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Moderate Atheroma of the Aortic Arch and the Risk of Stroke

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Key Words
Aorta · Cardiovascular diseases · Cerebral infarction · Echocardiography

Abstract

Background and Purpose: Severe atheroma ≥4 or 5 mm of the aortic arch is a risk factor for stroke. We investigated the most predictive characteristics of arch atheroma, including maximal plaque thickness, for subsequent cardiovascular events, and also examined whether moderate atheroma <4 mm is a risk of cerebral emboli. ***Methods:*** The maximal plaque thickness (MPT) and plaque morphologies of the aortic arch were evaluated by transesophageal echocardiography in 236 patients with ischemic stroke. We assessed the relationship between the incidence of cardiovascular events, recurrent stroke or myocardial infarction, and the characteristics of the atheroma. We also investigated the thickness of atheroma in patients with known causes of stroke (n = 148) and in patients with undetermined causes (n = 19). ***Results:*** Cardiovascular events occurred in 47 patients in the follow-up period with a mean of 3.5 years. MPT was a significant risk factor of the cardiovascular events, although plaque morphologies were not. For the receiver operator characteristics curve analysis, the suitable cutoff point of MPT associated with the cardiovascular events was 3.5 mm. Patients with MPT ≥3.5 mm had a higher risk of cardiovascular events than did those with MPT <3.5 mm. In addition, aortic atheroma with MPT ≥3.5 mm was more frequently observed in patients with undetermined causes of stroke than those with known causes at 68 vs. 39% (p = 0.024). ***Conclusions:*** MPT ≥3.5 mm is the best predictor of subsequent cardiovascular events and a possible cause of embolic stroke.

Aortic arch atheroma in patients with stroke, which is generally diagnosed using transesophageal echocardiography (TEE), is one of embolic sources. Several studies indicated that aortic arch atheroma is an important independent risk factor for stroke or vascular events. In these studies, thick atheroma, the presence of ulcerative or mobile plaques along the aortic arch or plaque extension to the branches was found to be associated with stroke [1–9]. However, the definition of thick atheroma related to ischemic stroke varies among these studies. Amarenco et al. [1] reported in a prospective case control study that the odds ratio for cerebral infarction increased with plaque thickness, especially for a thickness of 4 mm or more. Tunick et al. [4] reported in a prospective case control study that protruding ≥5 mm or mobile atheroma in the aortic arch was a predictor of vascular events. Toyoda et al. [8] defined aortic atheroma >3 mm with an irregular surface or broad acoustic shadow to be an echographi-

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cally significant lesion and showed that it has high sensitivity and specificity for permitting detection compared with histopathologically complicated lesions. However, most of these studies defined severe atheroma as a maximal intima-media thickness ≥4 mm.

Patients with embolic stroke may have only aortic atheroma less than 4 mm in thickness and no other embolic sources such as atrial fibrillation, right-left shunt or stenosis of the extracranial or intracranial artery. Even moderate atheroma less than 4 mm in thickness may be a possible risk of stroke. None of the previous studies evaluated the detailed thickness of aortic arch atheroma as a risk of stroke. The aims of our study were to determine the characteristics of arch atheroma including the plaque thickness, which have the most predictive value for the occurrence of ischemic stroke and other vascular events in patients with ischemic stroke, and to evaluate the clinical importance of these plaques as possible sources of cerebral emboli.

Table 1. Clinical diagnoses of the study patients

Clinical diagnosis	
Cardiogenic	81 (34.3)
Atherothrombotic	45 (19.1)
Lacunar	22 (9.3)
TIA	54 (22.9)
Other causes of stroke	34 (14.4)
Dissection of cerebral artery	7
Coagulopathy	2
Complication of conventional angiography	1
Moyamoya disease	1
Unknown	23
No definitive embolic source in the heart, ipsilateral carotid artery or ipsilateral intracranial artery	19

Figures in parentheses are percentages.

Methods

Between January, 1997, and July, 1999, we performed TEE in 296 Japanese stroke patients, all of whom were admitted into our institute, for extracranial embolic sources such as intracardiac thrombus and complicated atherosclerotic lesions in the aortic arch. Sixty (20.3%) of them were excluded from the study, since they were immediately lost on follow-up (n = 49) or full data were not available (n = 11). The study group comprised 236 patients, 163 men and 73 women with 64.3 ± 12.0 (mean ± SD) years of age.

We performed contrast TEE using a commercially available Model SSD-2200 real-time two-dimensional echocardiography system (Aloka, Tokyo) equipped with a 5.0-MHz phased array omniplane transesophageal transducer variable from 3.5 to 7.5 MHz. We evaluated the maximal plaque thickness of the aortic arch in the short-axis view (MPT), the presence of any mobile, ulcerative or calcified plaque along the aortic arch, and right-left shunts using TEE. Ulcerative plaque was defined to be the presence of surface defects showing a depth and length of 2 mm or more. Calcified plaque was defined to be focal increase in echo density within the aortic plaque combined with a broad acoustic shadow. Regarding right-left shunts, we first inspected the left atrium for debris appearing in its inside during the Valsalva maneuver and after release of the maneuver without any contrast medium. Next, the contrast medium, the mixture of 9 ml saline and 1 ml air, was infused into the right antecubital vein during the Valsalva maneuver. When the right atrium was opacified by the contrast medium as seen on the monitor, we asked patients to release the Valsalva maneuver. When contrast medium different from the debris was found in the left atrium after the release of the Valsalva maneuver, we diagnosed it to be the sign of right-left shunt.

The information obtained at the time of baseline TEE for each patient included their age, gender, referring diagnosis, history of hypertension, diabetes, hyperlipidemia, heart diseases of potential embolic sources such as atrial fibrillation, congestive heart failure, cardiopathy or valvular diseases, and finally carotid stenosis and intracranial artery stenosis. Carotid stenosis was evaluated by ultrasound sonography using a Model Sonos 5500 duplex color-coded ultrasonographic device (Philips Medical Systems, Massachusetts) equipped with a 7.5-MHz transducer, and intracranial stenosis was evaluated by magnetic resonance angiography or digital subtraction angiography. The percentage stenosis of arterial diameter was calculated by dividing the narrowest linear diameter at the stenotic segment by the distal diameter at the normal-looking vessel. In all patients, the follow-up information comprised the occurrence of cardiovascular events such as recurrent ischemic stroke or myocardial infarction during the period after TEE, therapeutic variables including anticoagulation or antiplatelet agents, and the occurrence and cause of death. The follow-up information was obtained by reviewing the patients' medical records until December, 2002 (n = 206) or by telephone interview (n = 30). When patients could not come to our hospital for more than 3 months, such as because of their removal or discontinuation of visits to our outpatient clinic, we determined their status by telephone interview with them or their family to ensure the survival of the patients. The mean follow-up period was 3.5 years (42 ± 13 months).

We investigated the relationship between the characteristics of the aortic arch atheroma and the occurrence of cardiovascular events, using two-tailed t tests for the comparison of means. For the comparison of proportions, we used χ^2 tests, which were replaced by Fisher's exact tests when the expected cell count was <5. We also used Cox proportional hazards analysis to assess the contribution of clinical and echocardiographic variables to the development of cardiovascular events. Receiver operator characteristics (ROC) curve analysis was used to establish a suitable cutoff point of MPT which was related to the cardiovascular events, cumulative event-free rates for the time until a cardiovascular event occurred were estimated by the Kaplan-Meyer product limit method, and the 2 groups with MPT cutoff points were compared by the log-rank test.

Table 2. Univariate analyses comparing patients with and without cardiovascular events

	Total (n = 236)	Cardiovascular event (n = 47)	No event (n = 189)	p value
Age, years	64.3 ± 12.0	68.6 ± 11.0	63.2 ± 12.0	0.006
Gender (male/female)	163/73	28/19	135/54	0.12
Atrial fibrillation	89 (38)	26 (45)	63 (33)	0.15
Right-left shunt	60 (25)	9 (19)	51 (27)	0.25
Diabetes	69 (29)	13 (28)	56 (28)	0.79
Hypertension	156 (66)	35 (74)	121 (83)	0.18
Hyperlipidemia	106 (45)	25 (53)	81 (53)	0.21
Carotid stenosis ≥ 50%	37 (16)	11 (23)	26 (14)	0.10
Cranial artery stenosis ≥ 50%	47 (20)	12 (26)	35 (19)	0.28
Antiplatelet therapy	134 (57)	30 (64)	104 (55)	0.28
Anticoagulation therapy	100 (42)	18 (38)	82 (43)	0.53
Characteristics of aortic atheroma				
MPT, mm	3.2 ± 1.7	3.8 ± 1.8	3.0 ± 1.6	0.009
Ulcerative plaque	24 (10)	8 (17)	16 (8)	0.08
Calcified plaque	15 (6)	3 (6)	12 (6)	0.99
Mobile component	4 (2)	1 (2)	3 (2)	0.99

Figures in parentheses are percentages.

Following this, we also investigated the relationship between the characteristics of aortic arch atheroma in patients with a known cause of cerebral infarction and in those with an undetermined cause. A patient with cerebral infarction of known cause was defined to be one who was diagnosed to have lacunar infarction, cardioembolic infarction or atherothrombotic infarction on the basis of typical onset, topography, the size of the infarct, and known risk factors [10, 11]. Patients with transient ischemic attack (TIA) were excluded. A patient with cerebral infarction of undetermined cause was defined to be one who was not classified into lacunar infarction, cardioembolic infarction, atherothrombotic infarction or TIA, had no heart disease as a potential embolic source, including atrial fibrillation, patent foramen ovale, congestive heart failure, cardiopathy or valvular diseases, or had no ipsilateral carotid stenosis of 30% or more, ipsilateral intracranial artery stenosis or coagulopathy (table 1). Two-tailed t tests were used for the comparison of means and Fisher's exact test was for the comparison of proportions. Multiple logistic-regression analysis was performed to compare the prevalence of aortic arch atheroma in patients with cerebral infarction of known cause with that in patients with cerebral infarction of undetermined cause. The data were analyzed using StatView software, where a probability value <0.05 was considered to indicate statistical significance.

Results

There were no significant differences in age, gender, cardiovascular risk factors, echocardiographic variables, and therapeutic variables between the study group and the patients who were immediately lost on follow-up.

Table 3. Multivariate analysis used to predict cardiovascular events (n = 236)

Variable	HR	95% CI	p value
Age	1.04	1.00–1.07	0.03
Gender (female)	1.75	0.96–3.15	0.06
MPT	1.07	0.87–1.29	0.51
Ulcerative plaque	1.37	0.59–2.56	0.45
Carotid stenosis ≥ 50%	1.48	0.69–3.14	0.30

Clinical characteristics of the study group are shown in table 2. Cardiovascular events occurred in 47 patients at 5.2%/person-years for 44 with recurrent stroke and 3 with myocardial infarction. MPT was <3.0 mm in 107 patients, 3.0–3.4 mm in 32 patients, 3.5–3.9 mm in 26 patients, 4.0–4.4 mm in 27 patients, 4.5–4.9 mm in 15 patients, and ≥ 5.0 mm in 29 patients. Increased MPT was a significant risk factor for cardiovascular events (table 2). Ulcerative, calcified, and mobile plaques were found in 24 (10.2%), 15 (6.4%), and 4 (1.7%) patients, respectively, but such plaque morphologies were not related to the cardiovascular events. Risk factors for cardiovascular events with $p < 0.15$ on univariate analyses were entered into a multivariate Cox proportional hazard model. Age was the only independent factor which contributed to the cardiovascular events (table 3), and MPT and age showed

Table 4. Multivariate analysis used to predict cardiovascular events in elderly patients with 65 years of age or older (n = 130)

Variable	HR	95% CI	p value
MPT ≥3.5 mm	2.40	1.07–5.38	0.03
Age	1.08	1.01–1.15	0.03
Gender, female	1.98	0.98–4.01	0.06

Table 5. Univariate analyses comparing stroke patients with known causes and those with undetermined causes

	Known (n = 148)	Undetermined (n = 19)	p value
Age, years	65.0 ± 10.5	67.2 ± 11.6	0.38
Gender, male/female	98/50	14/5	0.61
MPT ≥3.5 mm	57 (39)	13 (68)	0.02
Ulcerative plaque	13 (9)	1 (5)	0.99
Calcified plaque	9 (6)	1 (5)	0.99
Mobile component	2 (14)	0 (0)	0.99

Figures in parentheses are percentages.

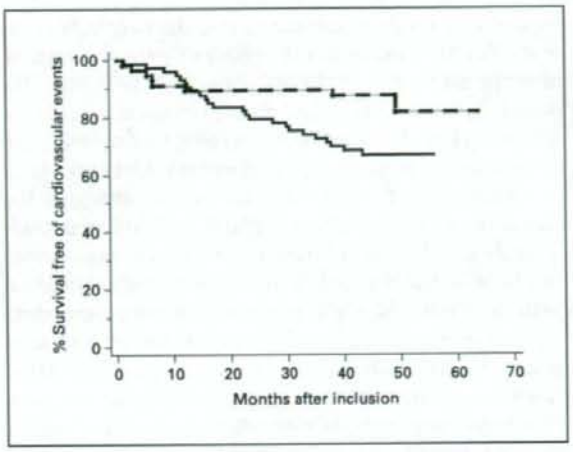

Fig. 1. Kaplan-Meyer curves showing that a significantly lower proportion of patients with MPT ≥3.5 mm (solid line) remained free of new cardiovascular events (p = 0.02, log-rank test) in elderly patients aged 65 years old or more (n = 130). The dashed line indicates MPT <3.5 mm.

a strong correlation by using quadratic regression analysis ($p < 0.001$). In the ROC curve analysis obtained using the cardiovascular event rates of each 0.5 mm MPT, the suitable cutoff point of MPT was 3.5 mm with a sensitivity of 57% and specificity of 63%. Patients with MPT ≥3.5 mm had a higher risk of cardiovascular events (hazard ratio [HR], 2.10; 95% confidence interval [CI], 1.18–3.74; p = 0.012). Next we focused on the subgroup of 130 patients with 65 years of age or older, in which 32 developed cardiovascular events (29 with recurrent ischemic stroke and 3 with myocardial infarction). In these elderly patients, MPT ≥3.5 mm was a significant risk factor for cardiovascular events even after adjusting for age and sex (HR, 2.40; 95% CI, 1.07–5.38; p = 0.034; table 4). Kaplan-Meyer curve analysis revealed a significant difference in the event-free survival between elderly patients with atheroma of MPT ≥3.5 mm and those without (fig. 1).

There were 148 patients with 65.0 ± 10.5 years of age composed of 98 men and 50 women with a known cause of stroke who were diagnosed to have cardioembolic infarction (81 patients), atherothrombotic infarction (45 patients) or lacunar infarction (22 patients), and 19 patients with 67.2 ± 11.6 years of age composed of 14 men and 5 women with undetermined causes of embolic stroke who had no heart diseases as a potential embolic source, ipsilateral carotid stenosis, ipsilateral intracranial artery stenosis or coagulopathy at the time of baseline TEE. In the group with undetermined causes, the MPT was <3.0 mm in 5 patients, 3.0–3.4 mm in 1, 3.5–3.9 mm in 5, 4.0–4.4 mm in 3, 4.5–5.0 mm in 2, and ≥5.0 mm in 3 patients. Aortic arch atheroma with MPT ≥3.5 mm was more frequently observed in patients with undetermined causes of stroke than in those with known causes at 68 versus 39%, and the difference between the two groups remained significant after adjusting for age and sex (p = 0.024; table 5). These two groups were not significantly different in terms of age and sex. When aortic arch atheroma was defined by another cutoff point, such as ≥3.0, ≥4.0, ≥4.5 or ≥5.0 mm, the significant difference between the two groups disappeared.

Discussion

Severe atheroma ≥4 or ≥5 mm along the aortic arch is associated with a high risk of subsequent vascular events, as shown in a study by the French Study of Aortic Plaques in Stroke Group [2], Tunick et al. [4], and several other studies. Our follow-up study showed that moderate atheroma between 3.5 and 4.0 mm also has an in-

creased risk of subsequent cardiovascular events. No other studies have examined the effect of critical thickness of aortic atheroma on the occurrence of cardiovascular events. The risk of aortic atheroma thickness <4 mm remains unclear. We used ROC analysis to determine the suitable cutoff point for aortic atheroma thickness which is associated with cardiovascular events, although the sensitivity and specificity of the ROC curve were not sufficiently high because of the contributions of many other risk factors. The plaque thickness was strongly associated with age. However, MPT ≥ 3.5 mm was an independent risk factor even after adjusting for age in patients 65 years of age or older. We believe that the cutoff point of 3.5 mm is an important index of extracranial atherosclerosis, which may be associated with subsequent cardiovascular events, especially in elderly patients with a history of ischemic stroke.

During the determination of aortic atheroma thickness, which may be critical for the association of cardiovascular events, race and ethnic differences should be taken into account. Most previous data regarding the relationship between aortic atheroma and stroke was derived from white populations. Gupta et al. [12] showed that white people tend to have increased complex plaques and plaques >4 mm thick along the thoracic aorta compared with African-Americans. Di Tullio et al. [13] reported on aortic atheroma morphology and the risk of stroke in a multiethnic population composed of whites, blacks, and Hispanics. However, few studies have described the differences in terms of aortic arch atherosclerosis between Asian and Caucasian populations. Angiographic and autopsy studies on stroke patients have shown that Japanese, Chinese, and Koreans tend to have more intracranial vascular lesions, whereas Caucasians tend to have more extracranial lesions [14–18]. Japanese may show less prevalence of severe aortic atheroma than do Caucasians, or smaller plaques may be more of a risk for vascular events in Japanese than in Caucasians.

In this cross-sectional study, we showed that aortic atheroma of MPT ≥ 3.5 mm is more frequently observed in patients with undetermined causes of stroke than those with known causes. Five patients with undetermined causes of strokes and MPT ≥ 3.5 mm developed recurrent ischemic strokes. Of them, 3 had recurrent embolic infarction, the embolic source of which was not identified other than aortic atheroma at the time of the second event. These results suggest that aortic atheroma of 3.5 mm or more may be a direct source of thromboemboli. Several studies using transcranial Doppler showed that embolic signals in the middle cerebral artery can be more frequently detected in patients with severe arch atheroma than in those without severe arch atheroma [19–21]. Severe atheroma along the aortic arch, therefore, can be a source of cerebral emboli. Moderate atheroma such as being 3.5–4.0 mm in thickness may also be a source of emboli. Ulcerated plaques were found in one-third of the aortic atheromas which were less than 4 mm in our study. A small unstable atheroma can cause dynamic changes such as plaque rupture and may cause cerebral embolism. Amarenco et al. [5] and others suggested that ulcerative plaques may be a cause of cerebral infarction. Mitusch et al. [3], Jones et al. [7] and others showed that mobile components are also a risk factor of cerebral infarction. A follow-up study by Cohen et al. [22] found that the risk of cerebral infarction associated with an aortic plaque thickness ≥4 mm is markedly increased by the absence of plaque calcification. However, we could not find any direct association between plaque morphology and stroke. A possible explanation is that the present study included fewer patients with ulcerative, mobile or calcified plaques than did previous studies and therefore failed to find any statistical differences in terms of such morphology.

The present study has several limitations. First, the study was performed in a retrospective manner, and most of the patients were originally recruited to assess embolic sources of the heart and thoracic aorta by TEE. The study population tended to have more patients with cardioembolic infarction and fewer patients with lacunar infarction than the general stroke population in our institution. Patients with known severe carotid artery stenosis may have been excluded from the study population. Thus, the subjects in the present study may not be representative of the general population of ischemic stroke. Second, the follow-up information obtained by telephone interview (13% of the study population) may not be highly accurate.

In conclusion, maximal plaque thickness ≥ 3.5 mm of the aortic arch is a predictor of subsequent cardiovascular events, especially in elderly patients with a history of ischemic stroke. Moderate atheroma with 3.5–4.0 mm in thickness may also be a possible cause of embolic stroke.

References

1 Amarenco P, Cohen A, Tzourio C, Bertrand B, Hommel M, Besson G, Chauvel C, Touboul PJ, Bousser MG: Atherosclerotic disease of the aortic arch and the risk of ischemic stroke. N Engl J Med 1994;331:1474–1479.

2 The French Study of Aortic Plaques in Stroke Group: Atherosclerotic disease of the aortic arch as a risk factor for recurrent ischemic stroke. The French Study of Aortic Plaques in Stroke Group. N Engl J Med 1996;334:1216–1221.

3 Mitusch R, Doherty C, Wucherpfennig H, Memmesheimer C, Tepe C, Stierle U, Kessler C, Sheikhzadeh A: Vascular events during follow-up in patients with aortic arch atherosclerosis. Stroke 1997;28:36–39.

4 Tunick PA, Nayar AC, Goodkin GM, Mirchandani S, Francescone S, Rosenzweig BP, Freedberg RS, Katz ES, Applebaum RM, Kronzon I: Effect of treatment on the incidence of stroke and other emboli in 519 patients with severe thoracic aortic plaque. Am J Cardiol 2002;90:1320–1325.

5 Amarenco P, Duyckaerts C, Tzourio C, Henin D, Bousser MG, Hauw JJ: The prevalence of ulcerated plaques in the aortic arch in patients with stroke. N Engl J Med 1992;326:221–225.

6 Fujimoto S, Yasaka M, Otsubo R, Oe H, Nagatsuka K, Minematsu K: Aortic arch atherosclerotic lesions and the recurrence of ischemic stroke. Stroke 2004;35:1426–1429.

7 Jones EF, Kalman JM, Calafiore P, Tonkin AM, Donnan GA: Proximal aortic atheroma. An independent risk factor for cerebral ischemia. Stroke 1995;26:218–224.

8 Toyoda K, Yasaka M, Nagata S, Yamaguchi T: Aortogenic embolic stroke: A transesophageal echocardiographic approach. Stroke 1992;23:1056–1061.

9 Kazui S, Levi CR, Jones EF, Quang L, Calafiore P, Donnan GA: Lacunar stroke: transoesophageal echocardiographic factors influencing long-term prognosis. Cerebrovasc Dis 2001;12:325–330.

10 Yamaguchi T, Minematsu K, Choki J, Ikeda M: Clinical and neuroradiological analysis of thrombotic and embolic cerebral infarction. Jpn Circ J 1984;48:50–58.

11 Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. Stroke 1990;21:637–676.

12 Gupta V, Nanda NC, Yesilbursa D, Huang WY, Li Q, Gomez CR: Racial differences in thoracic aorta atherosclerosis among ischemic stroke patients. Stroke 2003;34:408–412.

13 Di Tullio MR, Sacco RL, Savoia MT, Sciacca RR, Homma S: Aortic atheroma morphology and the risk of ischemic stroke in a multiethnic population. Am Heart J 2000;139:329–336.

14 Kieffer SA, Takeya Y, Resch JA, Amplatz K: Racial differences in cerebrovascular disease: angiographic evaluation of Japanese and American populations. Am J Roentgenol Radium Ther Nucl Med 1967;101:94–99.

15 Leung SY, Ng TH, Yuen ST, Lauder IJ, Ho FC: Pattern of cerebral atherosclerosis in Hong Kong Chinese: severity in intracranial and extracranial vessels. Stroke 1993;24:779–786.

16 Liu HM, Tu YK, Yip PK, Su CT: Evaluation of intracranial and extracranial carotid stenoocclusive diseases in Taiwan Chinese patients with MR angiography: preliminary experience. Stroke 1996;27:650–653.

17 Suh DC, Lee SH, Kim KR, Park ST, Lim SM, Kim SJ, Choi CG, Lee HK: Pattern of atherosclerotic carotid stenosis in Korean patients with stroke: different involvement of intracranial versus extracranial vessels. AJNR 2003;24:239–244.

18 Wong KS, Huang YN, Gao S, Lam WW, Chan YL, Kay R: Intracranial stenosis in Chinese patients with acute stroke. Neurology 1998;50:812–813.

19 Rundek T, Di Tullio MR, Sciacca RR, Titova IV, Mohr JP, Homma S, Sacco RL: Association between large aortic arch atheromas and high-intensity transient signals in elderly stroke patients. Stroke 1999;30:2683–2686.

20 Castellanos M, Serena J, Segura T, Perez-Ayuso MJ, Silva Y, Davalos A: Atherosclerotic aortic arch plaques in cryptogenic stroke: a microembolic signal monitoring study. Eur Neurol 2001;45:145–150.

21 Viguier A, Pavy le Traon A, Massabuau P, Valton L, Larrue V: Asymptomatic cerebral embolic signals in patients with acute cerebral ischaemia and severe aortic arch atherosclerosis. J Neurol 2001;248:768–771.

22 Cohen A, Tzourio C, Bertrand B, Chauvel C, Bousser MG, Amarenco P: Aortic plaque morphology and vascular events: a follow-up study in patients with ischemic stroke. FAPS Investigators. French Study of Aortic Plaques in Stroke. Circulation 1997;96:3838–3841.

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Microembolic Signals within 24 Hours of Stroke Onset and Diffusion-Weighted MRI Abnormalities

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Key Words
Acute stroke · Diffusion-weighted imaging · Transcranial Doppler sonography · Subcortical infarction

Abstract
Background: The clinical relevance of the microembolic signals (MES) detected by transcranial Doppler sonography (TCD) in acute stroke remains unclear. In a prospective study the authors analyzed the relationship between MES and the findings on diffusion-weighted magnetic resonance imaging (DWI) in acute stroke patients. ***Methods:*** We performed TCD for a period of 30 min to detect MES in patients within 24 h of stroke onset, and DWI was done within the initial 7 days. MES were assessed from Doppler waves obtained from the middle cerebral artery contralateral to the side of the neurological deficits. The acute ischemic lesions observed on DWI were classified by their diameter (small, medium or large) and by their site (cortical, superficial perforator territory, internal borderzone or deep perforator territory). ***Results:*** We obtained Doppler waves from 39 vessels in 37 patients; 2 patients had bilateral deficits. MES were detected in 12 vessels (MES-positive group) and not detected in 27 vessels (MES-negative group). No significant differences in clinical features were observed between the 2 groups. The number of small lesions was significantly higher in the MES-positive group than in the MES-negative group ($p = 0.02$). The numbers of cortical and superficial perforator infarcts were significantly higher in the MES-positive group than in the MES-negative group ($p = 0.002$ and 0.02, respectively). ***Conclusion:*** In acute ischemic stroke, MES detected by TCD in the acute phase may produce small cortical and subcortical lesions found on DWI.

Introduction

Microembolic signals (MES) have been detected by transcranial Doppler (TCD) in patients with carotid diseases, atrial fibrillation, prosthetic cardiac valves and carotid angiography, as well as intraoperatively in patients undergoing cardiac or carotid surgery [1–10]. MES is detected in 4–56% of acute stroke patients [11–17]. However, the clinical relevance of MES in acute stroke remains unclear. Although they are usually asymptomatic, small emboli may cause small ischemic brain lesions [18, 19].

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Diffusion-weighted magnetic resonance imaging (DWI) detects hyperacute ischemic lesions more sensitively than conventional CT and conventional magnetic resonance (MR) imaging [20, 21]. DWI has a high sensitivity and specificity in the acute setting of stroke [22]. It is also useful for the diagnosis of stroke within 6 h of symptom onset [23]. Although we and other authors have reported that there is a relationship between MES and DWI, in most of the studies, TCD was performed within ≥2 days of stroke onset, and the subjects were only those who had large artery disease or other emboligenic diseases [24, 25]. In these reports MES were obtained from both middle cerebral arteries (MCAs), which included the artery ipsilateral to the side of the deficits.

To evaluate the relationship between MES and the ischemic lesions more precisely, we did a prospective study to detect MES in patients with ischemic stroke or transient ischemic attack (TIA) by doing a TCD within 24 h of onset, and then by analyzing the relationship between the MES from only the MCA contralateral to the side of the deficits and the lesions found on DWI.

Patients and Methods

We conducted a prospective study of 56 consecutive patients with carotid ischemic stroke who were admitted to our hospital within 24 h of stroke or TIA onset between September 2002 and April 2003.

The following clinical data were collected from all patients: (1) patient age and gender; (2) the number of MES detected by TCD from the MCA contralateral to the side of the neurological deficits; (3) National Institutes of Health Stroke Scale (NIHSS) score [26] on admission; (4) the presence of vascular risk factors, including hypertension, diabetes mellitus, hypercholesterolemia and current cigarette smoking; (5) history of stroke; (6) emboligenic cardiac and aortic diseases; (7) significant arterial diseases corresponding to the neurological deficits; (8) laboratory parameters on admission (white blood cell count, hematocrit, platelet count, fibrinogen, C-reactive protein); (9) blood coagulation factors (thrombin-antithrombin III complex), D-dimer, antithrombin III on admission; (10) the interval of time between stroke onset and the TCD study or MR imaging; (11) the administration of anticoagulant agents, including heparin and warfarin, and antiplatelet agents, including aspirin and ticlopidine, at the time of the TCD study.

The TCD study was performed using a DWL Multidop X with a 2.5-MHz probe within 24 h of stroke or TIA onset. With the patients supine, bilateral MCA recordings for 30 min at a depth of 45–55 mm were done. The MES were based on the Doppler waves obtained from the MCA contralateral to the side of the neurological deficits. The TCD probe was held in place with an elastic headband to reduce the possibility of a movement artifact. MES were defined according to the standard consensus criteria [27] as being 6 dB above the background threshold. Blinded 'offline' validation of suspected MES was done by 2 of the authors (M.N. and A.S.), who reviewed the digital audiotape recordings, instead of relying solely on automatic counting by DWL software.

To detect potential cardiac sources of emboli, all patients were examined using a 12-lead electrocardiograph (ECG), 24-hour ECG monitoring and transthoracic echocardiography. The following potential emboligenic cardiac diseases were considered: nonvalvular atrial fibrillation, acute myocardial infarction, previous myocardial infarction with intraventricular thrombus, mitral valve diseases, prosthetic cardiac valve, dilated cardiomyopathy, patent foramen ovale and cardiac tumor. Potential aortogenic sources of emboli or patent foramen ovale were evaluated in 27 patients by transesophageal echocardiography. Localized raised lesions in the aorta with a maximal intima-medial thickness >4.0 mm and an obviously irregular surface, a broad acoustic shadow, or mobile plaque were defined as potential aortic sources of emboli.

All patients underwent color-flow duplex carotid ultrasonography on the day of admission. Conventional cerebral angiography and/or MR angiography was performed in all patients. The stenosis was graded according to the method used by the North American Symptomatic Carotid Endarterectomy Trial [28]. Significant arterial disease was identified if a stenosis >50% or an ulcerated plaque was found in the affected artery that corresponded to the neurological deficits.

MR imaging was performed within 7 days of stroke onset, using a 1.5-tesla system equipped with single-shot echo planar imaging to obtain rapid diffusion images. MR studies included axial T_1-weighted, axial T_2-weighted and DWI sequences. The imaging parameters were 4000/103 (TR/TE), 128 × 128 matrix, 230-mm field of view, and 4 mm slice thickness with a 2-mm gap between the slices. Two b-values were used: 0 and 1,000 s/mm^2. Diffusion gradients were applied in successive scans in each of the x, y and z directions, and DWI images were formed from the average of these values. The criterion for the diagnosis of acute infarcts on DWI was focal hyperintensity, judged not to be due to normal anisotropic diffusion or magnetic susceptibility artifacts. Each MRI was assessed by 2 authors (M.N. and K.K.); only those lesions that both assessors identified were judged to be new ischemic lesions. We classified the acute ischemic lesions found on DWI by size (small, <10 mm in diameter; medium, 10–30 mm in diameter, or large >30 mm in diameter) and by their locations (cortical, superficial perforator territory, internal borderzone or deep perforating artery territory). Cortical lesions were defined as the lesions including cortical ribbon (fig. 1A). Subcortical lesions were further split into superficial perforator lesions according to the templates of Bogousslavsky and Regli [29] (fig. 1B), and internal borderzone lesions according to the schematic templates by Del Sette et al. [30] and Lee et al. [31] (fig. 1C). The outermost limit of superficial perforator lesions was taken to be the cortical ribbon; the innermost limit was the corona radiata at the level of the deep perforator. Internal borderzone lesions were defined as hyperintense areas in the vascular internal borderzone, where the border between the deep and superficial perforating arteries divides the lesion into 2 approximately equal sections. We excluded lesions located within the borderzone area between the MCA and the anterior cerebral artery or the MCA and the posterior cerebral artery. The deep perforating artery territory was defined as the regions including deep corona radiata, putamen, globus pallidus, internal capsule and caudate head (fig. 1D).

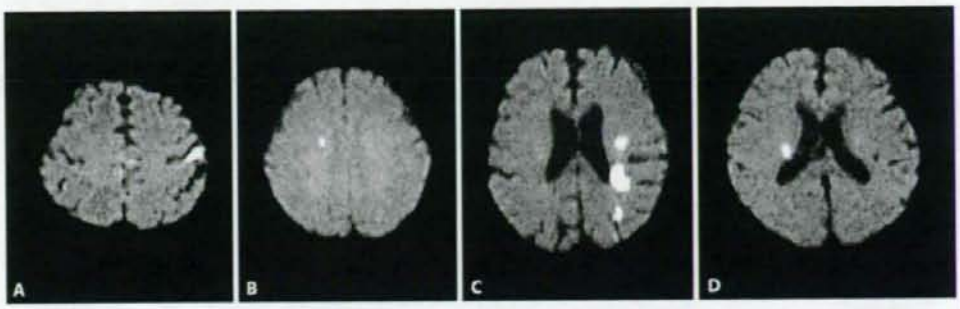

Fig. 1. Examples of location of infarct. **A** Cortical infarcts. **B** Superficial perforator infarcts. **C** Internal boderzone infarcts. **D** Deep perforating artery territory infarcts.

The Mann-Whitney U test was used to detect statistically significant differences in age, NIHSS score, and interval between stroke onset and TCD or MR assessment between the groups. All other findings were assessed by Fisher's exact test. In addition, we analyzed the relationship between the number of MES on TCD and the presence of lesions on DWI by Spearman's correlation test. Statistical analysis was performed using a commercially available software package (Stat-View, version 5, SAS Institute Inc., USA). p values <0.05 were considered statistically significant.

Results

We analyzed 39 MCAs in 37 patients (34 men, 3 women, age 69 ± 10 years); 19 patients were excluded from the initial 56 patients, as they lacked temporal windows on the side of interest (n = 16) or had no available MR images due to prior pacemaker implantation (n = 3). Both MCAs were evaluated in 2 of the 37 patients because they showed bilateral neurological deficits. The mean time interval between stroke onset and TCD assessment was 11.0 ± 6.4 h, (median 8.2, range 2.3–24.0); the mean time interval between stroke onset and MR assessment was 33.9 ± 29.7 h (median 27.9, range 12.3–183.5).

The MES-positive group consisted of 11 patients in whom MES were detected in 12 MCAs (30.8%), while the MES-negative group consisted of 26 patients in whom no MES were detected in 27 vessels (69.2%). No significant differences were observed between the 2 groups with respect to clinical features, including NIHSS score on admission, emboligenic diseases, arterial diseases and use of either oral or transvenous antithrombotic agents. Laboratory parameters and blood coagulation factors on admission did not differ between the 2 groups either (table 1). Transesophageal echocardiography was performed in 7 of 10 patients in the positive group and 20 of 26 patients in the negative group. Although certain emboligenic diseases (arterial diseases, heart diseases or complicated lesions in the aortic arch) were seen more frequently in patients in the MES-positive group, the differences between the MES-positive and -negative groups were not statistically significant.

The number of small lesions was significantly higher in the MES-positive group (3.5 ± 4.5) than in the MES-negative group (0.7 ± 1.0, $p = 0.02$), whereas the numbers of medium lesions (0.9 ± 1.5 MES-positive vs. 0.2 ± 0.4 MES-negative, $p = 0.07$) and large lesions (0.3 ± 0.5 MES-positive vs. 0.4 ± 0.5 MES-negative, $p = 0.83$) did not differ between the 2 groups (fig. 2). The number of cortical infarcts was significantly higher in the MES-positive group (3.8 ± 4.3) than in the MES-negative group (0.7 ± 0.8, $p = 0.002$). The number of superficial perforator lesions was significantly higher in the MES group (0.8 ± 0.9 vs. 0.3 ± 0.3, $p = 0.02$), while the number of internal borderzone lesions did not differ between the 2 groups (0.2 ± 0.4 vs. 0.3 ± 0.7, $p > 0.99$). No differences were seen in the number of deep perforator territory infarcts (0.2 ± 0.4 MES-positive vs. 0.3 ± 0.5 MES-negative, $p = 0.66$, fig. 3) between the 2 groups. The number of MES had a weak association with the number of DWI lesions ($p = 0.003$, $\rho = 0.605$, fig. 4).

MES was detected in 1 patient with a single deep perforating artery infarct. He is a 72-year-old man with hypertension and diabetes mellitus, who showed pure motor stroke on the right side. The coagulation and fibrinolytic markers showed no abnormality. His symptoms did not progress during his hospital stay.

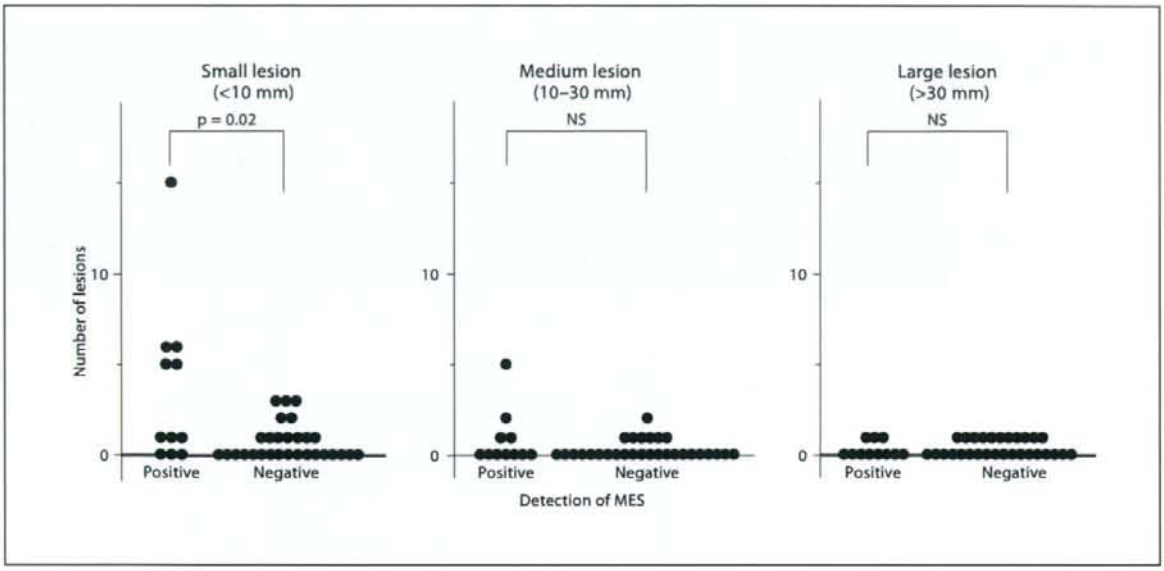

Fig. 2. On DWI, small ischemic lesions were more frequent in the MES-positive group than in the MES-negative group. However, the frequencies of medium lesions and large lesions did not differ between the 2 groups.

Table 1. Patient characteristics in MES-positive group and MES-negative group

	Detection of MES		p
	positive (n = 11)	negative (n = 26)	
Age[a]	68 (50–79)	68 (50–88)	0.48
Male sex	10 (91%)	24 (92%)	>0.99
Hypertension	10 (91%)	16 (62%)	0.12
Diabetes	3 (27%)	10 (38%)	0.71
Hyperlipidemia	3 (27%)	10 (38%)	0.71
Smoking	7 (64%)	18 (69%)	>0.99
Previous stroke	2 (18%)	12 (46%)	0.15
Arterial stenosis (≥ NASCET 50%)	3 (27%)	4 (15%)	0.40
Atrial fibrillation	4 (36%)	6 (23%)	0.44
Potential embolic source	4/7 (57%)	10/20 (50%)	>0.99
Intravenous antithrombotic agent	4 (36%)	12 (46%)	0.72
Oral antithrombotic agent	4 (37%)	13 (50%)	0.50
Time interval from onset to TCD study[a]	11.8 (2.5–24.0)	7.2 (2.3–24.0)	0.19
White blood cell, $\times 10^3/\mu l$[a]	9.1 (3.7–14.6)	7.4 (3.0–13.2)	0.61
Hematocrit, %[a]	41.2 (37.5–47.7)	39.7 (18.9–47.4)	0.34
Platelet, $\times 10^3/\mu l$[a]	182 (103–258)	210 (93–420)	0.27
Fibrinogen, mg/dl[a]	353 (251–455)	332 (179–582)	0.67
C-reactive protein, mg/l[a]	0.36 (0.11–10.40)	0.24 (0.06–5.39)	0.21
Thrombin-antithrombin III complex, μg/l[a]	1.6 (0.5–19.5)	1.7 (0.2–15.1)	0.59
D-dimer, ng/ml[a]	1.0 (0.5–14.5)	0.9 (0.1–11.0)	0.31
Antithrombin III, %[a]	84.7 (75.8–104.6)	86.0 (71.6–136.9)	0.87

[a] Median (range). NASCET = North American Symptomatic Carotid Endarterectomy Trial.

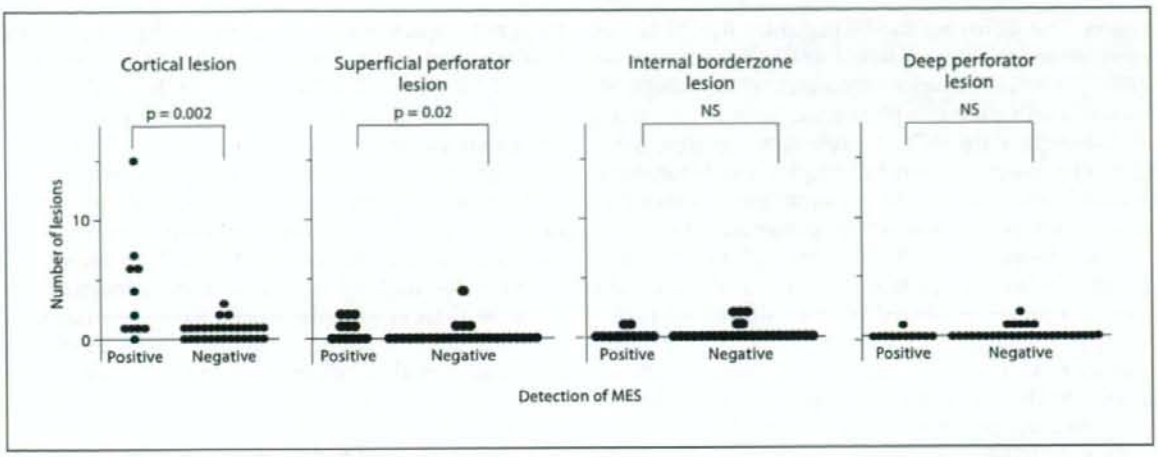

Fig. 3. Cortical lesions and superficial perforator lesions were more frequently seen on DWI in the MES-positive group than in the MES-negative group, while the frequencies of subcortical lesions and lesions in the perforating arterial territory did not differ between the 2 groups.

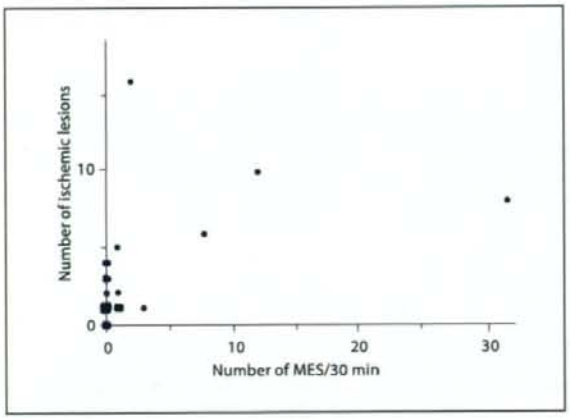

Fig. 4. Relationship between the number of MES and the number of ischemic lesions seen on DWI. A mild association is seen between them ($p = 0.003$, $\rho = 0.605$).

Discussion

The presence of MES indicates that a stroke may be embolic in origin. Multiple lesions on DWI would also relate to multiple emboli or the breakup of an embolus. Some authors have discussed the relationship between multiple lesions and stroke etiology [32, 33]. In this study, we confirmed the relationship between the detection of MES in the MCA contralateral to the side of the deficits and abnormal lesions found on DWI in the ipsilateral cerebrum. Our results are similar to the results of past studies suggesting that MES have a relationship with the small cortical lesions seen on DWI [24, 25], and our results extend this relationship to include patients with acute ischemic stroke within 24 h of stroke onset.

While the relationship between ischemic lesions in the cerebral cortex and an embolic mechanism is broadly recognized, subcortical infarcts may have a combined mechanism, including a hemodynamic mechanism. Our study showed a suggestive result: subcortical lesions in the superficial perforator associated with the detection of MES, while internal borderzone lesions did not. In previous reports, some subcortical infarcts including the centrum ovale are thought to be associated with cardiac or arterial diseases [33, 34]. Others have described that deep subcortical infarcts including the internal borderzone may be caused by a hemodynamic mechanism or a combination of hypoperfusion and microemboli in intracranial arteries with severe stenosis [35, 36]. Our result may emphasize the difference between superficial perforator infarcts and internal borderzone infarcts, although not confirmative because of small sample size.

We found that the association between the number of MES and the number of DWI lesions was weak ($\rho = 0.605$), while Wong et al. [35] reported a stronger asso-

ciation. This difference can be explained by differences between the 2 study populations; we assessed consecutive stroke patients with various etiologies, while Wong et al. evaluated only patients with stenotic lesions in the proximal segment of the MCA. Therefore, our result suggested an association not only between MES and embolism, but also between MES and other stroke mechanisms, that is, platelet hyperactivation or hypercoagulability.

One patient showed MES in the MCAs bilaterally, though he had no potential emboligenic diseases. On DWI this patient was found to have a single deep perforating artery infarct, indicating that embolism is the likely cause of a lacunar infarct. The mechanism of lacunar infarcts has been a matter of controversies, and at least in some patients, embolism causes lacunes [37].

Our study had some limitations. Firstly, the sample size was small. Some patients could not be studied because of an inadequate insonation window for TCD. It is known that the detection rate of the intracranial artery flow signal using TCD is lower in the Japanese population than in the Caucasian population [38]. The reason why the ratio of men was as high as >90% in this study is thought to be the lack of a temporal window in most old Japanese women, which may be attributed to osteoporosis and the characteristics of the race. Secondly, about 40% of all patients were treated with an anticoagulant and an antiplatelet agent at the time of the TCD study. Although there was no significant difference in anticoagulant and antiplatelet agent use between the MES-positive group and the MES-negative group, such treatment might have influenced the frequency of MES detection.

In conclusion, MES were detected more frequently in the acute phase of stroke in patients with a small cortical and subcortical infarction. The presence of MES may help determine the mechanism of stroke.

Acknowledgment and Funding

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References

1 Stork JL, Kimura K, Levi CR, Chambers BR, Abbott AL, Donnan GA: Source of microembolic signals in patients with high-grade carotid stenosis. Stroke 2002;33:2014–2018.

2 Sitzer M, Müller W, Siebler M, Hort W, Kniemeyer HW, Jäncke L, Steinmetz H: Plaque ulceration and lumen thrombus are the main sources of cerebral microemboli in high-grade internal carotid artery stenosis. Stroke 1995;26:1231–1233.

3 Siebler M, Sitzer M, Rose G, Steinmetz H: Microembolism in carotid artery disease. Echocardiography 1996;13:529–536.

4 Siebler M, Kleinschmidt A, Sitzer M, Steinmetz H, Freund HJ: Cerebral microembolism in symptomatic and asymptomatic high-grade internal carotid artery stenosis. Neurology 1994;44:615–618.

5 Siebler M, Sitzer M, Steinmetz H: Detection of intracranial emboli in patients with symptomatic extracranial carotid artery disease. Stroke 1992;23:1652–1654.

6 Levi CR, O'Malley HM, Fell G, Roberts AK, Hoare MC, Royle JP, Chan A, Beiles BC, Chambers BR, Bladin CF, Donnan GA: Transcranial Doppler detected cerebral microembolism following carotid endarterectomy: high microembolic signal loads predict postoperative cerebral ischaemia. Brain 1997;120:621–629.

7 Kumral E, Balkir K, Uzuner N, Evyapan D, Nalbantgil S: Microembolic signal detection in patients with symptomatic and asymptomatic lone atrial fibrillation. Cerebrovasc Dis 2001;12:192–196.

8 Grosset DG, Georgiadis D, Abdullah I, Bone I, Lees KR: Doppler emboli signals vary according to stroke subtype. Stroke 1994;25:382–384.

9 Georgiadis D, Grosset DG, Kelman A, Faichney A, Lees KR: Prevalence and characteristics of intracranial microemboli signals in patients with different types of prosthetic cardiac valves. Stroke 1994;25:587–592.

10 Babikian VL, Hyde C, Pochay V, Winter MR: Clinical correlates of high-intensity transient signals detected on transcranial Doppler sonography in patients with cerebrovascular disease. Stroke 1994;25:1570–1573.

11 Delcker A, Schnell A, Wilhelm H: Microembolic signals and clinical outcome in patients with acute stroke – a prospective study. Eur Arch Psychiatry Clin Neurosci 2000;250:1–5.

12 Serena J, Segura T, Castellanos M, Dávalos A: Microembolic signal monitoring in hemispheric acute ischaemic stroke: a prospective study. Cerebrovasc Dis 2000;10:278–282.

13 Batista P, Oliveira V, Ferro JM: The detection of microembolic signals in patients at risk of recurrent cardioembolic stroke: possible therapeutic relevance. Cerebrovasc Dis 1999;9:314–319.

14 Droste DW, Ritter M, Kemény V, Schulte-Altedorneburg G, Ringelstein EB: Microembolus detections at follow-up in 19 patients with acute stroke: correlation with stroke etiology and antithrombotic treatment. Cerebrovasc Dis 2000;10:272–277.

15 Daffertshofer M, Ries S, Schminke U, Hennerici M: High-intensity transient signals in patients with cerebral ischemia. Stroke 1996;27:1844–1849.

16 Sliwka U, Lingnau A, Stohlmann WD, Schmidt P, Mull M, Diehl RR, Noth J: Prevalence and time course of microembolic signals in patients with acute stroke: a prospective study. Stroke 1997;28:358–363.

17 Gao S, Wong KS, Hansberg T, Lam WW, Droste DW, Ringelstein EB: Microembolic signal predicts recurrent cerebral ischemic events in acute stroke patients with middle cerebral artery stenosis. Stroke 2004;35:2832–2836.

18 Spencer MP, Thomas GI, Nicholls SC, Sauvage LR: Detection of middle cerebral artery emboli during carotid endarterectomy using transcranial Doppler ultrasonography. Stroke 1990;21:415–423.

19 Müller M, Ciccotti P, Axmann C, Kreissler-Haag D: Embolic cerebral ischemia in carotid surgery: a model for human embolic stroke? Med Sci Monit 2003;9:CR411–CR416.
20 Warach S, Gaa J, Siewert B, Wielopolski P, Edelman RR: Acute human stroke studied by whole brain echo planar diffusion-weighted magnetic resonance imaging. Ann Neurol 1995;37:231–241.
21 Gass A, Ay H, Szabo K, Koroshetz WJ: Diffusion-weighted MRI for the 'small stuff': the details of acute cerebral ischaemia. Lancet Neurol 2004;3:39–45.
22 Lovblad KO, Laubach HJ, Baird AE, Curtin F, Schlaug G, Edelman RR, Warach S: Clinical experience with diffusion-weighted MR in patients with acute stroke. AJNR Am J Neuroradiol 1998;19:1061–1066.
23 Gonzalez RG, Schaefer PW, Buonanno FS, Schwamm LH, Budzik RF, Rordorf G, Wang B, Sorensen AG, Koroshetz WJ: Diffusion-weighted MR imaging: diagnostic accuracy in patients imaged within 6 h of stroke symptom onset. Radiology 1999;210:155–162.
24 Kimura K, Minematsu K, Koga M, Arakawa R, Yasaka M, Yamagami H, Nagatsuka K, Naritomi H, Yamaguchi T: Microembolic signals and diffusion-weighted MR imaging abnormalities in acute ischemic stroke. AJNR Am J Neuroradiol 2001;22:1037–1042.
25 Wong KS, Gao S, Chan YL, Hansberg T, Lam WW, Droste DW, Kay R, Ringelstein EB: Mechanisms of acute cerebral infarctions in patients with middle cerebral artery stenosis: a diffusion-weighted imaging and microemboli monitoring study. Ann Neurol 2002;52:74–81.
26 Lyden P, Brott T, Tilley B, Mascha EJ, Levine S, Haley EC, Grotta J, Marler J: Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. Stroke 1994;25:2220–2226.
27 Consensus Committee of the 9th International Cerebral Hemodynamic Symposium: Basic identification criteria of Doppler microembolic signals. Stroke 1995;26:1123.
28 North American Symptomatic Carotid Endarterectomy Trial Collaborators: Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991;325:445–453.
29 Bogousslavsky J, Regli F: Centrum ovale infarcts: subcortical infarction in the superficial territory of the middle cerebral artery. Neurology 1992;42:1992–1998.
30 Del Sette M, Eliasziw M, Streifler JY, Hachinski VC, Fox AJ, Barnett HJ: Internal border-zone infarction: a marker for severe stenosis in patients with symptomatic internal carotid artery disease. For the North American Symptomatic Carotid Endarterectomy (NASCET) Group. Stroke 2000;31:631–636.
31 Lee PH, Bang OY, Oh SH, Joo IS, Huh K: Subcortical white matter infarcts: comparison of superficial perforating artery and internal border-zone infarcts using diffusion-weighted magnetic resonance imaging. Stroke 2003;34:2630–2635.
32 Baird AE, Lovblad KO, Schlaug G, Edelman RR, Warach S: Multiple acute stroke syndrome: marker of embolic disease? Neurology 2000;54:674–678.
33 Kang DW, Chalela JA, Ezzeddine MA, Warach S: Association of ischemic lesion patterns on early diffusion-weighted imaging with TOAST stroke subtypes. Arch Neurol 2003;60:1730–1734.
34 Yonemura K, Kimura K, Minematsu K, Uchino M, Yamaguchi T: Small centrum ovale infarcts on diffusion-weighted magnetic resonance imaging. Stroke 2002;33:1541–1544.
35 Wong KS, Gao S, Lam WW, Chan YL, Kay R: A pilot study of microembolic signals in patients with middle cerebral artery stenosis. J Neuroimaging 2001;11:137–140.
36 Momjian-Mayor I, Baron JC: The pathophysiology of watershed infarction in internal carotid artery disease: review of cerebral perfusion studies. Stroke 2005;36:567–577.
37 Futrell N: Lacunar infarction: embolism is the key. Stroke 2004;35:1778–1779.
38 Itoh T, Matsumoto M, Handa N, Maeda H, Hougaku H, Hashimoto H, Etani H, Tsukamoto Y, Kamada T: Rate of successful recording of blood flow signals in the middle cerebral artery using transcranial Doppler sonography. Stroke 1993;24:1192–1195.

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REGULAR ARTICLE

Genotypes of vitamin K epoxide reductase, γ-glutamyl carboxylase, and cytochrome P450 2C9 as determinants of daily warfarin dose in Japanese patients

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Abstract The dose required for the anticoagulant effect of warfarin exhibits large inter-individual variations. This study sought to determine the contribution of four genes, vitamin K epoxide reductase (*VKORC1*), γ-glutamyl carboxylase (*GGCX*), calumenin (*CALU*), and cytochrome P450 2C9 (*CYP2C9*) to the warfarin maintenance dose required in Japanese patients following ischemic stroke. We recruited 93 patients on stable anticoagulation with a target International Normalized Ratio (INR) of 1.6–2.6. We genotyped eleven representative single nucleotide polymorphisms (SNPs) in the three genes involved in vitamin K cycle and the 42613A>C SNP in *CYP2C9*, known as *CYP2C9*3*, and then examined an association of these genotypes with warfarin maintenance doses (mean±SD=2.96±1.06 mg/day). We found an association of effective warfarin dose with the −1639G>A ($p=0.004$) and 3730G>A genotypes ($p=0.006$) in *VKORC1*, the 8016G>A genotype in *GGCX* ($p=0.022$), and the 42613A>C genotype in *CYP2C9* ($p=0.015$). The model using the multiple regression analysis including age, sex, weight, and three genetic polymorphisms accounted for 33.3% of total variations in warfarin dose. The contribution to inter-individual variation in warfarin dose was 5.9% for *VKORC1* −1639G>A, 5.2% for *CYP2C9*

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42613A>C, and 4.6% for *GGCX* 8016G>A. In addition to polymorphisms in *VKORC1* and *CYP2C9*, we identified *GGCX* 8016G>A, resulting in the missense mutation R325Q, as a genetic determinant of warfarin maintenance dose in Japanese patients.

Warfarin is the most widely prescribed anticoagulant for long-term prevention of thromboembolic events. The dose of warfarin required to achieve target levels of anticoagulation varies dependent on dietary intake and individual variations in pharmacokinetics. Management of warfarin therapy is difficult because of significant inter-individual and intra-individual variability and the narrow therapeutic range. The effectiveness and safety of warfarin must be monitored by serial determinations of prothrombin time using the standardized international normalized ratio (INR).

Warfarin exerts an anticoagulant effect by interfering with the regeneration of reduced vitamin K from the epoxide form, which is required for the enzymatic activity of vitamin K epoxide reductase subunit 1 (VKORC1) [1,2]. γ-Carboxylation of a wide variety of proteins, including numbers of factors in the clotting cascade, is catalyzed by γ-glutamyl carboxylase (GGCX), a vitamin K-dependent enzyme. This reaction incorporates a carbon dioxide molecule into specific glutamic acid residues with the help of the reduced form of vitamin K and oxygen, generating γ-carboxylglutamic acid and vitamin K 2,3-epoxide. When reduced vitamin K cannot be regenerated, the biosynthesis of vitamin K-dependent coagulation/anticoagulation factors, including prothrombin, factors VII, IX, and X, and proteins C and S, is suppressed. The endoplasmic reticulum resident protein calumenin (CALU) associates with γ-glutamyl carboxylase, inhibiting its activity [3]. Recent studies on the genetic aspects of the inter-individual variability of warfarin have demonstrated that single nucleotide polymorphisms (SNPs) in the *VKORC1* gene influence warfarin responses [4–15]. Haplotype analysis demonstrated that individuals who can be controlled by the low dose of warfarin showed the low hepatic expression of *VKORC1* mRNA [6].

The inter-individual variability of warfarin can also be explained by the genetic variability of the warfarin metabolizing enzyme, *CYP2C9*. The missense mutations R144C and I359L in the *CYP2C9* gene known as *CYP2C9*2* and *CYP2C9*3* are known to associate with warfarin dose [16]. These two genetic variations exhibited ethnic specificity. Asian population does not have the *CYP2C9*2* allele but carries the *CYP2C9*3* allele [17].

In this study, we investigated the influence of SNPs in four genes controlling γ-carboxylation (*VKORC1*, *GGCX*, *CALU*, and *CYP2C9*) on the inter-individual variability of warfarin dose requirements in Japanese patients. We identified SNPs in *VKORC1*, *GGCX*, and *CYP2C9* associated with the inter-individual differences in warfarin dosage.

Materials and methods

Subjects

The study population consisted of 93 unrelated Japanese patients admitted to the Cerebrovascular Division of the National Cardiovascular Center between November 2003 and March 2004. The patients had all experienced an ischemic stroke within the 7 days prior to admission. Stroke subtype consisted of cardioembolic infarction (n=48) and the embolic infarction of unknown origin with non-valvular atrial fibrillation (n=45). Anticoagulation of all patients was stably controlled with a target INR of 1.6–2.6 for the prevention of stroke recurrence [18,19]. Inclusion criteria were a confirmed date of initial exposure to warfarin, and current anticoagulation therapy. Data collection consisted of inpatient and outpatient medical records. The anticoagulant database was used to obtain information on daily warfarin doses. This study was approved by the Ethical Review Committee of the National Cardiovascular Center. All patients who participated in the study provided written informed consent for genetic analysis.

DNA analyses

We previously performed DNA sequence analyses of 3 genes (*VKORC1*, *GGCX*, and *CALU*) involved in vitamin K cycling in 96 Japanese stroke patients; that study identified genetic polymorphisms and pair-wise linkage disequilibrium (LD) [20]. Using the minor allele frequency (over 4%), LD (r^2 more than 0.5), and possible functional change (missense mutation) as guidance, we selected nine representative SNPs for genotyping: 523G>A, 1338A>G (H68R), and 3730G>A in *VKORC1*, 412G>A, 8016G>A (R325Q), and 8445C>T in *GGCX*, and 11G>A (R4Q), 344G>A, and 20943T>A in *CALU*. In *CYP2C9*, only the 42613A>C (I359L) SNP,

known as the *CYP2C9*3* genotype, was analyzed. In addition, recent studies have demonstrated the significant association of the *VKORC1* polymorphisms −1639G>A and 1173C>T with warf polymorphisms. We adopted the numbering standards of the Nomenclature Working Group, wherein the A of the initiator Met codon (ATG) is denoted nucleotide +1 [21].

The genotypes of the 12 SNPs in our subjects were identified by the TaqMan-PCR system. TaqMan genotyping methodology has been described previously [22]. The PCR primers and probes used for the TaqMan system are available on request.

Statistical analysis

The significance level for all statistical tests was set at $P<0.05$. Pair-wise LD between two polymorphisms was evaluated by r^2 using SNPAlyze v4.0 software (DYNACOM, Kanagawa, Japan). Statistical analyses were performed using JMP v 5.1 software and the SAS release 8.2 (SAS Institute Inc., Cary, NC). Associations between genotypes and warfarin daily doses were examined by one-way analysis of variance or univariate regression analysis. In addition, the relative contributions of age, sex, weight, and selected genetic variations to inter-individual variations in warfarin dose were estimated by using the multiple regression analysis. An index P_I, for estimating the relative contribution of a specific independent variable, x_I, was employed and given by

$$P_I = R^2 - R^2_{-I},$$

where R was the multiple correlation coefficient from the model with all of the selected independent variables (x_1, x_2,..., x_p) and R^2_{-I} was that of the model excluding x_I from the independent variables.

Results

We analyzed the frequency of 11 SNPs in three genes involved in the vitamin K cycle and one polymorphism in *CYP2C9* 42613A>C (*CYP2C9*3*) in 93 stroke patients under stable anticoagulation with warfarin. Characteristics of the patients are summarized in Table 1. The mean±SD daily warfarin dose was 2.96±1.06 mg/day (1.00–5.50 mg/day).

Table 1 Characteristics of patients

Number	93
Number of men (%)	66 (71.0)
Age (years)	68.1±10.6
Weight (kg)	59.8±9.7
Warfarin dose (mg/day)	2.96±1.06
Warfarin dose range (mg/day)	1.00–5.50

Age, weight, and warfarin dose are shown as mean±SD.

Table 2 Differences in daily warfarin dose for each genotype of the *VKORC1*, *GGCX*, and *CYP2C9* genes

Gene	SNP	Genotype	*n*	Mean±SD (mg/day)	*P*
VKORC1	−1639 G>A*	AA	79	2.83±1.00	
		GA	14	3.70±1.11	0.004
		GG	0	–	
VKORC1	1173 C>T*	TT	79	2.83±1.00	
		CT	14	3.70±1.11	0.004
		CC	0	–	
VKORC1	3730 G>A*	GG	79	2.84±1.00	
		GA	14	3.68±1.12	0.006
		AA	0	–	
GGCX	8016 G>A	GG	48	3.25±1.19	
	(R325Q)	GA	39	2.63±0.77	0.022
		AA	6	2.79±1.07	
CYP2C9	42613 A>C	AA	83	3.06±1.05	
	(CYP2C9*3)	AC	9	2.17±0.84	0.015
	(I359L)	CC	0	–	

P values were calculated by one-way ANOVA. *These SNPs were in linkage disequilibrium. Rieder et al. reported that the hepatic expression levels of *VKORC1* mRNA were significantly decreased in the carriers with the *VKORC1* −1639A allele [6]. As for the *GGCX* R325Q mutation, there were no available data on its function. CYP2C9 mutant carrying the missense mutation, I359L (*CYP2C9*3*), showed a markedly high *Km* for the 7-hydroxylation of *S*-warfarin [28].

We examined the association of the genotype data with maintenance warfarin doses by one-way analysis of variance (ANOVA). Of the 12 SNPs examined, five SNPs, −1639G>A, 1173C>T, and 3730G>A in *VKORC1*, 8016G>A (R375Q) in *GGCX*, and *CYP2C9*3* exhibited a significant association with daily warfarin dose (Table 2). The *VKORC1* 1338G>A allele could not be evaluated due to the low minor allele frequency. None of the other SNPs demonstrated a significant association with warfarin dosage.

The mean warfarin dose was higher ($p=0.004$) in patients with the *VKORC1* −1639GA or 1173CT genotypes (3.70 mg/day) than in those with the −1639AA or 1173TT genotypes (2.83 mg/day). The mean warfarin dose was higher ($p=0.006$) in patients with the *VKORC1* 3730GA genotype (3.68 mg/day) than in those with the 3730GG genotype (2.84 mg/day). For *CYP2C9*, the mean warfarin dose was higher ($p=0.015$) in patients with the *CYP2C9*1*1* (*CYP2C9* 42613AA) genotype (3.06 mg/day) than in those with the **1*3* (42613AC) genotype (2.17 mg/day).

A significant association was observed between warfarin dosage and the 8016G>A SNP of *GGCX*. The mean warfarin dose was higher ($p=0.022$) among patients with the *GGCX* 8016GG genotype (3.25 mg/day) than in those with the GA (2.84 mg/day) or AA (2.79 mg/day) genotypes. The *GGCX* 8016G>A SNP,

rs699664, leads to the substitution of Gln for Arg at amino acid 325.

We previously genotyped three SNPs, −1639G>A, 1173C>T, and 3730G>A in *VKORC1*, in 3652 population-based individuals [20]. This analysis obtained a minor allele frequency of 0.086 for all SNPs. Three SNPs were in tight LD with a pair-wise r^2 value of 0.98. Two SNPs in particular, −1639G>A and 1173C>T, were in complete LD in the study population. Therefore, −1639G>A and 3730G>A were used for additional analysis to estimate the influence of *VKORC1* genotypes of warfarin dosage.

To estimate the contribution of each SNP to variabilities in warfarin dosages, we performed univariate regression analyses for four SNPs, *VKORC1* −1639G>A and 3730G>A, *GGCX* 8016G>A, and *CYP2C9* 42613A>C (*CYP2C9*3*) (Table 3). The R^2 values determined for *VKORC1* −1639G>A and 3730G>A were 0.086 and 0.082, respectively. The equivalent R^2 value observed in the model of *GGCX* 8016G>A (R^2 = 0.081) was higher than that of *CYP2C9* 42613A>C (R^2 = 0.064).

Multiple regression analysis was performed to estimate the relative contributions of age, sex, weight, and three genetic polymorphisms to the inter-individual variations in warfarin dose. These results were shown in Table 4. The model included age, sex, weight, and three genetic polymorphisms, 6 variables in total, as the independent variables and accounted for 33.3% of total variations in warfarin dose. The contribution, P_i, to inter-individual variation in warfarin dose was 5.9% for *VKORC1* −1639G>A, 5.2% for *CYP2C9* 42613A>C, and 4.6% for *GGCX* 8016G>A.

Table 3 Univariate regression analyses for warfarin daily dosage

Variables	R^2	P
VKORC1-1639G>A*	0.086	0.004
VKORC1 3730G>A*	0.082	0.006
GGCX 8016G>A	0.081	0.022
CYP2C9 42613A>C	0.064	0.015

R^2 and P values were calculated by univariate regression analyses. *These two SNPs were in linkage disequilibrium.

Table 4 Multiple regression analysis for estimating the relative contributions of age, sex, weight, and selective genetic variations with warfarin dose

Independent	Std $\beta^\dagger$	$P_i \times 100$
Age	−0.141	1.69
Sex	0.786	8.12*
Weight	0.374	7.78*
VKORC1 −1639G>A	0.735	5.88**
GGCX 8016G>A	−0.451	4.60**
CYP2C9 42613A>C	−0.847	5.19**

†: Standardized regression coefficient.
*: $P<0.01$, **: $0.01 \le P<0.05$.

Discussion

In this study, we have examined the contribution of four genes to the warfarin maintenance dose required in Japanese patients following ischemic stroke. The patients were controlled in the target INR of 1.6–2.6. A previous study on the optimal intensity of warfarin therapy for secondary prevention of stroke in patients with non-valvular atrial fibrillation showed that the low-intensity warfarin (INR 1.5 to 2.1) treatment seemed to be safer than the conventional-intensity (INR 2.2 to 3.5) treatment [18]. The annual rate of ischemic stroke was low in both groups (1.1% per year in the conventional-intensity group and 1.7% per year in the low-intensity group) and did not differ significantly. Based on this result and the guideline of the Japanese Circulation Society for the treatment of atrial fibrillation, we adopted the target INR of 1.6–2.6. Daily warfarin dose of each patient was properly controlled to meet target INR. As a result, the range of the warfarin dose was between 1 and 10 mg.

Warfarin is the most prescribed oral anticoagulant. Warfarin targets VKORC1 and antagonizes vitamin K, an essential cofactor for the modification of specific glutamic acid to γ-carboxyglutamic acid in coagulation factors II, VII, IX and X. Warfarin is metabolized by CYP2C9. Patients with *CYP2C9*2* and *CYP2C9*3* alleles have lower mean daily warfarin doses and a greater risk of bleeding [16,23]. Recent studies on *VKORC1* showed that SNPs in *VKORC1* have a more important function than the *CYP2C9* variations in terms of inter-individual variability of warfarin. It has been reported that the *VKORC1* haplotype accounted for 21% of inter-individual variability of warfarin and the *CYP2C9* genotype explained 6% [6]. Subsequent studies reached the similar conclusion that the *VKORC1* genotype affects inter-individual variability of warfarin more greatly than the *CYP2C9* genotype [5,8–11]. Inclusion of non-genetic factors such as age, sex, body surface area, body weight, and drug interaction with genotype information accounted for up to 60% of inter-individual variability of warfarin [5,8–11]. The remaining 40% of warfarin dosing variability remains unexplained.

In our study, *VKORC1* −1639G>A explained 5.9% of the inter-individual variabilities in warfarin dose, while CYP2C9*3 explained 5.2% (Table 4). We also detected a significant association between *GGCX* 8016G>A (R325Q) and warfarin dosage, which explained 4.6% of the variability seen in our subjects (Table 4). We have recently reported that *GGCX* 8016G>A influences the inter-individual variations in