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Cerebrovascular disorders and genetic polymorphisms: mitochondrial DNA5178C is predominant in cerebrovascular disorders

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Abstract

We studied polymorphisms of mitochondrial DNA 5178cytosine/adenine (mt5178C/A) and angiotensin I-converting enzyme (ACE) genes (*DCP1*) in 127 cerebrovascular disorder (CVD) patients and 294 age-matched normal controls to clarify the genetic background of Japanese patients with CVD. Mt5178C was predominant in CVD patients compared with controls ($P<0.01$). The frequency of *DCP1* insertion (I) and deletion (D) alleles showed no significant difference between the CVD patients and controls or between each CVD subgroup. Although the number of CVD patients in the present study was too small to make a final conclusion, mt5178C might be one of the genetic factors to be considered in Japanese patients with CVD.

Keywords: Cerebrovascular disorders; ACE; Mitochondrial DNA; Aging; Risk factors

1. Introduction

Cerebrovascular disorder (CVD) is one of three common causes of death following circulatory disease and malignancy, and its prevention is an important health care problem in Japan [1]. Although CVD is the result of both genetic and environmental factors that are related to damage of cerebral vessels, aging is known to be the most important risk factor. Recent progress in molecular biology has disclosed the genes and genetic polymorphisms that are related to the aging process and to CVD [2–9].

Tanaka et al. [9] reported that the incidence of mitochondrial DNA 5178 (mt5178) cytosine (C)-to-adenine (A) polymorphism within the *ND2* gene of mtDNA was significantly high in centenarians. Matsunaga et al. [10] reported that the mt5178A genotype was associated with the mean intima-media thickness of bilateral carotid arteries in type 2 diabetic individuals and suggested that mt5178A had an antiatherogenic effect, at least in those patients.

The relationship between genetic polymorphisms of the human angiotensin I-converting enzyme (ACE) gene (*DCP1*) and several diseases, such as coronary artery disease [11–14], hypertension [15], dementia [21,22], CVD [6,7,18] and longevity [3,19], has been reported, but the relationship is still controversial [20]. The conflicting findings may be based on the selection of control subjects, methods of patient evaluation and the difference in genetic background in each population [21]. In Japanese patients with either coronary heart disease [22], dementia [23] or CVD [6,7,24,25], several studies have likewise reported *DCP1* polymorphisms. However, the effect of these polymorphisms is also controversial.

In the present study, we examined the relationship between polymorphisms of mitochondrial DNA 5178cytosine/adenine (mt5178C/A) and the ACE gene, and CVD in Japanese patients to clarify the genetic background of these patients.

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2. Methods and materials

2.1. CVD subjects

Subjects including 127 patients, between 50 and 80 years of age, were selected from consecutive patients admitted on an emergency basis to either Ookatsu or Tenyokai Chuo hospitals from 1994 to 1997. They were diagnosed as having cerebral hemorrhage or infarction based on clinical, brain CT, MRI and MRA findings and were able to be classified into the NINDS-III classification. All gave informed consent. The patients with cerebral infarction were classified into cerebral embolism, atherothrombotic infarction or lacunar infarction based on the NINDS-III classification [26] (Table 1). Clinical findings were stored in a database and used for analysis of CVD and genetic phenotype correlation.

2.2. Control subjects

Individuals who were admitted to the affiliated hospitals of our department for a physical checkup were evaluated neurologically by expert neurologists. Two hundred and ninety four, who had normal neurological examinations and between 50 and 80 years old, were included in this study. Clinical findings were stored in a database.

All studies were performed with informed consent, and the clinical findings and samples were analyzed anonymously. Permission for these studies was obtained from the Kagoshima University Faculty of Medicine Human Investigation Committee.

2.3. Analysis of risk factors for CVD

Blood pressure, smoking history, ECG and blood test were compared between the CVD and control subjects according to the genetic polymorphisms.

2.4. Detection of genetic polymorphisms

Genomic DNA was extracted from peripheral blood using a previously reported method [27]. Mt5178C/A polymorphism was determined by the method reported by Tanaka et al. [9]. The *DCP1* 288 bp insertion (I) or deletion (D) polymorphisms within intron 16 was determined by the method reported by Rigat et al. [28] and Shanmugam et al. [29].

Table 1
Age and sex classification of CVD and control groups enrolled in this study

Age	CVD group			Control group		
	Total	Men	Women	Total	Men	Women
50–60	9	5	4	24	16	8
60–70	50	34	16	110	64	46
70–80	68	36	32	160	87	73
All age	127	75	52	294	167	127
Average age	69.4 ± 6.9	68.5 ± 6.8	70.8 ± 6.9	69.4 ± 7.0	68.7 ± 7.2	70.4 ± 6.6

Table 2
Frequency of mt5178A and mt5178C in CVD and control groups

	Total			Men			Women		
	5178C	5178A	OR	5178C	5178A	OR	5178C	5178A	OR
CVD	89*	38	1.86	54**	21	1.86	35	17	1.84
Control	164	130		97	70		67	60	

OR: odds ratio.
* $P=0.0054$.
** $P=0.036$.

2.5. Statistical analysis

Distribution of ages in the case and control groups between 50 and 80 years old were matched accordingly frequency matching. Logistic analysis was conducted to compare the frequency of mt5178C and mt5178A, and was adjusted for age and sex. Characteristics of the CVD and control subjects were compared using Pearson's χ^2 test. All P values presented were two-sided. We set the level of statistical significance for the P value at less than 0.05.

3. Results

3.1. Mt5178C/A polymorphisms

Mt5178C and 5178A in controls were 164 and 130 (55.8% and 44.2%), respectively (Table 2). This proportion was similar to the 56.2% and 43.8% observed in the central part of Japan as reported by Tanaka et al. [9]. In the entire CVD group, the proportion of mt5178C and mt5178A were 70% and 30%, respectively. The proportion of mt5178C was predominant in patients with CVD compared to that in the control group ($P<0.01$) regardless of gender (Table 2). Mt5178C was predominant in each subgroup of CVD patients compared with the control group, although the differences were not significant. There were no significant

Table 3
Frequency of mt5178A and mt5178C in CVD subtypes

	No. of cases	mt5178C	mt5178A	C/A ratio
Total number of CVD	127	89	38	2.34
Cerebral hemorrhage	40	28	12	2.33
Cardiogenic brain embolism	18	14	4	3.50
Cerebral thrombosis	69	47	22	2.24
Atherothrombotic	29	20	9	2.22
Lacunar	40	27	13	2.08
Control	294	164	130	1.26

There was no significant correlation between the mt5178 genotype and CVD subclass.

Table 4
Frequency of ACE genotypes in CVD subtypes

	No. of cases	DD	ID	II	D	I
Total number of CVD	127	21	53	53	95	159
Cerebral hemorrhage	40	8	16	16	32	48
Cardiogenic brain embolism	18	2	7	9	11	25
Cerebral thrombosis	69	11	30	28	52	86
Atherothrombotic	29	6	14	9	26	32
Lacunar	40	5	16	19	26	54
Control	294	55	111	128	221	367

There was no significant difference between the whole CVD group and the control group or between each CVD subtypes in the allele frequencies.

differences between the CVD subgroups with regards to mt5178 polymorphisms (Table 3).

3.2. DCP1 insertion (I)/deletion (D) polymorphism

There was no significant difference between the entire CVD group and the control group or between each CVD subgroup with regards to allele frequencies (Table 4).

3.3. Risk factors for CVD and mt5178 genetic polymorphism

The prevalence rates of hypertension, electrocardiogram abnormalities and low HDL cholesterol in serum were significantly higher in the CVD group compared with the controls regardless of gender ($P<0.0001$). In addition to these risk factors, the prevalence rate of diabetes mellitus was significantly higher in men in the CVD group ($P<0.01$). In women in the CVD group, total cholesterol and triglyceride levels in serum were higher in patients with mt5178C than in patients with mt5178A ($P<0.05$) (Table 5). However, these levels were not different from the women in the control group. There was no significant difference in the incidence of risk factors in men in the CVD group with regards to the mt5178 genotype.

4. Discussion

Since aging is the most important risk factor for CVD in the general population, it is important to study the genetic background that is related to longevity or progeria in CVD patients. Epidemiological and genetic studies have suggested several genes and genetic polymorphisms related to longevity [3,4,9,19,30,31]. Tanaka et al. [9] reported that mt5178A polymorphism was predominant in Japanese centenarians. Gong et al. [32]suggested the protective effect of mt5178A against the occurrence of adult-onset diseases. Matsunaga et al. [10] reported that mt5178A genotype was associated with the mean intima-media thickness of bilateral carotid arteries in type 2 diabetic individuals. In the present study, we showed a new aspect of this mitochondrial DNA polymorphism as part of the genetic background in CVD, although the number of CVD patients in the present study was too small to make a final conclusion.

ND2, in which a Leu-to-Met replacement caused by mt5178C/A polymorphism is present, is a subunit of complex I which is one component of the respiratory chain/oxidative phosphorylation system. The system produces ATP and, simultaneously, reactive oxygen species (ROS) [33]. A block of complex I disturbs the oxidation of NADH formed in the Krebs cycle and causes several mitochondrial disorders. Although the role of mt5178C/A polymorphism in the *ND2* subunit of complex I is unclear, it is suggested that the ATP production and/or ROS generation activities are different in individuals with mt5178C compared to those with mt5178A.

The present study was performed in the Southern part of Japan, but the proportion of mt5178C and A in controls was similar to that obtained in a study undertaken in the central part of Japan [9]. Therefore, the present results are not geographically specific, but may be applicable to Japanese CVD patients in general.

The relation of *DCP1* insertion/deletion polymorphisms in intron 16 and coronary heart disease [11–14,22], dementia [16,17,23], CVD [5–7,18,24,34], longevity [3,19] or physical training [35] has been reported with some contra-

Table 5
Comparison of risk factors for CVD and mt5178C/A genotype in women

	CVD group			*P* value*	Control group			*P* value**
	Total	mt5178C	mt5178A	C vs A	Total	mt5178C	mt5178A	C vs A
Hypertension (%)	59.6	57.1	64.7		33.1	37.3	28.3	
Smoker (%)	2.0	0.0	5.9		1.6	0.0	3.3	
Diabetes Mellitus (%)	13.5	14.3	11.8		4.5	1.7	7.8	$P<0.05$
Abnormal ECG (%)	55.8	60.0	47.1		7.3	11.5	2.0	$P<0.05$
Total cholesterol (mg/dl)	202.4 ± 40.1	210.5 ± 36.7	185.8 ± 42.6	$P<0.05$	214.3 ± 28.4	210.4 ± 27.6	218.9 ± 28.8	
Triglyceride (mg/dl)	115.9 ± 42.6	124.3 ± 46.4	98.5 ± 27.0	$P<0.05$	107.6 ± 36.2	102.8 ± 33.2	112.3 ± 38.6	
HDL cholesterol (mg/dl)	46.6 ± 13.1	47.6 ± 12.1	44.5 ± 15.3		59.1 ± 14.1	58.8 ± 14.4	59.5 ± 13.0	
Uric acid (mg/dl)	5.2 ± 1.5	5.1 ± 1.4	5.2 ± 1.9		4.7 ± 1.1	4.8 ± 1.1	4.6 ± 1.0	

ECG: electrocardiogram.
* *P* value in comparison within CVD group.
** *P* value in comparison within control group.

dictions [20,25]. In Japanese patients with CVD, Doi et al. [7] demonstrated a significant association between the *DCP1* D allele and thrombotic brain infarction in Japanese patients aged 60 or younger. The main difference of the present findings from those reported by Doi et al. is the age range of the patients and controls enrolled in the studies. The *DCP1* allele frequencies in patients aged older than 60 were not different in both studies. Therefore, onset of brain infarction in patients aged younger than 60 may be related to *DCP1* alleles, but not to brain infarction in older patients. Large and longitudinal studies concerning atherosclerosis in cerebral vessels and the *DCP1* genotype have to be carried out to obtain new insights in this field.

In conclusion, the present preliminary study revealed that mt5178C was predominant in the CVD group, but *DCP1* alleles showed no difference among CVD patients in Japan. The number of CVD patients examined in the present study is too small to make final conclusions about the relation of the genotype and the onset of CVD. A longitudinal study in a large cohort is needed to clarify the genetic and environmental risk factors for CVD.

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□ORIGINAL ARTICLE□

Logistic Model Analysis of Neurological Findings in Minamata Disease and the Predicting Index

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Abstract

Objective **To establish a statistical diagnostic method to identify patients with Minamata disease (MD) considering factors of aging and sex, we analyzed the neurological findings in MD patients, inhabitants in a methylmercury polluted (MP) area, and inhabitants in a non-MP area.**

Materials and Methods **We compared the neurological findings in MD patients and inhabitants aged more than 40 years in the non-MP area. Based on the different frequencies of the neurological signs in the two groups, we devised the following formula to calculate the predicting index for MD: predicting index = $1/(1+e^{-x})\times 100$ (The value of x was calculated using the regression coefficients of each neurological finding obtained from logistic analysis. The index 100 indicated MD, and 0, non-MD).**

Results **Using this method, we found that 100% of male and 98% of female patients with MD (95 cases) gave predicting indices higher than 95. Five percent of the aged inhabitants in the MP area (598 inhabitants) and 0.2% of those in the non-MP area (558 inhabitants) gave predicting indices of 50 or higher.**

Conclusion **Our statistical diagnostic method for MD was useful in distinguishing MD patients from healthy elders based on their neurological findings.**

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Key words: epidemiology, statistical diagnostic method, methylmercury polluted area, non-methylmercury polluted area

Introduction

Minamata disease (MD) is diagnosed based on a combination of central and peripheral nervous signs that have been frequently seen in MD patients (1–6). However, it is sometimes difficult to diagnose the patients as having MD, when those patients have other diseases or due to the aging phenomenon (3, 5, 7). Our previous studies in inhabitants in the non-methylmercury polluted (non-MP) area showed that the neurological signs seen in MD were similar to those seen in the elders with aging (8–10), indicating that the diagnosis of atypical MD patients was rather difficult in the elders. In 1974, Igata et al (1) produced a statistical diagnostic method for MD with multivariate statistical analysis, and identified a questionable borderline group (1–4). However, their method does not give consideration to factors of aging and sex as well as neurological findings, and some problems remain to diagnose aged MD patients. From this points of view, a new statistical diagnostic method for MD considering the factors of aging and sex as well as the neurological findings was thought to be needed as an objective diagnostic method for MD. In this study, we compared the frequencies of neurological findings in MD patients with those in aged inhabitants in the methylmercury polluted (MP) and non-polluted (non-MP) areas, and tried to establish a new statistical diagnostic method for MD patients considering factors of aging and sex.

Materials and Methods

Subjects

The neurological findings were obtained from three groups. The first group included 95 MD patients (49 men and 46 women) who had been diagnosed as having MD during the period from 1972 to 1976 in Kagoshima prefecture. The second group included inhabitants of a MP area (245 men and 353 women) and the third group, inhabitants of a non-MP area (191 men and 367 women). The latter groups were not institutionalized and voluntarily underwent our health check-up. All data obtained from the three groups were semiquantitatively analyzed and databased. All of the subjects were over 40 years old, and the subjects with definite neurological diseases such

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as Parkinson's disease, cerebrovascular disorders and dementia were excluded from the analysis. The neurological findings in MD patients were obtained through the medical examination conducted by the Japanese government based upon the Pollution-related Health Damage Compensation Low to recognize Minamata disease patients for official compensation. The neurological findings were obtained from the inhabitants in MP area through the health check-up of residents which was conducted in 1994 (124 men and 167 women) and 1998 (121 men and 186 women), and from those in non-MP area in 1993 and 1994. Fifteen certified neurologists, authorized by The Japanese Society of Neurology, performed standardized physical examinations and neurological evaluation for all the subjects. For each year that the study was conducted, examinations were done for 4 days.

Predicting index

To establish a statistical diagnostic method to identify MD patients considering factors of aging and sex, we compared the neurological findings in MD patients and inhabitants in a non-MP area, and selected four signs (visual field abnormality, impairment in finger-to-nose test, glove and stocking type sensory disturbance, and decreased Achilles tendon reflex), in which the frequency of abnormal findings showed remarkable differences between the two groups. All of the subjects with at least two of these four signs (Group A) were found to be MD patients, and all subjects without any of these signs (Group B) were inhabitants of a non-MP area except one. Therefore we conducted multivariate statistical analysis using these two groups as index series, and ranked each neurological finding in order to distinguish the two groups. Based on these results, we established the predicting index for MD considering factors of aging and sex. More precisely, 1) a predicting index of 100 was given to Group A, and 0 to Group B, 2) the predicting index for subjects other than Group A or B was calculated using the following formulas:

$$\text{Predicting index} = 1/(1+e^{-x}) \times 100$$

$$\text{For men: } x = 14.45 + 2.397 \times x_1 + 3.049 \times x_2 + 3.833 \times x_3 + 3.780 \times x_4 + 1.777 \times x_5 - 8.095 \times x_6 + 3.150 \times x_7 + 1.758 \times x_8 + 1.554 \times x_9 - 0.3261 \times x_{10}$$

$$\text{For women: } x = 9.598 + 0.7787 \times x_1 + 1.210 \times x_2 + 4.637 \times x_3 + 2.406 \times x_4 + 1.930 \times x_5 + 0.6249 \times x_6 + 4.273 \times x_7 + 2.153 \times x_8 + 3.132 \times x_9 - 0.2408 \times x_{10}$$

x_1: abnormal eye movement, x_2: muscle weakness in lower limbs, x_3: ataxia in lower limbs, x_4: hearing impairment, x_5: dysdiadochokinesis, x_6: positive Romberg sign, x_7: dysarthria, x_8: poor tandem gait, x_9: smell disturbance, x_{10}: age. Each neurological finding was scored as 1 for abnormal and 0 for normal. The coefficients used in this formula were the regression coefficients obtained from the following logistic model (11) analyzing the distribution of abnormal neurological findings in Groups A and B:

$$\log (P/1-P) = a + b_1 x_1 + b_2 x_2 + \cdots + b_{10} x_{10}$$

The neurological findings in inhabitants with the predicting indices of 50 or higher in a MP area were compared with those in aged inhabitants with the indices below 50 in a non-MP area. We also analyzed the residential district of the inhabitants with the predicting indices of 50 or higher in a MP area.

Statistical methods

Logistic analysis was conducted to compare the prevalence of abnormal neurological findings in each sex among the three groups. Maximum likelihood parameter estimates and likelihood ratio statistics (LRS) in the logistic regression models were obtained with the use of a statistical package EPICURE (12). All of the p values presented were two-sided and statistical significance was defined as $p<0.05$.

Results

Using the formula to calculate the predicting index for MD, 94 out of 95 MD patients (99%; 100% in men, 98% in women) gave indices of 50 or higher. In the MP area, 16 of 291 inhabitants (5.5%) having a health check-up in 1994 and 14 of 307 (4.6%) in 1998 gave indices of 50 or higher. In the non-MP area, only one out of 558 inhabitants gave an index of 50 or higher (Fig. 1). The prevalence of inhabitants with an index of 50 or higher was not significantly different in the three residential districts, i.e., seashore, inland and intermediate distincts (Table 1). Among 29 inhabitants (one inhabitant overlapped at 1994 and 1998) with indices of 50 or higher in the MP area, low frequencies were observed in abnormal eye movement, smell disturbance, dysesthesia around the mouth, dysarthria, involuntary movement in upper limbs, and increased patellar tendon reflex, compared with MD patients. On the other hand, decreased patellar and Achilles tendon reflexes were frequently observed in the inhabitants of the MP area in comparison with those in MD patients (Table 2A, B). The frequency of sensory disturbance in the inhabitants in the MP area was significantly higher than that in the non-MP area.

Discussion

The statistical diagnostic method for MD proposed by Igata et al, which was based on a multivariate statistical analysis, i.e., discriminant analysis, was an epoch-making method to diagnose MD patients objectively (1–4). Using this method, they identified a borderline group of the inhabitants in the MP area. In the present study, we analyzed neurological data of MD patients that were the same as those used by Igata et al. However, the method of statistical analysis differed in the following points: 1) we used multivariate statistical analysis and logistic analysis considering the factors of aging and sex as well as neurological findings, 2) whereas Igata et al analyzed the neurological data obtained from inhabitants who had not been recognized as MD patients entitled for official compensation, we analyzed neurological findings in the inhabitants in

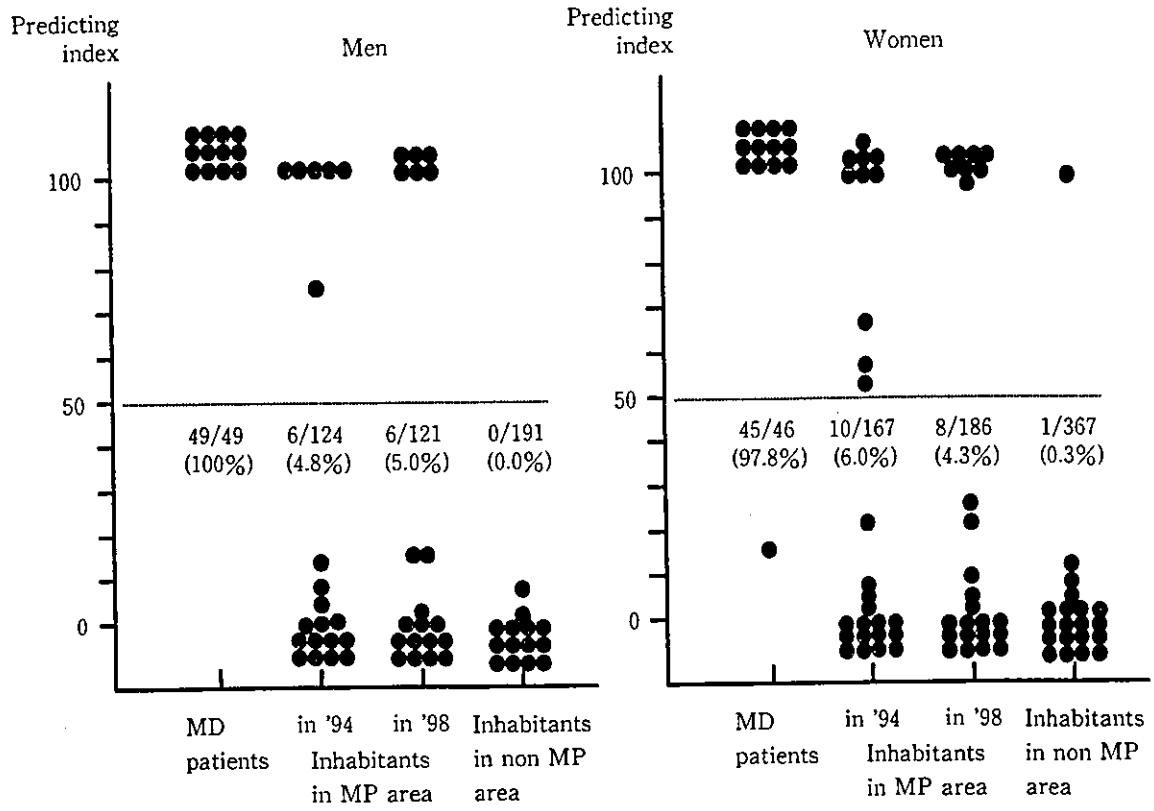

Figure 1. Predicting index scores in patients with Minamata disease (MD), inhabitants in methylmercury polluted (MP) area, and inhabitants in non-MP area. Proportion of subjects with predicting index scores of 50 or higher is presented in parentheses.

Table 1. Prevalence of Inhabitants with High Predicting Index Score for Minamata Disease in the Different Residential Districts in MP Area

Year of survey	Seashore	Inland	Intermediate	Total
1994	10/138	3/105	3/48	16/291
1998	7/123	5/128	2/56	14/307

the non-MP area. We previously studied the neurological findings in aged inhabitants in the non-MP area and found that some neurological findings such as muscle strength and sensory disturbance were greatly influenced by aging and sex (8–10). Meanwhile the diagnosis of atypical MD patients in the elders was difficult because it was obscure whether the neurological signs were due to methylmercury intoxication or aging effects (7).

In this study, we compared the frequencies of neurological findings in MD patients with those in aged inhabitants in the MP and non-MP areas, and created a new statistical diagnostic method for MD patients considering factors of aging and sex. Using the statistical diagnostic method, 99% of MD patients were identified as having MD, whereas 0.2% of the inhabitants in the non-MP area were identified to have a high predicting index for MD. Therefore, we consider that this statistical diagnostic method for MD patients is useful in distinguishing MD patients at least from the inhabitants in the non-MP area with normal aging.

Twenty-nine of the inhabitants (5%) in the MP area gave a high predicting index which was 50 or higher. The frequencies of abnormal neurological findings such as concentric constriction of visual field, incoordination, and sensory disturbance in the 29 inhabitants were significantly higher than those in the inhabitants of the non-MP area. In comparison with MD patients, the frequencies of abnormal neurological findings related to the central nervous system were relatively low in the 29 inhabitants, but the frequencies of abnormal neurological findings related to the peripheral nervous system were high in the inhabitants. These inhabitants in the MP area might belong to the borderline group proposed by Igata et al (1–4). Although it was obscure whether the neurological abnormalities detected in the 29 inhabitants in the MP area were due to methylmercury intoxication or other diseases, the prevalence of inhabitants with indices of 50 or higher did not show any statistical difference between the 2 residential districts. Our preliminary study showed that this statistical diagnostic method was not

Table 2A. Incidence of Neurological Signs (%) in Female Patients with Minamata Disease, Inhabitants in Methylmercury Polluted (MP) Area, and Inhabitants in Non-MP Area

	Women			
	Inhabitants with high predicting index score in MP area	Patients with Minamata disease	Inhabitants in MP area	Inhabitants in non-MP area
Number of subjects	N=17	N=46	N=353	N=367
Age: mean±S.D. (range)	70.5±7.4 (57–84)	57.9±11.5 (40–79)	68.2±5.9 (46–86)	71.5±7.6 (57–93)
Visual field abnormality	23.5*	100.0***	1.6	5.7
Abnormal eye movement	5.9	29.2***	2.7	5.7
Hearing impairment	35.3	77.1***	14.5	24
Smell disturbance	17.6	39.6***	4.8	7.4
Dysesthesia around mouth	0	50.0***	0	0.5
Dysphasia	0	10.4***	1.6	0.3
Dysarthria	0	43.8***	0.5	1.6
Neck pain on movement	29.4*	35.4***	11.8	8.4
Spurling sign	5.9	27.1***	3.3	4.9
Involuntary movement in upper limbs	17.6	39.6***	7.5	8.4
Muscle weakness in upper limbs	23.5***	56.3***	5.3	4.6
Increased muscle tonus in upper limbs	11.8*	6.3*	1.1	1.1
Muscle weakness in lower limbs	58.8***	66.7***	9.1	11.7
Increased muscle tonus in lower limbs	5.9	8.3*	0.5	2.4
Poor fine movement in upper limbs	23.5**	60.4***	3.2	3.0
Dysdiadochokinesis	29.4**	64.6***	4.3	5.7
Impairment in finger-to-nose test	23.5***	62.5***	2.7	0.8
Impairment in knee-to-heel test	11.8*	52.1***	1.6	1.4
Increased biceps reflex	17.6	22.9	21.5	13.9
Decreased biceps reflex	11.8	6.3	2.7	6.0
Increased radial reflex	17.6	18.8	22	15.8
Decreased radial reflex	17.6	10.4	4.8	7.6
Increased patellar tendon reflex	11.8	47.9*	22.5	24.5
Decreased patellar tendon reflex	47.1***	2.1	6.5	9.3
Increased Achilles tendon reflex	5.9	27.1*	11.8	13.4
Decreased Achilles tendon reflex	76.5***	29.2	16.7	17.4
Deep sensation abnormality	35.3***	89.6***	10.8	6.3
Glove-and-stocking type sensory disturbance	64.7***	93.8***	5.9***	0.5
Gait disturbance	23.5	37.5***	2.2	9.3
Poor tandem gait	41.2**	58.3***	7.5	12.3
Positive Romberg sign	11.8	12.5**	1.1	3.0

*: indicate the statistical significance determined by LRS in comparison with the incidence of abnormal neurological findings in inhabitants in non-MP area. ***p<0.001, **p<0.01, *p<0.05.

useful in distinguishing MD patients from patients with definite cervical spondylosis or peripheral neuropathy who have not been exposed to methylmercury. Therefore in addition to the clinical examination, electrophysiological and neuroimaging analyses, as well as epidemiological study on lifestyle were necessary to confirm the etiology of the abnormal neurological findings in the inhabitants in the MP area.

In this study, we proposed a new statistical diagnostic method for identifying MD patients and showed that this method was useful in distinguishing MD patients from inhabitants undergoing the normal aging process. Approximately 5% of inhabitants in the MP area, however, gave a high predicting index score. Continual neurological examination in the MP and non-MP areas is needed to clarify the neurological abnormalities of methylmercury intoxication.

Table 2B. Incidence of Neurological Signs (%) in Male Patients with Minamata Disease, Inhabitants in Methylmercury Polluted (MP) Area, and Inhabitants in Non-MP Area

	Men			
	Inhabitants with high predicting index score in MP area	Patients with Minamata disease	Inhabitants in MP area	Inhabitants in non-MP area
Number of subjects	N=12	N=49	N=245	N=191
Age: mean±S.D.	71.2±6.3 (62–80)	56.9±9.8 (41–79)	69.9±6.2 (52–85)	72.9±6.7 (59–89)
Visual field abnormality	25.0*	96.5***	3.3	5.8
Abnormal eye movement	0	36.8***	2.5	9.4
Hearing impairment	58.3**	80.7***	19.8	22
Smell disturbance	16.7	50.9***	9.9	12.6
Dysesthesia around mouth	8.3	36.8***	1.7	0
Dysphasia	0	10.5***	1.7	0
Dysarthria	0	42.1***	0.8	3.1
Neck pain on movement	33.3*	31.6***	5.8	8.9
Spurling sign	8.3	33.3***	5	2.6
Involuntary movement in upper limbs	25.0	45.6***	6.6	16.8
Muscle weakness in upper limbs	16.7	31.6***	5	4.7
Increased muscle tonus in upper limb	0	12.3***	2.5	0.5
Muscle weakness in lower limbs	41.7***	47.4***	6.6	4.2
Increased muscle tonus in lower limbs	0	8.8***	3.3	0.5
Poor fine movement in upper limbs	8.3	47.4***	6.6	8.9
Dysdiadochokinesis	33.3	64.9***	6.6	18.8
Impairment in finger-to-nose test	41.7***	70.2***	2.5	4.2
Impairment in knee-to-heel test	16.7	52.6***	1.7	3.1
Increased biceps reflex	0	19.3*	13.2	8.4
Decreased biceps reflex	8.3	8.8	12.4	11
Increased radial reflex	0	19.3**	12.4	6.3
Decreased radial reflex	33.3	19.3	15.7	11.5
Increased patellar tendon reflex	8.3	36.8***	19	12
Decreased patellar tendon reflex	33.3	12.3	5.7	17.3
Increased Achilles tendon reflex	0	21.2**	7.4	5.2
Decreased Achilles tendon reflex	75.0***	26.3	28.9	22
Deep sensation abnormality	41.7***	91.2***	10.7	7.3
Glove-and-stocking type sensory disturbance	66.7***	94.7***	6.6**	1
Gait disturbance	8.3	29.8***	7.4	4.2
Poor tandem gait	33.3	57.9***	11.6	13.1
Positive Romberg sign	0	8.8	1.7	6.3

*: indicate the statistical significance determined by LRS in comparison with the incidence of abnormal neurological findings in inhabitants in non-MP area. ***p<0.001, **p<0.01, *p<0.05.

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Cross-Sectional Analysis of Neurological Findings among Healthy Elderly: Study in a Remote Island in Kagoshima, Japan

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Key Words
Normal aging · Neurological findings · Healthy elderly · Cognitive impairment · Vibration sense · Handgrip strength · Mini-Mental State Examination

Abstract
We have conducted annual health checkup surveys of elderly subjects aged 60 years or older in a remote island of southwestern Japan. After excluding patients with neurological diseases and the subjects who needed help in activities of daily living, a cross-sectional analysis of the data obtained from 348 elderly people aged 60–89 years was made. We examined the age and sex distributions of abnormal neurological findings, including the scores of the Mini-Mental State Examination (MMSE). In this study, aging was associated with muscle weakness in the lower limbs, cerebellar dysfunction, hand tremor, decrease in handgrip, abnormality of deep sensation and a decrease in the MMSE score. These findings suggest a characteristic pattern of nervous system deterioration with age. We discuss the possible pathogenesis and significance of these findings that should contribute to a better understanding of normal aging in the nervous system.

Introduction

In 1931, Critchley [1] first reported the presence of various neurological signs among hospitalized elderly patients without neurological diseases. Thereafter, other studies noted neurological abnormalities in the elderly, such as essential tremor [2], absence of ankle jerk [2, 3], loss of vibration sense, particularly in the lower limbs [1–6], presence of primitive reflexes [7, 8] and instability of gait [1, 2, 9, 10]. The major drawbacks of those studies were the use of hospital-based subjects and the inclusion of patients with neurological diseases.

The increase in neurodegenerative diseases with advancing age makes it dfficult to understand normal aging [11]. In 1991, the Third Department of Internal Medicine, Kagoshima University Faculty of Medicine, started the Project for Healthy Aging. In this project, experienced neurologists conducted a biennial health checkup of residents aged 60 years or older in the town of Kasari, located on Amami island in Kagoshima prefecture, a remote island of southwestern Japan. The present study is a cross-sectional analysis of the data obtained from the project; we discuss the associations between age and abnormal neurological findings among Japanese elderly people without neurological diseases or neurodegenerative syndromes.

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Table 1. Age and sex distribution of the subjects and the population

Age	Subjects in the present study			Population[1]		
	male	female	all	male	female	all
60–69 years	46	117	163	488	607	1,095
70–79 years	56	86	142	353	505	858
80–89 years	16	27	43	141	285	426
Total	118	230	348	982	1,397	2,379

[1] According to the national census in 1995.

Subjects and Methods

The town of Kasari is located on the island of Amami in Kagoshima prefecture in southwestern Japan. Annually, since 1991, the Third Department of Internal Medicine, Kagoshima University Faculty of Medicine, has conducted health checkups of island residents 60 years old or older. This project was announced to all community dwellers by public information media. The total population of this area was 7,137 according to the national census in 1995 and the proportion of elderly residents over 65 years old was 26.2% compared with 14.1% for the nation. The study area was divided into two districts and the health checkup survey was conducted in either of the districts every year so that the residents could go for the health checkup of this project every other year. During the first 3 years of this project, there were several major changes in the procedures and questionnaires used. The survey in the current fashion started in 1994.

There were 593 elderly subjects (205 males and 388 females) aged 60 years or older who were not institutionalized and voluntarily underwent our biennial health checkup in 1994 or 1995. The examinees accounted for 24% of the population aged 60 or older in the study area. We excluded 241 subjects from statistical analysis after reviewing the medical history and neurological findings. Exclusions were due to the following: Parkinson disease, cerebrovascular diseases, peripheral nerve disorders, severe dementia, orthopedic disorders, vertebral deformity, history of diabetes mellitus, alcoholism and subjects showing a positive Babinski sign. We also excluded 4 subjects aged 90 or over since the number was too small to analyze the effect of age on neurological findings in that age group. The remaining 348 eligible subjects were included in the statistical analysis.

Neurological Examinations and Interview

Certified neurologists, authorized by the Japanese Society of Neurology, performed standardized physical examinations and neurological evaluation. The temperature of the physical examination rooms was kept at around 28°C. Neurological examinations were based on the procedures described in the *Mayo Clinic Examinations in Neurology*, 6th edition [12], and in the Japanese textbook *Physical Examination of the Nervous System*, 13th edition [13]. In subjects with diminished or absent reflexes, Jendrassic's reinforcement maneuver was used. A tuning fork (128 Hz) was used to examine vibration sense, measuring perception time (seconds) on both sides at the styloid apophysis of the ulna and fibula (internal malleolus). Loss of vibration sense was defined as vibratory perception time of less than 10 s. Strength of handgrip was measured with a dynamometer (kilograms). Cognitive impairment was assessed on the basis of the Mini-Mental State Examination (MMSE) [14].

Interviews using a standardized questionnaire were conducted to collect information on medical history, family history, education, occupation, physical activities, activities of daily living (ADL) and lifestyles, including smoking, drinking and dietary habits. Information on ADL included walking, going up and down the stairs, toileting, bathing and speaking.

Statistical Methods

Logistic analysis was conducted to compare the prevalence of abnormal neurological findings among three age groups (60–69, 70–79 and 80–89 years old) and two sexes. Each abnormal neurological finding was coded as 1 for abnormal and 0 for normal. Maximum likelihood estimates of odds ratios and their 95% confidence intervals were calculated. The p value for trend was calculated using age as a continuous variable in the logistic model.

As for strength of handgrip, vibration stimulus perception time and MMSE score, multiple linear-regression analyses were conducted to examine the association with age and sex. For the MMSE, the association with education was also examined. Educational level was classified in four groups based on schooling years: less than 6 years, 7–9 years, 10–12 years and longer than 13 years. The p value for trend was calculated using a consecutive integer of education levels. All of the p values presented were two-sided and statistical significance was defined as $p < 0.05$.

Results

Table 1 shows the age and sex distribution of the study subjects (118 males, 230 females) without neurological disease or degenerative syndromes. The proportion of each age- and sex-specific group was more than 15% of the population in this area except two groups, males aged 60–69 and females aged 80–89 years, which represented

Table 2. Frequencies and odds ratios of abnormal neurological findings among healthy subjects (according to age groups)

		Frequency, %				OR[1]			p for trend
		total	60–69	70–79	80–89	60–69	70–79	80–89	
Motor system									
Muscle atrophy	Upper limbs	0.9	1.3	0.7	0.0	1.0	–	–	–
	Lower limbs	1.2	0.6	1.4	2.4	1.0	1.9	3.5	0.9849
Muscle weakness	Upper limbs								
	Biceps	0.6	0.0	0.7	2.4	1.0	–	–	–
	Triceps	0.6	0.0	0.7	2.4	1.0	–	–	–
	Deltoid	0.6	0.0	1.4	0.0	1.0	–	–	0.4695
	Trapezius	–	0.0	0.0	0.0	1.0	–	–	–
	Lower limbs								
	Iliopsoas	3.3	1.3	4.4	7.3	1.0	3.9	6.4	0.0255
	Quadriceps femoris	3.3	1.3	3.6	9.8	1.0	3.3	9.2	0.0034
	Hamstrings	3.3	1.3	3.6	9.8	1.0	3.3	9.2	0.0045
	Tibialis anterior	1.5	0.6	2.2	2.4	1.0	3.4	3.8	0.6101
	Gastrocnemius	1.2	0.6	1.5	2.4	1.0	2.4	4.0	0.5120
Coordination									
Fine movement		3.0	1.3	5.0	2.4	1.0	3.8	1.9	0.0760
Dysdiadochokinesis		5.1	2.6	7.2	7.3	1.0	3.0	3.0	0.0118
Finger-nose test		2.7	1.9	3.6	2.4	1.0	1.6	1.2	0.7149
Finger-nose-finger test		3.6	1.9	5.1	4.9	1.0	2.5	2.5	0.2398
Tremor of the hands		5.3	2.5	5.8	14.6	1.0	2.4	6.6	0.0082
Involuntary movement of the legs		0.3	0.6	0.0	0.0	1.0	–	–	–
Sensory system									
Poor tactile sense	Upper limbs	3.3	3.8	2.9	2.5	1.0	0.7	0.6	0.7897
	Trunk	0.6	0.6	0.7	0.0	1.0	–	–	–
	Lower limbs	5.4	5.7	4.3	7.5	1.0	0.7	1.3	0.4257
Poor pain sense	Upper limbs	3.6	3.2	4.3	2.5	1.0	1.3	0.8	0.7723
	Trunk	0.6	0.6	0.7	0.0	1.0	–	–	–
	Lower limbs	6.0	4.5	6.5	10.0	1.0	1.4	2.3	0.1360
Poor posture sense	Upper limbs	0.9	0.0	2.3	0.0	1.0	–	–	–
	Lower limbs	1.2	0.0	2.3	2.5	1.0	–	–	0.1780
Vibration sense	Upper limbs								
(< 10 s)	Right	3.1	2.6	3.6	2.7	1.0	1.4	1.0	0.4985
	Left	3.4	2.6	3.6	5.4	1.0	1.4	2.1	0.4745
	Lower limbs								
	Right	33.2	25.0	35.0	59.5	1.0	1.6	4.4	0.0001
	Left	28.8	19.5	32.1	54.1	1.0	1.9	4.8	0.0001
Reflexes									
Absent	DTR of biceps	–	0.0	0.0	0.0	1.0	–	–	–
	DTR of triceps	0.3	0.6	0.0	0.0	1.0	–	–	–
	DTR of brachioradialis	0.9	1.3	0.7	0.0	1.0	–	–	–
	Knee jerk	2.4	1.9	2.9	2.4	1.0	1.4	1.2	0.4980
	Ankle jerk	3.9	2.5	5.8	2.4	1.0	2.3	1.0	0.4455
	Abdominal reflex	38.9	34.1	46.1	29.4	1.0	1.1	0.7	0.6063
Elicitation of	Jaw jerk	3.6	3.9	3.6	2.6	1.0	1.8	0.9	0.1995
	Palmomental reflex	13.5	11.3	16.3	12.8	1.0	1.7	1.2	0.6292
Station									
Standing		0.3	0.0	0.0	2.5	1.0	–	–	–
Squatting		10.1	5.1	13.7	17.5	1.0	3.4	4.2	0.0004
One foot		9.6	7.1	10.8	15.0	1.0	1.7	2.4	0.0401
On toe		4.2	2.6	5.0	7.5	1.0	2.0	3.1	0.1402
Tandem gait		5.4	1.9	5.8	17.5	1.0	3.3	11.2	<0.0001
Romberg test		0.6	0.0	0.0	5.0	1.0	–	–	0.0237
Mann test		6.9	4.5	8.0	12.5	1.0	1.8	3.0	0.0080

DTR = Deep tendon reflex.
[1] Odds ratio adjusted for sex.

Table 3. Sex-specific frequency of abnormal neurological finding among healthy subjects

		Frequency, %		p value[1]
		male (n = 118)	female (n = 230)	
Motor system				
Muscle atrophy	Upper limbs	2.7	0.0	-
	Lower limbs	2.7	0.4	0.0890
Muscle weakness	Upper limbs			
	Biceps	0.0	0.9	-
	Triceps	0.0	0.9	-
	Deltoid	0.9	0.4	0.6957
	Trapezius	0.0	0.0	-
	Lower limbs			
	Iliopsoas	1.8	4.1	0.1653
	Quadriceps femoris	0.9	4.5	0.0247
	Hamstrings	0.9	4.5	0.0255
	Tibialis anterior	1.8	1.3	0.7964
	Gastrocnemius	0.9	1.3	0.6661
Coordination				
Fine movement		4.4	2.3	0.3908
Dysdiadochokinesis		5.3	5.0	0.8739
Finger-nose test		6.1	0.9	0.0075
Finger-nose-finger test		6.2	2.3	0.1017
Tremor of the hands		5.3	5.4	0.7312
Involuntary movement of the legs		0.9	0.0	-
Reflexes				
Absent	DTR of biceps	0.0	0.0	-
	DTR of triceps	0.0	0.4	-
	DTR of brachioradialis	1.8	0.4	-
	Knee jerk	3.5	1.8	0.2999
	Ankle jerk	4.4	3.6	0.7756
	Abdominal reflex	23.8	47.3	0.0010
Elicitation of	Jaw jerk	0.9	5.0	0.0409
	Palmomental reflex	8.7	15.8	0.0632
Sensory system				
Poor tactile sense	Upper limbs	3.5	3.2	0.8950
	Trunk	0.0	0.9	-
	Lower limbs	7.0	4.5	0.4020
Poor pain sense	Upper limbs	4.4	3.2	0.6023
	Trunk	0.0	0.9	-
	Lower limbs	7.9	5.0	0.3886
Poor posture sense	Upper limbs	2.7	0.0	-
	Lower limbs	2.7	0.5	0.1281
Vibration sense	Upper limbs			
(< 10 s)	Right	2.8	3.2	0.7734
	Left	2.8	3.7	0.6158
	Lower limbs			
	Right	38.9	30.4	0.2788
	Left	32.4	27.0	0.6511
Station				
Standing		0.9	0.5	-
Squatting		3.5	13.5	0.0006
One foot		8.0	10.4	0.3592
On toe		4.4	4.1	0.9890
Tandem gait		3.5	6.3	0.1331
Romberg test		0.9	0.5	0.8201
Mann test		7.1	6.8	0.8099

DTR = Deep tendon reflex.
[1] p value adjusted for age.

relatively low percentages (9.4 and 9.5%, respectively, of the population).

From among 155 neurological findings obtained from a standardized neurological evaluation, we selected 44 neurological findings listed in table 2, which are frequently found even in normal elderly subjects [1–11]. Table 2 shows age-specific prevalence rates of abnormal neurological findings, and their odds ratios and p values obtained from logistic analysis, taking into account the effects of gender. Table 3 summarizes the results of the sex-specific analysis. Table 4 presents the results of handgrip strength.

Motor System

The frequency of the following neurological findings increased with advancing age: muscle weakness of the iliopsoas ($p = 0.0255$), quadriceps femoris ($p = 0.0034$) and hamstrings ($p = 0.0045$), dysdiadochokinesis ($p = 0.0118$) and tremor of hands ($p = 0.0082$) as shown in table 2. Female predominance was noted for muscle weakness of the quadriceps femoris ($p = 0.0247$) and hamstrings ($p = 0.0255$) as shown in table 3. On the other hand, poor performance of the finger-nose test was more common in men than in women ($p = 0.0075$).

Table 4 shows mean values and standard deviations (SDs) of the strength of handgrip and vibratory stimulus perception time by sex and age. The estimated coefficients were obtained using multiple linear-regression models. The major findings on handgrip are as follows: (1) males had a 1.5-fold greater handgrip strength than females; (2) the strength in both hands decreased with age; (3) the age association was not different in males and females, and (4) the right hand was 5% stronger than the left.

Sensory System

None of the prevalence rates of abnormal findings in the sensory system showed age or sex dependence except the loss of vibration sense in the lower limbs, which became more common with the advancement of age (table 2). Approximately 30% of the subjects had less than 10 s of vibration sense in the lower limbs, compared to 3% in the upper limbs. As shown in table 4, vibration stimulus perception time tended to be shorter in older subjects. Its age dependence was more marked in the lower limbs (fibula) than in the upper limbs (ulna). The mean perception time was approximately 3 s shorter in the lower limbs than that in the arms.

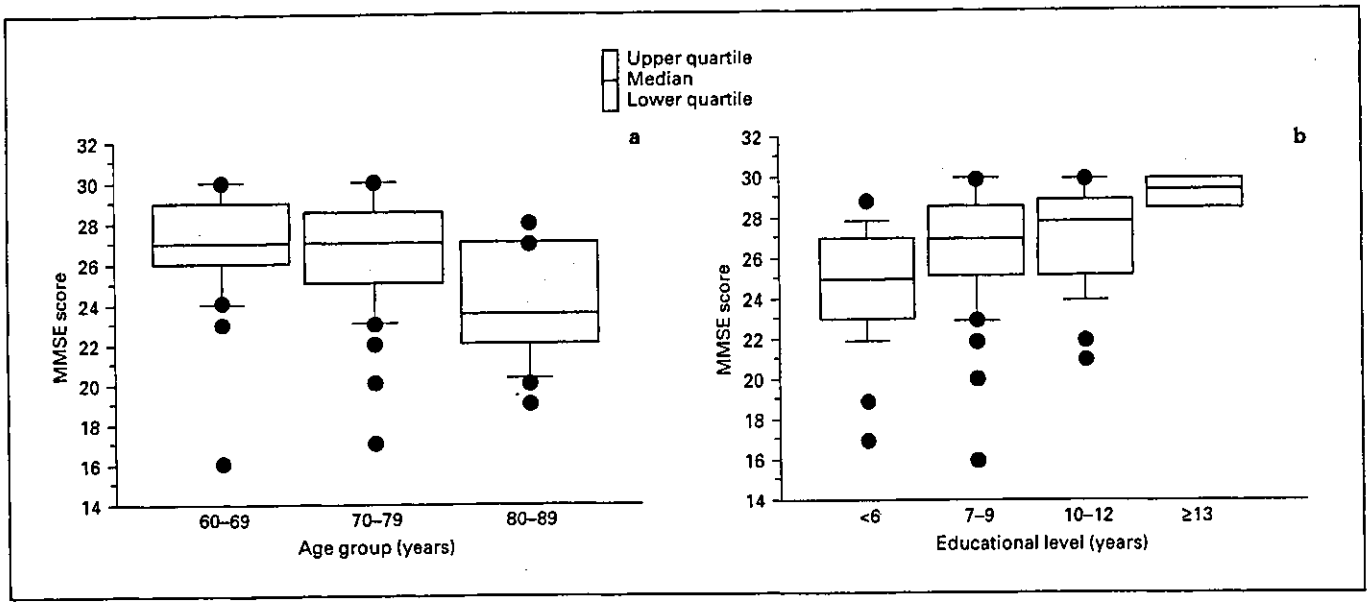

Fig. 1. Distribution of MMSE scores among healthy elderly subjects according to age group and educational level. **a** MMSE scores by age group. **b** MMSE scores by educational level. Bars show 95% confidence intervals.

Table 4. Strength of handgrip and vibratory stimulus perception times among healthy subjects

		Age group			All	β	SE	p value
		60–69	70–79	80–89				
Handgrip, kg								
Male	Right	32.4±7.6	25.8±6.0	23.1±6.5	27.9±7.5	−0.515	0.089	<0.0001
	Left	31.3±7.9	24.7±6.8	20.3±5.5	26.6±8.0	−0.591	0.093	<0.0001
Female	Right	20.6±4.5	17.0±4.5	13.1±4.5	18.3±5.1	−0.398	0.044	<0.0001
	Left	19.8±4.0	15.6±4.1	11.1±4.5	17.3±4.9	−0.438	0.040	<0.0001
Vibration stimulus perception times, s								
Male	Ulna							
	Right	14.5±2.6	13.4±2.2	14.7±2.8	14.0±2.5	−0.058	0.033	0.0847
	Left	14.7±2.6	13.5±2.2	14.7±2.6	14.1±2.5	−0.059	0.033	0.0800
	Fibula							
	Right	11.4±2.6	10.1±3.0	9.8±2.7	10.6±3.0	−0.134	0.039	0.0009
	Left	11.8±2.4	10.4±3.3	10.0±2.3	10.9±3.0	−0.142	0.041	0.0008
Female	Ulna							
	Right	14.5±2.3	14.2±2.5	12.8±2.5	14.2±2.4	−0.055	0.024	0.0226
	Left	14.4±2.4	14.6±2.7	12.6±2.2	14.3±2.6	−0.035	0.026	0.1690
	Fibula							
	Right	11.4±2.7	11.2±3.0	9.1±2.6	11.0±2.9	−0.081	0.028	0.0045
	Left	11.7±2.7	11.4±3.2	8.9±2.6	11.2±3.0	−0.092	0.029	0.0022

Data are means ± SD. β = Regression coefficient obtained with integral years of age as an independent variable; SE = standard error.

Reflexes

None of the prevalence rates of abnormal findings in reflexes showed age dependence (table 2). Female predominance was noted in an absent abdominal reflex ($p = 0.0010$) and the elicitation of jaw jerk ($p = 0.0409$).

Station

Age dependence was observed in poor performance of squatting ($p = 0.0004$) and one-foot standing ($p = 0.0401$), disability of tandem gait ($p < 0.0001$), positive signs of the Romberg test ($p = 0.0237$) and the Mann test ($p = 0.0080$). Difficulty in squatting was more common in women than in men ($p = 0.0006$).

Cognitive Impairment

The distributions of the MMSE score by age groups and educational levels are shown in figure 1a and b, respectively. In multiple regression analyses, the MMSE score decreased with age ($\beta = -0.097$, SE = 0.040, $p = 0.017$) and increased with the level of education ($\beta = 0.507$, SE = 0.140, $p = 0.0004$).

Discussion

The present study showed an age-dependent increase in motor dysfunction in the lower limbs that involved muscle weakness, decreased vibratory perception and cerebellar dysfunction. It has been reported that muscle weakness in the elderly is caused not by the failure of muscle activation [15] but by a decreased capacity to recover from age-related denervation injury [16]. Female predominance of instability of squatting can also be explained by proximal leg muscle weakness, because muscle weakness of the lower limbs was more common in females than in males. However, these abnormalities may not be explained completely by the age-dependent increase in motor dysfunction. Further studies are necessary to understand the cause of motor dysfunction in the lower limbs, which is the major cause of falls [17, 18].

The increase in dysdiadochokinesis and poor tandem gait we found in healthy elderly subjects is suggestive of cerebellar dysfunction, perhaps due to the loss of Purkinje cells since these cells are vulnerable to oxidative stress, which is considered to increase with aging [19, 20]. Hand tremor was associated with the advancement of age in this study. This so-called 'senile tremor' is thought to be a late-onset form of an essential tremor, different from a parkinsonian tremor. However, the precise mechanism of the essential tremor has not been clarified.

Michele et al. [21] and Meh and Denislic [22] reported that the perception time of a vibratory stimulus tended to be shorter with advancing age, although the tendency was less marked after the age of 60 [21, 22]. We obtained similar results in this study. The decrease in vibration sense observed in the present study may be explained by posterior-funiculus dysfunction or by loss of peripheral-nerve large myelinated fibers. Since the detection method for sensory disturbance in the present study was not quantitative – except for vibration sense – we cannot exclude the possibility that other sensory abnormalities could also occur with aging.

In our study, the mean handgrip strength of the right was stronger than that of the left, as previously reported [23]; the decline of handgrip strength with age (1.9%/year for males, 2.5%/year for females) was similar to the results of a previous study [23]. Interestingly, the magnitude of the decrease in handgrip strength in the left was slightly larger than that in the right in both males and females. More than 90% of our subjects were right handed [24].

The widely accepted associations of age and education level with the MMSE score [25, 26] were confirmed by our study. The median MMSE scores in the present study were similar to those reported by large-scale community-based studies in the USA and Japan [26, 27]. In the present study, 18.2% of the subjects had an MMSE score of less than 24 and 1.7% scored less than 18. These percentages are lower than those reported by other studies (3.4 and 6.0%) [27, 28]. These differences are probably related to our exclusion of subjects with other neurological diseases or severe dementia.

The effects of aging on deep tendon reflexes are controversial [2, 3, 29, 30]. Recently, Kumamoto et al. [29] have confirmed that deep tendon reflexes remained present regardless of age. Therefore, an absence of deep reflexes after reinforcement should probably be considered abnormal regardless of age; often these cases may be associated with diabetic neuropathy [30]. However, we observed absence of ankle jerk and knee jerk in 4.0 and 2.2%, respectively, in healthy elderly subjects without diabetes mellitus. We also found that 62.8% of the subjects with absent ankle and knee jerks had other diminished or absent deep tendon reflexes. Taken together, our results suggest that the absence of deep tendon reflexes in healthy elderly without diabetes mellitus is not related to aging but to other unknown factors.

Around 30% of the elderly subjects in this study had absent abdominal reflexes; this is approximately twice the frequency observed in the adolescents and young adults in Yngve's study [31]. However, the frequency of absent

abdominal reflex was not associated with aging in this study. Further research covering a wide age range is required to clarify the association between age and absent abdominal reflex. We found the palmomental reflex in 13.4% of subjects. The frequency in a community-based study in Japan was 22% [29] and in a US study 41% among volunteers [29, 32]. In 1980, Jacobs and Gossman [8] reported that the palmomental reflex was frequently found in healthy individuals (20–60%). The low frequency of the palmomental reflex in our study may be due to the restriction of our study subjects to healthy elderly. When the subjects with neurological diseases and the subjects who needed help in ADL were used in our analysis, the proportion went up to 20.8%, a similar value reported by other studies.

In conclusion, we estimated the prevalence rates of various neurological findings in the neurological examination of healthy elderly in Japan who had no definite neurological diseases and who were independent in ADL. Using strict eligibility criteria, our study showed that the frequencies of abnormal neurological findings in this population were much lower than those in other studies [3, 4, 7–10]. These data suggest that subjects with preclinical states of neurological diseases are fewer in this study than in the previous one, although it is virtually impossible to eliminate persons with preclinical conditions of neurological disorders. We found that age was associated with muscle weakness in the lower limbs, cerebellar dysfunction, hand tremor, decrease in handgrip, abnormality of deep sensation and decrease in MMSE score. We hope that our findings will contribute to the better understanding of normal aging and to the study of neurological function among the healthy elderly.

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