Table I. Baseline characteristics of the 3 randomized groups

Parameter	Placebo group (n = 49)	5 mg Group (n = 47)	20 mg Group (n = 77)	P
Age (y)	62 ± 12	59 ± 9	60 ± 12	.581*
Sex (male/female) (%)	82/18	77/23	74/26	.613†
Etiology of heart failure				
Nonischemic/ischemic (%)	76/24	75/25	71/29	.864†
NYHA class, II/III (%)	80/20	81/19	75/25	.735†
LVEF (%)	29 ± 7	30 ± 8	30 ± 7	.734*
Systolic BP (mm Hg)	121 ± 17	117 ± 16	119 ± 14	.418*
Diastolic BP (mm Hg)	72 ± 11	72 ± 11	73 ± 10	.660*
Heart rate (beats/min)	81 ± 14	74 ± 11	78 ± 17	.097*
Body weight (kg)	60 ± 10	60 ± 11	62 ± 14	.349*
Other medications				
ACE inhibitors (%)	80	81	70	.304†
Diuretics (%)	84	85	88	.743†
Digitalis (%)	59	70	65	.527†

Values presented as mean ± SD.
*Regression analysis.
†χ^2 Test.

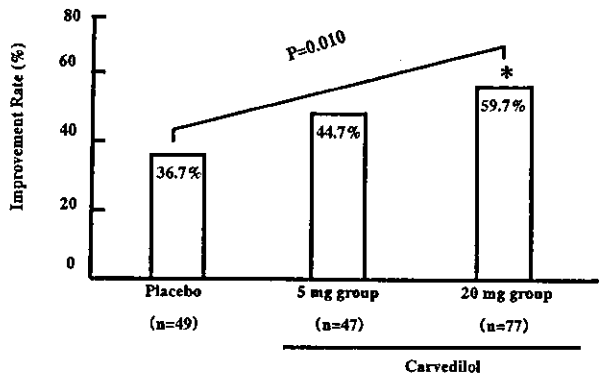
followed by uptitration to 50 mg/d for maintenance treatment if tolerated.[6] In Japan, the dose of carvedilol for the treatment of hypertension and angina pectoris is 10 to 20 mg/d,[7,8] which is less than half of that used in Western countries. However, the effectiveness of such a low-dose carvedilol regimen for Japanese patients with CHF remains unclear. The present study was designed to test the efficacy and safety of low-dose carvedilol regimens in Japanese patients with CHF.

Methods

Study design

The Multicenter Carvedilol Heart Failure Dose Assessment (MUCHA) trial was a randomized, multicenter, placebo-controlled, double-blinded study conducted in 5 phases: (1) screening, (2) challenge, (3) uptitration, (4) maintenance, and (5) downtitration. During the challenge phase after screening, eligible patients received open-label carvedilol (1.25 mg twice daily) for 1 to 2 weeks, and the dose was increased to 2.5 mg twice daily if tolerated. Patients were entered into the double-blinded uptitration phase after tolerating a dose of 2.5 mg twice daily for at least 2 weeks and were randomly assigned to placebo, carvedilol at 2.5 mg twice daily (5 mg group), or carvedilol at 10 mg twice daily (20 mg group) in the proportion of 1:1:2 by the dynamic allocation method. In the 20 mg group, the carvedilol dose was increased stepwise in a double-blinded fashion at 1- or 2-week intervals to reach 10 mg twice daily or the maximum tolerated dose of <10 mg twice daily. Patients then received placebo or carvedilol at a fixed dose for 24 to 48 weeks during the maintenance phase. When the last entered patient had completed 24 weeks of maintenance therapy, all patients were shifted into the downtitration phase.

Figure 1



Improvement rate in each group. Improvement rate is the percentage of patients with moderate or marked improvement of signs and symptoms of heart failure as assessed by the attending physician at the end of the maintenance phase in comparison with baseline. Improvement achieved with carvedilol treatment was evaluated by the Cochrane-Armitage test to assess the dose-response relation; pairwise comparisons with placebo were performed by means of χ^2 test. *$P < .05$ vs placebo.

Inclusion and exclusion criteria

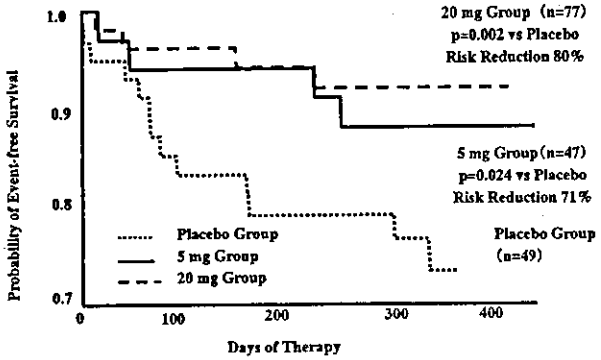
Patients who had ischemic or nonischemic cardiomyopathy with stable symptoms (New York Heart Association functional class [NYHA class] II or III) were eligible for enrollment if their left ventricular ejection fraction (LVEF) was ≤40% (measured by M-mode echocardiography or radionuclide ventriculography at the qualifying examination) and

Table II. Death and CVD hospitalization

Parameter	Placebo group (n = 49)	5 mg Group (n = 47)	20 mg Group (n = 77)	Placebo vs 5 mg Hazard ratio [95% CI]	P*	Placebo vs 20 mg Hazard ratio [95% CI]	P	P for linear trend†
Death or CVD hospitalization (%)	12 (24.5)	4 (8.5)	4 (5.2)	0.29 [0.10–0.91]	.024	0.20 [0.06–0.60]	.002	.002
CVD hospitalization (%)	12 (24.5)	2 (4.3)	3 (3.9)	0.14 [0.03–0.65]	.003	0.15 [0.04–0.52]	<.001	<.001
Worsening CHF	10 (20.4)	1 (2.1)	2 (2.6)	0.09 [0.01–0.69]	.004	0.12 [0.03–0.54]	<.001	<.001
Other CDV reasons	3 (6.1)	1 (2.1)	1 (1.3)	0.29 [0.03–2.75]	.229	0.20 [0.021–1.88]	.116	.111

*Log-rank test.
†Linear trend test using the Cox proportional hazards regression model.

Figure 2



Kaplan-Meier analysis of the probability of survival without death or CVD hospitalization in patients randomly assigned to placebo (*dotted line*), 5 mg/d carvedilol (*solid line*), or 20 mg/d carvedilol (*broken line*). Graph shows time to first event for each group. Dose-response relation was analyzed by means of Cox proportional hazards regression model; log-rank test was used to compare each carvedilol dose with placebo.

their age was between 20 and 79 years. Patients with the following conditions were excluded: valvular heart disease, hypertrophic obstructive cardiomyopathy, cardiogenic shock, systolic blood pressure <90 mm Hg, bradycardia (<60/min), grade II or III atrioventricular block, life-threatening arrhythmia, unstable angina, resting angina, cor pulmonale, asthma, Raynaud phenomenon, and intermittent claudication. Patients were also excluded if myocardial infarction or coronary artery bypass grafting had occurred within the preceding 3 months.

Diuretics, digitalis, angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, vasodilators, and antiarrhythmic agents could be used concomitantly. Drugs prohibited during the study were other β-blockers, αβ-blockers, α-blockers, inotropic agents other than digitalis, and intravenous diltiazem hydrochloride or verapamil hydrochloride.

The institutional review board of each participating hospital approved the study, and each subject gave written informed consent.

Parameters assessed

The primary end point of the study was improvement of the global assessment of CHF (signs and symptoms) by the attending physician. The secondary end points were as follows: all-cause death or hospitalization for cardiovascular disease (CVD), CVD hospitalization, hospitalization for worsening CHF, changes of LVEF, and changes of NYHA class.

Global assessment of heart failure was performed by entering CHF symptoms and objective findings at baseline and follow-up into a study form. Differences between the assessment at baseline and at last follow-up during maintenance treatment were rated according to the protocol and were assigned one of the following 6 grades: markedly improved, moderately improved, mildly improved, no change, worsened, or unassessable. The improvement rate (the primary end point) for each treatment group was defined as the proportion assigned a rating of "moderately improved" or "markedly improved." Data concerning CVD hospitalizations and deaths were reported prospectively by the investigators and were reviewed and classified by the End Point Committee.

The end point of changes in LVEF was defined as the difference between M-mode echocardiography measurements at baseline and last follow-up during the maintenance phase, with the left ventricular volumes being calculated by the method of Teichholz. The Data and Safety Monitoring Board prospectively monitored all serious adverse events.

Statistical analysis

On the basis of data from a Japanese pilot study and the US Carvedilol Heart Failure Trials Program,[9-12] it was projected that a sample size of 160 patients (40 for the placebo group, 40 for the 5 mg group, and 80 for the 20 mg group) would provide 80% power at the $P = .05$ level of significance to detect a dose-response effect among the three groups as well as assessing the efficacy of each carvedilol dose.

Data on the improvement rate from the physicians' global assessments were analyzed by the Cochrane-Armitage test to

Table III. Death or CVD hospitalization rates according to baseline characteristics

Subgroup	Event rate (%)				Hazard ratio (*P*, Log-rank test)	
	Placebo group (P)	5 mg Group (L)	20 mg Group (H)	*P*, linear trend test*	P vs L	P vs H
Sex						
Male (n = 133)	25.0	8.3	7.0	.013	0.27 (.036)	0.26 (.015)
Female (n = 40)	22.2	9.1	0.0	.034	0.39 (.419)	– (.023)
Age (y)						
<65 (n = 99)	24.0	0.0	4.4	.014	– (.005)	0.21 (.019)
≥65 (n = 74)	25.0	22.2	6.3	.041	0.81 (.745)	0.21 (.032)
Etiology						
Nonischemic (n = 127)	24.3	2.9	3.6	.002	0.10 (.007)	0.15 (.005)
Ischemic (n = 46)	25.0	25.0	9.1	.150	0.84 (.829)	0.29 (.156)
NYHA class						
II (n = 135)	28.2	7.9	6.9	.005	0.23 (.013)	0.23 (.006)
III (n = 38)	10.0	11.1	0.0	.215	1.05 (.970)	– (.180)
LVEF (%)						
<30 (n = 76)	33.3	10.0	2.9	.002	0.25 (.059)	0.08 (.003)
≥30 (n = 95)	18.5	7.4	7.3	.140	0.35 (.192)	0.34 (.127)
Systolic BP (mm Hg)						
<120 (n = 84)	15.0	14.8	10.8	.638	0.95 (.950)	0.70 (.633)
≥120 (n = 89)	31.0	0.0	0.0	<.001	– (.004)	– (.001)
Heart rate (beats/min)						
<75 (n = 78)	29.4	11.1	8.8	.102	0.35 (.135)	0.31 (.089)
≥75 (n = 95)	21.9	5.0	2.3	.004	0.18 (.073)	0.09 (.005)

*Linear trend test using the Cox proportional hazards regression model.

Table IV. Death or CVD hospitalization rates according to presence of concomitant disease at baseline examination

Subgroup	Event rate (%)				Hazard ratio (*P*, Log-rank test)	
	Placebo group (P)	5 mg Group (L)	20 mg Group (H)	*P*, linear trend test*	P vs L	P vs H
Hypertension						
Nonhypertensive (n = 132)	22.9	10.3	5.2	.014	0.38 (.104)	0.22 (.013)
Hypertensive (n = 41)	28.6	0.0	5.3	.041	– (.104)	0.15 (.052)
Diabetes						
Nondiabetic (n = 118)	24.2	6.1	7.7	.035	0.21 (.031)	0.30 (.035)
Diabetic (n = 55)	25.0	14.3	0.0	.011	0.49 (.401)	–(.010)
Hyperlipidemia						
Normolipidemic (n = 136)	26.8	8.6	6.7	.007	0.28 (.037)	0.24 (.008)
Hyperlipidemic (n = 37)	12.5	8.3	0.0	.140	0.53 (.648)	– (.145)
Cardiac rhythm						
Sinus rhythm (n = 132)	21.4	11.4	1.8	.002	0.46 (.188)	0.08 (.002)
Atrial fibrillation (n = 41)	42.9	0.0	13.6	.214	– (.014)	0.29 (.106)

*Linear trend test using the Cox proportional hazards regression model.

assess the dose-response relation. When a significant difference was found, pairwise comparisons with placebo were performed with the χ^2 test.

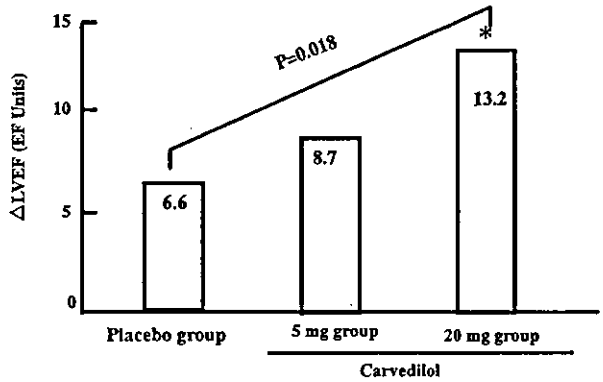
For death or CVD hospitalization, CVD hospitalization, and hospitalization for CHF, Kaplan-Meier curves were constructed as time-to-first event plots for each group, and data were analyzed to detect a dose-response relation by means of the Cox proportional hazards regression model. Then event-free survival curves were compared among the placebo group and carvedilol groups by means of the log-rank

Table V. Change of NYHA class from baseline to the end of the maintenance phase

Group	Improved, n (%)	No change, n (%)	Worsened, n (%)	P, Wilcoxon rank sum test	P, linear trend test*
Placebo	23 (48.9)	19 (40.4)	5 (10.6)		
5 mg	38 (80.9)	8 (17.0)	1 (2.1)	.037†	.046
20 mg	51 (70.8)	20 (27.8)	1 (1.4)	.051†	

*Linear trend test using the Cochrane-Mantel-Haenszel test.
†Versus placebo.

Figure 3



Changes of LVEF from baseline to end of maintenance phase. Dose-response relation was assessed by means of regression analysis; pairwise comparisons with placebo were done by Student *t* test. Change of LVEF was defined as the difference between baseline value and value at end of maintenance phase and is expressed in ejection fraction (EF) units.

test. All analyses were performed on the full analysis set,[13] and the level of significance was set at $P < .05$ (2-tailed).

Results

Open-label challenge phase

Treatment was started on October 28, 1996, and the trial was completed on March 17, 2000. One hundred ninety patients commenced the challenge phase and 174 patients (91.6%) were randomly assigned to the 3 treatment groups.

Baseline patient profile

Of 174 patients randomly assigned to the 3 treatment groups, one patient in the 20 mg group who received no study medication was excluded from the full analysis set. There were no significant differences in baseline characteristics among the three treatment groups (Table I). Seventy-four percent of patients in the 20 mg group and 100% in the 5 mg group achieved the target dose; the mean dose was 17.2 mg/d in the 20 mg group.

Global assessment of changes in CHF (primary end point)

The percentage of each treatment group with moderate or marked improvement in signs and symptoms of heart failure (improvement rate) ascertained by attending physicians showed a significant dose-response relation to carvedilol ($P = .010$), and in pairwise comparison, the improvement rate was significantly higher in the 20 mg group than in the placebo group ($P = .012$) (Figure 1).

Death or CVD hospitalization

The incidence of death or CVD hospitalization showed a significant dose-response relation ($P = .002$) and was significantly lower in both the 5 mg group ($P = .024$) and the 20 mg group ($P < .002$) on pairwise comparison with the placebo group (Table II and Figure 2). Risk reduction was 71% in the 5 mg group and 80% in the 20 mg group. Carvedilol treatment was associated with a highly significant decrease of the CVD hospitalization rate in the 5 mg group ($P = .003$) and the 20 mg group ($P < .001$), as well as for the linear trend ($P < .001$). Compared with placebo, the reduction in risk of CVD hospitalization was 86% in the 5 mg group and 85% in the 20 mg group (Table II). This included a risk reduction for hospitalization resulting from worsening heart failure of 91% for the 5 mg group and 88% for the 20 mg group.

Subgroup analysis showed that the risk of death or CVD hospitalization was lower in the carvedilol-treated groups regardless of age, sex, underlying cause of CHF, severity of CHF, LVEF, systolic blood pressure, or heart rate (Table III). The risk of death or CVD hospitalization in high-risk patients with hypertension, diabetes mellitus, hyperlipidemia, and atrial fibrillation was also lower in the carvedilol-treated groups (Table IV).

Left ventricular function

There was a significant dose-related increase of LVEF with carvedilol treatment ($P = .018$), and there was a significantly greater increase of LVEF in the 20 mg group compared with the placebo group ($P = .022$) (Figure 3).

NYHA class and adverse events

A significant dose-dependent improvement of NYHA class was observed with carvedilol treatment (Table V).

The incidence of all serious and nonserious adverse events was lower (but not significantly) in the carvedilol-treated groups (63.3%, 51.1%, and 59.7% in the placebo, 5 mg, and 20 mg groups, respectively). Adverse events with a higher incidence (P = NS) in the 20 mg group were dizziness, upper respiratory tract infections, worsening of diabetes, palpitations, headache, and hypotension.

Discussion

The MUCHA trial demonstrated a dose-related improvement in signs and symptoms of heart failure assessed by the attending physicians and improvement of LVEF in Japanese patients with CHF who received long-term carvedilol therapy at low doses of 5 or 20 mg/d. These low doses achieved a remarkable reduction in the risk of death or CVD hospitalization; surprisingly, the 5 mg/d dose achieved a reduction (71%) that was nearly as great as that for the 20 mg/d dose (80%).

In the current study, a dramatic reduction in the risk of hospitalization for CHF or CVD was observed with both carvedilol regimens. Although the majority of the patients were in NYHA class II, this was probably not the reason for the marked reduction of CVD hospitalization, because previous trials performed in the United States and Australasia[1-3] have shown that risk reduction is not related to the NYHA class.

In the current study, carvedilol reduced the risk of death or CVD hospitalization irrespective of the presence of hypertension, diabetes, hyperlipidemia, and atrial fibrillation. In the CIBIS-II study, the β_1-selective blocking agent bisoprolol decreased mortality and hospitalization rates in patients with sinus rhythm but not in patients with atrial fibrillation.[14] In contrast, we found that carvedilol reduced the risk of death or cardiovascular hospitalization irrespective of the presence of atrial fibrillation, consistent with the results of the US Carvedilol Heart Failure Trials Program.[15]

In previous studies of carvedilol therapy for patients with CHF, improvement of LVEF was accompanied by a reduction of rates for death or CVD hospitalization.[10] At a dose of 5 mg/d, however, a marked reduction in the risk of CVD hospitalization in the current study was observed without a statistically significant improvement of LVEF. In patients with CHF, Quaife et al[16] reported that carvedilol improved the right ventricular ejection fraction, with a decrease of the right ventricular end-diastolic and end-systolic volumes and a decrease of the pulmonary arterial pressure. They also suggested that the improvement of right ventricular systolic function was not secondary to the improvement of left ventricular function but was due to the direct effect of carvedilol.[16] Our results may be consistent with a direct effect of low-dose carvedilol on right ventricular function.

The recommended dose of carvedilol shown by the US MOCHA trial was 12.5 to 50 mg/d and that shown by the MUCHA trial in Japan was 5 to 20 mg/d. Since the pharmacokinetics of carvedilol are similar in healthy Japanese and American adults (unpublished observation), whereas β_1-receptor sensitivity is higher in Chinese than in white or black Americans,[17] the difference in the effective dose between patients in Japan and Western countries may depend on a difference in β_1-receptor sensitivity.

The current findings regarding the global improvement of heart failure, LVEF, and morbidity strongly suggest that the recommended dose of carvedilol for Japanese patients with CHF should range from 5 to 20 mg/d. In addition, our finding of reduced morbidity at a low dose of carvedilol (5 mg/d) suggested that low-dose therapy may have a beneficial effect in patients who cannot tolerate a standard regimen. A future large-scale study will be necessary to evaluate the impact of low-dose, long-term carvedilol therapy on survival in Japanese patients with CHF.

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Appendix

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Original Paper

European Neurology

Eur Neurol 2004;51:138–143
DOI: 10.1159/000077064

Received: June 27, 2003
Accepted: December 22, 2003
Published online: February 26, 2004

Association of Pontine Small Infarction (Lacuna) with Disturbance of Postural Stability

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Key Words
Postural stability · Pontine lacuna · Stabilometry

Abstract
Neuronal degeneration within the brainstem has been reported in patients with impaired postural stability. However, the functional significance of these abnormalities is unknown at present. In the present study, we evaluated the relationship between the presence of pontine lacunae and postural stability measured by stabilometry. A total of 209 consecutive patients without neurological signs were divided into three groups according to the territory of lacunae on magnetic resonance imaging: (1) non-lacunar group, (2) pontine lacunar group, and (3) non-pontine lacunar group. Stabilometry was performed and statokinesigram measures including each Romberg quotient were compared among the three groups. Using multivariate analysis, postural stability was found to be disturbed in the pontine lacunar group compared with the other groups. The data of stabilometry in this group were compatible with disturbance of the central controlling system for keeping postural stability. Pontine lacuna is associated with patients with postural instability. This result may be related to the deterioration of the central coordination system for posture and locomotion.

Introduction

Age-related structural deterioration of central coordination systems for posture and locomotion regulation may be responsible for postural control disturbance [1–4]. A neuronal loss within the brainstem vestibular nucleus complex and disturbed ventral tracts have been recognized depending on age [2–4]. The older individuals exhibit pontine small infarcts (lacunae) on brain magnetic resonance imaging (MRI). In the present study, we therefore assessed the relationships between the presence of pontine lacunar lesions and postural instability measured by stabilometry in elderly patients without neurological signs.

Subjects and Methods

Subjects

Between February 1998 and January 2001, 209 of 213 consecutive patients who complained of miscellaneous symptoms such as headache, tinnitus, and dizziness, and did not have any neurological abnormality were examined using the same protocol including neurological examination, MRI and stabilometry in the Department of Neurosurgery, Social Insurance Takaoka Hospital, Takaoka, Japan. Four patients did not undergo MRI and/or stabilometry. All 209 participants gave their informed consent after a full explanation of the aim of the present study. There were 90 male and 119 female

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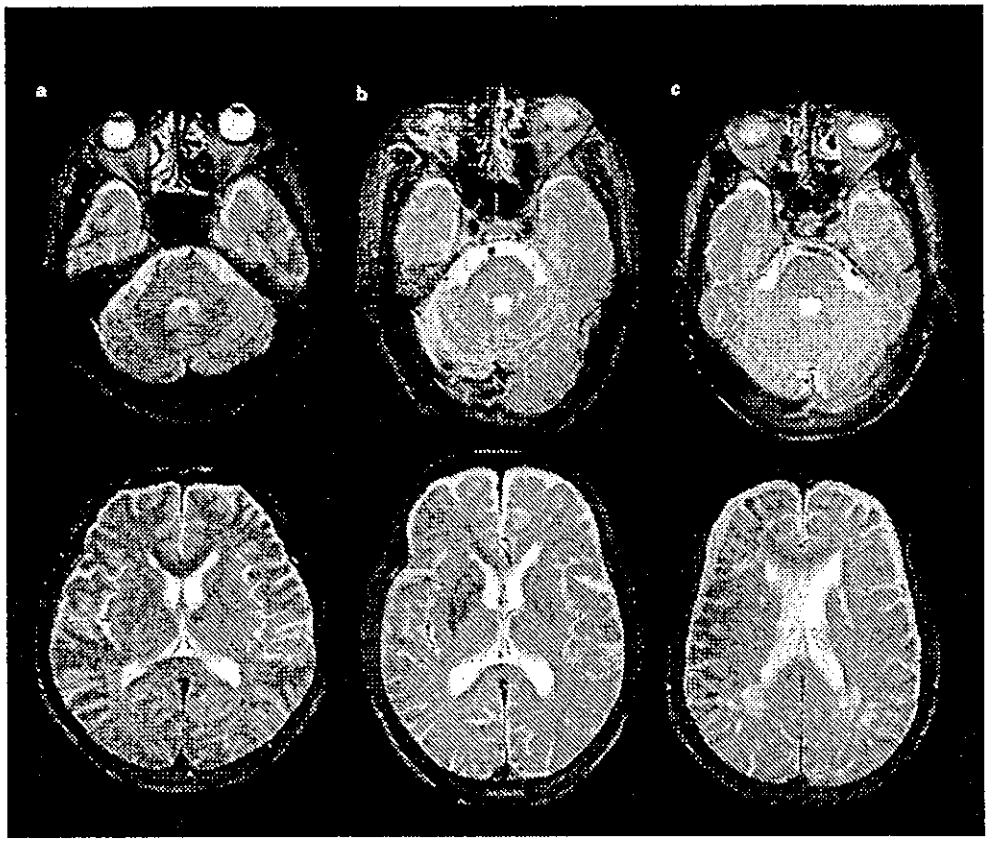

Fig. 1. Brain MRIs of patients representing the non-lacunar group (**a**), the pontine lacunar group (**b**), and the non-pontine lacunar group (**c**).

patients, ranging in age from 36 to 88 years (mean, 68.0 years). Information on their past history of hypertension, hyperlipidemia, diabetes mellitus (DM), and ischemic cardiac disease was also collected.

MRI Protocol

A standard MRI protocol was performed for all patients with a 1.5-tesla superconducting system (Shimazu, Kyoto, Japan). With a multislice technique, the brain was imaged in the axial plane at 5-mm intervals. The spin echo technique used a pulse repetition time (TR) of 2,000 ms and echo time (TE) of 120 ms for T_2-weighted images and a TR of 2,100 ms and TE of 25 ms for proton-weighted images. The inversion recovery technique used a TR of 2,400 ms, TE of 40 ms, and inversion time of 600 ms for T_1-weighted images. The image matrix was 256 × 256 pixels. A neuroradiologist divided the patients into three groups based on visual analysis of MRI (non-lacunar group, pontine lacunar group, and non-pontine lacunar group) (fig. 1). The pontine lacunar group included not only patients with pontine lacuna, but also patients with both pontine lacuna and non-pontine lacuna. For visual analysis, MRI did not reveal patient names nor data from examinations. Based on previous reports [5, 6], lacuna was defined as a lesion which is hypointense on T_1-weighted MRI and hyperintense on T_2-weighted MRI. Being wedge shaped vs. round shaped is also very helpful to distinguish lacuna from perivascular space (Virchow-Robin space), as well as if we can refer to proton density imaging.

Stabilometric Test Procedures

All stabilometric measurements were performed using a computerized force platform (Gravicorder™, Model G5500, Anima Corp, Tokyo, Japan) according to a standard protocol [7]. For stabilometry, we used vertical force transducers to determine instantaneous fluctuations in the center of pressure (COP). A statokinesigram (i.e. the sway path of the COP) was obtained from these vertical forces as the change in electrical signals during a 30-second upright stance. The standard test battery included the following measurements of sway for 30 s with eyes open and eyes closed: locus length (LNG), environmental area (ENV), rectangular area (REC), locus length per time (LNG/time), locus length per environmental area (LNG/ENV), and root mean square area (RMS). LNG was defined as the sum of the

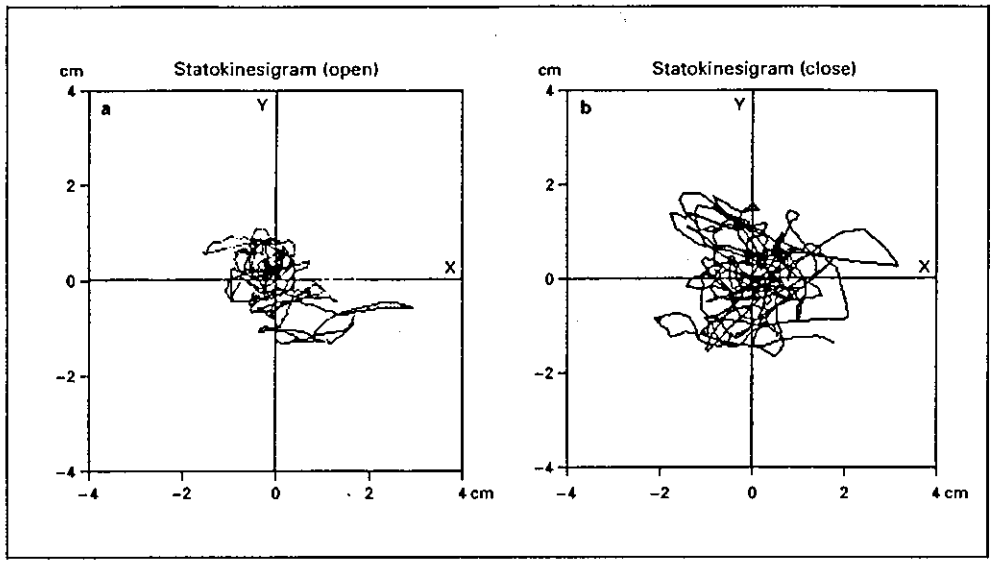

Fig. 2. Typical statokinesigrams of a 76-year-old male patient. His MRI exhibited pontine lacuna. **a** Measurement with eyes open: LNG = 58.0, LNG/time = 1.93, LNG/ENV = 15.5, ENV = 3.75, REC = 10.9, and RMS = 2.43. **b** Measurement with eyes closed: LNG = 119, LNG/time = 3.97, LNG/ENV = 15.3, ENV = 7.79, REC = 18.3, and RMS = 4.30. Romberg quotients of the parameters: LNG = 2.05, LNG/time = 2.05, LNG/ENV = 0.99, ENV = 2.07, REC = 1.67, and RMS = 2.33.

path length of the COP. ENV was the size of environmental area inside the path and REC was the rectangular area of the path. RMS was also calculated. Romberg quotients of the statokinesigram (measure with eyes closed/measure with eyes open) were also calculated. A typical statokinesigram chart is illustrated in figure 2. Each chart line indicates the path of the COP during the measurement. Actual real-time calculations were carried out by the built-in Gravicorder program, Model G5500.

Statistical Analyses

Values are presented as means ± SD. One-way analysis of variance followed by Fisher's protected least-squares difference test (ANOVA + Fisher) and χ^2 test were performed for multiple comparisons. Analysis of covariance was performed using multivariate analysis after adjustment of background factors, which exhibited imbalance among the three groups in univariate analysis. If there was a statistically significant difference among the three groups in multivariate analysis, pairwise comparison was performed. All statistical tests were performed at the 5% level of significance.

Results

We found no lacunar lesions in 73 (non-lacunar group), pontine lacunar lesions in 41 (pontine lacunar group), and lacunar lesions except in the pons in 95 (non-pontine lacunar group) of the 209 patients who were enrolled in the present study. Background data for these three groups including age, sex, and history of hypertension, hyperlipidemia, DM and ischemic cardiac disease were compared (table 1). Although no difference in sex, and history of DM or ischemic cardiac disease was observed among the three groups, there were significant differences in the history of hypertension and hyperlipidemia among them [hypertension: 15/73 (21%) in the non-lacunar group, 22/41 (54%) in the pontine lacunar group, and 42/95 (44%) in the non-pontine lacunar group; hyperlipidemia: 4/73 (5%) in the non-lacunar group, 4/41 (10%) in the pontine lacunar group, and 21/95 (22%) in the non-pontine lacunar group; table 1]. Furthermore, there were significant differences in age among them (61.2 ± 10.9 years in the non-lacunar group, 70.5 ± 7.80 years in the pontine lacunar group, 72.2 ± 7.70 years in the non-pontine lacunar group; pontine lacunar group > non-lacunar group, $p < 0.0001$; non-pontine lacunar group > non-lacunar group, $p < 0.0001$; table 1).

The results of measurements of the statokinesigram with eyes open and eyes closed are summarized in table 2. Univariate analysis of these data using the ANOVA +

Table 1. Summary of the characteristics of the 209 elderly patients

	Non-lacunar group (n = 73)	Pontine lacunar group (n = 41)	Non-pontine lacunar group (n = 95)	p value
Age (means ± SD), years	61.2 ± 10.9	70.5 ± 7.8	72.2 ± 7.7	$p < 0.0001^{a,c}$ $p < 0.0001^{b,c}$
Sex				
M	38 (52)	19 (46)	33 (35)	p = 0.072
F	35 (48)	22 (54)	62 (65)	
Hypertension				
+	15 (21)	22 (54)	42 (44)	$p = 0.0005^{c}$
–	58 (79)	19 (46)	53 (56)	
Hyperlipidemia				
+	4 (5)	4 (10)	21 (22)	$p = 0.0059^{c}$
–	69 (95)	37 (90)	74 (78)	
DM				
+	4 (5)	3 (7)	6 (6)	p = 0.926
–	69 (95)	38 (93)	68 (94)	
Ischemic cardiac disease				
+	3 (4)	4 (10)	6 (6)	p = 0.487
–	70 (96)	37 (90)	89 (94)	

Figures in parentheses indicate percentages.
[a] Comparison between the non-lacunar group and the pontine lacunar group.
[b] Comparison between the non-lacunar group and the non-pontine lacunar group.
[c] Statistically significant.

Table 2. Univariate analysis of statokinesigram data in the 209 elderly patients

	Non-lacunar group (n = 73)	Pontine lacunar group (n = 41)	Non-pontine lacunar group (n = 95)	p value
LNG, cm	47.8 ± 18.0	61.0 ± 24.1	48.8 ± 15.9	$0.0003^{a,c}$ $0.0005^{b,c}$
LNG/time, cm/s	1.69 ± 0.60	2.03 ± 0.80	1.64 ± 0.55	$0.0004^{a,c}$ $0.001^{b,c}$
LNG/ENV, 1/cm	23.0 ± 10.7	21.4 ± 9.80	21.0 ± 9.20	n.s.
ENV, cm^2	2.63 ± 2.19	3.77 ± 2.77	2.71 ± 1.34	$0.0039^{a,c}$ $0.0049^{b,c}$
REC, cm^2	7.28 ± 6.17	10.4 ± 8.50	7.50 ± 4.11	$0.0081^{a,c}$ $0.01^{b,c}$
RMS, cm^2	2.02 ± 2.12	2.26 ± 1.67	1.87 ± 0.96	n.s.
LNG, Romb	1.59 ± 0.414	1.67 ± 0.650	1.69 ± 0.506	n.s.
LNG/time, Romb	1.58 ± 0.416	1.67 ± 0.651	1.69 ± 0.566	n.s.
LNG/ENV, Romb	0.978 ± 0.377	1.11 ± 0.596	0.986 ± 0.356	n.s.
ENV, Romb	1.80 ± 1.04	1.89 ± 1.38	2.00 ± 1.23	n.s.
REC, Romb	1.86 ± 1.10	1.94 ± 1.54	2.10 ± 1.34	n.s.
RMS, Romb	1.63 ± 0.974	1.78 ± 1.21	1.82 ± 1.06	n.s.

Romb = Romberg quotient; n.s. = not significant. Values are means ± SD.
[a] Comparison between the non-lacunar group and the pontine lacunar group.
[b] Comparison between the pontine lacunar group and the non-pontine lacunar group.
[c] Statistically significant.

Fisher test revealed statistically significant differences among the three groups in measures with eyes open except for LNG/ENV and RMS, while no measures exhibited significant differences in the Romberg quotients (table 2). Age, and history of hypertension and hyperlipidemia were significantly different and sex was not significantly but considerably different among the three groups in univariate analysis. After adjustment for age, sex, hypertension and hyperlipidemia, the analysis of covariance demonstrated differences in LNG, LNG/time, ENV and REC among the three groups (table 3). Pairwise comparison revealed no difference in LNG, LNG/time, ENV or REC between the non-pontine lacunar group and the non-lacunar group, with all these measures greater in the pontine lacunar group than in the non-pontine lacunar group, as well as greater measures of LNG, LNG/time, and ENV in the pontine lacunar group compared with in the non-lacunar group (table 4).

Discussion

The localization of brain regions that are abnormal in patients with gait disturbance was reviewed by Masdeu [8]. To maintain postural stability, sensory information is gathered from various afferent sources, such as visual, vestibular and proprioceptive end organs [9]. Furthermore, this information is closely modulated by higher centers in the central nervous system including the brainstem and cerebellum [9–11]. Patients with ventral pontine lesions frequently show symptoms of disequilibrium, and corticospinal tracts, corticopontine tracts as well as rubrospinal tracts may also contribute to these symptoms [4]. According to clinical radiological analysis of pontine infarct boundaries, tegmental pontine infarcts were most frequently associated with vertigo [12]. However, lesions in other territories were also very often associated with vertigo [12]. In our series, 41 of the 209 patients (19.6%) exhibited pontine lacunae. Although the territory of lacunae was not taken into consideration, the lesions were located in the ventral part of the pons in many patients.

Using multivariate analysis of stabilometric measures, postural stability was shown to be disturbed in the pontine lacunar group compared with the non-pontine lacunar group and the non-lacunar group. When stabilometric tests show a disturbance of the postural stability, it is not always easy to explain the mechanism-evoking symptoms. Stabilometric measures of body sway with both eyes open and eyes closed were greater in the pontine lacunar group than in the non-pontine lacunar group and the non-lacunar group. However, the Romberg quotients for theses measures did not differ among the three groups.

Table 3. Least-square means of the three groups for several parameters adjusted for covariates

	Non-lacunar group (n = 73)	Pontine lacunar group (n = 41)	Non-pontine lacunar group (n = 95)	p value
LNG	53.2±26.0	63.4±25.2	48.6±22.1	0.0029[a]
LNG/time	1.77±8.71	2.10±0.845	1.61±0.742	0.0037[a]
ENV	2.95±2.87	4.17±2.78	2.61±2.45	0.0037[a]
REC	8.16±8.63	11.1±8.32	7.34±7.31	0.0263[a]

Values are means ± SD.
[a] Statistically significant.

Table 4. Pairwise comparisons of the least-square means for several parameters among the three groups

	Non-pontine lacunar group > non-lacunar group		Pontine lacunar group > non-pontine lacunar group		Pontine lacunar group > non-lacunar group	
	z value	p value	z value	p value	z value	p value
LNG	−1.21	0.225	3.26	0.0001[a]	2.05	0.041[a]
LNG/time	−1.21	0.226	3.19	0.0001[a]	1.99	0.047[a]
ENV	−0.815	0.415	3.11	0.0002[a]	2.21	0.027[a]
REC	−0.655	0.513	2.49	0.0013[a]	1.78	0.076

[a] Statistically significant.

This lack of differences of the Romberg quotients suggests that the patients of the pontine lacunar group are able to use the vestibular system. Therefore, the symptoms of these patients may be involved within the non-vestibular system.

Acknowledgments

The authors thank Tomomi Kiyohara and Yasuo Nakayama for preparing the manuscript.

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European Journal of Neurology 2005, 12: 218–222

Risk factors for occlusive lesions of intracranial arteries in stroke-free Japanese

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Keywords:
intracranial artery, MR angiography, risk factor

Received 7 March 2004
Accepted 7 July 2004

The aim of this study was to identify relevant risk factors for occlusive lesions of the intracranial arteries in stroke-free population. The subjects of this study were 425 patients without a history of stroke or transient ischemic attack and without any abnormality on a neurological examination who consecutively visited a neurology clinic between January 1994 and June 2001 requesting medical evaluation for possible cerebrovascular diseases. Subjects included 245 men and 180 women ranging in age from 33 to 89 years (mean ± SD = 64.0 ± 10.0 years). We performed cervical and intracranial magnetic resonance angiography (MRA) in all subjects. Using a validated rating scheme of MRA for occlusive lesions, we evaluated the degree of stenoses in the extracranial portion of the internal carotid artery (ICA) and the intracranial arteries including the intracranial portion of the ICA, middle cerebral artery (MCA) stem, intracranial portion of the vertebral artery (VA), and basilar artery (BA). More than 25% stenoses were regarded as significant lesions in this study. Multiple logistic regression analyses showed that significant and independent predictors for extracranial ICA lesions were age, hyperlipidemia, and ischemic heart disease (IHD), those for intracranial ICA lesions were age, hypertension, diabetes mellitus, and IHD, those for MCA lesions were age and hypertension, those for intracranial VA lesions were hyperlipidemia and IHD, and those for BA lesions were hypertension and diabetes mellitus. The present study suggested that atherosclerosis of the intracranial VA was related to hyperlipidemia and IHD as was the case for the extracranial carotid artery, whilst atherosclerosis of other sites of intracranial arteries was associated with hypertension and diabetes mellitus in stroke-free Japanese.

Introduction

In a previous study of stroke-free subjects using magnetic resonance angiographies (MRAs) (Uehara *et al.*, 1998), we found that the risk factors for occlusive lesions in the cervical carotid artery and intracranial arteries were different. Age and hyperlipidemia were risk factors for the former, and age and hypertension were risk factors for the latter. In that study we categorized the basilar artery (BA) into a common group of intracranial arteries together with the intracranial internal carotid artery (ICA) and the middle cerebral artery (MCA). However, some investigators (Caplan *et al.*, 1986; Yasaka *et al.*, 1993) have suggested that, in patients with ischemic stroke or transient ischemic attack (TIA), risk factors for BA lesions differ from those for MCA lesions. According to Caplan *et al.* (1986), extracranial ICA and BA lesions belong to a group closely related to hyperlipidemia and coronary heart disease, whilst MCA lesions belong to another group related to hypertension but not to hypercholesterolemia. They also pointed out that the intracranial ICA and the intracranial vertebral artery (VA) did not fall clearly into any of these groups because of a lack of information for these vessel sites (Caplan *et al.*, 1986). Yasaka *et al.* (1993) demonstrated that, in patients with ischemic stroke or TIA, MCA trunk atherosclerosis was related to advanced hypertension, and that atherosclerosis of both the BA and the extracranial ICA was associated with high serum lipid levels, coronary heart disease, and diabetes mellitus. However, until now, no studies have been carried out to examine risk factors for occlusive lesions in each site of the intracranial arteries in stroke-free subjects. We therefore looked for regional differences in the risk factors for occlusive lesions of the intracranial arteries in Japanese without stroke by using MRA.

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Materials and methods

Subjects

Subjects of this study were recruited from outpatients without stroke or TIA who consecutively visited the

clinic of the neurology service in our hospital between January 1994 and June 2001. All the patients without any history of stroke or TIA episode who requested medical evaluation for possible cerebrovascular diseases because of reasons including a simple fear of stroke, positive family history of stroke, vascular risk factors, and non-specific subjective symptoms such as headache or dizziness, and without a contraindication for magnetic resonance imaging (MRI) were invited to the study. Informed consent for the study was obtained from all the patients. Patients were carefully checked for their medical history and given a complete neurological examination, and cranial MRIs were employed. Patients whose examination was indicative of stroke or those whose scans revealed incidental significant lesions (except for asymptomatic lacunar infarcts in white matter, basal ganglia, or thalamus) were excluded (nine patients). Patients with migraine (four patients) and those with vertigo possibly caused by brainstem or cerebellar dysfunction (five patients) were not included in this study. Finally, the subjects of this study were 425 patients, including 245 men and 180 women ranging in age from 33 to 89 years (mean ± SD =64.0 ± 10.0 years). One hundred and fifty-six of these subjects also participated in the previous study (Uehara *et al.*, 1998).

Magnetic resonance angiography examinations

All MRA examinations were performed with a 1.0 tesla MR system (Magnetom Impact; Siemens, Erlangen, Germany). Image acquisition and reconstruction are described elsewhere (Uehara *et al.*, 1994, 1995). The extracranial portion of the ICA was evaluated based on the carotid MRA. The intracranial portion of the ICA, the horizontal portion of the MCA, the intracranial portion of the VA, and the BA were evaluated based on the intracranial MRA. Two investigators (T.U. and M.T.), who were blinded to all clinical information, independently reviewed the MRAs and rated occlusive lesions for each arterial portion into five grades depending on the narrowness of the arteries (Uehara *et al.*, 1994, 1995): <25% reduction of an arterial diameter was graded as normal, 25–49% reduction was graded as mild stenosis, 50–74% reduction was graded as moderate stenosis, 75–99% reduction was graded as severe stenosis, and no opening was graded as occlusion. When the judgment of the two readers was inconsistent, a decision was entrusted to a third investigator (E.M). To measure the percent stenosis of the extracranial portion of the ICA, we compared the diameter of maximal stenosis with that of the normal-appearing proximal ICA beyond the carotid bulb [North American Symptomatic Carotid Endarterectomy Trial (NASCET) Steering Committee, 1991]. Aplasia or hypoplasia of the VA is not uncommon, in which MRA assessment of stenosis is impractical. We regarded as VA aplasia/hypoplasia when fulfilled the followings: (i) the diameter of the VA in the dominant side being not smaller than the diameter of the BA, (ii) a smooth transition from the dominant VA to the BA, and (iii) the VA of the non-dominant side being not visible, constantly narrow through the whole length or terminated into the posterior inferior cerebellar artery.

The accuracy of MRA in detecting occlusive disease of extra- and intra-cranial ICA system was previously shown to be high (Uehara *et al.*, 1994, 1995). An additional validation study was carried out to evaluate the accuracy of MRA for the vertebrobasilar artery system, comparing MRA with conventional angiography. Subjects of this validation study consisted of 58 patients (44 men and 14 women, mean ± SD =60.7 ± 11.3 years old) selected from those who were admitted to our hospital for suspected ischemic cerebrovascular diseases (45 patients with ischemic stroke, 10 patients with TIA, two patients with cervical bruit, and one patient with transient global amnesia) between April 1992 and December 1993 and were given both MRA and conventional angiography studies within 1 month of each other. Seven vessels of the VA, which showed hypoplasia on both MRA and conventional angiography, were excluded because they were unable to estimate the degree of stenosis. The Spearman rank correlation coefficients between the conventional angiography rating and the MRA rating were 0.86 for the VA, 0.89 for the BA, and 0.80 for the posterior cerebral artery (PCA). When considering the normal-abnormal dichotomy, the sensitivity was 100% for the VA, 100% for the BA, and 83.3% for the PCA. The specificity was 93.9% for the VA, 96.0% for the BA, and 83.7% for the PCA. Because PCA lesions were uncommon, this portion was not considered in this study. Moreover, the proxymal portion of the VA was not also taken into consideration in this study, as the origin of the VA, a common site of occlusive lesions, was unable to evaluate on the cervical MRA.

Risk factors

Hypertension, diabetes mellitus, hyperlipidemia, smoking habit, and ischemic heart disease (IHD) were evaluated as risk factors. Hypertension was judged as present when either a systolic pressure of >140 mmHg or a diastolic pressure of >90 mmHg was demonstrated on repeated examinations or when a history of treatment for hypertension was present. Diagnosis of diabetes mellitus was made when the fasting blood glucose level was >126 mg/dl or when a history of treatment for diabetes mellitus was present. Hyperlipidemia was judged as present when laboratory

examination of the serum at presentation showed a high total cholesterol level of >220 mg/dl, a high triglyceride level of >150 mg/dl, a low high-density-lipoprotein cholesterol level of <40 mg/dl, or when a history of treatment was present. Smoking habit included previous history of smoking. IHD was defined as a known history of myocardial infarction or angina pectoris.

Statistical analyses

Multiple logistic regression analyses were used to estimate independent effects of the predictive variables on the cerebral arterial occlusive lesions. The contrast was between those with and without lesion in each site. All statistical analyses were carried out with StatView software (SAS Institute Inc., Cary, NC, USA). The level of significance was set at $P < 0.05$ for all statistical analyses.

Results

Two hundred five subjects (48.2%) were hypertensive, 91 subjects (21.4%) were diabetic, and 113 subjects (26.6%) were hyperlipidemic. One hundred thirty-nine subjects (32.7%) had a smoking habit. IHD was positive in 109 subjects (25.6%).

The results of MRA findings are summarized in Table 1. For estimation of MRA findings, the rate of agreement between two readers (T.U. and M.T.) was 94.6% (kappa = 0.92). Four vessels of the intracranial ICA and five vessels of the MCA were not assessable because of occlusion in their proximal portion. For the VA, 48 vessels were not assessable because of hypoplasia. Bilateral lesions were found in the extracranial ICA in 11 subjects, in the intracranial ICA in three subjects, in the MCA in two subjects, in the intracranial VA in two subjects. Fifteen subjects had both extracranial and intracranial lesions.

Table 1 Magnetic resonance angiography findings

	Extracranial	Intracranial			
Stenosis rating[a]	ICA	ICA	MCA	VA	BA
Normal	384	398	398	409	416
Mild stenosis	26	16	21	9	6
Moderate stenosis	7	7	0	3	1
Severe stenosis	4	3	5	3	2
Occlusion	4	1	1	1	0
Abnormal (%)[b]	9.6	6.4	6.4	3.8	2.1

[a]Based on the rating of more affected side in case of bilateral vessel lesions.
[b]Stenoses of more than 25%.
ICA, internal carotid artery; MCA, middle cerebral artery; VA, vertebral artery; BA, basilar artery.

Multiple logistic regression analyses showed that significant and independent predictors for lesions were age, hyperlipidemia, and IHD for the extracranial ICA, age, hypertension, diabetes mellitus, and IHD for the intracranial ICA, age and hypertension for the MCA, hyperlipidemia and IHD for the intracranial VA, and hypertension and diabetes mellitus for the BA (Table 2).

Discussion

Multiple logistic regression analyses showed that significant and independent predictors of the extracranial ICA lesions were age, hyperlipidemia and IHD, and

Table 2 Predictors for stenoses

Variable	Odds ratio	95% confidence interval	*P*-value
Extracranial ICA			
Age (>65 years)	2.67	1.30–5.48	0.0074
Male sex	1.88	0.93–3.79	0.0782
Hypertension	1.62	0.84–3.11	0.1492
Diabetes mellitus	1.37	0.66–2.86	0.3982
Hyperlipidemia	2.38	1.23–4.60	0.0099
Smoking habit	1.70	0.89–3.27	0.1110
Ischemic heart disease	3.95	2.05–7.64	<0.0001
Intracranial ICA			
Age (>65 years)	5.36	1.82–15.85	0.0024
Male sex	2.08	0.85–5.06	0.1069
Hypertension	5.01	1.85–13.55	0.0015
Diabetes mellitus	4.05	1.81–9.08	0.0007
Hyperlipidemia	2.14	0.95–4.80	0.0664
Smoking habit	1.55	0.69–3.48	0.2847
Ischemic heart disease	2.25	1.00–5.07	0.0496
MCA			
Age (>65 years)	3.36	1.33–8.51	0.0105
Male sex	0.91	0.42–2.00	0.8203
Hypertension	6.96	2.37–20.51	0.0004
Diabetes mellitus	1.90	0.82–4.38	0.1331
Hyperlipidemia	1.69	0.75–3.80	0.2084
Smoking habit	1.23	0.55–2.75	0.6206
Ischemic heart disease	1.78	0.79–4.01	0.1662
Intracranial VA			
Age (>65 years)	2.02	0.69–5.93	0.1984
Male sex	3.31	0.93–11.78	0.0651
Hypertension	2.49	0.85–7.29	0.0967
Diabetes mellitus	2.25	0.80–6.37	0.1256
Hyperlipidemia	13.39	3.74–47.95	<0.0001
Smoking habit	2.12	0.78–5.78	0.1410
Ischemic heart disease	6.98	2.37–20.59	0.0004
BA			
Age (>65 years)	7.41	0.92–59.77	0.0601
Male sex	1.48	0.37–6.00	0.5822
Hypertension	9.07	1.12–73.15	0.0385
Diabetes mellitus	7.67	1.88–31.32	0.0045
Hyperlipidemia	2.25	0.59–8.55	0.2322
Smoking habit	1.03	0.25–4.18	0.9677
Ischemic heart disease	1.46	0.36–5.95	0.5956

that those of the MCA lesions were age and hypertension. These findings were well consistent with the findings of previous studies (Heyden *et al.*, 1970; Crouse *et al.*, 1986, 1987; Salonen *et al.*, 1988; Craven *et al.*, 1990; Handa *et al.*, 1990; Howard *et al.*, 1990; Tanaka *et al.*, 1993; Yasaka *et al.*, 1993; Fabris *et al.*, 1994; Fine-Edelstein *et al.*, 1994; Uehara *et al.*, 1998). Heyden *et al.* (1970), who analyzed a group of patients with angiographically documented non-embolic cerebral artery occlusion, noted that patients with extracranial carotid lesions had a high frequency of associated IHD and hypercholesterolemia. Several ultrasonography studies have shown that extracranial carotid lesion is related to hyperlipidemia (Crouse *et al.*, 1987; Salonen *et al.*, 1988; Handa *et al.*, 1990; Fabris *et al.*, 1994; Fine-Edelstein *et al.*, 1994) and IHD (Crouse *et al.*, 1986, 1987; Craven *et al.*, 1990; Howard *et al.*, 1990; Tanaka *et al.*, 1993).

Although there have been fewer studies of MCA lesions than of extracranial ICA lesions, the results of the present study were consistent with those in the previous studies (Heyden *et al.*, 1970; Yasaka *et al.*, 1993; Uehara *et al.*, 1998; Takahashi *et al.*, 1999). Caplan *et al.* (1986) suggested that very common hypertension and relatively uncommon hypercholesterolemia could explain a predilection for occlusive lesions of the MCA and a low prevalence of occlusive extracranial ICA disease and coronary artery disease in Japanese. Yasaka *et al.* (1993) concluded that advanced hypertension was related to MCA trunk atherosclerosis. Takahashi *et al.* (1999) reported that hypertension and high serum levels of glycosylated hemoglobin A1c were significant and independent predictors of atherosclerotic lesions of the MCA detected by MRA in Japanese.

Like the MCA lesions, the intracranial ICA lesions had age, hypertension, and diabetes mellitus as significant and independent predictors. In addition, we found a weak but significant correlation between intracranial ICA lesions and IHD. Ingall *et al.* (1991) demonstrated that significant and independent predictors of intracranial ICA atherosclerosis found by conventional angiography were duration of cigarette smoking, age, hypertension, and diabetes mellitus. Marzewski *et al.* (1982), who followed up >66 patients with more than 50% stenosis of the intracranial ICA for an average of 3.9 years, concluded that intracranial ICA stenosis was a marker of extensive cerebrovascular and systemic atherosclerotic disease, especially coronary artery disease. Little is known about the risk factors for intracranial ICA occlusive lesions.

In the present study, atherosclerosis of the intracranial VA was related to hyperlipidemia and IHD as was the case for the extracranial carotid artery, whilst atherosclerosis of BA was associated with hypertension and diabetes mellitus. Our results clearly suggested that intracranial VA lesions belong to the same class as extracranial ICA lesions, which are closely related to hyperlipidemia and IHD. Although no studies comparable to the present study have examined the risk factors for intracranial VA occlusive lesions, the New England Medical Center Posterior Circulation Registry (Muller-Kuppers *et al.*, 1997; Shin *et al.*, 1999) reported that the prevalences of hypertension, hyperlipidemia, diabetes mellitus, smoking, and IHD were high in patients with symptomatic intracranial VA occlusive lesions. In addition, the prevalence of coronary artery disease in patients with symptomatic intracranial VA occlusive disease was reportedly quite high, ranging from 20 to 36% (Bogousslavsky *et al.*, 1986; Moufarrij *et al.*, 1986; Muller-Kuppers *et al.*, 1997; Shin *et al.*, 1999), which supports our findings. However, our results failed to verify the previous view. Caplan *et al.* (1986), in a review of occlusive cerebrovascular disease, found that atherosclerosis of the large arteries including extracranial ICA and BA, was closely related to hyperlipidemia and coronary artery disease. Yasaka *et al.* (1993) demonstrated that atherosclerosis of extracranial ICA and BA was strongly associated with high serum lipid levels, coronary heart disease, and diabetes mellitus in patients with ischemic stroke. This discrepancy may be attributable to the different characteristics of the cohorts, a difference between patients with ischemic stroke and stroke-free subjects, or to the small number of subjects with BA stenosis. In the present study, the proxymal segment of the VA was not studied, as it was not accessible on the cervical MRA we used. As the origin of the VA is a critical site, risks for the proxymal VA lesions should be elucidated in future.

Finally, limitations of the present study have to be mentioned. The subjects of this study were patients without a history of stroke or TIA and without any abnormality on a neurological examination who visited a neurology service requesting medical evaluation for possible cerebrovascular diseases. To minimize the selection bias as far as possible, subjects of this study were prospectively recruited from consecutive outpatients. The cohort in the present study is a part of stroke-free general population. However, this kind of study is prone to referral or selection bias. The prevalence derived from such a hospital-based study should be carefully interpreted and applied to general population. Nevertheless, at least the association between risk factors and vascular lesions demonstrated could be generalizable, as the association would be universal. Another weakness is that the individual lesion numbers were all low, which may introduce type II errors. The ideal study method is to conduct a population-based study rather than a hospital-based study,

and larger population-based studies are evidently needed to confirm our findings. Low prevalence also affects the creditability of the MRA rating results. False positives, which may unavoidably occur in MRA, are of concern especially in a low-risk population. The most vulnerable site for MRA is the intracranial ICA, where the false positive rate is considerably high. Signal discontinuity caused by tortuosity of the vessel in this region would be often judged as 'severe' stenosis (Uehara *et al.*, 1994). However, in the present study, as the occasion of 'severe' rating was very few, most of the stenotic ratings should be true.

In conclusion, the present study suggested that atherosclerosis of the intracranial VA was related to hyperlipidemia and IHD as was the case for the extracranial carotid artery, whilst atherosclerosis of other sites of major intracranial arteries was mainly associated with hypertension and diabetes mellitus in stroke-free Japanese. Our results might shed light into the important question why there were ethnic differences in the distribution of atherosclerotic lesion. In the future, a study investigating the correlation between the severity of the occlusive lesions and risk factors is needed to determine the predictors of the development of atherosclerosis.

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J Hum Genet (2004) 49:455–462
DOI 10.1007/s10038-004-0174-z

ORIGINAL ARTICLE

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High throughput multiple combination extraction from large scale polymorphism data by exact tree method

Received: 15 March 2004 / Accepted: 18 May 2004 / Published online: 11 August 2004

Abstract Single nucleotide polymorphisms (SNPs) are increasingly becoming important in clinical settings as useful genetic markers. For the evaluation of genetic risk factors of multifactorial diseases, it is not sufficient to focus on individual SNPs. It is preferable to evaluate combinations of multiple markers, because it allows us to examine the interactions between multiple factors. If all the combinations possible were evaluated round-robin, the number of calculations would rapidly explode as the number of markers analyzed increased. To overcome this limitation, we devised the exact tree method based on decision tree analysis and applied it to 14 SNP data from 68 Japanese stroke patients and 189 healthy controls. From the obtained tree models, we succeeded in extracting multiple statistically significant combinations that elevate the risk of stroke. From this result, we inferred that this method would work more efficiently in the whole genome study, which handles thousands of genetic markers. This exploratory data mining method will facilitate the extraction of combinations from large-scale genetic data and provide a good foothold for further verificatory research.

Keywords Multifactorial disease · Genetic polymorphism · SNP · Interaction · Multiple factor · Combination · Data mining · Exact tree

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Introduction

Biological researchers face an explosion of data arising from human genome projects and recent high throughput experiments. There are many traditional techniques for analyzing data, including statistics and epidemiological approaches. Data mining methods, however, offer new approaches to data analysis by using techniques based on machine learning that has been developed in research on artificial intelligence. These techniques work by learning patterns in data, and then sometimes discover new information overlooked by conventional analyses.

The amount of single nucleotide polymorphism (SNP) data as useful genetic polymorphism markers in clinical settings has been increasing, because SNPs are widespread and frequent in the human genome and high throughput typing technology has become established. To evaluate the risk of multifactorial diseases in detail, the information of single polymorphism alone is insufficient. It is preferable to evaluate combinations of multiple markers, since they reflect interactions between multiple factors. If all the combinations were evaluated round-robin, the amount of computation would easily explode as the number of markers analyzed increased at an exponential rate. To overcome this limitation, we developed the exact tree method based on decision tree analysis (Kass 1980), which we previously used and evaluated in another epidemiological study (Miyaki et al. 2002). The decision tree analysis is the popular classification method in data mining algorithms. This method evaluates all predictor variables and stratifies the study population with the best variable to contrast the high risk group and the low risk group as much as possible. This process is repeated, and the obtained "only one" tree, which is multiply stratified by best variables, becomes an empirical rule for predicting the class of an object form values of predictor variables.

On the other hand, the "exact tree" we devised aims not for constructing the best classification model but for

extracting hidden combinations that are statistically significant. The main principle of multiple stratifications is the same as the former "decision tree," but this method constructs plural tree models in contrast to the one-tree model by regular decision tree algorithm.

In this article, we introduce an example of application of this new exploratory data mining algorithm to our SNPs data of stroke patients and healthy controls focusing upon the combination extraction.

Materials and methods

The patients and healthy controls analyzed here were from the same population published in our previous studies (Ishii et al. 2004; Ito et al. 2002; Oguchi et al. 2000; Sonoda et al. 2000 etc.). We recruited 235 unrelated Japanese patients under 70 years of age with symptomatic ischemic cerebrovascular disease (CVD) from Keio University Hospital and 189 age- and gender-matched healthy controls. CVD patients with cardioembolic cerebral infarction and cerebral hemorrhage were excluded. Control subjects were enlisted from people who came to the hospital for regular checkups; those who had a clinical history of CVD or myocardial infarction or peripheral vascular diseases were excluded. Informed consent was obtained from all subjects. Brain CT and/or MRI were performed in all CVD patients. MR angiography and/or extracranial duplex ultrasonography were available for more than 80% of CVD patients. On the basis of classification of CVDs III (ad hoc committee of the National Institute of Neurological Disorders and Stroke 1990), the CVD patients were divided into three clinical categories: an atherothrombotic infarction group (69 patients), a lacunar infarction group (142 patients), and a transient ischemic attack group (24 patients). In this study, we focused on the atherothrombotic infarction group to clarify the differences from healthy controls by limiting the cases to one pathophysiological state. We typed 14 genetic polymorphism markers in 69 atherothrombotic stroke patients and 189 healthy controls. These 14 markers were chosen because they were already known to be related to stroke by effecting blood coagulation, platelet function, and lipid metabolism (target gene approach). These genetic polymorphism markers and their abbreviations used in this analysis are shown in Table 1, and the characteristics of the stroke patients and healthy controls are compared in Table 2.

For conventional statistical analysis, we used SPSS 11.0 and Clementine 7.1 (Chicago, IL, USA). For advanced statistical analysis, we devised the exact tree method based on decision tree analysis. The procedure of the exact tree method is described below. First, we made cross-tabs of genotypes between the groups of stroke patients and healthy controls. Then, we compared the prevalence of these alleles between the groups and calculated the P value of Fischer's exact test (See "Appendix"). We defined these P values as splitting indices of the data set to make the decision tree models. The smaller the P value, the more influential the allele was assumed to be. In regular decision tree methods, we adopt the best splitting point that shows the best index score (e.g., split the first data set by the marker that shows the smallest P value). But this method discards the second-best or the third-best onward, even when they are also meaningful. In the exact tree method, we also adopt the second-best or third-best onward as well as the first-best, as the occasion demands. How many best points we decide to adopt is the number of the tree models obtained later. For the effectiveness in data exploration, we must decide the number of the tree analytically. One simple approach is counting the variables that show the P value at an arbitrary statistical significance level depending on the number of variables analyzed. Another approach we adopted here was to make the talus plot of P values of each variable. The talus plot is a line graph of P values and is plotted in ascending order (Fig. 1). Using this plot, we could figure out the point where the slope changed sharply. If we chose the variables plotted on the left side of the large slope change, we adopted the variables that contained relatively larger amounts of information efficiently. With reference to Fig. 1, we chose the first five variables visually and made five tree models. In each tree model, we adopted the genetic polymorphism marker that had the smallest P value as the second splitting point and grew the trees with the next smallest P values continuously. Such a repetitive process needs stopping rules. As is common for all decision tree methods, the more the root node was split deeply, the smaller the number of subjects becomes in each stratified node, and it tends to be difficult to be interpreted. How many times the tree model should be split depends on the number of study subjects. In this report, we empirically defined the depth of tree as four, which is the level less than

Table 1 List of genetic polymorphism markers and abbreviations used for analysis. Genetic polymorphism markers are listed in alphabetical order by abbreviations

Genetic polymorphism markers	Abbreviations
5HT2A receptor (Exon1 102 T/C)	5HT2A
Angiotensin-converting enzyme (Exon 16)	ACE
Human apolipoprotein E	APO-E
Cholesteryl ester transfer protein	CETP
Fibrinogen β	FIB-B
Factor XII gene (Exon1 46 C/T)	F-XII
Glycoprotein Ib (145 Thr/Met)	GPIb (145Thr/Met)
Glycoprotein Ib kozak sequence (5 T/C)	GPIb (kozak)
Mitochondrial DNA mutation (5178 A/C)	Mitochondria
Methylene tetra hydoro folate reductase (677 C/T)	MTHFR
Endothelial nitric oxide synthase (Intron 4)	NOS
NADPH oxidase p22phox	p22phox
Plasminogen activator inhibitor-1 (promotor region)	PAI-1
Paraoxonase (192 Arg/Gln)	PON

Table 2 Comparative table of characteristics

	All participants ($n=257$)	Stroke patients ($n=68$)	Healthy controls ($n=189$)
Demographic			
Age (years)[a] **	60.3 (5.2)	63.4 (7.7)	59.5 (3.7)
Male/female	201/56	56/12	145/44
Clinical			
Body-mass index (kg/m^2)[a] *	22.8 (2.7)	23.7 (2.6)	22.6 (2.7)
Smoking (%) never/ex or current **	65.4/34.6	42.1/57.9	72.5/27.5
Hypertension, yes/no *	82/173	30/38	52/135
Diabetes, yes/no **	35/222	22/46	13/176
Hyperlipemia, yes/no **	93/138	25/17	68/121
Family history of cerebellar vein disease, yes/no	37/141	13/39	24/102
Family history of cardiac artery disease, yes/no	17/163	4/51	13/112
Biochemical			
Fast blood sugar (mg/dl)[a] *	101.1 (18.8)	109.1 (31.7)	99.2 (13.7)
Total cholesterol (mg/dl)[a]	203.4 (32.7)	201.1 (37.1)	204.1 (31.1)
Triglyceride (mg/dl)[a] **	117.3 (77.4)	160.0 (90.8)	108.5 (71.5)
Serum uric acid (mg/dll)[a]	5.7 (1.3)	5.6 (1.4)	5.7 (1.3)
Platelet count (μl)[a]	235.5 (70.0)	229.4 (101.4)	237.5 (55.4)
High density lipoprotein (mg/dl)[a] **	56.2 (15.3)	44.0 (11.6)	57.5 (15.1)
CETP density (μg/ml)[a]	2.5 (0.6)	2.4 (0.6)	2.5 (0.6)

[a]mean (SD)
*$P<0.05$, t-test; **$P<0.01$, chi-square test

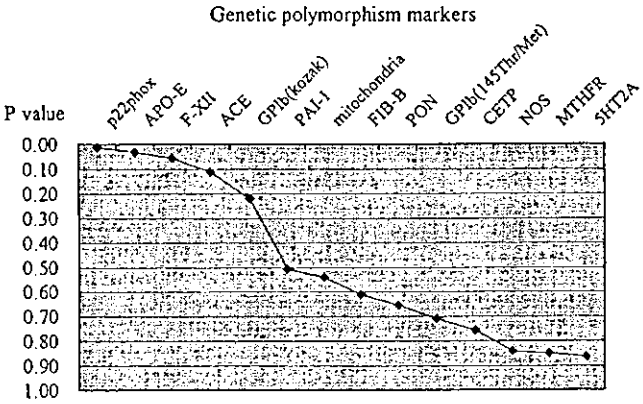

Fig. 1 Talus plot of P values for Fischer's exact test

half of the terminal nodes that consist of 30 subjects. Before reaching the defined depth, the extension was terminated when the number of cases or controls in the node became zero.

In addition, we calculated the odds ratios (OR) of each node with 95% confidence intervals (Figs. 2, 3, 4, 5, 6). Each OR stands for odds ratio of each node to "root node," which is the whole population we analyzed shown as Node #0 in each figure. It thus makes it possible to remove the influence of the sampling ratio of cases to controls on the estimated OR. We added the P value of each OR in addition to the 95% confidence interval of each OR. The OR shows the estimate of the risk ratio of the disease, so the odds ratio in this study indicates the risk of stroke. We circled the node with statistically significant odds ratio in each tree. These processes (which we call the "exact tree" method) enabled us to appraise risks quantitatively and extract meaningful combinations from the tree models constructed.

Results

The alleles used as variables in this analysis are listed in Table 3. The P values according to Fischer's exact test in the case-control tables are listed in Table 4, and the talus plot of these P values, which was plotted in ascending order, is shown in Fig. 1. From the view point of P value, p22phox (P value = 0.012) and APO-E (P value = 0.031) had statistically significant differences in allele frequencies between stroke patients and healthy controls. Based on the talus plots, we adopted F-XII (P value = 0.056), ACE (P value = 0.112), and GPIb (kozak) (P value = 0.220) as useful genetic polymorphism markers for the first node splitting in the tree models, since it is more likely to be statistically significant in some combinations than markers with lower P values. By exact tree method described above, we constructed five exact tree models (Figs. 2, 3, 4, 5, 6). As to the second or later splitting point in each exact tree, we adopted the variables that had the smallest P value continuously. Subsequently, we calculated the OR of each node of the trees and drew circles around nodes whose 95% confidence interval of the odds ratio was not across 1 (i.e., significant at 5% alpha-error level). In the first exact tree (Fig. 2), which is the usual decision tree in itself, we could not extract any significant combination, since the only circled Node #2 indicated the elevated risk [Odds ratio: 2.34 (1.14–4.81)] in p22phox t allele-positive people (single polymorphism). Significant combination extraction needs more than twice-split circled node. In the second exact tree (Fig. 3), we observed three significant combinations in three nodes (Node #5, #8 and #11). In Node #5, for example, when with a combination of APO-E E4 allele-positive and PAI-1 5G allele-negative, the odds ratio became 3.24 (1.05–9.99). In Node #8,