研究計画書

未破裂脳動脈瘤患者の意思決定支援に関する研究

作成日： 2004 年 12 月 2 日　Version 0.9
修正日： 2004 年 12 月 20 日　Version 1.0

目次

1. 研究の概要

[目的]

1) 未破裂脳動脈瘤をもつ患者とその家族が、治療方針を決定する際にどのような情報を求めているかを明らかにする。(以下ニーズアセスメントと記す)
2) 未破裂脳動脈瘤をもつ患者の選好(utility)を評価する。(以下utility測定と記す)

[実施内容]

研究計画書作成、インタビューガイドの作成、インタビュー調査、インタビュー逐語録の作成、データ分析、結果の解釈、報告書および論文の作成

[調査対象]

ニーズアセスメント、Utility測定、それぞれで経過観察中の患者10例、クリッピング術を行った患者10例、血管内治療を行った患者10例、計30例ずつ。ニーズアセスメントは患者の家族(特に配偶者、他の親族でも可)も対象とする。

[調査実施施設]

京都大学付属病院脳神経外科

[調査内容]

1) 患者背景(性別、年齢、家族構成、職業の有無など)
2) 治療方針を決定した際に得た情報
3) 治療方針を決定する際に求める情報
4) 治療方針を決定する要因
5) Utility (時間得失法 Time Trade Off)

[調査実施方法]

1) 半構造化面接法(調査内容2)～4))
2) 時間得失法インタビュー(調査内容5))

[調査実施期間]

2004年12月~2005年2月

準備期間: 2004年12月

倫理委員会審査および協力施設との連絡調整: 2004年12月~2005年1月

調査の実施: 2005年1月~2月

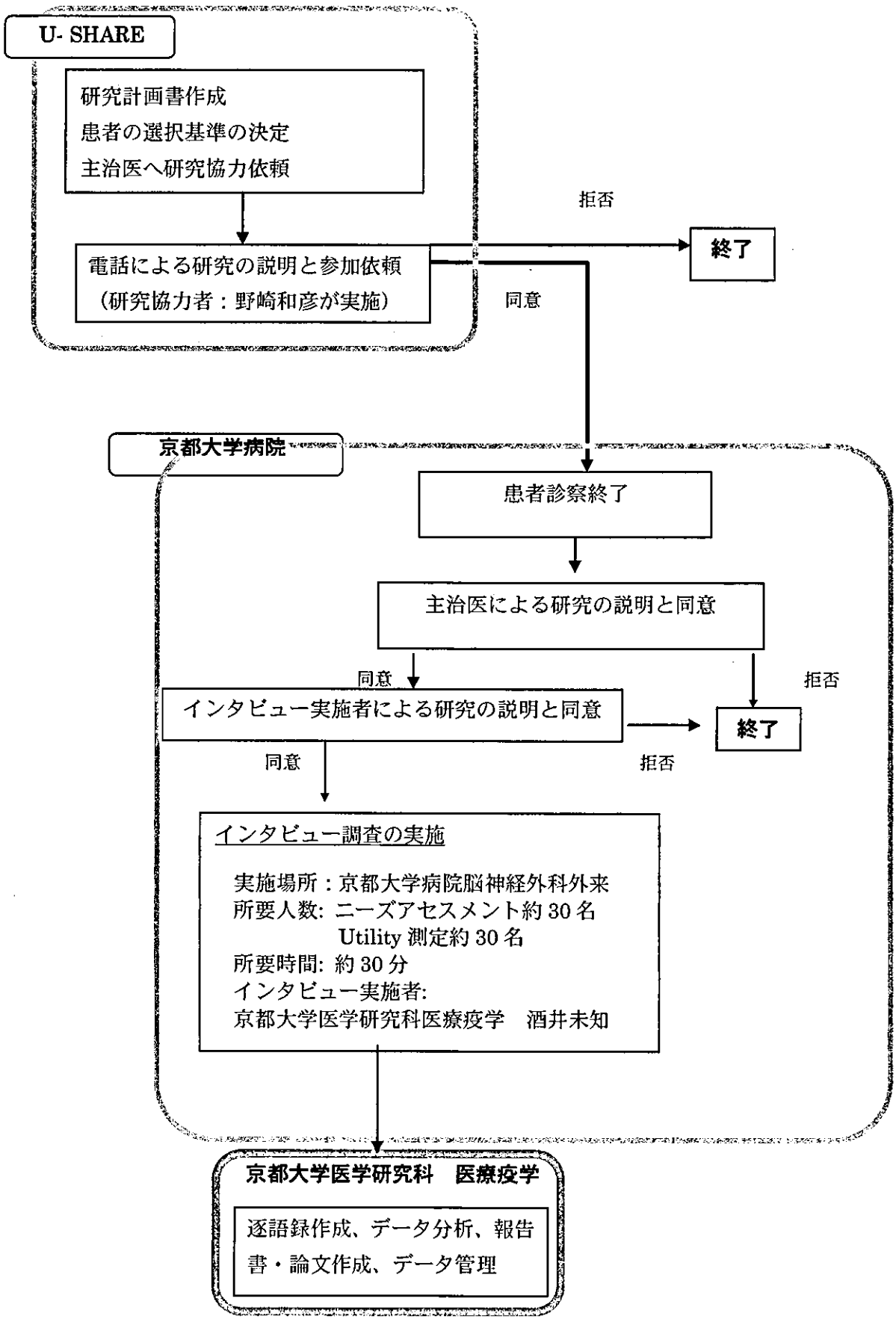
データ分析: 2005年1月~3月

報告書および論文作成: 2005年3月

[調査の主体] 循環器疾患等総合研究事業「未破裂脳動脈瘤の要因、治療法選択における リスク・コミュニケーションに関する研究」班Ubiquitously - Support and Heal Aneurysmal patients with Risk Communication and Empowerment: 以下U-SHAREと記す

[調査の実施] 京都大学大学院医学研究科　医療疫学分野

2. 研究全体の流れ


U- SHARE
研究計画書作成
患者の選択基準の決定
主治医へ研究協力依頼
電話による研究の説明と参加依頼
（研究協力者：野崎和彦が実施）
拒否
終了
同意
京都大学病院
患者診察終了
主治医による研究の説明と同意
同意
拒否
インタビュー実施者による研究の説明と同意
終了
同意
拒否
インタビュー調査の実施
実施場所：京都大学病院脳神経外科外来
所要人数: ニーズアセスメント約 30 名
Utility 測定約 30 名
所要時間: 約 30 分
インタビュー実施者:
京都大学医学研究科医療疫学　酒井未知
京都大学医学研究科　医療疫学
逐語録作成、データ分析、報告書・論文作成、データ管理

3. 背景

未破裂脳動脈瘤は成人の約4～6％位がこれを有すると言われており、比較的頻度が高い疾患である。外来受診や積極的な健診(脳ドック)によって発見されるものは年間約5000例に上り、近年は脳ドックの普及によってその数が増加している。未破裂脳動脈瘤の破裂により、クモ膜下出血を来しうる。一般にクモ膜下出血の予後は悪く、40%は初回出血で即死または瀕死の状態に陥り、20～30％は社会復帰を見込めないほど重症になるとされている。

未破裂脳動脈瘤の治療法は、予防的治療または経過観察である。予防的治療にはクリッピング術と血管内治療がある。クリッピング術は、開頭により脳動脈瘤の頸部をクリップで挟むもので、血管内治療は、脳動脈瘤の中にコイルを血管内から詰めて血栓化させる方法である。

国内では予防的治療が推奨されてきたが、国際共同研究 ISUIA の報告 (1998 年)[1] から、1cm 未満の未破裂脳動脈瘤の破裂率は極めて低く、予防的治療の妥当性を再検討する必要があることが示唆された。また、予防的治療による障害や死亡も報告されている。クリッピング術は侵襲が高く、手術による全身状態への影響が大きい。血管内治療は、血管内にカテーテルを長時間留置するため、術中および術直後の血栓、塞栓による虚血性合併症が問題になっている。[2][3][4] 国内でも、日本未破裂脳動脈瘤悉皆調査UCAS Japan (2001年～2004年)[5] など、未破裂脳動脈瘤の自然歴、予防的治療のリスクを明らかにする研究が行われている。しかし未だ確たるエビデンスが不足しているため、患者の価値観や心理社会的状態についても十分に考慮し、治療方針を決定しているのが現状である。

従って、医療者と患者が情報を共有した上で意思決定を行うこと(Shared decision-making)は極めて重要である。しかしながら、未破裂脳動脈瘤をもつ患者が治療方針を決める際にどのような情報を必要としているかを明らかにした研究は行われていない。また青木らの研究(1998 年、2001 年)[6][7] から、患者の意思決定においては、患者個人の選好(utility)が重要な要素であることが示唆されている。utility はその定義から、患者個人を対象に調査すべきである。しかしながら先行研究では、医療専門家の意見に基づいて未破裂脳動脈瘤をもつ患者の utility を決定しており、患者を対象にした研究は我々の知る限り存在しない。

4. 目的

1) 未破裂脳動脈瘤をもつ患者とその家族が、治療方針を決定する際にどのような情報を求めているかを明らかにする。(以下ニーズアセスメントと記す)
2) 未破裂脳動脈瘤をもつ患者の選好(utility)を評価する。(以下utility測定と記す)

5. 対象と方法

5.1 ニーズアセスメント

5.1.1 対象

未破裂脳動脈瘤をもち、京都大学附属病院脳神経外科で経過観察中の患者、クリッピング術を行った患者、血管内治療を行った患者のうち、下記の選択基準を満たす者を対象とする。理論的飽和をもって終了とするため、対象者数を現時点で厳密に決定することはできないが、各治療法につき各10名、合計30名程度を目安として実施する。さらに、研究参加に同意が得られた場合、各患者の家族(配偶者、いない場合は治療方針の決定に関わった親族)も対象とする。

選択基準および除外基準

下記の条件を満たす患者を対象とする。

5.1.1.1 選択条件

1) 年齢20歳以上70歳以下の患者
2) 外来患者
3) 調査に参加する意思、判断能力を有する患者
4) 研究参加に関して文書で同意が得られた患者

5.1.1.2 除外条件

主治医が以下の条件に該当すると判断した患者は除外する。

1) インタビューを実施できない程度に言語に関する障害がある
2) 同意文書を判読できない程度に視覚に関する障害がある
3) 同意の意思、判断能力がない
4) その他インタビューを30分間継続し難いと考えられる患者(うつ病の症状がある患者等)

5.1.2 方法

半構造化面接(予め作成された質問リストに基づいて、自由に回答してもらう面接方法)による個人インタビューを行う。インタビューは、事前にトレーニングを行った研究者(京都大学医学研究科医療疫学分野の酒井未知)が行う。実施場所は京都大学病院脳神経外科の外来診察室の隣室、所要時間は1件につき約30分である。

インタビューは参加者の了承を得た上で録音し、逐語録を作成する。逐語録は内容分析(コード化とカテゴリー化)、テキストマイニングの手法で分析する。テキストマイニングとは、テキストデータを単語レベルまで分割し、単語の出現頻度や相関関係などを分析する手法である。
本研究では、インタビュー参加者が多く使った単語やそれらの関連性を分析することで、参加者が表現した言葉の根底にある考えを抽出する目的で、テキストマイニングを行う。

5.1.3 調査項目

＜治療方針を決定した際に得た情報＞

1) 治療方針を決定する際、どのような情報を得て、何を参考にしたか
2) 家族や友人の意見

＜治療方針を決定する要因＞

1) 治療方針を決定する決め手となった要因は何か
2) 意思決定を困難にした要因は何か

＜治療方針を決定する際に求める情報＞

1) 治療方針を決定する前に、特に何を知りたかったか
2) 今、治療方針を決める前に、何を知っておけばよかったと感じているか
3) それを知っていたら意思決定の結果は変わったか
4) どのような情報があれば意思決定しやすくなると考えているか

＜研究参加者の背景情報＞

1) 年齢
2) 性別
3) 家族構成
4) 職業
5) 未破裂脳動脈瘤が見つかった理由
6) 重症度(modified Rankin Scale)
7) 脳動脈瘤の家族歴
8) 脳神経外科への通院歴
9) 既往症
10) 主治医が推奨する治療方針

5.2 Utility測定

5.2.1 対象

未破裂脳動脈瘤をもち、京都大学附属病院脳神経外科で経過観察中の患者、クリッピング術を行った患者、血管内治療を行った患者を対象とする。各治療法につき各10名、合計30名を選択する。選択基準および除外基準はニーズアセスメントと同じである。

5.2.2 方法

時間得失法（Time Trade-Off: TTO）（健康状態への選好を時間に換算して評価する方法）[8]のインタビューを行う。インタビューは、事前にトレーニングを行った研究者(京都大学医学研究科医療疫学分野の酒井未知)が行う。実施場所は京都大学附属病院脳神経外科の外来診察室の隣室、所要時間は1名につき約30分である。

5.2.3 調査項目

1) modified Rankin Scale(脳卒中の重症度を評価する尺度)[9]で評価される各状態への選好

スコア	状態
0	全く症状なし
1	何らかの症状はあるが障害はない；通常の仕事や活動は全て行える
2	軽微な障害；これまでの活動の全てはできないが身のまわりのことは援助なしでできる
3	中等度の障害；何らかの援助を要するが援助なしで歩行できる
4	中等度から重度の障害；援助なしでは歩行できず，身のまわりのこともできない

5	重度の障害; ねたきり, 失禁, 全面的な介護
6	死亡

2) Mini Mental State Examination (認知機能を評価する方法)[10] スコアで評価される各状態への選好

スコア	状態
25 以上	正常な認知機能
20-24	軽度の認知機能障害
15-19	軽度～中程度の認知機能障害
10-14	中程度の認知機能障害
9 以下	重篤な認知機能障害

3) 患者背景情報

ニーズアセスメントと同じ

6. 同意の取得

u-SHARE 研究協力者：野崎和彦が、患者本人 (ニーズアセスメントはその家族も) に対し、来院予定日前に電話で参加を依頼する。患者来院時、主治医が患者本人に対し、再度インタビュー調査の説明、参加依頼を行う。同意が得られた場合、インタビュアーが文書で研究参加の同意を得る。説明文書および同意書 (ニーズアセスメント:別紙①、Utility 測定:別紙②) の記述は以下の通りである。

1) この研究は、未破裂脳動脈瘤をもつ患者さまが治療方針を決める際にどのような情報を必要としているか (Utility 測定では「健康状態をどのように価値づけているか」) を明らかにするために行われます。
2) インタビューの所要時間は約 30 分です。
3) インタビューの内容は録音され、その後テープおこしをして文書化されます。もし回答してもよいが、録音はしてほしくないと思われるようでしたら録音は致しません。(ニーズアセスメントのみ)
4) 研究へのご参加は任意であり、ご参加を頂けない場合でも治療への影響は一切ありません。
5) 研究へのご参加を撤回することはいつでも可能です。
6) プライバシーは厳重に保護され、個人のお名前が公になることはありません。
7) 研究で得られた内容は、研究目的以外に使用することはありません。
8) この研究は国の研究費で行われ、京都大学で倫理審査を受けたものです。
9) ご参加頂いた場合、謝礼 (図書カード 1000 円) を差し上げることになっております。
10) 研究について不明な点はいつでも質問して下さい。

了解が得られた場合には、研究参加了解への覚書への署名を頂く。署名した覚書は複写し、研究参加者本人、京都大学医学研究科医療疫学分野が保管する。

7. 倫理的配慮

7.1 研究による健康被害の可能性と対策

本研究では、研究参加者に対してインタビューを行うのみであり、研究に参加する個人への身体的害はないと考えられる。ただし、インタビューによって患者の不安感が増大することも考えられる。その場合主治医がコンサルテーションを行うこととする。また、インタビュー実施中に

患者の体調が悪くなった場合は調査を中止する。

7.2 研究対象者の選択

本研究は未破裂脳動脈瘤をもつ患者を研究対象としている。しかし特に手術直前の患者など、状態の不安定な患者に対してインタビューを行うべきではない。そのため主治医の協力のもと、状態の安定している患者のみを対象に行うこととし、その選択に慎重を期す。

7.3 インタビュアーのトレーニング

インタビューはインタビューガイド(別紙③)に従って行われる。インタビューは、半構造化面接のインタビュアーの経験を有する者が行う。またインタビュー実施前には3名を対象にパイロットテストを行い、参考文献[11][12]に基づくインタビュアートレーニング(別紙④)をした上で、インタビューを実施する。

7.4 インタビュー実施環境

インタビューは、京都大学病院脳神経外科の外来診察室の隣の部屋で行う。インタビュー実施中に室内の声が外部に届くことはないため、参加者が話しやすい環境である。

7.5 インフォームド・コンセントと拒否する権利

本研究は、研究参加者本人から個別に、文書でインフォームド・コンセントを得る。患者来院時には、主治医が参加依頼を行うが、参加者をインタビュアーに紹介した後退室し、インフォームド・コンセントおよびインタビューを行う場には同席しない。従って研究参加への強制はないと考えられる。またインタビューの途中中断も保証しているため、研究対象者の自発的意思は尊重される。

7.6 個人情報保護

インタビューに参加した個人の発言内容は、研究者のみが閲覧し、厳重に保管される(責任者:京都大学大学院医学研究科医療疫学分野:福原俊一)。研究者は調査後、研究成果をまとめて学術雑誌に報告するが、個人名、医療機関名が特定できる情報は公表しない。研究終了後、録音記録は破棄されるため、研究の対象となる個人のプライバシーは保護される。

8. 研究スケジュール

準備期間: 2004年12月

倫理委員会審査および協力施設との連絡調整: 2004年12月~2005年1月

調査の実施: 2005年1月~2月

データ分析: 2005年1月~3月

報告書および論文作成: 2005年3月

9. 研究実施体制

[研究代表者]

福原俊一　京都大学大学院医学研究科医療疫学分野　教授

[協力研究者]

野崎和彦　京都大学医学部脳神経外科　助教授

青木則明　The University of Texas Health Science Center At Huston School of Health Information Sciences Assistant professor

中山健夫　京都大学大学院医学研究科医療情報学　助教授
森田智視　京都大学大学院医学研究科医療疫学分野　講師
酒井未知　京都大学大学院医学研究科医療疫学分野　大学院博士課程

連絡先　〒606 - 8501　京都市左京区吉田近衛町
京都大学大学院医学研究科　社会健康医学科社会健康医学系　医療疫学分野内
Tel : 075 - 753 - 4645
Fax: 075 - 753 - 4644

10. 論文化に際しての著者資格

本研究の成果を論文化する際の著者資格は、研究代表者と協力研究者の合議によって決定する。9. 研究実施体制で挙げた研究者以外の著者資格についても状況に応じて適宜考慮する。

11. 研究資金

厚生労働科学研究　循環器疾患等総合研究事業
(未破裂脳動脈瘤の要因、治療法選択におけるリスク・コミュニケーションに関する研究
主任研究者: 橋本信夫)

12. 文献

1) International Study of Unruptured Intracranial Aneurysms Investigators: Unruptured Intracranial Aneurysms-risk of rupture and risks of surgical intervention. N Engl J Med 340: 1439-1442, 1998

2) Pelz DM, Lownie SP, Fox AJ. Thromboembolic events associated with the treatment of cerebral aneurysms with Guglielmi detachable coils. AJNR. 1998 Sep;19(8):1541-7.

3) Rordorf G, Bellon RJ et al., Silent thromboembolic events associated with the treatment of unruptured cerebral aneurysms by use of Guglielmi detachable coils: prospective study applying diffusion-weighted imaging. AJNR. 2001 Jan;22(1):5-10.

4) Roy D, Milot G, Raymond J. Endovascular treatment of unruptured aneurysms. Stroke. 2001 Sep;32(9):1998-2004.

5) UCAS Japan 事務局. 日本未破裂脳動脈瘤悉皆調査(UCAS Japan)中間報告(III). 脳神経外科ジャーナル 13(3):163-169

6) Aoki N, et al. Management of unruptured intracranial aneurysm in Japan: a Markovian decision analysis with utility measurements based on the Glasgow Outcome Scale. Med Decis Making. 1998 Oct-Dec;18(4):357-64.

7) Aoki N, et al. Reanalysis of unruptured intracranial aneurysm management: effect of a new international study on the threshold probabilities. Med Decis Making. 2001 Mar-Apr;21(2):87-96.

8) 坂巻弘之. やさしく学ぶ薬剤経済学. じほう. 2003

9) Bonita R, Beaglehole R. Modification of Rankin Scale: Recovery of motor function after stroke. Stroke 1988 Dec;19(12):1497-1500.

10) Cockrell JR and Folstein MF (1988) Mini Mental State Examination (MMSE), Psychopharmacology bulletin, 24: 689-692.

11) Bowling A. Research methods in health: Investigating health and health services. Philadelphia: Open University Press, 1999.

12) キャサリン・ホープ. 質的研究実践ガイドー保健・医療サービス向上のために. 医学書院. 2001

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamada S et al	Genome-wide scan for japanese familial intracranial aneurysms: Linkage to several chromosomal regions.	Circulation	110	3727-3733	2004
Shojima M et al	Magnitude and Role of Wall Shear Stress on Cerebral Aneurysm. Computational Fluid Dynamic Study of 20 Middle Cerebral Artery Aneurysms	Stroke	35	2500-2505	2004
Houkin et al	Inadequate websites disclosure of the surgical outcome of intracranial aneurysms---Survery of 1225 sites in Japan	Neurologia Medico-chirurugica	in press		2005
Morita A et al	The risk of rupture of unruptured cerebral aneurysms in the Japanese population: A systematic review of the literature from Japan.	J Neurosurg	in press		2005
宝金清博　他	脳神経外科におけるリスクマネジメント－脳神経外科は安全か？－	脳神経外科	32	111-119	2004
UCAS Japan 事務局	日本未破裂脳動脈瘤悉皆調査(UCAS Japan)：中間報告III	脳外誌	13	163-169	2004
UCAS Japan事務局	日本未破裂脳動脈瘤悉皆調査(UCAS Japan)：中間報告IV	Mt. Fuji Workshop Proceeding			2005

Genome-Wide Scan for Japanese Familial Intracranial Aneurysms

Linkage to Several Chromosomal Regions

Shigeki Yamada, MD*; Maki Utsunomiya, MPH*; Kayoko Inoue, MD, MPH; Kazuhiko Nozaki, MD, PhD; Sumiko Inoue, PhD; Katsunobu Takenaka, MD, PhD; Nobuo Hashimoto, MD, PhD; Akio Koizumi, MD, PhD

Background—Genetic factors have an important role in the pathogenesis of intracranial aneurysm (IA). The results of previous studies have suggested several loci.

Methods and Results—From 29 IA families with ≥3 individuals affected by IA, we used nonparametric (model-free) methods for linkage analyses, using GENEHUNTER and Merlin software. Genome-wide linkage analyses revealed 3 regions on chromosomes 17cen (maximum nonparametric logarithm of the odds score [MNS] = 3.00, nominal P=0.001), 19q13 (MNS=2.15, nominal P=0.020), and Xp22 (MNS=2.16, nominal P=0.019). We tested 4 candidate genes in these regions: the microfibril-associated protein 4 gene (*MFAP4*) and the promoter polymorphism of the inducible nitric oxide synthase gene (*NOS2A*) on chromosome 17cen, the epsilon genotypes of the apolipoprotein E gene (*APOE*) on chromosome 19q13, and the angiotensin I converting enzyme 2 gene (*ACE2*) on chromosome Xp22. Associations of their polymorphisms with IA were evaluated by a case-control study (100 cases: 29 probands from IA families and 71 unrelated subjects with IAs, 100 unrelated control subjects [unaffected members with IAs and absence of family history of IAs]). However, the case-control study showed that none of the polymorphisms of the examined genes had associations with IA.

Conclusions—A genome-wide scan in 29 Japanese families with a high degree of familial clustering revealed 1 suggestive linkage region on chromosome 17cen and 2 potentially interesting regions on chromosomes 19q13 and Xp22. These regions were consistent with previous findings in various populations. (***Circulation.* 2004;110:3727-3733.)**

Key Words: aneurysm ■ cerebrovascular disorders ■ genes ■ stroke

Family members of patients with subarachnoid hemorrhage (SAH) have been documented to have a high risk of SAH and a high prevalence of unruptured intracranial aneurysms (IAs).[1,2] The risk of ruptured IAs in first-degree relatives of patients with aneurysmal SAH is ≈4 times higher than that in the general population.[2] Genetic and environmental factors play important roles in the pathogenesis of IA, and recent progress in molecular genetics enables the genetic determinants to be approached directly.

Genome-wide linkage analyses for familial IA have been reported by 2 groups.[3,4] Onda et al[3] suggested linkage to regions on chromosomes 5q22–31 (maximum LOD score [MLS] 2.24), 7q11 (MLS 3.22), and 14q22 (MLS 2.31) in 104 Japanese affected sibling pairs. The linkage to 7q11 was replicated by another study in a white population,[5] although we could not confirm the linkage to this locus in Japanese.[6] Olson et al[4] found suggestive linkages on chromosomes 19q12–13 (MLS 2.58) and Xp22 (MLS 2.08) in 48 Finnish affected sibling pairs, a linkage confirmed by another study.[7] These results suggest that multiple genes determine susceptibility to IA.

In the present study, we conducted a family-based approach because high degrees of familial clustering of IAs can raise relative risks and thereby provide us a better chance to isolate the major locus.[8] We recruited 29 families with ≥3 affected members and report here the results of genome-wide linkage analysis.

Methods

Families

From collaborating hospitals in the western part of Japan, we recruited patients with IA who had a family history of IAs or SAH.

Received May 20, 2004; revision received July 15, 2004; accepted August 2, 2004.

From the Department of Health and Environmental Sciences (S.Y., M.U., K.I., S.I., A.K.) and the Department of Neurosurgery (S.Y., K.N., N.H.), Kyoto University Graduate School of Medicine, Kyoto, Japan; and the Department of Neurosurgery, Takayama Red Cross Hospital (K.T.), Gifu, Japan.
*Drs Yamada and Utsunomiya contributed equally to this work.

The online-only Data Supplement, which contains Tables I through III, is available with this article at http://www.circulationaha.org.

Correspondence to Dr Akio Koizumi, Department of Health and Environmental Sciences, Graduate School of Medicine Kyoto University, Konoe-cho, Yoshida, Sakyo-ku, Kyoto, 606-8501, Japan. E-mail koizumi@pbh.med.kyoto-u.ac.jp

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000143077.23367.18

If they had ≥3 family members with IAs or SAH and ≥2 including the proband were alive, their families were regarded as suitable subjects for the present study. "Affected" status of participants was determined in 2 ways. First, if participants had been diagnosed with IAs or SAH, they were confirmed as having saccular IAs from their medical records. Second, if participants aged ≥30 years did not know whether they had had IAs, they underwent magnetic resonance angiography (MRA) for screening of IAs. All MRA images were examined by ≥3 neurosurgeons or neuroradiologists. If IAs were suspected by MRA, additional examinations, such as digital subtraction angiography and 3-dimensional computed tomography, were conducted. Families with known heritable diseases associated with IAs, such as Ehlers-Danlos syndrome type IV, Marfan syndrome, neurofibromatosis type 1, or autosomal dominant polycystic kidney disease, were excluded from this study.[9,10] Details of the methods of participation and data collection have been reported previously.[6] The methods used in this study conformed to the tenets of the Declaration of Helsinki and received approval from the Ethics Committee of Kyoto University.

Genotyping

Genomic DNA was extracted from blood samples (in 2 cases, from a preserved umbilical cord) with a QIAamp DNA Blood Mini Kit (Qiagen Inc). Polymerase chain reaction (PCR) amplification from genomic DNA was performed with fluorescence-labeled (6-FAM, HEX, NED) and tailed primers. PCR primers to analyze microsatellite markers comprised an ≈10-cM human index map (ABI Prism Linkage Mapping Set Version 2: 382 markers for 22 autosomes and 18 markers for the X chromosome), and other microsatellite fine markers were designed according to information from the UniSTS map.[11] PCR reactions were carried out in 7.5 μL with 50 ng genomic DNA, using AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a 2-step amplification program. DNA fragments were analyzed on an ABI Prism 3100 Avant Genetic Analyzer. Genotyping errors and inconsistent relationships were checked with the use of SimWalk2 and Merlin software.[12,13] If the results of genotyping were missed or ambiguous, we treated them as an unknown genotype for the linkage analysis.

Linkage Analysis

Unaffected members who were ≥60 years of age and underwent MRA screening and affected members were included for the linkage analysis. The inheritance patterns of familial IA have not been determined, though autosomal dominant, recessive, and undetermined modes have been reported in familial IA[9,14]; we thus used only a nonparametric method. In addition, the phenotype of unaffected members was assigned as "unknown" in this study. The purpose of including unaffected members was to increase the accuracy of haplotype estimation in affected members, although inclusion did not increase the statistical power. Multipoint nonparametric analyses for autosomes and X chromosome were run with 1-tailed probability values (P), using GENEHUNTER (Version 2.0 and 1.3) and Merlin software.[13,15] Population allele frequencies for each microsatellite marker were estimated from the founders of IA families. We used a 2-stage design: First, all chromosomal regions were screened by genotyping at an ≈10-cM density (screening), and the region, of which nominal $P<0.05$ of the nonparametric logarithm of the odds (NPL) score, was considered as a potentially interesting region. Second, these regions were further finely mapped at ≈1- to 2-cM densities (fine mapping). Nominal $P<0.05$ regions were again considered as potentially interesting regions or nominal $P<0.001$ regions were considered as suggestive linkage regions.[15]

We evaluated statistical power through the use of simulations, as previously reported.[6] Simulations were run 1000 times by GENEHUNTER to obtain a false-negative rate (ie, sensitivity, percentage) when the threshold of nominal P was equal to 0.05 under conditions of 75%, 50%, and 25% of locus heterogeneities among families for the linkage analysis.

Case-Control Study

To test associations of polymorphisms of candidate genes (described below) in suggestive linkage or potentially interesting regions with IA, we conducted a case-control study. In the case-control study, 100 cases and 100 control subjects were enrolled from collaborating hospitals in western Japan. Control subjects had the following characteristics: (1) confirmation of not harboring IA by digital subtraction angiography, 3-dimensional computed tomography, or MRA, (2) age at the time of diagnosis ≥40 years, (3) no medical history of any stroke including IAs or SAH, and (4) no family history of IAs or SAH in first-degree relatives. Cases were composed of unrelated subjects whose presence of IA had been confirmed by angiography or operation and family probands.

The allele frequencies of candidate genes in cases and control subjects were compared by the contingency table of $\chi 2$ test statistics, with the use of SAS software (Version 8.2, SAS Institute Inc).

Candidate Genes

To search for mutations or polymorphisms of the microfibril-associated protein 4 gene (*MFAP4*, GenBank accession number = NT_030843) and the angiotensin I converting enzyme (*ACE*) 2 gene (ACE2, NT_011757), genomic DNA from the probands was used as a template to generate PCR products, which then were sequenced directly. Forward and reverse PCR primers for each coding exon were selected in an intronic sequence ≥50 bp away from the intron/exon boundaries (Data Supplement Table I). PCR products were run on 2% agarose gel, and the appropriate bands were excised and then purified with the use of the QIAquick Gel Extraction Kit (Qiagen). PCR and sequencing primers are shown in Data Supplement Table I. Sequencing results were analyzed on an ABI Prism 3100 Avant DNA sequencer (Applied Biosystems). Any polymorphic sites identified through our sequencing were searched for registered single nucleotide polymorphisms (SNPs) at the web site of the National Center for Biotechnology Information database SNP (dbSNP).[16]

Restriction enzymes to determinate the genotypes of found polymorphisms were *BbvCI* for the *MFAP4*, *AluI* for the *ACE2*, and *HhaI* for the apolipoprotein E (*APOE*, *NT*_011109),[17] respectively. The bi-allelic AAAT-repeat located 2.45 kbp upstream from the start codon of the inducible nitric oxide synthase (iNOS) gene (*NOS2A*, NT_010799) was determined by the length of the PCR products amplified by using primers designed according to a DNA sequence found from GenBank (D29675).[18]

Results

Characterization of Families

Twenty-nine families met our criteria (≥3 affected members in a family) and were enrolled in this study (Figure 1). In the 29 families, 116 asymptomatic family members (59 men, 57 women, 30 to 82 years of age; mean, 50.0 years) without a history of IAs or SAH underwent MRA examinations. Of the 116 examinees, 22 (8 men [13.6%] and 14 women [24.6%]) were found to have IAs. Of a total 105 affected members (35 men, 70 women) in the 29 families, 70 affected members (66.7%) had SAH and 35 affected members (33.3%) have or have had unruptured IAs. The mean age at the time of diagnosis with SAH among the 70 affected members was 55.4 years (51.7 among 23 men, 57.3 among 47 women). Eighteen affected members had died of SAH.

Linkage Analysis

Eighty-seven living affected members were genotyped through blood DNA; 2 affected members who had died (IV-1 in Pedigree 5 and III-10 in Pedigree 29) were genotyped through DNA from their preserved umbilical cords. Genotypes of 4 deceased affected members (II-4 in Pedigree 1, II-5

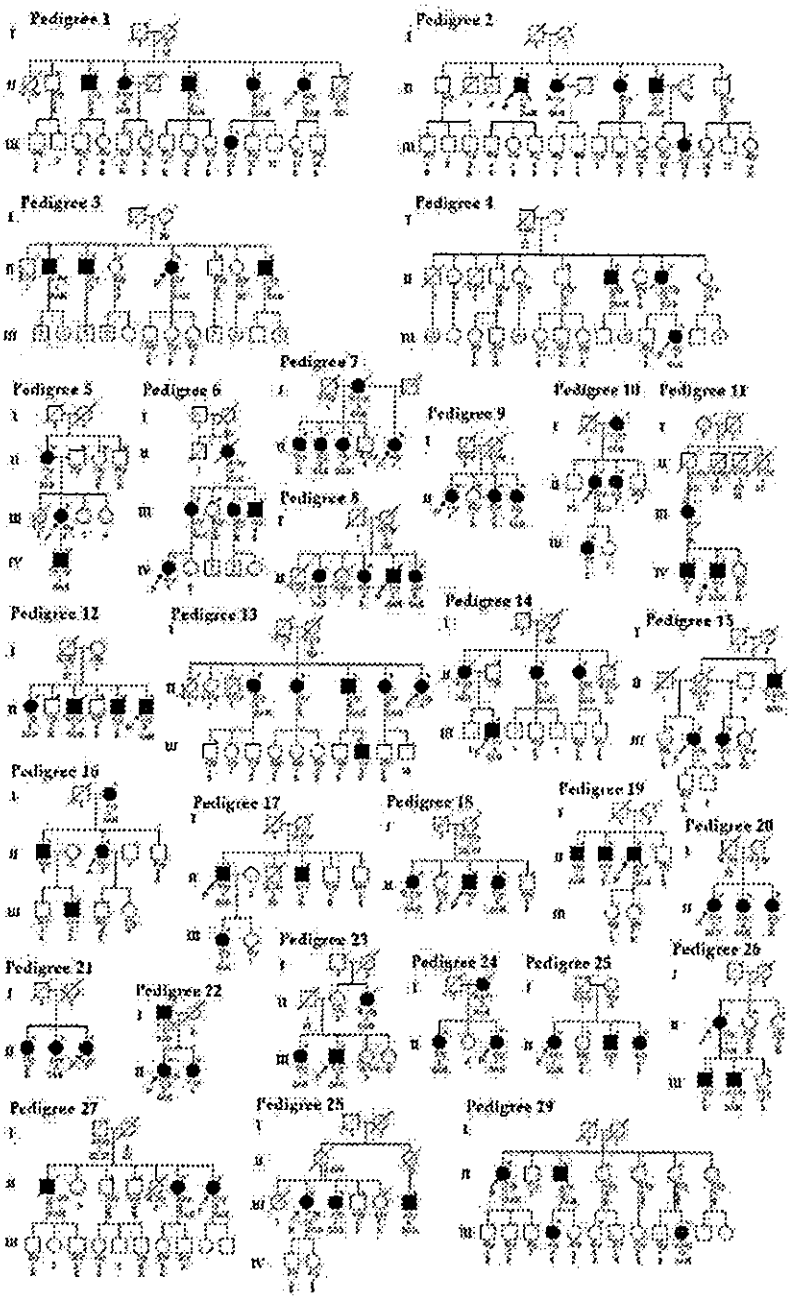


Figure 1. Twenty-nine families in genetic linkage analysis. □, Male; ○, female; ■, affected members (IA or SAH); ●, individual died. *Participation in linkage analysis: P, proband; SAH, subarachnoid hemorrhage; ST, stroke; SD, sudden death by unknown cause; E, examination with MRA; and d, age at death.

in Pedigree 2, II-1 in Pedigree 14, and II-1 in Pedigree 27) were reconstructed from the genotypes of offspring and spouses. In total, 93 affected members (31 men, 62 women; mean age at diagnosis, 55.2 years) and 27 unaffected members (13 males, 14 females, aged ≥60 years) were included in the linkage analysis. Characteristics of these members are shown in Table 1. The genome-wide linkage results in the screening are shown in Figure 2. Regions of potentially interest (nominal $P<0.05$) by multipoint NPL scores were observed on chromosomes 12q11–13, 15q21, 17cen, 19q13, and Xp22 (Table 2 and Data Supplement Table II).

The statistical power of this screening was 52%, 93%, and 99% when the locus heterogeneity was 75%, 50%, and 25%, respectively.

After fine mapping, 2 of 5 regions, 19q13 (maximum NPL score [MNS] = 2.15, nominal $P=0.020$) and Xp22 (MNS=2.16, nominal $P=0.019$), remained potentially interesting regions (Table 2 and Data Supplement Table III). The region on chromosome 17cen turned out to be a suggestive linkage region (MNS=3.00, nominal $P=0.001$). The sizes of regions with nominal $P<0.05$ were 17.7 cM (D17S921–D17S1800) on chromosome 17, 7.9 cM (D19S198–

TABLE 1. Characteristics of Family Members in the Linkage Analysis

	Affected			Unaffected		
	Male (n=31)	Female (n=62)	Total (n=93)	Male (n=13)	Female (n=14)	Total (n=27)
Age at diagnosis, y, mean±SD	53.6±13.8	56.0±11.7	55.2±12.4	66.2±3.9	67.1±7.2	66.7±5.7
Hypertension, %	36.7	44.3	40.9	38.5	35.7	37.0
Current or ex-smoker, %	70.0	29.5	41.9	76.9	7.1	40.7
Subarachnoid hemorrhage, %	61.3	62.9	62.4	...	...	...
Multiple IAs, %	12.9	19.4	17.2	...	...	...

Those classified as "Affected" were diagnosed as harboring IAs. Those classified as "Unaffected" were diagnosed as free from IAs by MRA and were ≥60 y of age.

D19S596) on chromosome 19, and 10.1 cM (DXS987–DXS7593) on chromosome X. Physical localization of microsatellite markers in these regions and candidate genes are shown in Table 3.

Case-Control Study for Candidate Genes

We searched putative candidate genes in 1 suggestive linkage and 2 potentially interesting regions after considering physiological functions and documented evidence: chromosomes

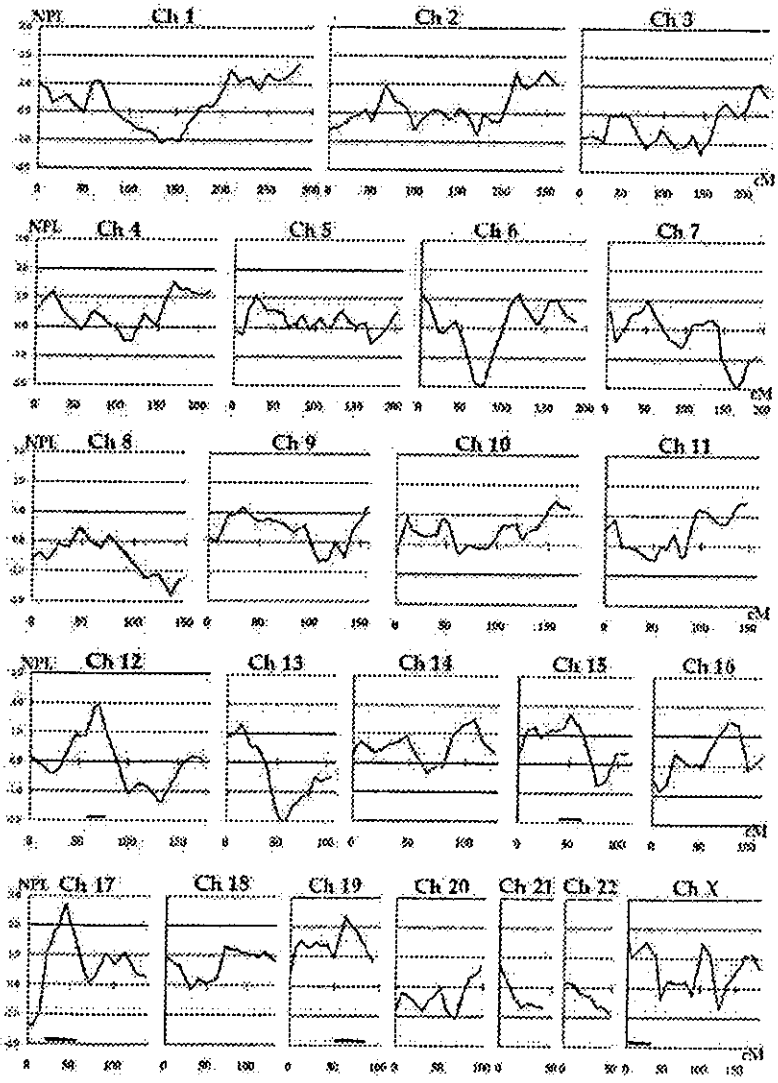


Figure 2. Multipoint nonparametric logarithm of odds score in genome-wide screening. Bars at bottom of chart indicate regions genotyped further in fine mapping.

TABLE 2. Maximum Multipoint NPL Scores

Region	Screening		Fine Mapping	
	NPL Score	Nominal *P*	NPL Score	Nominal *P*
12q11–13	1.96	0.032	1.15	0.126
15q21	1.71	0.043	0.80	0.209
17cen	2.74	0.003	3.00	0.001
19q13	2.33	0.014	2.15	0.020
Xp22	1.80	0.040	2.16	0.019

Regions were those exceeding the threshold (nominal $P<0.05$) of the NPL score in the screening. Screening was genotyping all chromosomal regions at ≈10-cM density. Fine mapping was genotyping at ≈1- to 2-cM densities.

17cen (*NOS2A* and *MFAP4*), 19q13 (*APOE*), and Xp22 (*ACE2*) (Table 3). In *NOS2A*, there was the 4-bp (AAAT) deletion (R4)/insertion (R5) polymorphism in the regulatory region, and R5 was a high-risk allele of hypertension and coronary artery stenosis.[18–20] We therefore tested the association of this polymorphism. Characteristics of the case versus control subjects are shown in Table 4. All cases and control subjects had an R4/R4 genotype (100%) (Table 5).

We next checked whether the epsilon (ε) genotypes of *APOE* were involved in IA because the *APOE* ε-4 genotype has been reported as a risk factor for SAH among Japanese.[21] The relevant allele frequencies in 100 cases were ε−2=4%, ε−3=85%, and ε−4=11%, which were not different from 100 control subjects (Table 5) and the Japanese general population.[22] Six genotype frequencies were ε−2/ε−2=0%, ε−2/ε−3=7.0%, ε−2/ε−4=0.5%, ε−3/ε−3=69.9%, ε−3/ε−4=20.6%, and ε−4/ε−4=2.0% in cases, being the same in control subjects. These did not differ significantly from those expected from the Hardy-Weinberg equilibrium. Furthermore, there was no family in which the ε−4 allele of *APOE* was segregated with IA (data not shown).

TABLE 3. Physical Localization of Microsatellite Markers and Candidate Genes

Ch	Position, kb	Marker/Gene	NPL	Nominal *P*
17	14461	D17S921	1.42	0.080
17	15356	D17S918	2.50	0.006
17	16616	D17S1857	2.88	0.002
17	17465	D17S2196	3.00	0.001
17	**19450**	*MFAP4*		
17	19731	AFMa126yd5	2.78	0.003
17	20924	D17S1871	2.40	0.008
17	21493	D17S783	1.95	0.030
17	**26250**	*NOS2A*		
17	26806	D17S1824	1.95	0.030
17	28528	D17S1294	1.94	0.030
17	30082	D17S1800	1.41	0.080
19	46845	D19S198	0.96	0.200
19	48501	D19S420	1.73	0.040
19	49815	D19S574	1.83	0.030
19	**50100**	*APOE*		
19	51703	D19S412	2.15	0.020
19	52665	D19S596	1.56	0.060
X	14071	DXS987	1.33	0.090
X	**14960**	*ACE2*		
X	17103	DXS8019	2.16	0.020
X	21755	DXS7593	1.61	0.050

Positions should be referred to human genome sequence information at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/genome/guide/human/).

TABLE 4. Characteristics of Cases vs Controls

	Cases*	Controls†
No. (male/female)	100 (34/66)	100 (39/61)
Age at diagnosis, y		
Mean±SD	58.7±9.4	58.4±10.1
Range	38–78	40–83
Family history of IA, %	50	0
Hypertension, %	45	26
Current or ex-smoker, %	40	26

*Cases were 29 family probands and 71 unrelated subjects confirmed with IAs by operation or digital subtraction angiography.

†Controls were diagnosed as without IAs by digital subtraction angiography or MRA.

We sequenced entire coding regions of *MFAP4* and *ACE2* in the 29 probands of the IA families (Data Supplement Table I). However, we found no polymorphism in the coding regions of these genes. One novel SNP was identified in intron 4 of *MFAP4*, of which the allele frequency (G/A) was essentially the same between 200 case and 200 control chromosomes (Table 5). One registered SNP (dbSNP: rs2285666) was identified in intron 3 of *ACE2*, in which allele frequency (C/T) did not differ between 200 case and 200 control chromosomes (Table 5).

Discussion

Linkage Analysis

We found 1 suggestive linkage region on chromosome 17cen and 2 potentially interesting (nominal $P<0.05$) regions on chromosomes 19q13 and Xp22. The suggestive linkage region on chromosome 17cen was in accord with the results of a previous Japanese sib-pair analysis (nominal $P=0.027$),[3] whereas 2 potentially interesting regions on chromosomes 19q13 and Xp22 were reported as candidate regions in a Finish population.[4] The region on chromosome 19q13 was also replicated by another Finish study.[7] The region on chromosome 19q13 may thus not be specific to Finnish population. Collectively, candidate regions that have to date been replicated in >1 study include 7q11 (Onda et al and Farnham et al) and 19q13 (Olson et al and Van Der Voet et al).[3–5,7] The regions of 17cen (Onda et al[3]) and Xp22 (Olson et al[4]) are extended in the present study. Such concordant regions should be considered as high-priority loci and provide promising scaffolds for future studies to identify the exact genetic mechanisms for IA. On the other hand, scattering over various chromosomes may suggest some complexity to the pathophysiology of IAs; such complexity represents the

TABLE 5. Case-Control Study for Candidate Genes

Gene	Base Position*	Location	Allele	Allele Frequency,† n		χ^2	P
				Cases	Controls		
MFAP4	+1824	Intron 4	G	193	188	1.38	0.240
			A	7	12		
NOS2A	−2453	Promoter	$(AAAT)_4$	200	200	...	...
			$(AAAT)_5$	0	0		
APOE	+2059, +2197	Exon 4	ϵ−2	8	7	1.493	0.474
			ϵ−3	171	164		
			ϵ−4	21	29		
ACE2	+8686	Intron 3	C	75	80	0.666	0.414
			T	91	81		

*The number from the A of the start codon (ATG) in the genomic DNA reference sequence.
†Allele frequency was described chromosome number.

complexity of interactions among many genetic and environmental risk factors that contribute in different degrees with different populations.

It is well known that female sex is a risk factor for IA. The reasons for this increased prevalence are unknown, but there could be a genetic basis as demonstrated in this study. It has recently been shown that many genes ("escapees") on chromosome Xp22 escape inactivation,[23] which may explain the sex differences in susceptibility to IA by gene dosage effects.

The present study has several limitations. First, because we took a family-based approach, it was hard to narrow down the candidate regions to 1-cM resolution. These candidate regions still have ≈8- to 18-cM sizes, and further efforts will be needed to find susceptibility genes for IA. For this goal, linkage disequilibrium (LD) mapping will be required. The second limitation is associated with the statistical power and specificity. Although the statistical power is highly dependent on the locus heterogeneity, it is hard to predict what degrees of locus heterogeneity exist among the 29 families. Simulation could, however, provide a prediction of the statistical power; >90% power was obtainable if the locus heterogeneity was <50%.

Candidate Genes

At least 2 mechanisms are hypothesized to play critical roles in the development of IA. These include defects in the maintenance of extracellular matrix and in remodeling. These hypotheses suggest *MFAP4* and *iNOS* on chromosome 17cen[9,10,24–26] and *ACE2* on chromosome X p22[27–29] as candidate genes. On the other hand, an epidemiological study among Japanese ranked *APOE* high as a candidate gene on chromosome 19q13.[21] However, in the case-control study, albeit with very limited numbers of plausible genes, candidate genes including *APOE* failed to show positive associations with IA. Obviously, LD mapping covering entire regions is required to search for clues to susceptibility genes.

Conclusions

Linkage analyses in 29 IA families with ≥3 affected members showed 1 suggestive linkage region on chromosome 17cen (17.7 cM) and potentially interesting regions on chromosomes 19q13 (7.9 cM) and Xp22 (10.1 cM). These 3 loci provide promising scaffolds for searching for genes determining susceptibility to IA. We also showed evidence that 4 candidate genes, *MFAP4*, *ACE2*, a promoter variant of *NOS2A*, and *APOE* ϵ genotypes, did not have LD with an unknown susceptibility gene for IA. Further efforts are clearly needed to identify susceptibility genes for IA.

Acknowledgments

This work was supported by a grant from the Ministry of Education, Science, Sports, and Culture of Japan (Kiban Kenkyuu A: 14207016) and a grant from the Japan Society for the Promotion of Science (15012231). We are grateful to Dr Mark G. Lathrop (The Centre National de Genotypage, Evry, France) for critical reading of the manuscript. We thank Miho Yoshida and Norio Matsumra for technical assistance and the following doctors for patient recruitment and help in ascertaining MRA examinations: Susumu Miyamoto (National Cardiovascular Center), Shiro Nagasawa and Nobuhisa Mabuchi (Soseikai General Hospital), Yasuhiko Tokuriki and Tomoo Tokime (Fukui Red Cross Hospital), Takaaki Kaneko and Nozomu Murai (Hikone Municipal Hospital), Shunichi Yoneda and Yoshito Naruo (Nihonbashi Hospital), Sen Yamagata (Kurashiki Central Hospital), Kenji Hashimoto (Hyogo Prefectural Tsukaguchi Hospital). Atsushi Okumura and Yoshihiko Uemura (Kyoto City Hospital), Tomohiko Iwai (Gifu Municipal Hospital), Hiroyasu Yamakawa (Geroonsen Hospital), Shingo Sugimoto (Sumi Hospital), Atsushi Kawarazaki (Kawarazaki Hospital), Kiyohiro Houkin and Osamu Honmon (Sapporo Medical University School of Medicine), Akira Ogawa and Miyuki Abe (Iwate Medical University), Masayuki Matsuda (Shiga University of Medical Science), Michiyasu Suzuki and Sadahiro Nomura (Yamaguchi University School of Medicine), Izumi Nagata (Nagasaki University School of Medicine), Masatsune Ishikawa (Kitano Hospital), Shinichiro Okamoto (Osaka Red Cross Hospital), Yoshinori Akiyama (Tenri Hospital), Takeshi Nishihara (Kouseikai Takeda Hospital), Hiroshi Kajikawa and Shinichi Wakabayashi (Kajikawa Hospital), Akihiro Doi and Junji Yoshioka (Okayama Kyokuto Hospital), Kazunori Kajihara and Yuji Okamoto (Saiseikai Yahata Hospital), Ichiro Nakahara and Toshio Higashi (Kokura Memorial Hospital), and Takashi Yoshizawa and Kenjiro Ito (Yokohama Shintoshi Neurosurgical Hospital).

References

1. Okamoto K, Horisawa R, Kawamura T, Asai A, Ogino M, Takagi T, Ohno Y. Family history and risk of subarachnoid hemorrhage: a case-control study in Nagoya, Japan. *Stroke*. 2003;34:422–426.

2. Ronkainen A, Miettinen H, Karkola K, Papinaho S, Vanninen R, Puranen M, Hernesniemi J. Risk of harboring an unruptured intracranial aneurysm. *Stroke*. 1998;29:359–362.
3. Onda H, Kasuya H, Yoneyama T, Takakura K, Hori T, Takeda J, Nakajima T, Inoue I. Genome-wide linkage and haplotype-association studies map intracranial aneurysm to chromosome 7q11. *Am J Hum Genet*. 2001;69:804–819.
4. Olson JM, Vongpunsawad S, Kuivaniemi H, Ronkainen A, Hernesniemi J, Ryynanen M, Kim LL, Tromp G. Search for intracranial aneurysm susceptibility gene(s) using Finnish families. *BMC Med Genet*. 2002;3:7.
5. Farnham JM, Camp NJ, Neuhausen SL, Tsunoda J, Parker D, MacDonald J, Cannon-Albright LA. Confirmation of chromosome 7q11 locus for predisposition to intracranial aneurysm. *Hum Genet*. 2004;114:250–255.
6. Yamada S, Utsunomiya M, Inoue K, Nozaki K, Miyamoto S, Hashimoto N, Takenaka K, Yoshinaga T, Koizumi A. Absence of linkage of familial intracranial aneurysms to 7q11 in highly aggregated Japanese families. *Stroke*. 2003;34:892–900.
7. Van Der Voet M, Olson JM, Kuivaniemi H, Dudek DM, Skunca M, Ronkainen A, Niemela M, Jaaskelainen J, Hernesniemi J, Helin K, Leinonen E, Biswas M, Tromp G. Intracranial aneurysms in Finnish families: confirmation of linkage and refinement of the interval to chromosome 19q13.3. *Am J Hum Genet*. 2004;74:564–571.
8. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037–2048.
9. Schievink WI. Genetics of intracranial aneurysms. *Neurosurgery*. 1997; 40:651–663.
10. Schievink WI, Parisi JE, Piepgras DG, Michels VV. Intracranial aneurysms in Marfan's syndrome: an autopsy study. *Neurosurgery*. 1997;41: 866–871.
11. National Center for Biotechnology Information. Summary of Maps in UniSTS. Available at: http://www.ncbi.nlm.nih.gov/genome/sts/. Accessed March 1, 2004.
12. Sobel E, Papp JC, Lange K. Detection and integration of genotyping errors in statistical genetics. *Am J Hum Genet*. 2002;70:496–508.
13. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin: rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet*. 2002;30:97–101.
14. Wills S, Ronkainen A, van der Voet M, Kuivaniemi H, Helin K, Leinonen E, Frosen J, Niemela M, Jaaskelainen J, Hernesniemi J, Tromp G. Familial intracranial aneurysms: an analysis of 346 multiplex Finnish families. *Stroke*. 2003;34:1370–1374.
15. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet*. 1996;58:1347–1363.
16. National Center for Biotechnology Information. Single Nucleotide Polymorphism. Available at: http://www.ncbi.nlm.nih.gov/SNP/. Accessed March 1, 2004.
17. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. 1990;31: 545–548.
18. Kunnas TA, Mikkelsson J, Ilveskoski E, Tanner MM, Laippala P, Penttila A, Perola M, Nikkari ST, Karhunen PJ. A functional variant of the iNOS gene flanking region is associated with LAD coronary artery disease: an autopsy study. *Eur J Clin Invest*. 2003;33:1032–1037.
19. Glenn CL, Wang WY, Morris BJ. Different frequencies of inducible nitric oxide synthase genotypes in older hypertensives. *Hypertension*. 1999;33: 927–932.
20. Morris BJ, Glenn CL, Wilcken DE, Wang XL. Influence of an inducible nitric oxide synthase promoter variant on clinical variables in patients with coronary artery disease. *Clin Sci (Lond)*. 2001;100:551–556.
21. Kokubo Y, Chowdhury AH, Date C, Yokoyama T, Sobue H, Tanaka H. Age-dependent association of apolipoprotein E genotypes with stroke subtypes in a Japanese rural population. *Stroke*. 2000;31:1299–1306.
22. Zaman MM, Ikemoto S, Yoshiike N, Date C, Yokoyama T, Tanaka H. Association of apolipoprotein genetic polymorphisms with plasma cholesterol in a Japanese rural population: the Shibata Study. *Arterioscler Thromb Vasc Biol*. 1997;17:3495–3504.
23. Carrel L, Cottle AA, Goglin KC, Willard HF. A first-generation X-inactivation profile of the human X chromosome. *Proc Natl Acad Sci U S A*. 1999;96:14440–14444.
24. Fukuda S, Hashimoto N, Naritomi H, Nagata I, Nozaki K, Kondo S, Kurino M, Kikuchi H. Prevention of rat cerebral aneurysm formation by inhibition of nitric oxide synthase. *Circulation*. 2000;101:2532–2538.
25. Iwashina M, Shichiri M, Marumo F, Hirata Y. Transfection of inducible nitric oxide synthase gene causes apoptosis in vascular smooth muscle cells. *Circulation*. 1998;98:1212–1218.
26. Sadamasa N, Nozaki K, Hashimoto N. Disruption of gene for inducible nitric oxide synthase reduces progression of cerebral aneurysms. *Stroke*. 2003;34:2980–2984.
27. Ohkuma H, Suzuki S, Fujita S, Nakamura W. Role of a decreased expression of the local renin-angiotensin system in the etiology of cerebral aneurysms. *Circulation*. 2003;108:785–787.
28. Slowik A, Borratynska A, Pera J, Betlej M, Dziedzic T, Krzyszkowski T, Czepko R, Figlewicz DA, Szczudlik A. II genotype of the angiotensin-converting enzyme gene increases the risk for subarachnoid hemorrhage from ruptured aneurysm. *Stroke*. 2004;35:1594–1597.
29. Takenaka K, Yamakawa H, Sakai H, Yoshimura S, Murase S, Okumura A, Nakatani K, Kimura T, Nishimura Y, Yoshimi N, Sakai N. Angiotensin I-converting enzyme gene polymorphism in intracranial saccular aneurysm individuals. *Neurol Res*. 1998;20:607–611.

Magnitude and Role of Wall Shear Stress on Cerebral Aneurysm

Computational Fluid Dynamic Study of 20 Middle Cerebral Artery Aneurysms

Masaaki Shojima, MD; Marie Oshima, PhD; Kiyoshi Takagi, MD, PhD; Ryo Torii, PhD; Motoharu Hayakawa, MD, PhD; Kazuhiro Katada, MD, PhD; Akio Morita, MD, PhD; Takaaki Kirino, MD, PhD

Background and Purpose—Wall shear stress (WSS) is one of the main pathogenic factors in the development of saccular cerebral aneurysms. The magnitude and distribution of the WSS in and around human middle cerebral artery (MCA) aneurysms were analyzed using the method of computed fluid dynamics (CFD).

Methods—Twenty mathematical models of MCA vessels with aneurysms were created by 3-dimensional computed tomographic angiography. CFD calculations were performed by using our original finite-element solver with the assumption of Newtonian fluid property for blood and the rigid wall property for the vessel and the aneurysm.

Results—The maximum WSS in the calculated region tended to occur near the neck of the aneurysm, not in its tip or bleb. The magnitude of the maximum WSS was 14.39 ± 6.21 N/m^2, which was 4-times higher than the average WSS in the vessel region (3.64 ± 1.25 N/m^2). The average WSS of the aneurysm region (1.64 ± 1.16 N/m^2) was significantly lower than that of the vessel region ($P<0.05$). The WSSs at the tip of ruptured aneurysms were markedly low.

Conclusions—These results suggest that in contrast to the pathogenic effect of a high WSS in the initiating phase, a low WSS may facilitate the growing phase and may trigger the rupture of a cerebral aneurysm by causing degenerative changes in the aneurysm wall. The WSS of the aneurysm region may be of some help for the prediction of rupture. (***Stroke.*** **2004;35:2500-2505.)**

Key Words: aneurysm ■ biomechanics ■ hemodynamics ■ shear strength

Unruptured cerebral aneurysms are diagnosed with greater frequency since the development of increasingly accurate noninvasive cerebrovascular imaging techniques. Among 400 adult volunteers (39 to 71 years old; mean age, 55 years) who underwent clinical and radiological evaluations, Nakagawa et al reported the incidence of unruptured intracranial aneurysms to be as high as 6.5%.[1] Because the rupture of aneurysms results in subarachnoid hemorrhage, which has a dismal prognosis,[2,3] it is desirable to be able to determine whether a particular aneurysm has a high risk of rupture so that it can be treated before bleeding occurs. Aneurysms of a larger size (>10 mm) and/or a higher aspect ratio (>1.6) have a high risk of bleeding.[4–6] However, the majority of the unruptured aneurysms do not meet these criteria,[5] and it is difficult to predict the likelihood of their rupture.

Hemodynamic stresses are considered to have profound effects on the development of cerebral aneurysms.[7,8] One of these, the wall shear stress (WSS), acts directly on the vascular endothelium as a biological stimulator that modulates the cellular function of the endothelium.[9,10] Thus, the focus of the study presented here was aneurysm WSS. The close relationship between high WSS and the initiation of cerebral aneurysm formation has already been demonstrated in animal experiments.[11] The WSS may also play an important role in the growth and rupture of cerebral aneurysms.

The measurement of WSS in vivo is becoming feasible; however, it remains very difficult, especially in small and tortuous intracranial arteries.[12,13] With recent advances in computer technology, the magnitude and distribution of WSSs in complex arterial models have been observed with the aid of the computational fluid dynamics (CFD) technique, which is also a useful clinical tool for planning endovascular treatment.[14,15] The use of this technique has been limited to just a few cases; therefore, a statistical analysis of the results has not been possible. Here, we present the results of a

Received May 4, 2004; final revision received July 2, 2004; accepted August 10, 2004.

From the Department of Neurosurgery (M.S., A.M., T.K.), Faculty of Medicine, and the Institute of Industrial Science (M.S., M.O., R.T.), University of Tokyo, Japan; the Department of Neurosurgery (K.T.), Faculty of Medicine, Teikyo University, Japan; and the Departments of Neurosurgery (M.H.) and Radiology (K.K.), Faculty of Medicine, Fujita Health University, Japan.

Correspondence to Dr Masaaki Shojima, Department of Neurosurgery, Faculty of Medicine, University of Tokyo, Japan. 7-3-1, Hongo, Bunkyo-ku, Tokyo, Japan 113-0033. E-mail mshoji-tky@umin.ac.jp

Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000144648.89172.of