topographical analysis

画像判定委員会において、初回出血時の頭部 CT 等から、初回出血点の位置推定が行われた。P 群では、出血点の多くが側脳室三角部・体部後方の上衣下、すなわち脈絡叢動脈の灌流域に集中していた。

図 5. P 群における初回出血点

(全て左側にプロット。再出血例を赤で示す)



D. 考察

サブ解析 2 の結果からは、後方出血群の脳血管撮影上特徴は、脈絡叢動脈からの側副路発達と後大脳動脈の狭窄であり、P 群出血点の多くは脈絡叢動脈灌流域に生じることが示唆された。総合すると、後大脳動脈狭窄進行と、それに伴う脈絡叢動脈側副路の発達・破綻が、P 群出血の代表的臨床像と考えられる。

予後不良とされる後方出血群は、同じもやもや病でも前方出血群と極めて異なる血管構築を有することが確認された。特に脈絡叢動脈側副路から髄質動脈へ吻合するもやもや病特有の側副路は、出血に強く関係する dangerous anastomosis である可能性が示唆される。今後、JAM Trial 登録例と登録施設からの虚血症例との血管撮影所見の比較を行う case-control study や、非手術群による retrospective cohort study など、出血の機序解明や再出血予測に関する更なる研究が、JAM Trial group にて行われる予定である。

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(2) Takahashi JC, Funaki T, Houkin K, Inoue T, Ogasawara K, Nakagawara J, Kuroda S, Yamada K, Miyamoto S; JAM Trial Investigators.
Significance of the Hemorrhagic Site for Recurrent Bleeding: Prespecified Analysis in the Japan Adult Moyamoya Trial.
Stroke. 2016 Jan;47(1):37-43

D. 知的財産権の出願・登録状況

なし

[症例登録22施設]
中村記念病院、北海道大学医学部附属病院、札幌医科大学医学部附属病院、東北大学医学部附属病院、長岡中央総合病院、岩手医科大学付属病院、秋田県立脳血管研究センター、東京女子医科大学病院、北里大学病院、千葉大学医学部附属病院、群馬大学医学部附属病院、名古屋市立大学医学部附属病院、岐阜大学医学部付属病院、京都大学医学部附属病院、奈良県立医科大学付属病院、天理よろず相談所病院、国立循環器病センター、徳島大学医学部付属病院、中国労災病院、倉敷中央病院、国立病院九州医療センター、長崎大学医学部附属病院

平成 27 年度発表論文（国立循環器病研究センター）

Takahashi JC, Funaki T, Houkin K, Inoue T, Ogasawara K, Nakagawara J, Kuroda S, Yamada K, Miyamoto S; JAM Trial Investigators.
Significance of the Hemorrhagic Site for Recurrent Bleeding: Prespecified Analysis in the Japan Adult Moyamoya Trial.
Stroke. 2016 Jan;47(1):37-43.

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

新規患者レジストリーの構築

北海道大学北海道大学病院・臨床研究開発センター
佐藤　典宏

研究要旨

成人もやもや病に関する診断および治療法を確立するため、新規患者レジストリーの構築に関する検討を行った。本研究班で実施している既存研究も含めた統一的な収集項目、情報収集に使用するシステムについて検討した。これらの検討結果を元に、次年度は研究開始と研究データ収集システム稼動に向けた検討、すなわち、患者追跡方法を含む運用上の問題点・解決策の検討を行っていきたい。

A. 研究目的

成人もやもや病に関する診断および治療法を確立するため、新規患者レジストリーの構築方法について検討を行った。具体的には、本研究班で実施している既存研究も含めた統一的な収集項目、研究データ収集に使用するシステムについて検討した。

B. 研究方法

新規レジストリーの構築の内、収集項目について、本研究班で実施している各種研究およびNINDS（National Institute of Neurological Disorders and Stroke）のStrokeに関するCommon Data Elementsを参考に、統一的な収集項目を検討した。実施中の各種研究は、AMORE研究（富山大学・黒田）、COSMO Japan研究（京都大学・高木）、JAM研究（京都大学・舟木）、MODEST研究（東北大学・藤村）である。検討の際、本研究班の班員から意見を収集した。また、研究データの収集に使用するシステムについて、継続可能性を含め検討を行った。

C. 研究結果

収集項目について、本研究班で実施している各種研究の収集項目のほとんどはNINDSの収集項目として示されており、NINDSで示されていないものは各研究特有の情報であった。そのため、新規レジストリーでは各研究特有の情報は含めず、NINDSの項目を基準に必要な項目を選定した。なお、疾患診断情報、生体試料情報等、新規レジストリーとして必要だが各研究およびNINDSのいずれにもない情報は個別に項目を作成した。班員へのレビューの結果、特に異論はなく、情報の過不足はないものと考えられた。

研究データ収集システムについては、北海道大学病院と企業で共同開発したEDC（Electronic Data Caputure）システムを使用することとした。その選定理由として、システムの継続可能性が挙げられた。レジストリーは長期に亘り運用することが必須であるが、研究毎に個別にシステムを外注すると、外注費用を払えなくなった時点でシステムを使用できなくなる。開発したEDCシステムであれば、例え

ば研究班の班員が運用していくことも可能である。少なくとも研究期間中は北海道大学病院臨床研究開発センターにて支援を行い、研究の質の担保および将来を見据えたデータ管理手順の整備を行う予定である。

D. 結論

今回検討を行った統一的な収集項目について、必要な情報は網羅されていると考えられた。また、研究データ収集システムについて、システムの継続可能性を含め問題はないものと考えられた。これらを元に、次年度は１．研究開始と２．研究データ収集システム稼動に向けた検討が必要である。具体的には、１については患者追跡方法や匿名化番号表の管理方法を含む運用上の問題、２については統一的な収集項目のシステムへの反映の際の問題が考えられるため、それらの問題点・解決策の検討を行っていきたい。

E. 文献

１．NINDS Common Data Elements: https://commondataelements.ninds.nih.gov/Stroke.aspx#tab=Data_Standards

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3. 佐藤典宏：＜文部科学省 橋渡し研究加速ネットワークプログラム 第2回海外TRセンターワークショップ 米国及び日本の TR センターにおける成功と挑戦－国際協力体制の構築に向けて－＞北海道大学 TR センターの取り組みと実績 臨床評価 Vol.43, No.1 2015 175-177

研究班構成員名簿

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A novel application of four-dimensional magnetic resonance angiography using an arterial spin labeling technique for noninvasive diagnosis of Moyamoya disease☆

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ABSTRACT

Background: Noncontrast-enhanced time-resolved four-dimensional magnetic resonance angiography using an arterial spin labeling technique (ASL-4D MRA) is emerging as a next generation angiography for the management of neurovascular diseases. This study evaluated the feasibility of ASL-4D MRA for the diagnosis of Moyamoya disease (MMD) and MMD staging by using digital subtraction angiography (DSA) and time-of-flight MRA (TOF MRA) as current standards.
Methods: Eleven consecutive non-operated patients who underwent DSA for the diagnosis of MMD were recruited. Two independent observers evaluated the three tests. The data were analyzed for inter-observer and inter-modality agreements on MMD stage. Nine of 22 hemispheres underwent surgical revascularization and ASL-4D MRA was repeated postoperatively.
Results: Time-resolved inflow of blood through the cerebral vessels, including moyamoya vessels, was visualized in all the 22 non-operated hemispheres. MMD stages assessed by DSA and ASL-4D MRA were completely matched in 18 hemispheres, with a significant positive correlation between these modalities ($r=0.93$, $P<0.001$). Inter-observer agreement with ASL-4D MRA ($\kappa=0.91\pm0.04$, $P<0.001$) and inter-modality agreement between ASL-4D MRA and DSA ($\kappa=0.93\pm0.04$, $P<0.001$) were both excellent. MMD stages assessed by ASL-4D MRA have also a significant positive correlation with those assessed by TOF MRA ($r=0.68$, $P=0.004$). Repeated ASL-4D MRA clearly demonstrated the bypassed arteries and changes in the dynamic flow patterns of cerebral arteries in all the nine hemispheres after surgical revascularization. Of these, postoperative focal hyperperfusion was detected by single photon emission tomography in 7 hemispheres. In five of the seven hemispheres (71%) with postoperative hyperperfusion, ASL-4D MRA demonstrated focal hyperintense signals in the bypassed arteries, although TOF MRA did not.
Conclusions: Noninvasive ASL-4D MRA is feasible for the diagnosis of MMD staging. This next generation angiography may be useful for monitoring disease evolution and treatment response in cerebral arteries after revascularization surgery in MMD.

1. Introduction

Moyamoya disease (MMD) is an idiopathic cerebrovascular disease characterized by chronic progressive stenosis of the terminal portion of the bilateral internal carotid arteries (ICAs) around the circle of Willis, which leads to the formation of collateral vascular networks that look like "a puff of smoke" (moyamoya vessels) at the base of the brain [14]. Although digital subtraction angiography (DSA) has been recommended for a definitive diagnosis of MMD and MMD staging (known as a 6-grade Suzuki's stage system), especially in candidates of surgical revascularization [12], this procedure is known to carry a potential risk of persistent neurological deficits [1]. When certain findings are fulfilled on time-of-flight (TOF) imaging conducted using a ≥1.5-Tesla scanner, magnetic resonance angiography (MRA) can also provide a definitive diagnosis

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[12]. However, TOF MRA does not have temporal resolution. It would be ideal if dynamic flow patterns within the cerebral vasculature were demonstrated noninvasively to monitor clinical course of MMD. Growing evidence revealed that a 3.0-Tesla MR scanner offers a higher signal-to-noise ratio, allowing a higher spatial resolution and a refined visualization of features of intracranial neural and vascular structures, including pathological vasculature in patients with MMD [9,13]. Recently, noncontrast-enhanced time-resolved four-dimensional MRA using an arterial spin labeling technique (ASL-4D MRA) was developed to delineate dynamic flow patterns within the cerebral vasculature [18]. Although conventional contrast agent-enhanced dynamic MRA with some temporal resolution has received considerable attention [11], this imaging technique still has limitations, including requirements of intravenous contrast agent injection as well as the low temporal resolution (only on the order of seconds) [18]. ASL-4D MRA can be performed without any contrast agent by labeling circulating protons in arterial blood of the targeted vessels. The dynamic inflow pattern of arteries, including intracranial ones, can be visualized with higher temporal resolution (on the order of milliseconds) [17]. Growing number of studies has already tested ASL technique to measure cerebral perfusion parameters, including cerebral blood flow and arterial transit time in various type of central nervous system pathology, including MMD [16]. On the other hand, this novel technique has been tested as a time-resolved cerebral angiography only for cerebral arteriovenous malformations [17]. Thus, it has not been tested in patients with MMD. In the present study, therefore, the feasibility of ASL-4D MRA was evaluated for the diagnosis of MMD and MMD staging by using DSA and TOF MRA as current standards. In patients who underwent surgical revascularization, ASL-4D MRA was further repeated after surgery and the changes in the dynamic flow patterns within the cerebral vasculature were tested.

2. Materials and methods

2.1. Study subjects

This prospective study included 11 consecutive patients with MMD who were treated at the Hokkaido University Hospital between June 2012 and February 2013. They all met the criteria for definitive MMD as determined by the Suzuki's stage classification based on the DSA findings [14]. MRI examinations, including ASL-4D MRA and TOF MRA, were performed on all the patients before surgery. The MRA stage was also assigned for all the 22 hemispheres in 11 patients according to the individual TOF MRA total score described elsewhere [5]. The mean period between DSA and ASL-4D MRA examination was 4.5 months (range: 0–13). In 7 cases with a period longer than 1 month between DSA and ASL-4D MRA, we confirmed that the TOF MRA stage did not progressed. All the 11 patients cover all six types of clinical events or symptoms of MMD reported previously: [12] cerebral infarction, transient ischemic attack, intracerebral hemorrhage, headache, epilepsy, and asymptomatic.

2.2. Protocol for ASL-4D MRA

All MR scanning were performed using a 3.0-Tesla scanner (Achieva 3T TX Release 3.2.1.0; Philips Medical Systems, Bests, Netherlands) with a 32-channel head coil. The Pulsed ASL was performed using echo planar imaging and signal targeting with alternating radiofrequency (EPI-STAR) technique [2]. Labeling was achieved by applying section-selective 180° radiofrequency pulses in a 30.0-mm-thick labeling slab that was located below the imaging plane. Image acquisition was performed using Look-Locker sampling with an excitation pulse of 10° [7], and various delay times; post-labeled delay of 200 ms after labeling and a subsequent constant phase interval of 150 ms were used. Imaging plane was located sufficiently to cover the circle of Willis and the associated main branches in all the patients. As a result, a total of eight phases were acquired (200, 350, 500, 650, 800, 950, 1100, and 1250 ms after labeling). A turbo-field echo-planar imaging (TFEPI) sequence was used as readout. Other imaging parameters were set as follows to adjust scanning time to approximately 5 min: TR, 13 ms; TE, 5.1 ms; cycle duration, 1460 ms; FOV, 230 mm × 230 mm; slab thickness, 105 mm; matrix, 192 × 192; slice thickness, 0.7 mm; voxel size, 1.2 mm × 1.2 mm × 0.7 mm; turbo field echo (TFE) factor, 13; EPI factor, 5; sensitivity encoding (SENSE) factor, 3; and Flip angle, 10°. After ASL-4D MRA was completed, a routine MRI scan, including TOF MRA was performed as part of the routine diagnostic protocol, which was reported elesewhere [5].

Table 1
Protocol for imaging evaluation to determine the Suzuki's stage by ASL-4D MRA.

Suzuki's stage	Angiographic findings
Stage I	Narrowing of the carotid fork
Stage II	Dilated major cerebral artery and a slight moyamoya vessel network
Stage III	Discontinuity of the proximal portion of ACA and/or MCA with distinct basal moyamoya vessels
Stage IV	Disappearance of ACA and/or MCA and/or PCA and narrowing of basal moyamoya vessels
Stage V	Disappearance of all the main cerebral arteries arising from the ICA system without basal moyamoya vessels
Stage VI	Complete disappearance of the intracranial ICA and main cerebral arteries arising from the ICA system without basal moyamoya vessels

2.3. Determination of Suzuki's disease stage by ASL-4D MRA

ASL-4D MRA was performed on all the non-operated 11 patients prior to surgical revascularization, within an average of 4.4 months (range: 0–13 months) after the most recent DSA exam. Two authors (HU and MI) who are expertized at radiological diagnosis for Moyamoya disease and also are certificated as board neurosurgeons by Japan Neurosurgical Society (more than 7- and 11-years experience, respectively) used ASL-4D MRA data to independently diagnose the MMD stage of each patient (22 hemispheres). A board diagnostic-neuro-radiologist of Japan Radiological Society (third author, NF) confirmed these two raters to be satisfied for intra-rater agreement indices of the staging of Moyamoya disease. Based on Suzuki's stage classification, cerebral angiography on ASL-4D MRA were analyzed bilaterally for stenosis, occlusion of the terminal portion of ICA or the proximal portions of the anterior, middle and/or posterior cerebral artery (ACA, MCA and/or PCA), as well as for the development of basal moyamoya vessels [12,14]. In brief, each six-grade stage was assigned when ASL-4D MRA demonstrated each of findings as described in Table 1. First, we compared inter-observer differences in the diagnosis of Suzuki's stage classification determined by time-resolved ASL-4D MRA. Next, inter-observer disagreements on the stage determined by ASL-4D MRA were resolved during a consensus meeting with all the co-authors, including abovementioned board diagnostic-neuro-radiologist of the present study. Third, the inter-modality differences in the diagnosis of the stage between time-resolved modalities (i.e.; DSA and ASL-4D MRA) were also compared. In addition, six-grade-Suzuki's stage determined by ASL-4D MRA was compared to four-grade-MRA stage determined by TOF MRA, however, it was impossible to calculate kappa coefficient for the inter-modality agreement between these modalities due to the difference of the number of grades. Finally, the sensitivity, specificity, true/false predictive value, and accuracy of the diagnosis were calculated for each Suzuki's stage classification.

Table 2
Summary of the demographic and clinical data at the diagnosis of the patients with Moyamoya disease (MMD). Suzuki and MRA stage for MMD staging were determined on the basis of the DSA and TOF MRA findings, respectively.

Case	Age, *y*	Gender	Disease type	Suzuki's stage determined by DSA		MRA stage determined by TOF MRA		Risk factors
				Right	Left	Right	Left	
1	40	Male	Infarction-type	V	VI	4	4	Hypertension
2	60	Female	Headache → TIA type	IV	V	2	3	None
3	12	Female	TIA-type	II	III	1	2	None
4	7	Female	Infarction-type	II	III	2	3	None
5	38	Female	Hemorrhagic-type	V	III	3	3	None
6	3	Male	Infarction-type	IV	III	3	2	None
7	65	Male	Epileptic-type	III	IV	2	2	Hypertension, carotid stenosis
8	49	Female	Epileptic-type	IV	III	3	3	None
9	40	Female	Hemorrhagic → infarction-type	III	V	3	4	Hypertension, diabetes mellitus
10	21	Female	Asymptomatic-type	III	I	2	1	None
11	40	Female	infarction-type	III	III	3	1	Hypertension, smoking

TIA, transient ischemic attack; DSA, digital subtraction angiography; TOF MRA, time-of-flight magnetic resonance angiography.

2.4. Analysis of postoperative change in the dynamic flow patterns

Seven of the 11 patients underwent direct or combined direct and indirect surgical revascularization [4,6]. ASL-4D MRA were repeated in nine hemispheres of the seven patients at postoperative Day 0 and Day 7 to visualize postoperative changes in the dynamic flow patterns within the cerebral vasculatures. In the nine hemispheres, each phase of ASL-4D MRA was inspected to compare the phase in which inflow of blood through the cerebral vessels after labeling was visualized first (defined as "appearing phase" in this study), as well as the maximum intensity of the bypassed donor and recipient arteries qualitatively.

2.5. Statistical analysis

All continuous and ranked data were expressed as mean ± SE. Correlation coefficient between pairs of ranked variables was calculated by Spearman's test. The parameters between two groups were compared using Wilcoxon's signed rank test for ranked variables. Cohen's kappa coefficients (κ) of concordance were calculated to determine the inter-observer and inter-modality agreements on the Suzuki's stage classification of MMD. Agreements were rated as excellent when $\kappa > 0.8$. The significance level was set at $P < 0.05$. Statistical analysis was completed with Excel (EXEL-TOUKIEI 2012^{R}, Social Survey Research Information Co., Ltd., Tokyo, Japan).

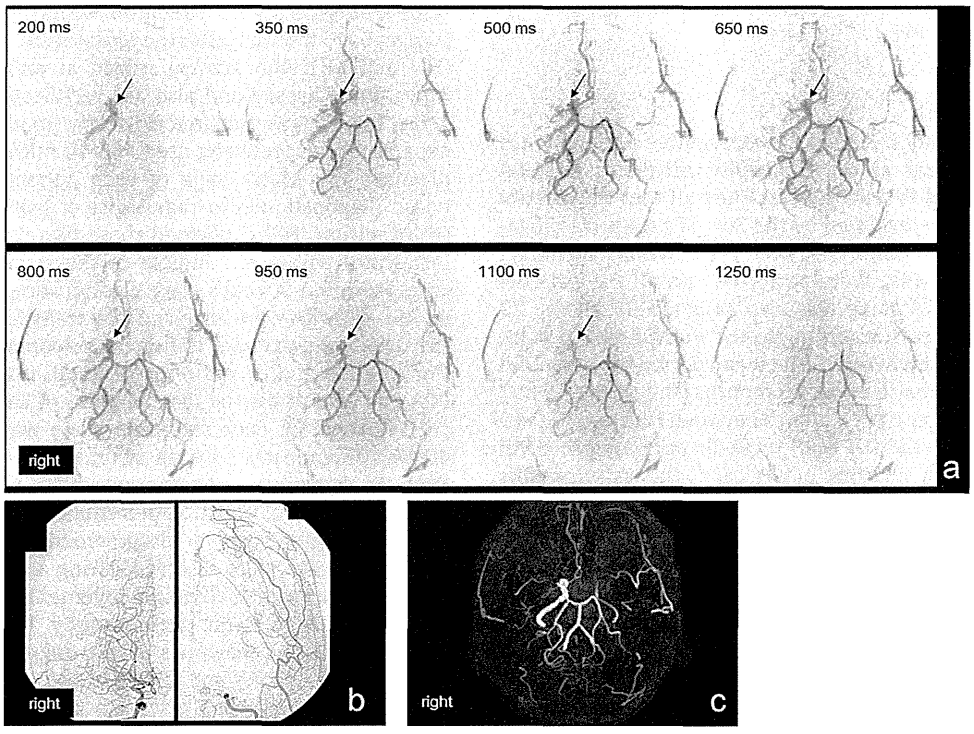

Fig. 1. Representative serial images of a patient with Moyamoya disease (Case 2) obtained by noncontrast-enhanced time-resolved four-dimensional magnetic resonance angiography using an arterial spin labeling technique (ASL-4D MRA, Panel a) and digital subtraction angiography (DSA, Panel b). Panel c showing the static TOF MRA image. Note that ASL-4D MRA clearly demonstrates abnormal vascular nets around the right carotid fork (arrow, basal moyamoya vessels). In addition, severe stenosis of the right ICA and MCA, respectively, as well as the occlusion of the left ICA and MCA is shown. Based on DSA and ASL-4D MRA, the Suzuki's stage of this 60-year-old woman with transient ischemic attack is rated as stage IV on the right hemisphere and stage V on the left hemisphere. Based on TOF MRA, MRA stages are 2 for the right hemisphere and 3 for the left hemisphere.

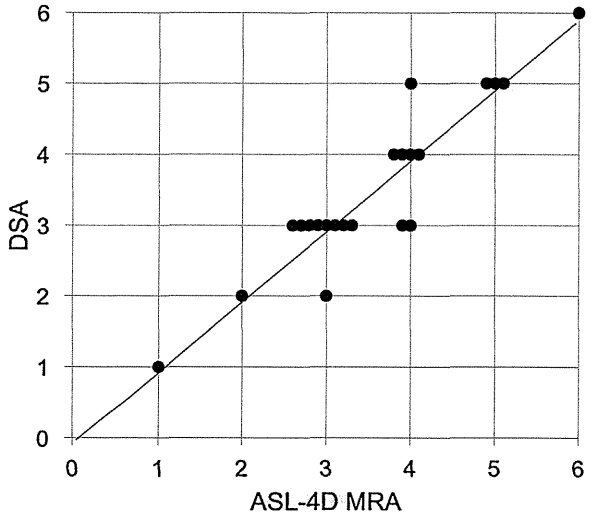

Fig. 2. Scatter plots of Moyamoya disease stages diagnosed by DSA and ASL-4D MRA. A positive linear correlation is confirmed ($r = 0.93$, $P < 0.001$).

3. Results

3.1. Baseline characteristics of the patients

In total, 11 consecutive patients diagnosed with MMD using DSA were included in this study, three males and eight females with a mean age of 34.1 years (range: 3–65 years). The patients' demographic and clinical data were summarized in Table 2. All patients underwent ASL-4D MRA examinations prior to surgical revascularization.

3.2. Diagnostic accuracy of disease staging by ASL-4D MRA

ASL-4D MRA visualized time-resolved inflow of blood through the large cerebral and moyamoya vessels in 22 non-operated hemispheres of the 11 patients with MMD. A representative image was presented in Fig. 1. MMD stages assessed by time-resolved modalities (i.e., ASL-4D MRA and DSA) and non-time-resolved modality (i.e., TOF MRA) were summarized in Table 3. DSA diagnosed 1 hemisphere with stage I, 2 hemispheres with stage II, 10 hemispheres with stage III, 4 hemispheres with stage IV, 4 hemispheres with stage V, and 1 hemisphere with stage VI. TOF MRA diagnosed 3 hemispheres with stage 1, 7 hemispheres with stage 2, 9 hemispheres with stage 3, and 3 hemispheres with stage 4. Suzuki's stage determined by ASL-4D MRA by two independent observers completely matched in 17 of the 22 hemispheres. A positive linear correlation was also observed between the stages determined by the observers using ASL-4D MRA ($r = 0.93$, $P < 0.001$). Accordingly, there was an excellent inter-observer agreement for the diagnosis of the Suzuki's stage by ASL-4D MRA ($\kappa = 0.91 \pm 0.04$, $P < 0.001$).

The disease stages that were finally determined by ASL-4D MRA were compared with those obtained by analysis of DSA and TOF MRA, respectively (Table 3). There was a perfect match in the disease stage between ASL-4D MRA and DSA in 18 of the 22 hemispheres. A positive linear correlation was observed between the disease stages determined by using the two modalities ($r = 0.93$, $P < 0.001$: Fig. 2), and an excellent inter-modality agreement ($\kappa = 0.93 \pm 0.04$, $P < 0.001$) was observed. Notably, in 3 of the 4 hemispheres causing a disagreement, ALS-4D MRA overestimated Suzuki's stage compared to DSA. Thus, ASL-4D MRA diagnosed three hemispheres with stage III or IV, although conventional DSA diagnosed those with stage II or III. On the other hand, there was a positive linear correlation between the Suzuki's stage and MRA stage determined by ASL-4D MRA and TOF MRA, respectively ($r = 0.68$, $P = 0.004$). As aforementioned, it was difficult to compare the 6-grade Suzuki's stage and 4-grade MRA stage systems directly and kappa coefficients for the inter-modality agreement could not be calculated.

The impact of disease severity on the validity of MMD staging determined by ASL-4D MRA was verified at each stage (Table 4). Analysis of the most common stage (stage III) indicated 80% inter-modality agreement between DSA and ASL-4D MRA. The sensitivity and specificity of ASL-4D MRA for the diagnosis of MMD stage III were 0.8 and 0.92, respectively. The positive predictive value (PPV) and negative predictive value (NPV) of ASL-4D MRA for MMD stage III were 0.89 and 0.85, respectively. As a result, the diagnostic accuracy of MMD stage III by ASL-4D MRA was 0.86. Taken together, the diagnostic accuracy of ASL-4D MRA was >0.86 (ranging from 0.86 to 1) for all the Suzuki's stage.

3.3. Postoperative changes in the dynamic flow patterns after revascularization

Seven patients (nine hemispheres) underwent combined revascularization surgery, including direct bypass [4,6], as well as pre- and post-operative ASL-4D MRA. In all cases, ASL-4D MRA clearly detected the bypassed donor and recipient arteries i.e., superficial temporal artery (STA) and MCA, respectively), postoperatively, at the phase ranging between 200 and 500 ms (Table 5). Preoperatively, these arteries were visualized at 200–650 ms. In addition, most donor STA and recipient MCA cortical branches were observed at earlier phases after surgery. The donor STA branches were observed at 317 ± 33 ms and 217 ± 17 ms, before and after surgery, respectively. The recipient MCA branches were observed at 533 ± 33 ms and 367 ± 17 ms, before and after surgery, respectively. Significant differences were observed between them ($P = 0.02$, $P < 0.001$, respectively). Therefore, ASL-4D MRA demonstrated the postoperative changes in the dynamic flow patterns within the cerebral vasculatures after successful direct or combined revascularization surgery for MMD.

In the cases of postoperative hyperperfusion, including hyperperfusion syndrome after surgical revascularization for MMD [15], ASL-4D MRA demonstrated marked focal hyperintense signals in the bypassed arteries. In 7 of the 9 hemispheres with surgical revascularization and repeated ASL-4D MRA, postoperative focal hyperperfusion was observed by SPECT. Five of these 7 hemispheres exhibited marked focal hyperintense signals in the bypassed arteries by ASL-4D MRA. ASL-4D MRA did not demonstrate these findings in the other two hemispheres without postoperative focal hyperperfusion. In addition, non-time-resolved TOF MRA did not demonstrate these findings in any of the seven hemispheres. Representative ASL-4D MRA images were shown for a 12-year-old girl who developed a transient ischemic attack (right hemiparesis) and underwent left-sided combined direct and indirect surgical revascularization (Fig. 3). As shown in Fig. 3, repeated ASL-4D MRA demonstrated the findings of postoperative focal hyperperfusion revealed by the serial measurement of CBF by SPECT. She did not develop any clinical sign of hyperperfusion. Thus, she did not develop postoperative hyperperfusion syndrome.

4. Discussion

This study presents a novel application of noncontrast-enhanced time-resolved ASL-4D MRA for noninvasive staging of patients with MMD in comparison with DSA and TOF MRA. DSA was known as a current standard time-resolved angiography with relative invasiveness. Non-time-resolved TOF MRA was known as another current standard for the diagnosis of MMD. The first

Table 3
Summary of Suzuki's stage determination for MMD made by examining the DSA and ASL-4D MRA findings as well as estimated by TOF MRA stage.

Case		Suzuki's stage (six-grade system)				MRA stage (four-grade system)
		DSA	ASL-4D MRA			TOF MRA
			Observer A	Observer B	In agreement	
1	Right	V	V	V	V	4
	Left	VI	VI	VI	VI	4
2	Right	IV	V	IV	IV	2
	Left	V	V	V	V	3
3	Right	II	I	II	II	1
	Left	III	III	III	III	2
4	Right	II	III	III	III	2
	Left	III	IV	IV	IV	3
5	Right	V	V	IV	IV	3
	Left	III	III	III	III	3
6	Right	IV	IV	IV	IV	3
	Left	III	III	III	III	2
7	Right	III	IV	IV	IV	2
	Left	IV	IV	IV	IV	2
8	Right	IV	IV	IV	IV	3
	Left	III	IV	III	III	3
9	Right	III	III	III	III	3
	Left	V	V	V	V	4
10	Right	III	III	III	III	2
	Left	I	I	I	I	1
11	Right	III	IV	III	III	3
	Left	III	III	III	III	1

Table 4
Validity for the diagnosis of the Suzuki's stage by ASL-4D MRA.

Suzuki's stage	*N*	Sensitivity	Specificity	PPV	NPV	Accuracy
Stage I	1	1	1	1	1	1
Stage II	2	0.5	0	1	0.95	0.95
Stage III	10	0.8	0.92	0.89	0.85	0.86
Stage IV	4	1	0.83	0.57	1	0.86
Stage V	4	0.75	1	1	0.95	0.96
Stage VI	1	1	1	1	1	1

PPV, positive predictive value; NPV, negative predictive value.

attempt of ASL-4D MRA based on TFEPI readout with Look-Locker sampling after EPI-STAR labeling [2] clearly demonstrates the high accuracy of ASL-4D MRA for MMD staging.

The time-resolved imaging modalities studied in the present study (i.e., DSA and ASL-4D MRA) differ in terms of invasiveness, scanning time, and temporal/spatial resolutions. The DSA approach has the advantage of high temporal and spatial resolution and is now considered as a current standard for evaluating dynamic flow patterns within the cerebral vasculature. Nonetheless, DSA has been recommended for a definitive diagnosis of MMD, especially in candidates of surgical revascularization [12]. However, DSA is hard to be repeated frequently for monitoring disease evolution and treatment response, especially in pediatric patients or unstable patients exhibiting frequent transient neurological symptoms (intractable headaches or neurological deteriorations) or postoperative transient neurological deteriorations, because of the potential invasiveness. In contrast, ASL-4D MRA noninvasively captures dynamic cerebral blood flow patterns repeatedly, without any contrast agents, and within a clinically relevant scanning time. Although TOF MRA also provides high-resolution images of

Table 5
Characteristic findings observed on ASL-4D MRA after revascularization surgery in patients with Moyamoya disease. "Appearing phase" means the phase in which inflow of blood through the cerebral vessels was visualized first on ASL-4D MRA.

Case	Revascularization	Side	"Appearing phase" on ASL-4D MRA (ms)				Postoperative hyperperfusion on SPECT	Focal hyperintense signal on bypassed arteries
			Pre Op		Post Op (Day 0)			
			STA	MCA	STA	MCA		
1	Combined	Right	200	500	200	350	Yes	Yes
	Direct	Left	200	350	200	350	Yes	No
2	Combined	Right	350	650	200	350	Yes	Yes
3	Combined	Left	350	500	200	350	Yes	Yes
5	Combined	Right	350	500	200	350	Yes	Yes
6	Combined	Right	500	500	200	350	No	No
8	Combined	Right	200	650	200	350	Yes	Yes
9	Combined	Left	350	650	200	350	Yes	No
	Direct	Right	350	500	350	500	No	No

MCA, middle cerebral artery; STA, superficial temporal artery; Op, operation; SPECT, single-photon emission computed tomography.

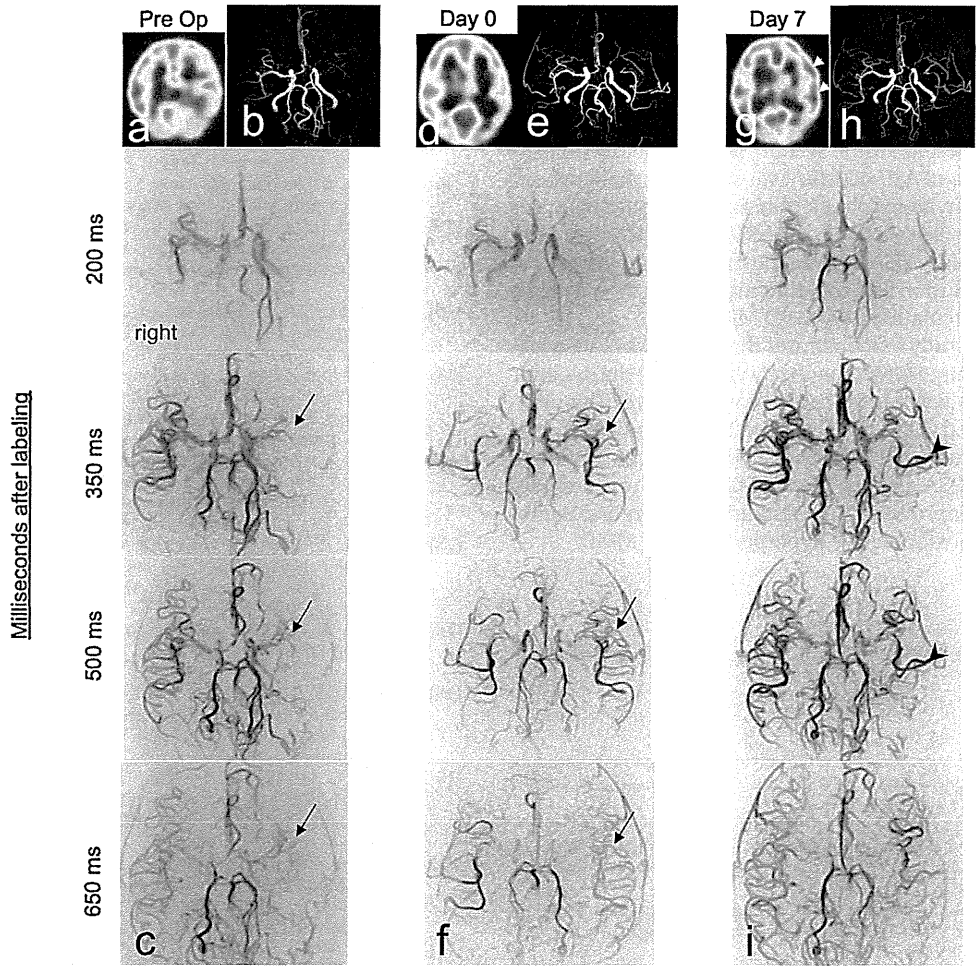

Fig. 3. Representative images of SPECT (a, d, g), conventional TOF MRA (b, e, h) and ASL-4D MRA (c, f, i) in a patient with focal hyperperfusion after combined revascularization for the left hemisphere (Case 3). Preoperative SPECT shows decreased CBF in the left hemisphere (a). Serial images of preoperative ASL-4D MRA (c) shows a relatively weak and delayed signal for the MCA in the left hemisphere (arrow; c) compared with the opposite hemisphere. SPECT, TOF MRA, and ASL-4D MRA immediately after surgery (Day 0; d–f) shows improvement in CBF and visualization of the left MCA branches (arrow; f). At postoperative Day 7, SPECT detects focal hyperperfusion in the left frontotemporal cortex around the bypassed site (white arrowheads; g). ASL-4D MRA shows focal hyperintense signals on the bypassed arteries (arrow heads; i) at phases of 350 and 500 ms. The detection site and days after surgery for these findings by ASL-4D MRA are consistent with those for focal hyperperfusion shown by SPECT. There is no change in TOF MRA between postoperative Day 0 (e) and Day 7 (h).

the cerebral vasculature non-invasively and repeatedly, it is basically different from above mentioned time-resolved modalities. Indeed, the correlation coefficient between ASL-4D MRA and DSA is different in comparison with that between ASL-4D MRA and TOF MRA (0.93 and 0.68, respectively). Based on these considerations, this study suggests not only that ASL-4D MRA has a potential to determine disease stage with proper accuracy but noninvasive, repeatable and time-resolved nature of ASL-4D MRA might make an new epoch as a next generation angiography.

This study also demonstrates that ASL-4D MRA can detect postoperative changes in dynamic flow patterns within the cerebral vasculature of patients with MMD after revascularization surgery. Thus, ASL-4D MRA detects a sign of hyperperfusion within the bypassed arteries in 5 of 7 cases (71%) in whom SPECT demonstrates postoperative focal hyperperfusion. As mentioned above, ASL-4D MRA has a high temporal resolution, comparable to that of DSA [17,18]. Accordingly, ASL-4D MRA could compare the phase of the first appearance of the arteries of interest, and arterial blood flow velocity before and after surgery. In addition, the bypassed arteries with postoperative focal hyperperfusion on SPECT had focal hyperintense signals by ASL-4D MRA. Although the precise mechanism remains unclear, this finding may suggest that the blood flow through these bypassed STAs was too high for the recipient MCA arterial bed. Therefore, ASL-4D MRA may be able to detect whether blood flow congestion develops around the site of bypass. Further accumulation of study population may give us some insights to elucidate the pathophysiology of postoperative focal hyperperfusion in MMD. Further quantitative studies, including signal-intensity and time course analysis should be conducted.

There are several limitations in the present study. First, there are some technical limitations in ASL-4D MRA. One of them is the relatively lower spatial resolution. The spatial resolution (1.2 mm × 1.2 mm × 0.7 mm in ASL-4D MRA) was 2.5- to 5.0-fold lower than that of DSA (0.25 mm × 0.25 mm) and TOF MRA (0.45 mm × 0.45 mm). A compromise must be made between the optimal scanning time and temporal and spatial resolution. Recently, rapid MRI using the compressed sensing technique was developed to enable scanning with a higher spatial resolution in a shorter scanning time [8]. Therefore, it may be possible to further improve the spatial and temporal resolution of ASL-4D MRA through technical innovation. Another technical limitation is that ASL-4D MRA could only demonstrate the arterial blood inflow because of the labeling attenuation effect of T1 relaxation. Because the T1 relaxation time of arterial blood with the 3.0-Tesla MR scanner is approximately 1.6 s, it is difficult to capture the labeled signal after that [10]. On an average, the normal cerebral circulation

time is 3.5 ± 0.5 s [3]. We expect it to be longer in patients with MMD having reduced cerebral perfusion pressure. Therefore, ASL-4D MRA cannot show delayed arterial blood flow and venous circulation. We estimate that this may be the reason why ASL-4D MRA overestimated the MMD stage compared to DSA in 3 of the 22 hemispheres in the present study. Second, the number of subjects selected for both DSA and ASL-4D MRA was small. Further analyses need to be conducted in larger population. Finally, while this study demonstrates the feasibility of ASL-4D MRA for MMD staging, it may be difficult to replace DSA as a diagnostic standard because of the lower spatial resolution and the difficulty to visualize delayed arterial and venous circulation in ASL-4D MRA.

In conclusion, this study demonstrates that ASL-4D MRA is a feasible method to determine MMD staging by visualizing the dynamic arterial flow patterns within the cerebral vasculature. This non-invasive, time-resolved imaging technique has some potential to monitor the clinical course of MMD, including surgical treatment responses instead of DSA. Finally, the feasibility and repeatability of ASL-4D MRA may improve our understanding of cerebral hemodynamics, including postoperative hyperperfusion in MMD.

Conflict of interest

The authors declare that they have no conflict of interest.

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もやもや病の遺伝研究最前線

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もやもや病は，内頚動脈終末部の進行性狭窄と脳底部を中心とする異常血管網の発達を特徴とする原因不明の希少疾患である．2011年にわが国で感受性遺伝子*RNF213*が同定され，その臨床的意義や生理的機能の解明に向けた研究が続けられている．ただし，*RNF213*変異単独では，本疾患の発症に関してすべてを説明することはできていない．そのため，その他の因子の関与が強く想定され，エピジェネティクスの観点からも病態研究が始められつつある．本稿では，もやもや病の疫学的，病理学的背景のほか，もやもや病遺伝研究の歴史的変遷と最新の知見について述べる．

KEY WORDS：もやもや病，遺伝子，*RNF213*，エピジェネティクス，miRNA

Ⅰ．はじめに

もやもや病は，内頚動脈終末部および近傍主幹動脈の進行性狭窄と，脳底部の異常な微細血管網の発達を特徴とする原因不明の脳血管疾患である．本疾患は1957年にTakeuchiらにより，両側内頚動脈の閉塞をきたす疾患として初めて報告された[55)]．1969年には，発達した異常血管網が“タバコの煙のたちこめる様子”に似ていることから，Suzukiらにより「もやもや病」と国際的に発表され，疾患概念として確立した[53)]．以降，約50年にわたり，外科的血行再建術による治療法確立のほか，疫学的研究，診断法研究など包括的な疾患研究が，わが国を含め世界的に精力的に行われてきた[36)]．病気の原因を探る病態研究に関しては1980年代から，家系調査と連鎖解析，相関解析といった手法により，遺伝性素因の探索が行われてきた．分子遺伝学的解析手法の技術革新により，genome-wide association study（GWAS）のような全ゲノム網羅的解析が可能となった．すなわち多数サンプルのゲノム情報を，短時間で，効率的かつ網羅的に解析可能となったことも相まって，2011年に，もやもや病感受性遺伝子*RNF213*の発見に至った[28, 37)]．この発見は，もやもや病の疫学的・病態学的研究におけるブレークスルーであり，もやもや病の病態解明の歴史におけるマイルストーンとなったことは間違いない．ただし，*RNF213*同定後の現在でも，もやもや病の発症機序は完全には解明されたとは言えない．

本稿ではもやもや病の疫学的，病理学的背景を振り返り，これまで行われてきたもやもや病の遺伝研究を，最新の知見を踏まえ，述べていきたい．

Ⅱ．疫学的背景

近年では世界各国から本疾患に関する報告が相次いでいるが，日本，韓国といった東アジアにおける有病率・罹患率が圧倒的に高い[9, 56, 59)]．わが国では，全国規模の疫学調査が複数回行われて

きた．その結果，日本における10万人あたりの有病率および罹患率は，それぞれ3.16～10.5，0.35～1.13人であった[4, 17, 35, 57]（図1）．MRIの技術向上および普及により，無症候性もやもや病を含めた診断率が向上し，実際はさらに有病率や罹患率が高い可能性もある[4, 19, 22]．男女比はどの調査でもおおむね一致した結果であり，女性が男性の約2倍である．発症年齢は2峰性の分布をとり，第一のピークは5～10歳，第二のピークは40歳前後に認める．家系内発症の割合は10～15％で[4, 35, 57]，家族例では男女比が1：5.0と孤発例より女性の割合が高く，発症年齢も孤発例より低い傾向にある（11.8歳 vs 30.0歳）[36, 47]．

韓国や中国での罹患率は日本と同程度と考えられる[2, 23, 31]．アメリカ合衆国（カリフォルニア州とワシントン州）の疫学調査によると，罹患率は全体で10万人あたり0.086人と日本に比べ低い数字となっているが，人種ごとに見るとアジア系アメリカ人での罹患率は日本と同程度であった[56]．アジア系人種の多いハワイの疫学調査では，有病率および罹患率はアメリカ本土より高い数値であった[10]．また，ヨーロッパにおける罹患率は日本の約1/10と報告されている[59]．東アジア以外のアジア地域，南アメリカ，アフリカやオセアニア地域などに関しては，まとまった疫学的データはない．

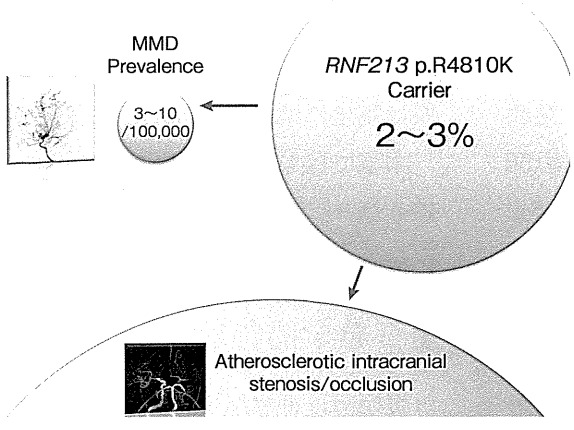

図1 *RNF213* p.R4810K保因率ともやもや病有病率
日本人において*RNF213* p.R4810K保因率は2～3％であるのに対し，もやもや病の有病率は10万人あたり3～10人で，約1/1,000ほど低い値である．また，動脈硬化性脳動脈狭窄症の一部にもp.R4810Kを認める．

Ⅲ．病理学的背景

もやもや病における内頚動脈終末部の典型的な病理像は，内膜の線維細胞性肥厚，内弾性板の異常（wavingなど），中膜の菲薄化である[8]．これらの病理学的変化は，脳表末梢動脈壁にも認められる[54]．また，内膜肥厚には過剰な平滑筋様細胞の集簇が関与していることが知られており[39]，炎症性変化や動脈硬化性変化を伴わないのも特徴である．さらに動脈硬化性疾患と異なり，狭窄部の血管外径そのものが縮小している点も注目されている[27, 36]．過去には中膜の平滑筋細胞が内膜側へ遊走し，内膜の線維細胞性肥厚と中膜の菲薄化をきたすという説が一般的であったが，現在は内膜肥厚をもたらす細胞成分の起源が必ずしも平滑筋由来とは言えないと考えられている．すなわち，endothelial progenitor cell（EPC）やsmooth muscle progenitor cell（SMPC）といった血液中を循環するvascular progenitor cell（VPC）の関与も指摘されている[1, 11, 18]．

これらの病理組織学的検討では，多くの手がかりが得られているものの，疾患の本態が，どの細胞や血管組織にあるのかいまだ不明である．また複数の研究により髄液中におけるb-FGF，HGFの上昇や，血中TGF-βの上昇が確認されているが[15, 20, 46]，これらの結果が原因なのか，結果（虚血に対する応答）なのかは結論が得られていない．