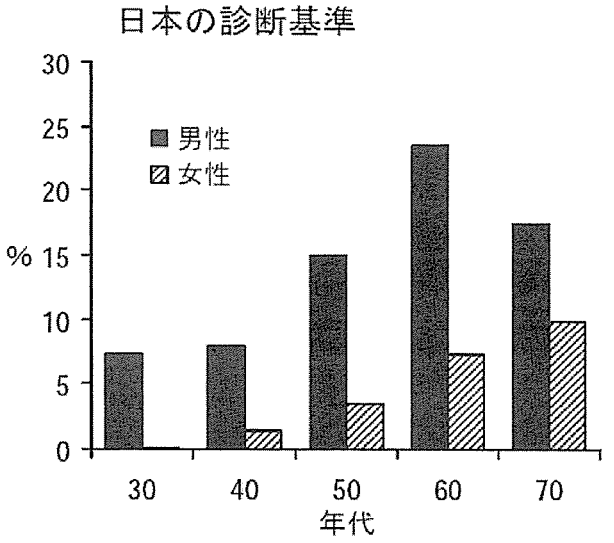

図 4. 性年代別メタボリック症候群の頻度

表 1. ベースライン男女別健診結果

	男性 (n=2,737)	女性 (n=3,122)
年齢(歳)	56.1±13.3	54.5±12.9
BMI(kg/mm2)	22.8±2.9	22.3±3.2
収縮期血圧(mmHg)	128.7±20.9	125.2±22.1
拡張期血圧(mmHg)	79.3±12.2	75.5±11.8
総コレステロール(mg/dl)	201.4±34.5	213.2±38.9
HDLコレステロール(mg/dl)	49.3±13.2	56.5±13.4
中性脂肪(mg/dl)	142.8±105.7	108.2±74.9
ウェスト周囲長(cm)	82.5±8.1	77.6±10.4
空腹時血糖(mg/dl)	101.3±20.8	96.3±16.5
現病歴		
高血圧(%)	34.0	28.6
高脂血症(%)	29.1	42.2
糖尿病(%)	5.5	2.8
現在喫煙(%)	50.2	11.9
現在飲酒(%)	74.9	32.7

表2. メタボリック症候群と脳卒中・心筋梗塞との関係

	全脳卒中		脳梗塞		心筋梗塞	
	RR	95% CL	RR	95% CL	RR	95% CL
男女						
日本診断基準	1.74	1.14-2.66	2.57	1.37-4.83	2.43	1.60-3.70
NCEP 診断基準	1.91	1.30-2.79	3.20	1.85-5.55	2.32	1.10-4.90
男性						
日本診断基準	1.66	1.02-2.71	2.57	1.34-4.93	2.40	1.47-3.93
NCEP 診断基準	1.59	0.98-2.57	2.56	1.29-5.08	4.02	1.73-9.40
女性						
日本診断基準	2.03	0.85-4.85	2.17	0.63-7.49	2.53	1.12-5.75
NCEP 診断基準	2.71	1.45-5.07	4.85	1.93-12.18	-	-

多変量調整：性年齢、喫煙、飲酒により調整、RR:ハザード比、95%CL:95%信頼区間

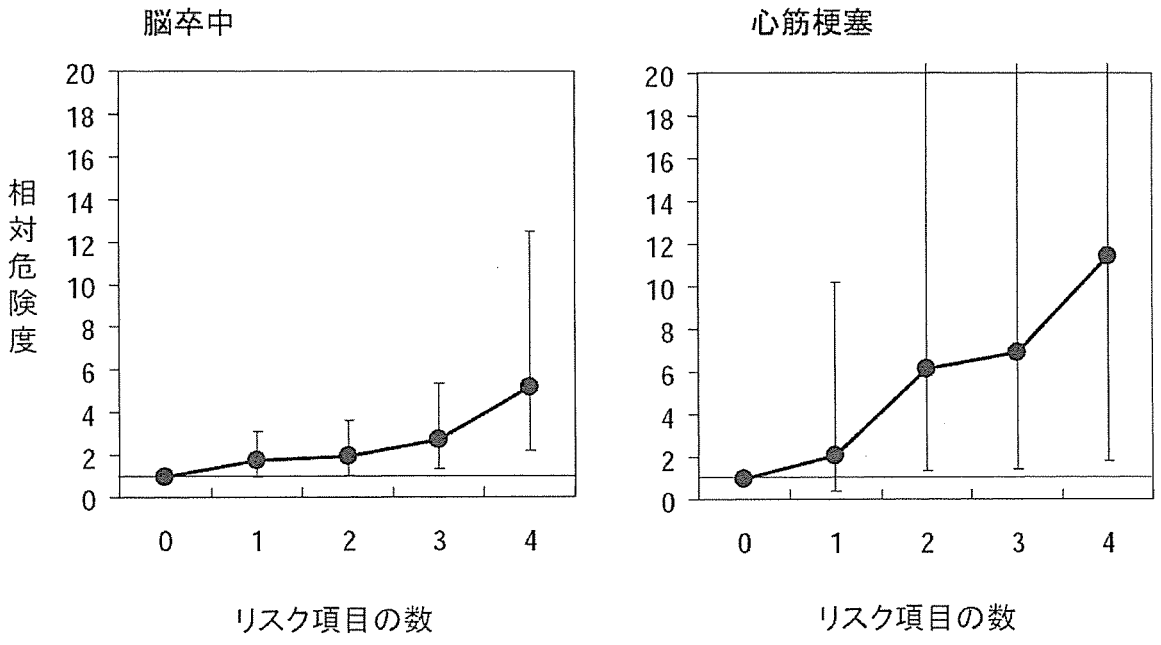

図 5. メタボリック症候群構成要因数と循環器病の危険度

(1.27-4.45)、2.57(1.37-4.83)であったが、出血性脳卒中では統計的に有意ではなかった(表 2)。コンポーネントの該当する数と全脳卒中との関連は、コンポーネント数が 0 を基準にして、性年齢、飲酒、喫煙で調整された相対危険度は、1 から 4 と増加するに連れてそれぞれ、1.91 (1.07-3.43)、2.00 (1.08-3.69)、2.91 (1.49-5.66)、5.73 (2.47-13.30)、トレンド $p<0.0001$ であり、心筋梗塞では 2.15 (0.44-10.59)、6.46 (1.42-29.31)、7.17 (1.45-35.59)、12.59 (2.01-78.75)、トレンド $p=0.0055$ であった(図 5)。

表 3. BMI・ウェスト周囲と脳卒中の相対危険度

	BMI			ウェスト周囲長		
	四分位	RR	95% CL	四分位	RR	95% CL
男女	-20.36	1		-73	1	
	20.37-22.31	1.06	0.61-1.84	74-80	1.31	0.72-2.38
	22.32-24.38	1.23	0.74-2.05	81-86	1.67	0.95-2.97
	24.39-	**1.65**	1.03-2.70	87-	**2.41**	1.41-4.11
	P for trend		***0.003***	*P for trend*		***<0.0001***
男性	-20.83	1		-72	1	
	20.84-22.73	0.98	0.51-1.90	73-83	2.23	0.87-5.71
	22.74-24.61	1.48	0.82-2.69	84-88	2.21	0.83-5.89
	24.62-	1.37	0.73-2.56	89-	**3.75**	1.45-9.71
	P for trend		***0.044***	*P for trend*		***0.006***
女性	-20.02	1		-70	1	
	20.03-21.92	1.02	0.43-2.40	71-77	2.70	0.87-8.29
	21.93-24.12	0.70	0.29-1.73	78-85	2.07	0.65-6.52
	24.13-	1.44	0.68-3.09	86-	**3.87**	1.33-11.31
	P for trend		***0.028***	*P for trend*		***0.016***

(性)年齢による調整

表 3 は BMI、ウェスト周囲長を四分位にわけて、第 1 四分位を基準に脳卒中の相対危険度を求めたものである。BMI ではトレンド P が男女とも 0.05 未満であるが、男女合わせた第4四分位で脳卒中の相対危険度が 1.65(1.03-2.70)倍であった。ウェスト周囲長は、第 4 四分位で、男性で(89cm 以上)3.75(1.45-9.71)倍、女性で(86cm 以上)3.87(1.33-11.31)倍であった。

Ⅲ. 吹田コホート研究の現状

人口動態調査死亡票(磁気テープ転写分)の目的外利用申請で承認を受けて、平成元年から平成 17 年分までの原死因のデータを整理している。平成元年から平成 6 年までに 155 名の死亡が、また平成 7 年から平成 17 年までに 1047 名の死亡が確認された。合計 1,202 名の死亡が確認され、その原死因が同定された。循環器疾患についての内訳は、心筋梗塞が 72 名、可能性のある心筋梗塞も含めると 92 名であった。脳卒中については、脳梗塞が 63 名、脳出血が 22 名、くも膜下出血が 12 名、分類不能の脳卒中が 2 名であった。可能性のある脳

	合計
心筋梗塞	72
可能性のある心筋梗塞	20
脳卒中	99
くも膜下出血	12
脳内出血	22
脳梗塞	63
分類不能の脳卒中	2
可能性のある脳卒中	3
その他の循環器疾患	125
全循環器疾患	319

卒中も含めると脳卒中は102例であった。

エンドポイントが発症の対象者については、吹田市基幹病院にほぼ調査完了している。遠隔の医療機関においての発症登録を依頼中である。また、吹田地域循環器疾患発症登録制度は、1月25日に市と国循との間に協定書を締結した。次年度においては、発症、死亡をエンドポイントに健診の各項目において脳卒中、心筋梗塞との関連性が見られるかどうか解析を進めていく予定である。

Metabolic Syndrome and the Risk of Stroke and Myocardial Infarction in a Japanese General Population: the Suita Cohort Study

Yoshihiro Kokubo[1], Yasunao Yoshimasa[2], Hitonobu Tomoike[1], Katsuyuki Kawanishi[3], Yasushi Kotani[3], Hiroko Ueda[1], Mikiko Kasahara[1], Mikiko Kojima[1], Keiko Yamaguchi[1], Mayumi Yoshimura[1], and Akira Okayama[1]
[1]Department of Preventive Cardiology and [2]Division of Atherosclerosis, Metabolism and Clinical Nutrition, National Cardiovascular Center, Osaka, Japan; [3]Suita City Medical Association, Osaka, Japan.

Background: The metabolic syndrome, a clustering of disturbed glucose and insulin metabolism, obesity and abdominal fat distribution, dyslipidemia, and hypertension is associated with cardiovascular diseases in Caucasian. However, the few studies have examined prospective studies in Japan.
Objective: The aim of this study was to examine the association of metabolic syndrome, as defined by Japanese criteria, with the risk for stroke and myocardial infarction.
Design and Methods: Population-based cohort study with an average follow-up of 5.8 years from Japan. A total of 5,847 men and women without history of cardiovascular disease at baseline participated. Seventy-nine strokes and thirty-nine myocardial infarctions occurred. The relative risks were calculated after adjusting for sex, age, smoking, and drinking by Cox proportional-hazards models.
Results: Metabolic syndrome had a 1.74-fold (95% CI, 1.14 to 2.66) risk for all strokes, 2.48-fold (1.40 to 4.39) risk for ischemic stroke, and 2.35-risk (1.12-4.95) risk for myocardial infarction, after adjusting for confounding variables. Compared with 0 component of metabolic syndrome, the multivariate adjusted risk ratios for all strokes and myocardial infarction with the number of components were 1.91 (1.07 to 3.43) and 2.15 (0.44-10.59) for 1 component, 2.00 (1.08-3.69) and 6.46 (1.42-29.31) for 2 components, 2.91 (1.49-5.66) and 7.17 (1.45-35.59) for 3 components, and 5.73 (2.47-13.30) and 12.59 (2.01-78.75) for 4 components (trend $p<0.0001$ and $p=0.0055$), respectively.
Conclusions: The risks of stroke and myocardial infarction are increased with metabolic syndrome, in a Japanese general population. Prevention of the metabolic syndrome may reduce risks for cardiovascular disease.
(246 words for abstract; 19 words for title; 46 words for authors)

The 21st Scientific Meeting of the International Society of Hypertension
October 15-19, 2006 Fukuoka, Japan

都市部一般住民を対象とした脳卒中と肥満指数との7年追跡研究：吹田コホート研究

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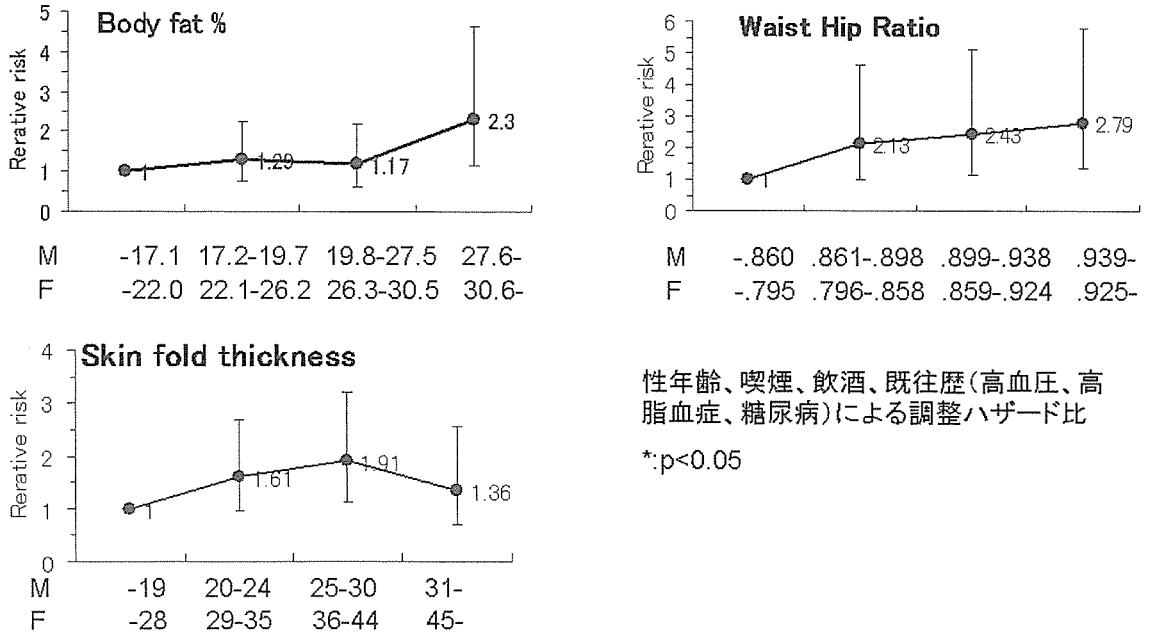
【目的】近年、肥満が生活習慣病の危険因子であるといわれているが、脳卒中と肥満との関係についてあまり報告がない。そこで今回、都市部一般住民を対象とした7年追跡研究により、脳卒中と肥満指数との関係について明らかにすることを目的とする。
【方法】平成元年に大阪府吹田市の住民台帳から性年齢別に無作為抽出され、初診時健診で脳卒中の既往のない追跡可能の男性2,768名(平均年齢56.1歳)、女性3,127名(同54.5歳)を今回の解析対象とした。平成元年～平成4年度の初診時健診後、2年毎の健診、毎年の問診、発症登録制度、病院カルテ調査により、1997年度末まで新規脳卒中の発症があるかについて追跡した。各種肥満指数と脳卒中との関係は、性年齢で調整されたCox比例ハザードモデルを用いて解析した。
【成績】男性15,724人年、女性18,244人年の観察より(平均追跡期間5.8年間)、脳梗塞55人、脳出血18人、くも膜下出血6名の発症が確認された。全脳卒中の年齢調整相対危険度はBMIの第1四分位を基準にして、第4四分位(24.39 kg/m2以上)で1.65 (1.03-2.70)、ウェストの第4四分位(87cm以上)で2.41 (1.41-4.11)、体脂肪率の第4四分位(27.3%以上)で2.15 (1.08-4.26)、ウェストヒップ比の第2-4四分位の順に2.12 (1.01-4.44)、2.54 (1.24-5.18)、3.13 (1.57-6.24)であった。男女別では、全脳卒中年齢調整相対危険度は、ウェストの第4四分位(男性89cm、女性86cm以上)で男女の順に3.75 (1.45-9.71)、3.87 (1.33-11.31)であった。脳梗塞の年齢調整相対危険度は、ウェストの第1四分位を基準にして、第4四分位で2.14 (1.01-4.59)、ウェストヒップ比の第4四分位(0.933以上)で4.22 (1.25-14.28)であった。
【結論】都市部一般住民で、BMI、ウェスト、体脂肪率が全脳卒中の危険因子であった。ウェストは男性89cm以上、女性86cm以上で全脳卒中の危険因子であることが認められた。
研究協力者：奈倉淳子,上田博子, 笠原美紀子, 小島美紀子, 真砂智子, 山口啓子, 吉村真由美, 友池仁暢(国立循環器病センター), 川西克幸, 小谷泰(吹田市医師会)

肥満指数四分位別全脳卒中ハザード比



性年齢、喫煙、飲酒、既往歴(高血圧、高脂血症、糖尿病)による調整ハザード比

*:p<0.05

第 31 回日本脳卒中学会総会:シンポジウム—メタボリックシンドローム(平成 18 年 3 月 19 日 横浜)

5.　久山町研究

分担研究者：九州大学大学院医学研究院環境医学分野　清原　裕

研究協力者：九州大学大学院医学研究院環境医学分野　有馬久富

1）コホートの現況

久山町研究は、九州大学大学院医学研究院環境医学および病態機能内科学が中心となり、1961年より継続しておこなわれてきた前向きコホート研究である。40歳以上の一般住民を対象とし、病歴調査（既往歴、家族歴など）、生活習慣調査（飲酒、喫煙など）、身体計測、血圧測定、多項目の血液検査、尿検査、心電図検査などを含む包括的な健診が行われてきた。同時に健診受診者を追跡し、心血管病の発症や死因別死亡などに関する調査を継続してきた。心血管病発症が疑われる者に対しては、研究スタッフが往診し、病歴、理学所見、検査所見などから診断を確定している。また、死亡例に対しては、80%以上において剖検を施行し、死因、臓器病変を特定している。このような徹底した追跡調査システムを用いることにより、追跡脱落率は0.2%以下と極めて低く抑えられている。このように、久山町研究は、世界でも類をみない極めて精度の高い疫学研究であり、今後も継続されていく予定である。

2) 最新の研究成果

Kubo M, Kiyohara Y, Ninomiya T, Tanizaki Y, Yonemoto K, Doi Y, Hata J, Oishi Y, Shikata K, Iida M. Decreasing incidence of lacunar vs other types of cerebral infarction in a Japanese population. Neurology 2006 ;66:1539-44.

日本人におけるラクナ梗塞対その他の脳梗塞の発症率低下

脳梗塞サブタイプ別の発症率や生存率の時代的変化を見た論文はきわめて少ない。久山町研究では、40 歳以上の久山町住民から 1961 年（1618 名）、1974 年（2038 名）、1988 年（2637 名）の 3 つのコホート集団を創設した。これらの集団を各々12 年間追跡し、脳梗塞サブタイプ別の発症率・生存率を比較した。全てのコホートにおいて剖検または画像診断による形態学的検査が、ほぼ全ての脳梗塞例について行なわれた。年齢標準化ラクナ梗塞発症率は、第 1 集団から第 2 集団にかけて男性で 59%、女性で 28%と有意に低下した。第 2 集団から第 3 集団にかけて男性では 41%と発症率低下が持続したが、女性では低下傾向が鈍化した。年齢標準化アテローム血栓性脳梗塞発症率は男女とも第 1 集団から第 2 集団にかけて低下傾向を示したが、第 2 集団から第 3 集団にかけて発症率は同程度であった。年齢標準化心原性塞栓症発症率は、全ての集団において変化を認めなかった。3 つのコホートにおいて、高血圧者の平均血圧レベルと喫煙者の頻度は時代とともに減少したが、高血圧者の頻度に時代的変化は見られなかった。ラクナ梗塞発症後の 5 年生存率は 3 集団の間で有意に改善したが、アテローム血栓性脳梗塞や心原性塞栓症の生存率の改善は見られなかった。これらのデータは、日本人において過去 40 年間の間にラクナ梗塞発症率が急速に低下したことを示唆する。高血圧管理の改善や喫煙者の頻度の低下がこの傾向に関与したことがうかがえる。

HIGH LIGHT

Decreasing incidence of lacunar vs other types of cerebral infarction in a Japanese population

M. Kubo, MD, PhD; Y. Kiyohara, MD, PhD; T. Ninomiya, MD, PhD; Y. Tanizaki, MD, PhD; K. Yonemoto, PhD; Y. Doi, MD, PhD; J. Hata, MD; Y. Oishi, MD; K. Shikata, MD; and M. Iida, MD, PhD

Abstract—*Background:* There is scant information on secular trends in the incidence and survival of ischemic stroke subtypes. *Methods:* The authors established three cohorts of Hisayama residents age ≥40 years in 1961 (1,618 subjects), 1974 (2,038 subjects), and 1988 (2,637 subjects). They followed up with each cohort for 12 years, comparing the incidence and survival rate of ischemic stroke subtypes. Morphologic examinations by autopsy or brain imaging was performed on most of the ischemic stroke cases in all cohorts. *Results:* The age-standardized incidence of lacunar infarction significantly declined by 59% for men and by 28% for women from the first to the second cohort. It continued to decline by 41% for men, but the decline decelerated for women between the second and third cohort. The age-standardized incidence of atherothrombotic infarction tended to decline from the first to the second cohort, whereas it was sustained between the second and third cohort for both sexes. The age-standardized incidence of cardioembolic infarction was unchanged throughout the cohorts. In these cohorts, mean blood pressure levels among hypertensive subjects and the prevalence of current smoker decreased with time, though the prevalence of hypertension remained stable. The 5-year survival rate after lacunar infarction significantly improved among the cohorts, but those of atherothrombotic and cardioembolic infarction did not. *Conclusions:* These data suggest that, in the Japanese population, the incidence of lacunar infarction steadily declined for the last 40 years. The improvement of hypertension control and decreasing prevalence of smoking might be responsible for this trend.

NEUROLOGY 2006;66:1539–1544

Stroke is the major cause of mortality and the third leading cause of death in Japan and in Western countries.[1] Ischemic stroke is the most common type of stroke in developed countries, and it can be further divided into several subtypes based on the size and location of the affected cerebral arteries and their pathogenesis: that is, lacunar (LI), atherothrombotic (ATI), and cardioembolic (CEI) infarction.[2] A few cohort studies in Japan, including ours, have shown that the incidence of ischemic stroke significantly declined in the 1970s, but that in recent years, the rate of decline has decreased.[3,4] As risk factors, prognosis, and treatment among subtypes of ischemic stroke are different,[5-7] it would be informative to examine trends in the incidence and long-term survival of ischemic stroke by subtypes to improve our understanding of its pathogenesis and assist in establishing preventive measures. However, there has been very little information on this issue, as the definitive classification of ischemic stroke into subtypes requires detailed clinical data, including information on the disease course, neurologic symptoms, and morphologic features.

The Hisayama study is a population-based study that has established three cohorts at times corresponding to periods of remarkable lifestyle changes in Japan.[3,8-10] In this study, study-team physicians performed physical and neurologic examinations on the subjects who developed stroke and collected detailed clinical information. Furthermore, morphologic examinations by autopsy or brain imaging were performed in most of the stroke cases in each cohort.[3,6] These characteristics of the study design enabled us to examine secular trends in the incidence and survival rate of ischemic stroke subtypes.

Methods. *Study population.* Hisayama Town is a suburban community adjacent to Fukuoka City, a metropolitan area on Kyushu Island in southern Japan. The population of the town has been stable for many years (annual variation rate <5%)[8] and has been shown to be representative of Japan as a whole on the basis of data from the national census.[8,10] The study design and characteristics of the subject population have been described in detail elsewhere.[8-10] In brief, we established three study cohorts from Hisayama residents age ≥40 years in 1961, 1974, and 1988 after screening examinations. In 1961, a total of 1,658 subjects in that age group consented to participate in the screening examination (participation rate 90.1%). After the exclusion of 28 subjects with

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Supported in part by a Grant-in-Aid for Scientific Research C (no. 13670370), a Special Coordination Fund for Promoting Science, and a Fund for Technology and Innovative Development Project in Life Sciences from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Disclosure: The authors report no conflicts of interest.

Received July 20, 2005. Accepted in final form January 31, 2006.

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a history of stroke or myocardial infarction and 12 subjects who died or moved out of town during the examination, 1,618 subjects were enrolled as the first cohort. In the same manner, we established the second cohort consisting of 2,038 subjects from 2,135 participants (participation rate, 81.2%) in 1974 and the third cohort of 2,637 subjects from 2,742 participants (participation rate, 80.9%) in 1988.

Follow-up. We followed up with each cohort for 12 years by repeated health examinations. Health status was checked every year by mail or telephone for any subjects who did not undergo a regular examination or who moved out of town. We also established a daily monitoring system organized by the study team, local physicians, and members of the local health and welfare office. When the subjects died, autopsy examinations were performed at the Department of Pathology of Kyushu University. During the follow-up period of each cohort, autopsy examinations were performed on 372 subjects (81.6% of the deceased subjects) in the first cohort, 342 subjects (86.2%) in the second cohort, and 366 subjects (75.5%) in the third cohort. Only two subjects in the first cohort, two in the second cohort, and one in the third cohort were lost to follow-up.

Definition of ischemic stroke subtype. The diagnosis of stroke was determined on the basis of clinical information and autopsy findings. In principle, stroke was defined as a sudden onset of nonconvulsive and focal neurologic deficit persisting for >24 hours and was classified as ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage, or undetermined type.[3] Two stroke neurologists reviewed all gathered information about stroke cases and made the diagnoses of ischemic stroke subtypes separately on the basis of the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke[2] as well as on the basis of the diagnostic criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) Study[11] and Cerebral Embolism Task Force.[12] Their diagnoses agreed in 94% of cases, and in the remaining cases, the diagnoses were determined by a detailed panel discussion. When sufficient clinical and morphologic information was obtained, a diagnosis of ischemic stroke subtype was defined as "definite." When the amount of either type of information was insufficient, the diagnostic level was defined as "probable."

Details of the diagnostic criteria of ischemic stroke subtypes have been described previously.[6] In brief, LI was diagnosed as the presence of a relevant brainstem or subcortical hemispheric lesion with a diameter of <1.5 cm demonstrated on brain imaging or autopsy and no evidence of cerebral cortical or cerebellar impairment. ATI was diagnosed when the subjects had significant stenosis (>50%) or occlusion of a major cerebral artery with infarct size ≥1.5 cm on brain imaging or autopsy. The diagnosis of CEI was made on the basis of primary and secondary clinical features suggestive of CEI as reported by the Cerebral Embolism Task Force.[12] The category of undetermined subtype (UND) included all ischemic stroke cases for which the subtype could not be determined because of insufficient clinical or morphologic information. We considered morphologic findings significant and used clinical features as reference information.

During the follow-up period of each cohort, first-ever ischemic stroke developed in 122 subjects (78 cases of LI, 26 of ATI, 13 of CEI, and 5 of UND) in the first cohort, 124 in the second cohort (67 of LI, 26 of ATI, 28 of CEI, and 3 of UND), and 137 in the third cohort (67 of LI, 37 of ATI, 33 of CEI, and 0 of UND). Among these, morphologic examinations by autopsy or brain imaging were performed on 110 patients (90.2%) in the first cohort, 118 (95.2%) in the second cohort, and 137 (100%) in the third cohort. In this study, we present the data regarding definite and probable ischemic stroke subtype cases together, as these combined data were almost identical to those for definite cases only.

Risk factors. Recumbent blood pressures were measured three times at every examination, and hypertension was defined as a mean systolic blood pressure of ≥140 mm Hg or a mean diastolic blood pressure of ≥90 mm Hg or a current use of antihypertensive agents. Glucose intolerance was defined by an oral glucose tolerance test in the subjects with glycosuria in 1961, by fasting and postprandial glucose concentrations in 1974, and by a 75-g oral glucose tolerance test in 1988, in addition to medical history of diabetes. Serum cholesterol levels were measured by the Zak-Henly method with a modification by Yoshikawa in 1961, by the Zurkowski method in 1974, and by the enzymatic method in 1988.[6] Hypercholesterolemia was defined as total cholesterol level of ≥6.2 mmol/L (240 mg/dL). Body height and weight were measured in light clothing without shoes, and obesity was defined as body mass index of ≥25.0 kg/m^2. Information on antihypertensive treatment, alcohol intake, and smoking habits was obtained with the use of a standard questionnaire and was categorized as current habitual use or not.

Statistical analysis. The incidence rates of ischemic stroke and its subtypes were calculated by the person-year method and adjusted for the age distribution of the World Standard Population by the direct method. The differences in the incidence among the three cohorts were tested by sex with the use of the Cox proportional hazards model after adjustment for age. Subjects who developed ischemic stroke were also followed up for the subsequent 5 years or to the end of the follow-up in every cohort, and survival rates were estimated with the Cox proportional hazards model. All statistical analyses were performed with the SAS program package. Values of $p < 0.05$ were considered significant in all analyses.

Results. *Trends in risk factors.* We compared the prevalence of cardiovascular risk factors at the baseline examination among the three cohorts by sex (table 1). In both sexes, the prevalence of hypertension was not different among the cohorts, but the proportion of individuals using antihypertensive agents consistently increased with time. As a result, among hypertensive subjects, mean blood pressures significantly decreased from the first to the third cohort in both sexes. The prevalence of glucose intolerance, hypercholesterolemia, and obesity increased progressively with time. The proportion of current smokers in both sexes and that of male drinkers declined linearly over the cohorts.

Trends in incidence of ischemic stroke subtype. The age-standardized incidence of ischemic stroke for men declined throughout the cohorts (table 2; $p < 0.05$). For women, the incidence also declined from the first to the second cohort ($p < 0.05$), but this declining trend was slowed between the second and third cohort. The age-standardized incidence of LI for men declined by 59% from the first to the second cohort ($p < 0.01$), and it continued to decline by 41% from the second to the third cohort ($p < 0.05$). The age-standardized incidence of LI for women also declined by 28% from the first to the second cohort, but the decline decelerated between the second and third cohort (15%). The age-standardized incidence of ATI declined by 41% from the first to the second cohort for both sexes, but the difference was not significant probably owing to the small number of events. The age-standardized incidence of ATI for women was slightly decreased in the third cohort (11%), but that for men was not. The age-standardized incidence of CEI did not change significantly among the cohorts for either sex.

The proportions of ischemic stroke subtypes among the cohorts by sex are shown in table 3. For men, the proportion of the subjects with LI steadily decreased from the first to the third cohort, whereas those of ATI and CEI increased. For women, the proportion of the subjects with CEI increased slightly from the first to the third cohort, but the proportions of the other subtypes were constant among the cohorts.

Trend in age-specific incidence of ischemic stroke subtype. The age-specific incidence rates of ischemic stroke subtypes for men and women combined among the three cohorts are shown in figure 1. The incidence of each subtype of ischemic stroke increased with advancing age in every cohort. The incidence of LI consistently decreased from the

Table 1 Prevalence of cardiovascular risk factors at baseline among three cohorts in 1961, 1974, and 1988 of the Hisayama study by sex

Variables	Men				Women			
	First cohort, n = 705	Second cohort, n = 855	Third cohort, n = 1,110	p for trend	First cohort, n = 913	Second cohort, n = 1,183	Third cohort, n = 1,527	p for trend
Age, y	55 ± 11	56 ± 11	57 ± 12	<0.001	57 ± 12	58 ± 12	59 ± 12	0.002
Hypertension, %	38.6	40.4	41.5	0.22	37.4	44.0	38.4	0.98
Antihypertensive agents, %	2.1	8.5	14.3	0.001	2.2	8.3	15.5	0.001
Systolic blood pressure,* mm Hg	161	158	152	<0.001	163	162	155	<0.001
Diastolic blood pressure,* mm Hg	91	87	84	<0.001	88	86	81	<0.001
Glucose intolerance, %	12.1	13.8	31.9	0.001	4.8	8.1	27.2	0.001
Hypercholesterolemia, %	1.7	5.3	14.9	0.001	3.2	9.6	25.9	0.001
Obesity, %	7.4	11.6	23.2	0.001	12.9	20.8	23.4	0.001
Current smoker, %	76.3	73.0	49.9	0.001	16.8	10.7	6.9	0.001
Current drinker, %	69.4	64.0	60.2	0.001	8.3	5.6	8.7	0.41

Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or a current use of antihypertensive agents. Hypercholesterolemia was defined as total cholesterol level ≥6.2 mmol/L (240 mg/dL). Obesity was defined as body mass index ≥25.0 kg/m^2.

* Mean systolic and diastolic blood pressures among hypertensive subjects in each cohort.

first to the third cohort mainly in the aged subjects. The incidence of ATI in the subjects age <80 decreased from the first to the second cohort but was unchanged in the third cohort. In contrast, the incidence of ATI remained high and showed no significant trend in the subjects age ≥80. The incidence of CEI showed no significant change in any age group.

Trends in survival of ischemic stroke subtype. Age- and sex-adjusted 5-year survival curves after ischemic stroke by its subtypes are shown in figure 2. The 5-year survival after LI was better than those after other subtypes and improved from the first (54%) to the third cohort (86%; $p < 0.05$). The 5-year survival after ATI tended to improve from the first (17%) to the second cohort (40%; $p = 0.08$) but showed no further improvement in the third cohort (40%). The 5-year survival after CEI was lowest among ischemic stroke subtypes and remained low throughout the study period (16% in the first, 24% in the second, and 26% in the third cohort).

Discussion. To our knowledge, this is the first report to examine secular trends in the incidence and survival rates of ischemic stroke by its subtype. Among three cohorts established at different times in a Japanese community, the incidence of LI declined significantly from the first to the third cohort, especially for men. The incidence of ATI tended to decline from the first to the second cohort, but it was sustained in the third cohort for both sexes. The incidence of CEI was unchanged throughout the study period. As a result, for men, the proportion of individuals with LI decreased from the first to the third cohort, and an opposite trend was observed for ATI and CEI. The 5-year survival rate after LI improved significantly among the cohorts, but those of ATI and CEI did not.

In our three cohorts, blood pressure levels were significantly decreased with time as a result of the

*Table 2 Age-standardized incidence rate (per 100,000 person-years) of ischemic stroke and its subtypes among three cohorts of the Hisayama study by sex, with a 12-year follow-up in each cohort**

	Men						*Women*					
	First cohort, 1961–1973		*Second cohort, 1974–1986*		*Third cohort, 1988–2000*		*First cohort, 1961–1973*		*Second cohort, 1974–1986*		*Third cohort, 1988–2000*	
	n	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*
Ischemic stroke	63	801	59	506*	60	357*†	59	450	65	304*	77	260*
Lacunar	44	559	28	229*	24	134*†	34	259	39	186	43	158*
Atherothrombotic	12	165	12	98	19	116	14	105	14	62	18	55*
Cardioembolic	6	67	18	169	17	107	7	57	10	47	16	47
Undetermined	1	10	1	10	0	0	4	29	2	9	0	0

* $p < 0.05$ vs first cohort; † $p < 0.05$ vs second cohort.

Table 3 Proportion of subjects with subtypes of ischemic stroke among three cohorts of the Hisayama study by sex

	Men						Women					
	First cohort, 1961–1973		Second cohort, 1974–1986		Third cohort, 1988–2000		First cohort, 1961–1973		Second cohort, 1974–1986		Third cohort, 1988–2000	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Lacunar	44	(69.9)	28	(47.5)	24	(40.0)	34	(57.6)	39	(60.0)	43	(55.8)
Atherothrombotic	12	(19.0)	12	(20.3)	19	(31.7)	14	(23.7)	14	(21.5)	18	(23.4)
Cardioembolic	6	(9.5)	18	(30.5)	17	(28.3)	7	(11.9)	10	(15.4)	16	(20.8)
Undetermined	1	(1.6)	1	(1.7)	0	(0.0)	4	(6.8)	2	(3.1)	0	(0.0)

sevenfold increment in the use of antihypertensive medication, though the prevalence of hypertension remained stable. The prevalence of smoking habits for men was 4.5-fold higher than that for women in the first cohort, and it decreased significantly for both sexes in the third cohort. Contrary to these declining trends of the risk factors, the prevalences of glucose intolerance, hypercholesterolemia, and obesity were greatly increased over the study period for both sexes. These changes in risk factors might have affected trends in the incidence of ischemic stroke subtype.

In our Japanese population, LI was the most common subtype of ischemic stroke, contrary to the previous reports of Western populations.[13,14] An autopsy study comparing small intracerebral arteriosclerosis between Japanese and Japanese American men demonstrated that small intracerebral artery lesions

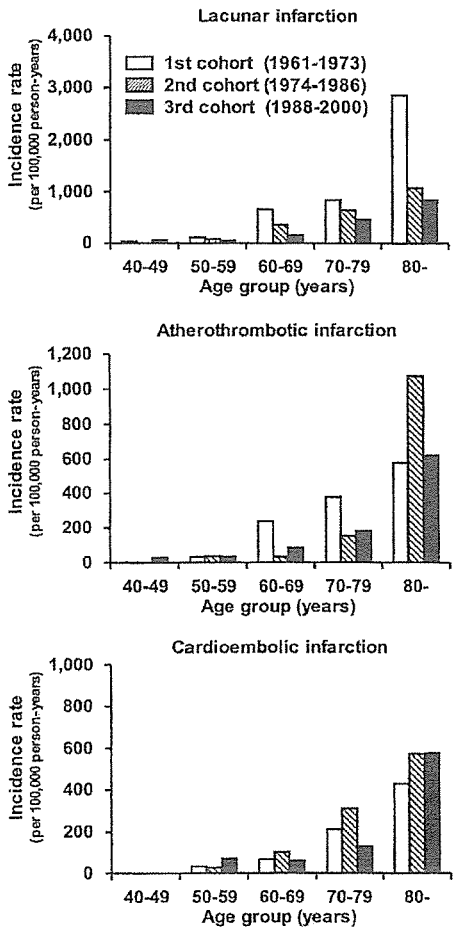

Figure 1. Age-specific incidence of ischemic stroke subtype of men and women combined among three cohorts of the Hisayama study, with a 12-year follow-up in each cohort.

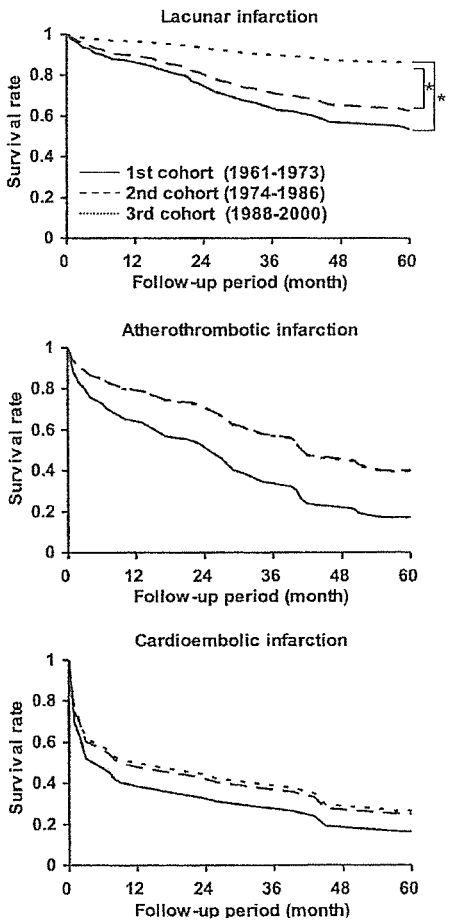

*Figure 2. Age- and sex-adjusted 5-year survival rates after ischemic stroke subtype among three cohorts of the Hisayama study. *p < 0.01.*

were more common in Japanese at every age.[15] Moreover, high blood pressure and a typical Asian diet were significantly associated with small intracerebral artery lesions.[15] The differences in race and lifestyle-related factors might contribute to the difference in the proportion of ischemic stroke subtypes between Japan and Western countries.

During the study period, the incidence of LI declined steeply, especially in men. The improvement of hypertension control and the decreasing prevalence of smoking may have been responsible for this finding. In contrast to the dynamic changes in the incidence of LI, the incidence of ATI has remained stable in recent years. One of the reasons for this finding may have been that the steep increase in metabolic disorders, such as glucose intolerance, dyslipidemia, and obesity, hindered the beneficial effects of the secular improvement of hypertension control and the cessation of smoking. Another possible reason is that hypertension control might be less effective for prevention of ATI. The Systolic Hypertension in the Elderly Program has also shown that the active treatment of hypertension significantly reduced the risk of LI, whereas such treatment appeared to have no effect on the occurrence of ATI.[16]

Despite the marked changes in cardiovascular risk factors among the cohorts, the incidence of CEI showed no significant change in this study. The effect of cardiovascular risk factors on the incidence of CEI was weaker than the effect on other subtypes.[6] In addition, the prevalence of atrial fibrillation, the most common risk factor for CEI, increased from 0.7% in the first cohort to 1.4% in the third cohort. These factors may have contributed to the sustained incidence of CEI. As a result of dynamic changes in risk factors, the proportion of ischemic stroke subtypes in our subjects has become closer to that of Western populations in recent years. However, it is important to note that this trend was caused not by the increase in the incidence of ATI and CEI, but by the steep decrease in the incidence of LI.

Consistent with previous studies,[17-19] we found that the 5-year survival rate was higher for LI, and lower for CEI in each cohort. Moreover, the survival rate improved significantly with time in the subjects with LI, but not in the subjects with ATI or CEI. Stroke is more severe in subjects with ATI and CEI than in those with LI. In addition, the incidence of coronary heart disease, a more common comorbidity in ATI and CEI,[19] is increasing among elderly individuals in Japan.[3] These factors may have contributed to the sustained low survival rate in ATI and CEI.

Our study had several possible limitations. First, the method for diagnosing stroke has been remarkably changed by the improvement of diagnostic techniques, and this may have affected the incidence rate.[20,21] It is possible that the decrease in the LI incidence could be artificial, that is, correspond to inclusion of the same patients into another category, for example, small deep infarction due to cardioembolism. In this study, however, methods for case ascertainment and the criteria for ischemic stroke subtypes were consistent among the cohorts, and the classification of ischemic stroke subtype was confirmed by detailed clinical and morphologic examination, the latter of which was performed in most of the ischemic stroke cases (90 to 100%). These facts make it unlikely that this bias invalidates the findings of the current study. Second, we established three cohorts independently in the same manner, but the subjects in later cohorts included many survivors of the former cohorts. This may have affected the development of stroke; however, we enrolled most of the unselected residents in every cohort, and the prevalence rate of cardiovascular risk factors in the third cohort was similar to that of the National Nutritional Survey of Japan.[3] Third, there were a small number of cases in each cohort, indicating a larger chance of bias in the results of this study. Nonetheless, we believe that the findings of our study represent precise secular trends, as we performed this study using a highly accurate method for determining all cardiovascular events.

Our findings indicate that correction of increasing metabolic disorders such as obesity, dyslipidemia, and glucose intolerance as well as strict management of hypertension have become more important to prevent ischemic stroke in contemporary Japanese individuals.

Acknowledgment

The authors thank the residents of Hisayama Town for their participation in the survey and the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study.

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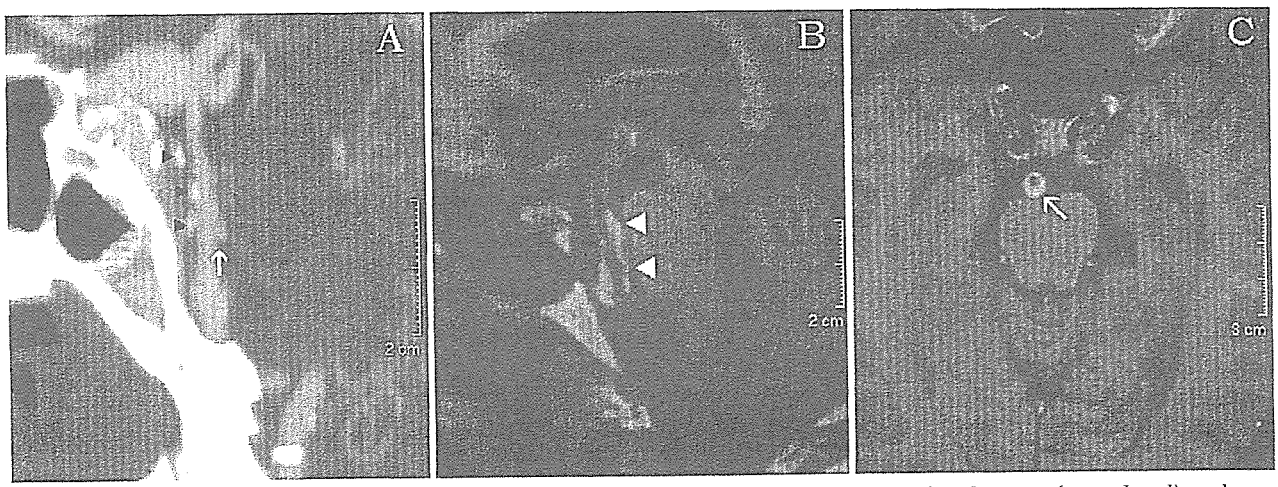

Figure. Sagittal computed tomographic angiography image depicts basilar artery (A) with atheroma (arrowhead) and proximal segment of dissection (arrow). T1-weighted images reveal clot in the atheromata (arrowheads, B) and T1 fat-suppressed image depicts circumferential clot in the vessel wall (arrow, C).

Intraplaque dissection of the basilar artery

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A 61-year-old right-handed man with hypertension, hyperlipidemia, and tobacco abuse presented with sudden dysarthria, left hemiparesis, and hemianesthesia. Examination also revealed left hemiataxia and hemianopsia. MRI revealed multiple acute infarctions in the right posterior cerebral artery territory. Magnetic resonance angiography revealed a narrowed and irregular basilar artery. Computed tomographic angiography demonstrated extensive calcific atherosclerotic changes with an intraluminal filling defect in the mid-basilar artery (figure). Fat-suppressed axial T1-weighted images confirmed intraplaque dissection (figure); T2 images showed low signal consistent with subacute intraplaque clot.

MRI can characterize complicated atheroma and distinguish intraplaque from juxtaluminal thrombosis in the anterior circulation.[1,2] In this case, CT and MRI were complementary for the characterization of the symptomatic lesion and helped guide choice of antithrombotic therapy.

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Disclosure: The authors report no conflicts of interest.

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PRKCH(タンパク質キナーゼCη)の非同義置換SNPが脳梗塞の危険度を増す

脳梗塞は、その発症や進展に複数の環境要因と遺伝要因が複雑に組み合わさって関与する多因子疾患である。これまでに多くの脳梗塞の候補遺伝子が検討されているが、残念ながらその遺伝要因については未だほとんど解明されていない。本研究では、脳梗塞関連遺伝子を探索する目的で、九州大学病院を含む7つの関連施設を受診した脳梗塞患者1,126例と2002-03年に行われた久山町ゲノム疫学研究参加者のうち性・年齢を対応させた同数の者の血液サンプルを用いて、ゲノムワイド関連解析を行った。2段階のスクリーニングにより脳梗塞に関連する12ヶ所の候補領域を同定した。ラクナ梗塞において有意な関連を示したマーカーSNPについてfine mapping、LD解析を用いて検討した所、Protein kinase C-eta (PKCη) をコードする遺伝子PRKCHを脳梗塞関連遺伝子として新たに同定した。PRKCH遺伝子内には374番目のアミノ酸がバリンからイソロイシンに変わるSNP (1425G/A) があり、このSNPはラクナ梗塞と有意な関連を示した ($p=9.84 \times 10^{-6}$、オッズ比1.66)。また、バイオバンクジャパンに登録されているラクナ梗塞1,137例と対照群1,875例においても同様の関連が認められた($p=9.89 \times 10^{-4}$)。このアミノ酸置換はATP結合部位に位置しており、イソロイシン型のPKCηはバリン型に比べPKC活性が1.6倍高かった。久山町剖検例の病理学的検討では、PKCηは血管内皮細胞および泡沫化したマクロファージに発現しており、その程度は動脈硬化の重症度と強く相関していた。さらに、1988年の健診を受診した久山町住民1,642

人を 14 年間追跡し、この SNP と脳梗塞発症との関連を検討した所、SNP が AA の群は GG 群に比べ脳梗塞発症率が有意に高かった（p=0.03、ハザード比 2.83）。以上の結果より、PRKCH は日本人の脳梗塞関連遺伝子であり、遺伝子内のアミノ酸置換を伴う SNP は脳梗塞発症の遺伝的危険因子であると考えられた。

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A nonsynonymous SNP in *PRKCH* (protein kinase C η) increases the risk of cerebral infarction

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Cerebral infarction is the most common type of stroke and often causes long-term disability. To investigate the genetic contribution to cerebral infarction, we conducted a case-control study using 52,608 gene-based tag SNPs selected from the JSNP database. Here we report that a nonsynonymous SNP in a member of protein kinase C (PKC) family, *PRKCH*, was significantly associated with lacunar infarction in two independent Japanese samples ($P = 5.1 \times 10^{-7}$, crude odds ratio of 1.40). This SNP is likely to affect PKC activity. Furthermore, a 14-year follow-up cohort study in Hisayama (Fukuoka, Japan) supported involvement of this SNP in the development of cerebral infarction ($P = 0.03$, age- and sex-adjusted hazard ratio of 2.83). We also found that PKCη was expressed mainly in vascular endothelial cells and foamy macrophages in human atherosclerotic lesions, and its expression increased as the lesion type progressed. Our results support a role for *PRKCH* in the pathogenesis of cerebral infarction.

Stroke is a major cause of long-term disabilities, leading to very serious public health issues. Once a stroke has occurred, most affected individuals suffer from disability, cognitive dysfunction and other complications and have a higher risk of death[1]. In Japan, stroke mortality rate has decreased significantly in the last three decades, but the incidence of stroke has remained high in recent years, especially in the elderly[2]. As the proportion of elderly individuals is increasing rapidly worldwide, primary prevention of stroke is becoming an important medical and social issue requiring urgent attention.

Cerebral infarction is the most common form of stroke and is classified into the following subtypes based on clinical and neuroimaging data: lacunar infarction due to presumed arteriosclerosis of small penetrating arteries, atherothrombotic infarction due to atherosclerosis involving the external and major intracranial arteries, cardioembolic infarction due to a cardiac source of the embolus and undetermined subtype[3]. Twin- and family-based studies indicate a

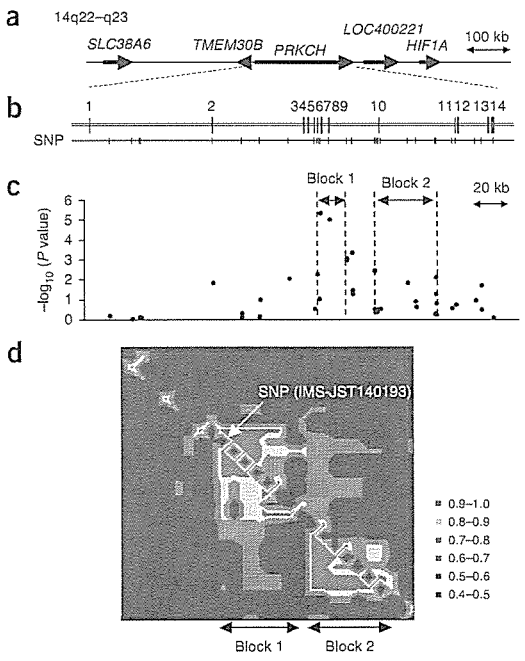

Figure 1 Genomic structure, case-control association results and linkage disequilibrium map of the *PRKCH* locus. (**a**) Genomic structure around *PRKCH*. (**b**) Exon-intron structure of *PRKCH*. Genotyped SNPs in *PRKCH* are indicated below the gene (vertical line). (**c**) Case-control association study results for lacunar infarction. The $-\log_{10}$-transformed *P* values for an allele frequency model are plotted on the *y* axis. (**d**) Pairwise linkage disequilibrium map between SNPs, as measured by D′ (lower left) and Δ (upper right).

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Received 26 July 2006; accepted 27 November 2006; published online 7 January 2007; doi:10.1038/ng1945

Table 1 Case-control study showing association between nonsynonymous SNP 1425G/A in *PRKCH* and cerebral infarction

	Case				Control				Minor allele frequency		*P* value (adjusted *P*)		Odds ratio (95% c.i.)	
Samples	AA	AG	GG	Sum	AA	AG	GG	Sum	Case	Control	A vs. G	AA+AG vs. GG	A vs. G	AA+AG vs. GG
Screening														
Cerebral infarction	57	390	662	1,109	40	332	724	1,096	0.227	0.188	1.31×10^{-3}	1.98×10^{-3}	1.27 (1.10–1.47)	1.31 (1.11–1.56)
Lacunar	27	178	286	491	11	130	344	485	0.236	0.157	9.84×10^{-6} (0.0004)	3.47×10^{-5} (0.0009)	1.66 (1.33–2.09)	1.75 (1.34–2.28)
Atherothrombotic	14	132	220	366	16	108	238	362	0.218	0.194	0.234	0.115	1.17 (0.90–1.50)	1.27 (0.94–1.72)
Cardioembolic	7	39	90	136	9	48	77	134	0.195	0.246	0.150	0.141	0.74 (0.49–1.11)	0.69 (0.42–1.13)
Undetermined	9	41	66	116	4	46	65	115	0.254	0.235	0.625	0.952	1.11 (0.73–1.70)	0.98 (0.59–1.66)
BioBank Japan														
Lacunar	56	416	665	1,137	81	575	1,219	1,875	0.232	0.197	9.89×10^{-4}	3.34×10^{-4}	1.24 (1.09–1.40)	1.32 (1.13–1.54)

Adjusted *P* values were obtained from case-control samples with 10^4 permutation tests in lacunar infarction. c.i.: confidence interval.

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role for genetic factors in cerebral infarction, although their contribution is not very large[4]. There have been several approaches to identifying genetic variants associated with susceptibility to common diseases[5]. With the availability of a large volume of SNP information and large-scale genotyping methods, genome-wide association studies have successfully identified genetic variants associated with susceptibility to common diseases such as myocardial infarction[6], rheumatoid arthritis[7,8] and Crohn disease[9].

In regard to the genetic risk for stroke, *PDE4D* has been reported as a candidate for cerebral infarction through a genome-wide linkage study[10]. Here we report identification of *PRKCH* as a candidate risk locus for cerebral infarction through a case-control study by means of large-scale gene-based SNP analysis. We replicated this association in independent samples from BioBank Japan and confirmed it by a 14-year population-based follow-up study.

To identify variants associated with susceptibility to cerebral infarction, we performed a genome-wide case-control study using 1,112 Japanese individuals with cerebral infarction and 1,112 age- and sex-matched controls. First, we genotyped 188 individuals with cerebral infarction and 188 age- and sex-matched controls using 52,608 gene-based tag SNPs selected from the JSNP database[11]. We compared allele frequencies of 48,083 successfully genotyped SNPs (overall success rate of 91.4%) between the two groups and identified 1,098 SNPs showing *P* values of < 0.01. In a second round of screening, we genotyped the remaining cases and controls for these SNPs. As the subjects included subtypes of cerebral infarction, we also analyzed data by subgroups. Through this analysis, we found that SNP_15 in *PRKCH* (IMS_JST140193) was strongly associated with lacunar infarction ($P = 4.73 \times 10^{-6}$ for allele frequency model). We did not find any significant association of this SNP with atherothrombotic infarction, probably because of the small number of subjects. SNP_15 retained a significant association with lacunar infarction ($P = 0.0036$) after a permutation test. We therefore concluded that the susceptibility locus for lacunar infarction was likely to be in a region including *PRKCH*.

We subsequently attempted to construct a fine linkage disequilibrium (LD) map of the *PRKCH* locus, and we defined the region showing a strong association with lacunar infarction. We genotyped 45 SNPs among 491 cases with lacunar infarction and age- and sex-matched controls (**Supplementary Table 1** online), and we constructed a high-resolution LD map consisting of 27 SNPs that had minor allele frequencies of >0.2 (**Fig. 1**). Of two LD blocks defined, SNP_15 was located within block 1, and the association with lacunar infarction peaked at SNPs located in block 1, suggesting that *PRKCH* is the most likely candidate for harboring variants associated with susceptibility to lacunar infarction. We further sequenced all exons in *PRKCH* using DNA from 48 affected individuals and 48 controls, and we identified four SNPs: 695A/G in exon 2 (rs3742633), 1425G/A in exon 9 (rs2230500), 1427A/C in exon 9 (rs2230501) and 1979C/T in exon 12 (rs1088680). Among them, the first and the last SNPs were not located within block 1 and showed no association. We considered the remaining two SNPs to be in absolute LD from the DNA sequence results. The 1425G/A SNP caused an amino acid substitution (V374I). We then genotyped the 1425G/A SNP for all cases and controls by direct sequencing, and we confirmed its significant association with lacunar infarction ($P = 9.84 \times 10^{-6}$, odds ratio (OR) = 1.66; 95% confidence interval (c.i.) = 1.33–2.09 for allele frequency model; **Table 1**). We replicated this association in an independent case-control

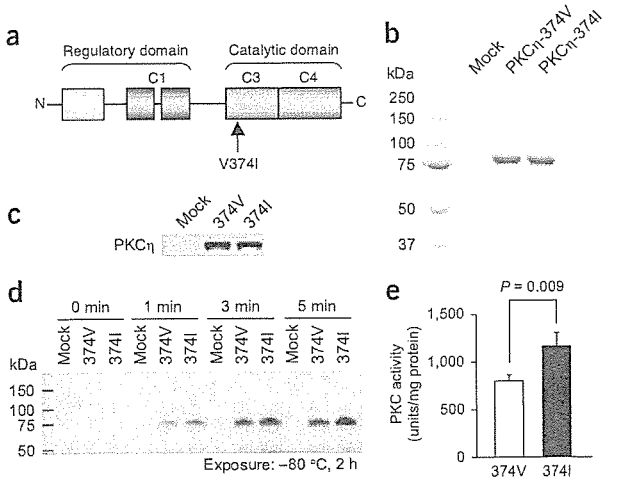

Figure 2 Comparison of the PKC activity of PKCη-374V and PKCη-374I. (**a**) Domain structure of *PRKCH*. Arrow indicates the position of 1425G/A. (**b**) Immunoprecipitates of mock, PKCη-374V and PKCη-374I by Coomassie brilliant blue staining. (**c**) Protein blotting using equal amounts of immunoprecipitates of mock, PKCη-374V and PKCη-374I. (**d**) Autophosphorylation assay of mock, PKCη-374V and PKCη-374I after stimulation with 10 μM phosphatidylserine and 100 nM PDBu. (**e**) PKC activity of PKCη-374V and PKCη-374I using myelin basic protein peptide as a substrate after 3-min stimulation with 10 μM phosphatidylserine and 100 nM PDBu.

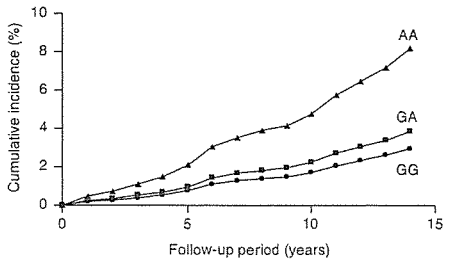

Figure 3 Age- and sex-adjusted cumulative incidence of cerebral infarction by nonsynonymous SNP 1425G/A in *PRKCH* (leading to amino acid substitution V374I) during a 14-year follow-up period in the Hisayama study.

group of 1,137 cases with lacunar infarction and 1,875 controls selected from BioBank Japan project ($P = 9.89 \times 10^{-4}$, OR of 1.24, with 95% c.i. of 1.09–1.40). Combined analysis of two case-control samples showed that the A allele was significantly associated with lacunar infarction under a dominant model, with an OR of 1.40 (95% c.i., 1.23–1.59, $P = 5.1 \times 10^{-7}$). Although the association was slightly stronger at SNP_15 than at 1425G/A SNP, these SNPs were in almost absolute LD ($D' = 0.99$, $\Delta^2 = 0.98$). Therefore, either one or a combination of these SNPs in block 1 might have functional significance by altering the quality or quantity of the gene product.

To further clarify an independent effect of these SNPs, we performed multivariate analysis with adjustment for clinical risk factors using a conditional logistic regression model in 491 individuals with lacunar infarction along with age- and sex-matched controls. The genotypic risk for lacunar infarction was substantially unchanged after adjustment for age, sex, hypertension, hyperlipidemia and diabetes (**Supplementary Table 2** online). We also evaluated the impact of population stratification on all 1,112 individuals with cerebral infarction, along with age- and sex-matched controls using STRUCTURE[12] and did not find any significant population stratification (**Supplementary Fig. 1** online).

Among the SNPs in block 1, a 1425G/A SNP (leading to V374I) is located within an ATP-binding site of PKCη (ref. 13). Therefore, we first examined the effect of V374I on PKCη kinase activity (**Fig. 2**). We constructed expression vectors of Flag-tagged PKCη corresponding to a valine-encoding allele (Flag-PKCη-374V) and to an isoleucine-encoding allele (Flag-PKCη-374I). We transfected these vectors to 293T cells and immunoprecipitated the Flag-tagged proteins. We subjected equal amounts of immunoprecipitates to SDS-PAGE and examined the purity of the immunoprecipitates by Coomassie brilliant blue staining. We also checked the amount of the two forms of Flag-PKCη by protein blot analysis (**Fig. 2b,c**). As PKCη was reported to be activated by autophosphorylation[14], we examined the kinase activity of these two proteins by autophosphorylation assay. After stimulation with 10 μM phosphatidylserine and 100 nM phorbol 12,13-dibutyrate, we observed autophosphorylation of PKCη 1 min later; the degree of autophosphorylation was higher for PKCη-374I than for PKCη-374V (**Fig. 2d**). To further confirm these results, we examined PKC activity using myelin basic protein as a substrate and found that PKCη-374I has 1.6 times the activity of PKCη-374V ($P = 0.009$, Student's *t*-test; **Fig. 2e**). These results suggest that the amino acid substitution of V374I in PKCη results in higher autophosphorylation and kinase activity after stimuli and activates its signaling pathway.

Replication of the association using different populations is critical. However, the minor allelic frequencies of SNP_15, which is in absolute LD with the candidate 1425G/A SNP, were reported in the HapMap database as 0.239 in Japanese in Tokyo, 0.178 in Han Chinese in Beijing, 0.008 in CEPH samples (Utah residents with ancestry from northern and western Europe) and 0.00 in Yoruba from Ibadan, Nigeria. As these data suggest that this candidate SNP is likely to be specific to Asian populations, we attempted to confirm the association of this SNP using a population-based prospective cohort[2] established in 1988. During a 14-year follow-up period, 67 individuals experienced their first cerebral infarction (42 cases of lacunar infarction, 18 cases of atherothrombotic infarction and 7 cases of cardioembolic infarction) among 1,642 subjects without a history of stroke at baseline examination. The age- and sex-adjusted cumulative incidence of cerebral infarction was 2.96% in the GG genotype, 3.86% in GA and 8.18% in the AA (**Fig. 3**), and we found a significant difference between the GG and AA genotypes ($P = 0.030$, age- and sex-adjusted hazard ratio of 2.83, with a 95% c.i. of 1.11–7.22; **Table 2**). This relationship remained significant even after adjustment for baseline clinical risk factors. We estimated the population attributable risk of the AA genotype to be 30 per 100,000 person-years in this cohort (population attributable risk percentage of 10.1%). We also examined the impact of the 1425G/A SNP on the development of lacunar infarction in particular. A Kaplan-Meier estimate showed similar results to those seen for cerebral infarction, but the differences among the genotypes were not significant, probably owing to the small number of events (**Supplementary Fig. 2** online). In addition, when we examined the effect of the 1425G/A SNP on the development of coronary heart disease among 1,661 subjects without a history of coronary heart disease, we found a similar result ($P = 0.024$, age- and sex-adjusted hazard ratio of the AA genotype was 3.31 (95% c.i., 1.17–9.36) compared with the GA and GG genotype combined; **Supplementary Fig. 3** online). These findings indicate that the 1425G/A SNP is a common genetic risk factor for the development of atherosclerotic diseases.

Table 2 Hazard ratios for the incidence of cerebral infarction

			Age- and sex-adjusted			Multivariate-adjusted		
Genotype of nonsynonymous SNP 1425G/A	Total number of subjects	Number of cerebral infarctions	Hazard ratio	95% c.i.	*P* value	Hazard ratio	95% c.i.	*P* value
GG	1,063	39	1.00			1.00		
AG	518	23	1.31	0.78–2.19	0.309	1.31	0.78–2.20	0.317
AA	61	5	2.83	1.11–7.22	0.030	2.91	1.14–7.47	0.026
GG+AG	1,581	62	1.00			1.00		
AA	61	5	2.58	1.03–6.44	0.043	2.66	1.06–6.68	0.038

Multivariate analysis was performed with adjustment for age, sex, hypertension, diabetes, cholesterol and smoking and drinking habits. c.i.: confidence interval.

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