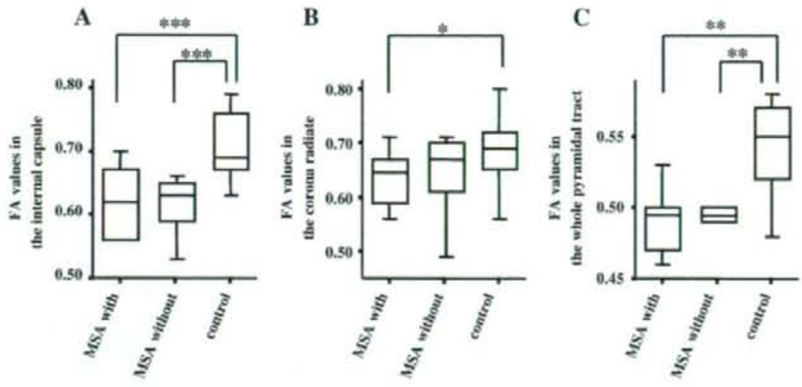

Fig. 4. Correlation between FA values and clinical pyramidal signs. FA values in MSA patients with or without pyramidal signs, and controls are shown for the internal capsule (A), corona radiate (B), and whole pyramidal tract (C). MSA with: MSA with pyramidal signs. MSA without: MSA without pyramidal sings. ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$.

with only pyramidal signs. Although FA values in all three regions of interest were slightly more decreased in MSA patients showing pyramidal signs than those without these signs, however, there were no significant differences between the two groups in all three areas. FA values were decreased even in the MSA patients without pyramidal signs.

4. Discussion

FA values specifically measure the degree of anisotropy of the diffusing water along different axes of an image, enabling useful quantitative estimation of decreased tissue anisotropy reflecting degeneration [12]. Decreased FA values could reflect destruction of this tissue architecture resulting from neuronal loss or gliosis, enhancing random mobility of free water molecules within the tissue; decreasing FA values can be interpreted to show tissue degeneration in normal aging [13] or various diseases [14–17], and demyelination is a possible process underlying FA changes [25]. More recently, decreased FA values in cerebellopetal fibers and the pyramidal tract have been reported in some MSA-C patients [18]. In addition, it was reported that FA values in the pons, cerebellum, putamen and middle cerebellar peduncles were useful in distinguishing MSA from PD [19,20]. FA values were useful in detecting tissue architecture degenerations in MSA; and our results supported these previous observations.

To our knowledge, this is the first systematic study to demonstrate the beneficial utility of FA values in identifying pyramidal tract degeneration in MSA. We evaluated FA values in the internal capsule, corona radiate, and whole pyramidal tract to identify pyramidal tract degeneration; FA values in MSA patients were significantly lower than in controls in all anatomical regions. Especially, FA values in the whole pyramidal tract analyses were more useful in detecting pyramidal tract degeneration than analyses of the internal capsule and the corona radiate. FA values in the internal capsule and the corona radiate evaluated one region in the pyramidal tract. However, in the whole pyramidal tract analyses, we evaluated multiple regions constituting the pyramidal tract. Thus, the whole pyramidal tract analyses could detect more sensitive values than the internal capsule and the corona radiate.

A limitation of the present study is that correction of brain atrophy was not assessed for analysis of FA values. Further comparative study with or without correction will strengthen our result.

In addition, though FA values in cases of MSA were similar to those seen in ALS patients' internal capsule and corona radiate, they were lower than in ALS in the whole pyramidal tract of ALS patients. These results suggest that pyramidal tract degeneration in MSA is more severe than reported to date. Previous pathological studies have demonstrated more frequent and extensive involvement of the pyramidal tract than that observed at clinical examination in MSA [4–6,9], and the level was similar to that of ALS. Despite previous findings of extensive pathological involvement in MSA, it has been noted that it is more difficult to detect pyramidal tract degeneration in MSA by clinical examinations or electrophysiological techniques (such as central motor nerve conduction study) compared with evaluations of degeneration in ALS patients [9–11]. These discrepancies may be explained by the size of the neurons and axons which degenerate in MSA. According to our previous pathological studies of the lateral corticospinal tracts or precentral gyrus in MSA, small- or middle-sized neurons and small-sized axons are predominantly affected, while large-sized neurons and axons are relatively well preserved [9], leading to normal central motor conduction. On a theoretical basis, the smaller the fiber diameter, the higher the FA values in those fibers, particularly as compared to large diameter fibers. Hence, degeneration of small-sized neuron and axon in MSA may readily show low FA values. In contrast to electrically detected central motor conduction, FA values can detect the small-sized neuron and axon involvement characteristic to pyramidal tract pathology in MSA. Extensive involvement including large-sized neurons may lead to a significant reduction of FA values, but excessive FA values reduction would not be enough to visualize pyramidal tracts as documented in our one patient with severe MSA-C. However, to our knowledge, there were no references that suggested smaller axons had higher FA values and degenerating smaller axons showed lower FA values compared with larger axons. We speculated that small-diameter axons would lead to higher anisotropy of the diffusing water than large-sized axons based on our present observations. In future, we intended to examine the correlation between this point and pathological study.

In this study, only 50% of MSA patients showed pyramidal signs; this frequency is almost consistent with previous reports [7,8]. It is worth nothing that not only the MSA groups with pyramidal signs, but also those without these signs, showed significant low FA values in the pyramidal tract. The mechanism behind the Babinski sign is still unclear [26,27], and a corticospinal tract lesion is not always sufficient to produce a Babinski sign, as previously reported [26,27]. Furthermore, it is not known whether involvement of small fibers in the pyramidal tract contributes to manifestation of a Babinski sign or hyperreflexia. Thus, discrepancies between clinical pyramidal signs and pathological changes of the corticospinal tracts could present in these patients. Our findings suggest that several underlying factors could contribute to elicit clinical pyramidal signs, even in MSA patients with extensive pathological pyramidal tract involvement. Determination of FA values may enable us to detect pyramidal tract damage with higher sensitivity, particularly when we compare this measurement to current clinical and electrophysiological procedures.

In our study, there were six MSA patients with precentral gyrus hyperintensity in FLAIR images; this frequency is almost consistent with previous reports [21]. Strikingly, MSA patients even without precentral gyrus hyperintensity showed lower FA values than controls. In addition, FA values with precentral gyrus hyperintensity in the internal capsule were significantly lower than those without it. These findings suggest that FA values can be used to detect early pyramidal tract degeneration and that precentral gyrus hyperintensity appears when pyramidal tract degeneration becomes more advanced.

In summary, pyramidal tract involvement in MSA was present at a similar level to ALS using FA values measures. In addition, FA values could detect early pyramidal tract degeneration prior to the appearance of clinical pyramidal signs or MR signal changes in MSA. Our findings further support the view that more extensive degeneration of the pyramidal tract occurs in MSA than so far believed.

Acknowledgement

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Cognitive impairments in multiple system atrophy

MSA-C vs MSA-P

Y. Kawai, MD
M. Suenaga, MD
A. Takeda, MD
M. Ito, MD
H. Watanabe, MD
F. Tanaka, MD
K. Kato, MD
H. Fukatsu, MD
S. Naganawa, MD
T. Kato, MD
K. Ito, MD
G. Sobue, MD

Address correspondence and reprint requests to Dr. G. Sobue, 65 Tsurumai-cho Showa-ku, Nagoya 466-8550, Japan
sobueg@med.nagoya-u.ac.jp

ABSTRACT

Objective: We evaluated comprehensive neuropsychological tests and regional brain blood flow to compare cognitive dysfunction between two types of multiple system atrophy: predominant cerebellar ataxia (MSA-C) and predominant parkinsonism (MSA-P).

Methods: Twenty-one patients with MSA-C, 14 patients with MSA-P, and 21 age- and education-matched control subjects were subjected to neuropsychological tests and SPECT. The neuropsychological tests examined general cognition, verbal and visual memory, working memory, visuospatial and constructional ability, language, executive function, depression, and anxiety, while SPECT analysis examined brain perfusion.

Results: Patients with MSA-P showed severe involvement of visuospatial and constructional function, verbal fluency, and executive function compared with control subjects. Patients with MSA-C showed involvement only in visuospatial and constructional function compared with control subjects and a milder degree of involvement compared with patients with MSA-P. Patients with MSA-P tended toward a wide and severe impairment in cognitive function compared with patients with MSA-C. In addition, neuropsychological impairment in patients with MSA-P was significantly correlated with a decrease in prefrontal perfusion. This significant relation was not correlated to other factors such as age, education, and severity of cerebellar ataxia and parkinsonism, which are relevant factors associated with cognitive performance.

Conclusions: Patients with multiple system atrophy-parkinsonism show more severe and more widespread cognitive dysfunctions than patients with multiple system atrophy-cerebellar ataxia. Our results also indicate that cognitive dysfunction in patients with multiple system atrophy-parkinsonism may be associated with prefrontal involvement. *Neurology*® **2008;70:1390-1396**

GLOSSARY

MSA = multiple system atrophy; **MSA-C** = MSA-cerebellar; **MSA-P** = MSA-parkinsonism.

Multiple system atrophy (MSA) is a sporadic neurodegenerative disease that presents with parkinsonism, cerebellar ataxia, autonomic failure, and pyramidal signs of varying severity during the course of the illness.[1-3] Neuronal loss, gliosis, and demyelination with widespread involvement—particularly in the striatonigral region, olivopontocerebellar region, and autonomic nervous system—are characteristic. Diagnostic criteria for MSA proposed by a Consensus Conference in 1998[4] recommend designating patients as having MSA-P if parkinsonian features are predominant or MSA-C if cerebellar features are predominant.

Although dementia consistent with the *Diagnostic and Statistical Manual of Mental Disorders* is an exclusion criterion for the diagnosis of MSA,[4] some studies have reported that patients with MSA reveal a cognitive decline when compared with control subjects.[5-9] Furthermore, several studies reported that patients with MSA exhibited more severe cognitive impairment, particularly with executive function, compared with that of

Supplemental data at www.neurology.org

From the Departments of Neurology (Y.K., M.S., M.I., H.W., F.T., G.S.) and Radiology (K.K., H.F., S.N.), Nagoya University Graduate School of Medicine, Nagoya, Japan; and Departments of Neurology (A.T.) and Radiology (T.K., K.I.), National Hospital for Geriatric Medicine, Aichi, Japan.

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Disclosure: The authors report no conflicts of interest.

Table 1	Clinical characteristics of patients with MSA (MSA-C and MSA-P) and control subjects			
	MSA (n = 35)	MSA-C (n = 21)	MSA-P (n = 14)	Control subjects (n = 21)
Sex (male/female)	19/16	12/9	7/7	7/14
Age at examination, y	61.0 ± 8.1 (47–76)	60.3 ± 8.3 (48–74)	62.0 ± 7.9 (47–76)	63.1 ± 9.4 (48–79)
Age at onset, y	58.0 ± 8.1 (41–73)	58.1 ± 8.8 (45–73)	57.9 ± 7.2 (41–68)	
Disease duration, y	2.9 ± 1.7 (1–7)	2.6 ± 1.6 (1–7)	3.2 ± 2.0 (1–7)	
Education, y	11.8 ± 2.4 (9–17)	12.1 ± 2.2 (9–17)	11.3 ± 2.5 (9–16)	12.5 ± 2.9 (8–16)
UPDRS part-III	24.2 ± 13.0 (4–54)	16.5 ± 8.8* (4–34)	35.2 ± 9.7* (22–54)	
ICARS	26.1 ± 11.9 (7–51)	27.7 ± 11.8 (11–48)	23.8 ± 12.2 (7–51)	
MMSE	27.4 ± 2.6 (21–30)	28.1 ± 2.4 (21–30)	26.4 ± 2.7† (22–30)	29.1 ± 1.0 (27–30)

Data are presented as mean ± SD (range).
*$p < 0.001$: MSA-P vs MSA-C.
†$p < 0.01$ compared with control.
MSA = multiple system atrophy; MSA-C = MSA-cerebellar; MSA-P = MSA-parkinsonism; UPDRS = Unified Parkinson's Disease Rating Scale; ICARS = International Cooperative Ataxia Rating Scale; MMSE = Mini-Mental State Examination.

severity- and age-matched patients with Parkinson disease.[6-11] In addition, neuroradiologic and pathologic studies have documented a variable extent of frontal and/or temporal lobe involvement in patients with MSA.[12-22] These observations suggest that cognitive function may be more widely and more severely impaired in patients with MSA than has been considered.[5-11] However, the differences in the degree and features of cognitive dysfunction between patients with MSA-C and those with MSA-P are not well understood. Furthermore, the relation between these cognitive dysfunctions and regional cerebral perfusion has not been clarified.

In the present study, we evaluated a wide range of cognitive functions and examined the relation between these impairments and brain regional perfusion, as assessed by SPECT, to reveal the background mechanism of cognitive dysfunction in patients with MSA-C and those with MSA-P.

METHODS Subjects. Twenty-one patients with MSA-C (12 male, 9 female; age, 60.3 ± 8.3 years; range, 48–74 years), 14 patients with MSA-P (7 male, 7 female; age, 62.0 ± 7.9 years; range, 47–76 years), and 21 control subjects (7 male, 14 female; age, 63.1 ± 9.4 years; range, 48–79 years) were enrolled in this study (table 1). Diagnoses of all patients with MSA were "probable" according to established diagnostic criteria.[4] We examined all patients with MSA by MRI before inclusion, and other neurologic diseases were excluded by MRI. We observed a hyperintense rim at the lateral edge of the dorsolateral putamen and a "hot cross bun" sign in the pontine basis in most of the patients with MSA. All subjects were native Japanese speakers. The severity of ataxia and parkinsonism was rated on the International Cooperative Ataxia Rating Scale[23] and the Unified Parkinson's Disease Rating Scale part-III.[24] Age- and education-matched paid volunteers were recruited as control subjects for neuropsychological tests and the perfusion study. Control subjects had no history of any neurologic or psychiatric disease that influenced cognition. Written informed consent was received in advance from both patients with MSA and control subjects. This study was approved by the ethics committee of the Nagoya University Hospital.

Neuropsychological tests. Each subject underwent a standard cognitive status assessment. All of a subject's neuropsychological tests were given on the same day. The Mini-Mental State Examination (MMSE) was used for overall assessment of cognition.[25] To evaluate attention, the Digit Span Subtest of the Revised Wechsler Adult Intelligence Scale was used.[26] Visual memory was examined using Visual Paired Associates Subtests 1 and 2 of the Revised Wechsler Memory Scale.[27] Verbal memory was examined using Logical Memory Subtests 1 and 2 of the Revised Wechsler Memory Scale.[27] To evaluate verbal fluency as measures of language functions, we asked subjects to name as many items as possible within 1 minute from a semantic category (animals) and from a phonemic category (Japanese nouns starting with the Japanese Kana character "Ka"). To assess executive function, simplified versions of the Wisconsin Card-Sorting Test[28] and the Rule Shift Cards test of the Behavioral Assessment of the Dysexecutive Syndrome[29] were used. Constructional ability was tested using the Block Design Subtest of the Revised Wechsler Adult Intelligence Scale.[26] All subjects completed a self-reporting instrument concerning anxiety and depression (Hospital Anxiety and Depression scale).[30]

Assessment of regional brain perfusion with SPECT. ^{99m}Tc-Ethylcysteine dimer (Neurolite; Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan), 600 MBq, was injected IV while the subjects were in a supine position with eyes closed in a quiet dimly lit room. SPECT scanning was carried out between 5 and 30 minutes after injection using a triple-head GCA 9300A gamma camera (Toshiba, Tokyo, Japan) equipped with low-energy, super high-resolution, fan-beam collimators. The data were acquired in a 128 × 128 matrix through a 120° rotation at an angle interval of 4°. The projection data were prefiltered through a Butterworth filter and reconstructed using filtered backprojection with a Ramp filter. No attenuation correction was performed. The in-plane spatial resolution was 8 mm in full width at half maximum. The final image slices were set parallel to the orbitomeatal line and were obtained at an interval of 6.9 mm through the entire brain.

Data analysis. For the statistical analysis of the neuropsychological tests, SPSS version 11.0 for Windows was used.

Table 2 Results of neuropsychological tests in patients with MSA (MSA-C and MSA-P) and control subjects

	MSA (n = 35)	MSA-C (n = 21)	MSA-P (n = 14)	Control subjects (n = 21)
Logical memory 1	16.5 ± 8.3	17.5 ± 7.2	14.8 ± 9.9	18.7 ± 4.8
Visual paired associates 1	9.7 ± 4.3	10.5 ± 4.5	8.6 ± 4.0	11.4 ± 4.0
Logical memory 2	12.0 ± 7.3	13.3 ± 6.7	9.9 ± 8.0	13.8 ± 5.0
Visual paired associates 2	4.3 ± 1.7	5.0 ± 1.3	3.3 ± 1.7	4.6 ± 1.7
Block Design	27.8 ± 11.6†	28.9 ± 12.1*	26.0 ± 11.1*	38.4 ± 7.5
Phonemic fluency	7.2 ± 3.0*	7.6 ± 2.9	6.6 ± 3.1*	9.8 ± 2.7
Semantic fluency	12.4 ± 3.9*	13.0 ± 3.5	11.4 ± 4.4*	15.9 ± 3.4
Wisconsin Card Sorting Test				
Categories	2.8 ± 2.0	3.1 ± 2.2	2.4 ± 1.6	2.8 ± 1.8
Total errors	23.1 ± 10.0	22.8 ± 11.2	23.8 ± 7.7	22.7 ± 8.1
Perseverative errors	8.6 ± 8.5	8.3 ± 9.5	9.1 ± 6.8	5.7 ± 4.6
Rule Shift Cards test	2.7 ± 1.6*	3.1 ± 1.2	2.1 ± 1.9*‡	3.8 ± 0.7
Digit Span	12.5 ± 5.0	12.7 ± 4.8	12.1 ± 5.4	12.5 ± 2.8
Depression	8.9 ± 3.4†	9.1 ± 3.2†	8.7 ± 3.8*	4.6 ± 2.7
Anxiety	5.7 ± 3.8	5.8 ± 4.0	5.4 ± 3.6	5.2 ± 3.6

Data are presented as mean ± SD. Unpaired *t* test for normally distributed data or Mann-Whitney *U* test for non-normally distributed data between MSA and control group. One-way analysis of variance and Tukey-Kramer test for data with normal distribution or the Kruskal-Wallis test and Steel-Dwass test for data with non-normal distribution among MSA-C, MSA-P, and control groups.

$^*p < 0.01$.

$^†p < 0.001$ compared with control.

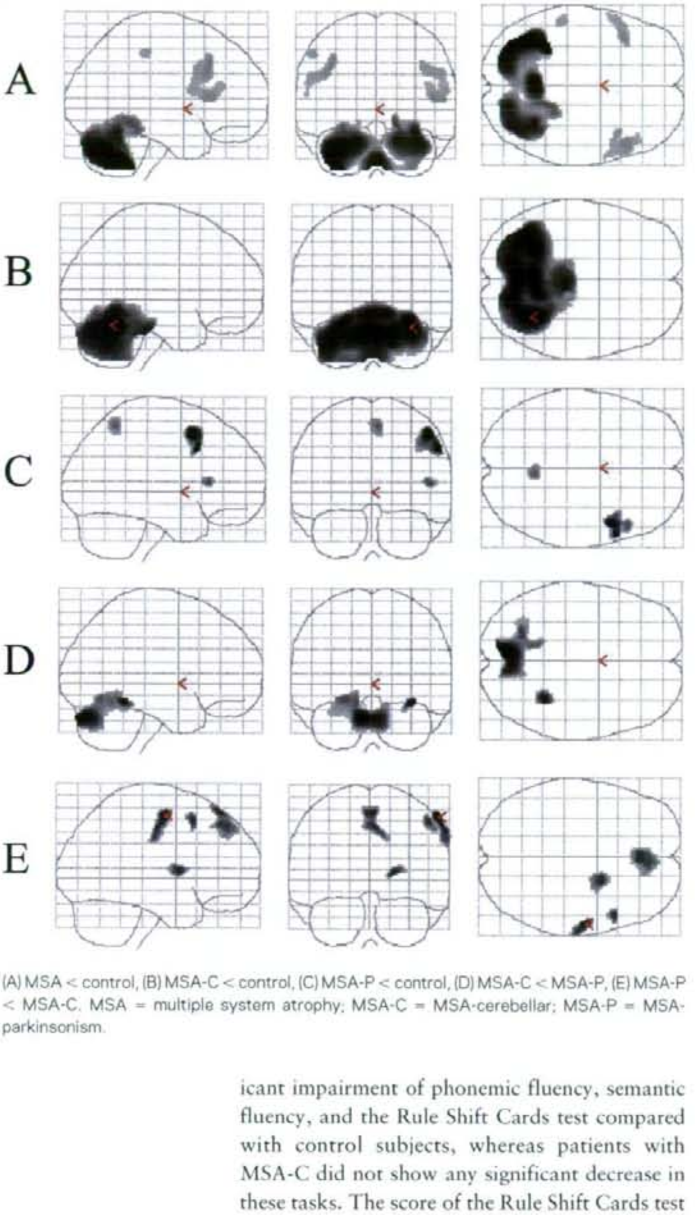
$^‡p < 0.05$ compared with MSA-C.

MSA = multiple system atrophy; MSA-C = MSA-cerebellar; MSA-P = MSA-parkinsonism.

The Shapiro-Wilk test was used to assess the normality of continuous variables. For comparison between the MSA group and the control group, we performed the unpaired *t* test for normally distributed data or the nonparametric Mann-Whitney *U* test for non-normally distributed data. For comparison among the MSA-C group, MSA-P group, and control group, we performed one-way analysis of variance for data with normal distribution or the Kruskal-Wallis test for data with non-normal distribution. When the *p* value for this overall comparison was significant (< 0.05), post hoc tests were done with the Tukey-Kramer test for data with normal distribution or Steel-Dwass test for data with non-normal distribution. The *p* values were subsequently adjusted for multiple comparisons using the Holm-Sidak method. In an attempt to assess the cognitive components of performance independently of motor skills and speed, we created a composite measure of processing speed for each subject by averaging T-transformed values of the time (seconds) demanding Design 1 on the Block Design task and the time (seconds) demanding the preshift phase of the Rule Shift Cards test.[31] Analyses of covariance were also conducted with this composite measure as a covariate when analyzing group differences on those tests in which speed, motor skill, or rapid speech was an important aspect of performance (e.g., Block Design task, phonemic and semantic fluency task). The SPECT data were analyzed using Statistical Parametric Mapping 2 (SPM2; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) implemented in MATLAB 6.5.1 (Math Works, Sherborn, MA). In a preprocessing step, data sets were spatially normalized to a standard stereotactic three-dimensional space and smoothed with an isotropic Gaussian kernel of full width at half maximum 12 mm. All images resulting from the normalization procedure were visually acceptable. In the following analyses, proportional scaling was applied to adjust the mean whole brain activity to 50 mL/100 g per minute to avoid interindividual variation in global cerebral blood flow. The gray matter threshold was 0.8. The normalized images of the patients with MSA-C, patients with MSA-P, and control subjects were compared by voxel-by-voxel *t* statistics. Confounding effects may arise from age or education differences, which could influence the regional cerebral blood flow change in MSA. Therefore, age and education were inserted as covariates in the SPM analyses. In addition, we compared the images of the patients with MSA-C and those with MSA-P to detect voxels in which the regional cerebral blood flow was significantly correlated with the scores of neuropsychological tests on which patients with MSA-C and those with MSA-P showed impairment. Each value of the neuropsychological tests was used as a covariate of interest, and the values of the global regional cerebral blood flow, age, education, International Cooperative Ataxia Rating Scale, and Unified Parkinson's Disease Rating Scale part-III, which are the relevant factors associated with cognitive performance, were excluded as nuisance variables. Regions were reported as significant if they contained voxels with a value of at least $p < 0.001$, uncorrected for multiple comparisons, with cluster extent threshold (k_E) < 10.

RESULTS Comparisons of neuropsychological tests in MSA-C and MSA-P patients. Patients with MSA did not differ significantly from control subjects with regard to MMSE (table 2). However, patients with MSA as a whole showed significant cognitive impairment in several tasks compared with control subjects. Visuospatial and constructional function as tested using Block Design, naming nouns from phonemic and semantic categories, and the Rule Shift Cards test of the Behavioral Assessment of the Dysexecutive Syndrome showed significant impairment. No significant differences were observed between patients with MSA and control subjects in attention (Digit Span) or memory functions, including Logical Memory Subtests 1 and 2 and Visual Paired Associates 1 and 2 of the Revised Wechsler Memory Scale as well as Wisconsin Card-Sorting Test. Scores for depression in patients with MSA were also significantly higher than those of control subjects. The visuospatial and constructional function test assessed by the Block Design task were impaired in both patients with MSA-P and those with MSA-C; however, the corrected *p* values did not reach the significance level in patients with MSA-C. Visuospatial and constructional function tended to be more severely impaired in patients with MSA-P, although the difference was insignificant. Patients with MSA-P showed signif-

Figure 1 SPM maps comparing brain perfusion among patients with MSA-C, patients with MSA-P, and control subjects (uncorrected $p < 0.001$)



(A) MSA < control, (B) MSA-C < control, (C) MSA-P < control, (D) MSA-C < MSA-P, (E) MSA-P < MSA-C. MSA = multiple system atrophy; MSA-C = MSA-cerebellar; MSA-P = MSA-parkinsonism.

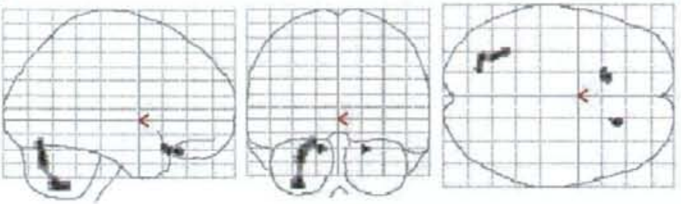
icant impairment of phonemic fluency, semantic fluency, and the Rule Shift Cards test compared with control subjects, whereas patients with MSA-C did not show any significant decrease in these tasks. The score of the Rule Shift Cards test in patients with MSA-P was lower than that of patients with MSA-C ($p < 0.05$). All of the 12 cognitive function tests performed in this study showed a tendency of more severe impairment in patients with MSA-P as compared with patients with MSA-C, although they did not show a significant difference (table 2). After a composite measure of processing speed by averaging T-transformed values of the time (seconds) demanding Design 1 on the Block Design task and the time (seconds) demanding the preshift phase of the Rule Shift Cards test were excluded, patients with MSA still showed impairments on the Block Design, phonemic fluency, and semantic fluency compared with control subjects. After removal of effects of processing speed, the Block Design, phonemic, and semantic fluency tests were impaired in patients with MSA-P compared with control subjects, whereas the impairment of the Block Design test in patients with MSA-C was not significant. Taken together, these results indicate that patients with MSA as a whole are impaired in wide-ranging cognitive functions, whereas patients with MSA-P show more severe and more widespread impairment in cognitive function than patients with MSA-C.

Regional brain perfusions and cognitive dysfunction in MSA-C and MSA-P patients. See tables e-1, e-2, and e-3 (on the *Neurology*® Web site at www.neurology.org) and figures 1, 2, and 3. According to SPM analyses, cerebellar and dorsolateral prefrontal perfusion in patients with MSA was significantly lower than that of the control subjects. Patients with MSA-C showed significantly reduced perfusion in the cerebellar hemisphere compared with control subjects. In contrast, patients with MSA-P showed reduced brain perfusion in the medial frontal cortices and the right dorsolateral prefrontal cortex compared with control subjects. The putamen, pallidum, and caudate nucleus also showed lower perfusion, but the differences were not significant.

When we compared brain perfusion between patients with MSA-C and those with MSA-P, cerebellar perfusion of the patients with MSA-C was decreased significantly compared with that of patients with MSA-P, whereas perfusion in the medial frontal cortices, right motor cortex, and right putamen of patients with MSA-P was decreased significantly compared with that of patients with MSA-C.

When cognitive dysfunction was correlated with regional brain perfusion, there was a positive correlation between the Block Design score and the perfusion in the bilateral prefrontal cortices and left cerebellar hemisphere in patients with MSA-C after age, education, and severity of cerebellar ataxia and parkinsonism were excluded statistically (figure 2). In patients with MSA-P, there was a positive correlation between the Block Design score and the perfusion in the dorsolateral prefrontal cortex, between phonemic fluency score and the perfusion in the dorsolateral prefrontal cortex, and

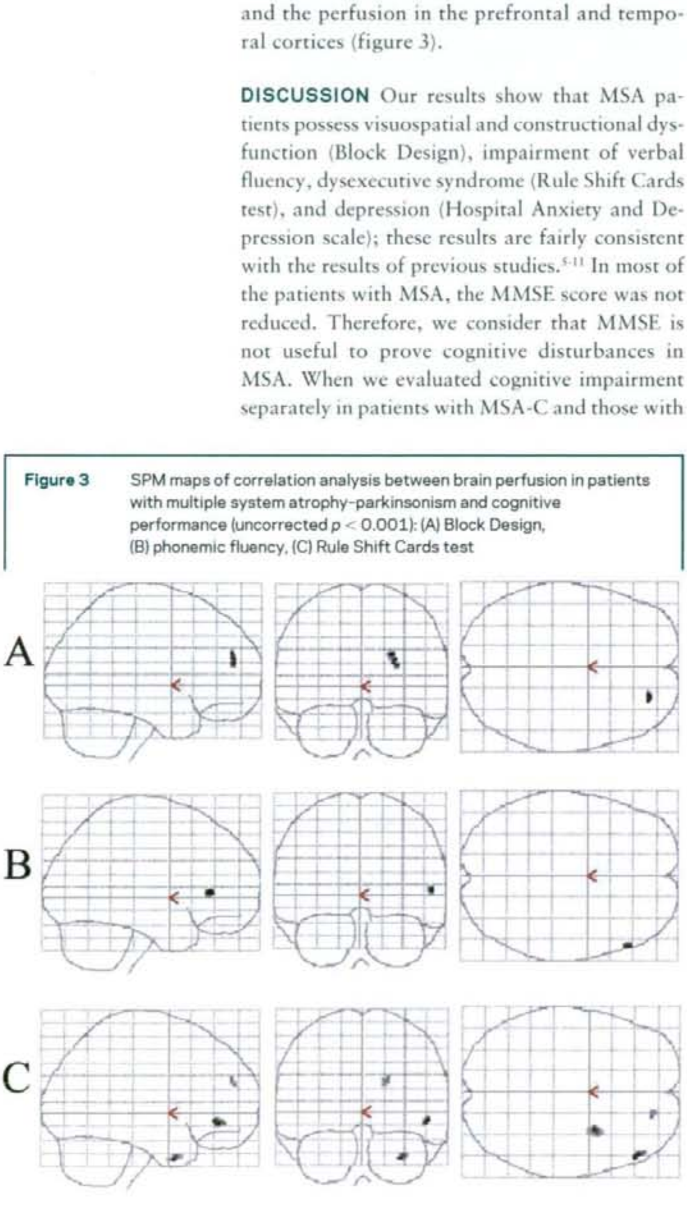
Figure 2 SPM maps of correlation analysis between brain perfusion in patients with multiple system atrophy-cerebellar and cognitive performance (uncorrected $p < 0.001$): Block Design



between the score of the Rule Shift Cards test and the perfusion in the prefrontal and temporal cortices (figure 3).

DISCUSSION Our results show that MSA patients possess visuospatial and constructional dysfunction (Block Design), impairment of verbal fluency, dysexecutive syndrome (Rule Shift Cards test), and depression (Hospital Anxiety and Depression scale); these results are fairly consistent with the results of previous studies.[5-11] In most of the patients with MSA, the MMSE score was not reduced. Therefore, we consider that MMSE is not useful to prove cognitive disturbances in MSA. When we evaluated cognitive impairment separately in patients with MSA-C and those with MSA-P, patients with MSA-P showed wide-ranging cognitive dysfunctions such as visuospatial and constructional dysfunction, impairment of verbal fluency, dysexecutive syndrome, and depression, while patients with MSA-C showed only visuospatial and constructional dysfunction and depression. Furthermore, the cognitive dysfunction in patients with MSA-P showed a tendency to be more severe than that of patients with MSA-C, particularly in executive functions. Thus patients with MSA-P may have more widespread and severe cognitive impairments than patients with MSA-C, although both showed a similar profile of cognitive dysfunction.

Figure 3 SPM maps of correlation analysis between brain perfusion in patients with multiple system atrophy-parkinsonism and cognitive performance (uncorrected $p < 0.001$): (A) Block Design, (B) phonemic fluency, (C) Rule Shift Cards test



Several neurophysiologic reports have shown significant cognitive decline suggesting frontal lobe impairment in patients with MSA. However, it has not been clarified whether frontal lobe or basal ganglia plays a primary role in the manifestation of cognitive impairment, because basal ganglia, which are associated with executive dysfunction, and the frontal or prefrontal area are both involved in MSA, particularly in MSA-P.[32,33] Our results show clearly that patients with MSA-P have significant hypoperfusion in the medial frontal cortices and dorsolateral prefrontal cortex, and the severity of cognitive impairments was significantly correlated with the hypoperfusion in the dorsolateral prefrontal cortex. Furthermore, the scores on the Block Design, phonemic and semantic fluency, and the Rule Shift Cards test were significantly correlated with brain perfusion in the prefrontal cortices after age, education, and severity of cerebellar ataxia and parkinsonism were excluded statistically. Our results support the view that frontal lobe involvement is responsible for the cognitive dysfunction in patients with MSA-P.

In patients with MSA-C, cognitive impairment in visuospatial and constructional dysfunction, assessed by Block Design task, was revealed, although the tendency to be more severely impaired in MSA-C was insignificant after age, education, and severity of cerebellar ataxia and parkinsonism were excluded. Removing only the effect of motor and speech on neuropsychological performances is difficult, because movement disorders and cognitive dysfunctions may progress simultaneously in neurodegenerative diseases and no established method exists. In patients with MSA-C, cognitive impairment in visuospatial and constructional function, assessed by the Block Design task, was correlated with the perfusion in the prefrontal cortex and cerebellum. Involvement of cerebellocortical circuits may also have influence on the cognitive decline in patients with MSA-C as demonstrated in patients with pure cerebellar involvement.[32-36] The Block Design task is related to spatial visualization, visual–motor coordination, and abstract conceptualization, which require frontal lobe function. Voxel-based morphometry revealed that the frontal lobes, including Brodmann areas 8, 10, 11, 46, and 47, were significantly correlated with the Wechsler Adult Intelligence Scale subtest, including the Block Design.[37] These previous results are consistent with our results, indicating that frontal lobe involvement can be responsible for cognitive dysfunction in patients with MSA-C. We suggest that the performance on cognitive tasks in patients with MSA-C was influenced by various lesions, including those in the cerebellum, cerebellocortical circuits, and the frontal lobe.

In this study, we showed that not only the frontal lobe, but also the posterior parietal lobe, exhibited a significant hypoperfusion in patients with MSA compared with that in control subjects. We also reported a varying degree of progressive cerebral atrophy, particularly in the frontal and temporal lobe, in patients with MSA in an advanced phase.[3] Furthermore, variable atrophy of the corpus callosum is present in a subgroup of patients even at a relatively early course of the illness.[3] More recently, a study based on longitudinal voxel-based morphometry demonstrated brain atrophy of widespread cortical regions such as the primary sensorimotor cortex, supplementary motor area, lateral premotor cortex, medial frontal gyrus, middle frontal gyrus, orbitofrontal cortex, insula, posterior parietal cortex, and hippocampus.[38] In addition, a recent pathologically confirmed case of MSA presenting with frontal executive and semantic language deficits was demonstrated.[39] Taken together, much earlier and more widespread cerebral involvement is present in patients with MSA than has been understood. The present study strongly suggests that parkinsonism, cerebellar ataxia, autonomic failure, and pyramidal tract sign are early and cardinal features of MSA, but cognitive decline is also present widely in MSA, particularly in MSA-P. The pathologic background of these cognitive impairments would be the widespread cortical involvement. Our study also demonstrated that the extent of cognitive involvement is significantly variable among the individual patients, even in those of MSA-P. Factors determining the evolution of cognitive involvement and cerebral involvement in MSA are not clear at present, but certain genetic factors need further study.

It is important to determine whether the cognitive impairments in patients with MSA have any influence on daily life activities. Because many neuropsychological tests and questionnaires are a significant burden to patients with MSA, we can evaluate only limited items at one time. In this study, we evaluated the differences in the cognitive function between patients with MSA-C and patients with MSA-P and the relation to brain perfusion; thus, we could not include the questionnaires to assess daily life activities, particularly in relation to cognitive functions. This relation of daily life activity to cognitive function is an important study to be performed.

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CAG repeat size correlates to electrophysiological motor and sensory phenotypes in SBMA

Keisuke Suzuki,[1] Masahisa Katsuno,[1,2] Haruhiko Banno,[1] Yu Takeuchi,[1] Naoki Atsuta,[1] Mizuki Ito,[1] Hirohisa Watanabe,[1] Fumitada Yamashita,[1,3] Norio Hori,[1,3] Tomohiko Nakamura,[1,3] Masaaki Hirayama,[1,3] Fumiaki Tanaka[1] and Gen Sobue[1]

[1]Department of Neurology, Nagoya University Graduate School of Medicine, [2]Institute for Advanced Research, Nagoya University and [3]Central Neurophysiological Laboratories, Nagoya University Hospital, Nagoya, Japan

Correspondence to: Gen Sobue, MD, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
E-mail: sobueg@med.nagoya-u.ac.jp

Spinal and bulbar muscular atrophy (SBMA) is an adult-onset, lower motor neuron disease caused by an aberrant elongation of a CAG repeat in the androgen receptor (AR) gene. The main symptoms are weakness and atrophy of bulbar, facial and limb muscles, but sensory disturbances are frequently found in SBMA patients. Motor symptoms have been attributed to the accumulation of mutant AR in the nucleus of lower motor neurons, which is more profound in patients with a longer CAG repeat. We examined nerve conduction properties including F-waves in a total of 106 patients with genetically confirmed SBMA (mean age at data collection = 53.8 years; range = 31–75 years) and 85 control subjects. Motor conduction velocities (MCV), compound muscle action potentials (CMAP), sensory conduction velocities (SCV) and sensory nerve action potentials (SNAP) were significantly decreased in all nerves examined in the SBMA patients compared with that in the normal controls, indicating that axonal degeneration is the primary process in both motor and sensory nerves. More profound abnormalities were observed in the nerves of the upper limbs than in those of the lower limbs. F-waves in the median nerve were absent in 30 of 106 cases (28.3%), but no cases of absent F-waves were observed in the tibial nerve. From an analysis of the relationship between CMAPs and SNAPs, patients were identified with different electrophysiological phenotypes: motor-dominant, sensory-dominant and non-dominant phenotypes. The CAG repeat size and the age at onset were significantly different among the patients with motor- and sensory-dominant phenotypes, indicating that a longer CAG repeat is more closely linked to the motor-dominant phenotype and a shorter CAG repeat is more closely linked to the sensory-dominant phenotype. Furthermore, when we classified the patients by CAG repeat size, CMAP values showed a tendency to be decreased in patients with a longer CAG repeat ($\geqslant$47), while SNAPs were significantly decreased in patients with a shorter CAG repeat ($<$47). In addition, we found that the frequency of aggregation in the sensory neuron cytoplasm tended to inversely correlate with the CAG repeat size in the autopsy study, supporting the view that the CAG repeat size differentially correlates with motor- and sensory-dominant phenotypes. In conclusion, our results suggest that there are unequivocal electrophysiological phenotypes influenced by CAG repeat size in SBMA.

Keywords: CAG repeat; spinal and bulbar muscular atrophy; electrophysiological phenotypes; motor-dominant; sensory-dominant

Abbreviations: CMAP = compound muscle action potential; MCV = motor conduction velocity; SBMA = spinal and bulbar muscular atrophy; SCV = sensory conduction velocity; SNAP = sensory nerve action potential

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Introduction

Spinal and bulbar muscular atrophy (SBMA) is a hereditary lower motor neuron disease affecting adult males (Kennedy *et al.*, 1968; Sobue *et al.*, 1989, 1993; Fischbeck *et al.*, 1997). The cause of SBMA is an aberrant elongation of a CAG repeat in the androgen receptor (AR) gene. Normally, 9–36 CAGs are observed in the AR gene in normal subjects, but 38–62 CAGs are observed in SBMA patients (La Spada *et al.*, 1991; Tanaka *et al.*, 1996; Andrew *et al.*, 1997). A similar gene mutation has been detected in Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA) and several types of spinocerebellar ataxia (Gatchel *et al.*, 2005). Since CAG is translated to glutamine, these disorders, including SBMA, are called polyglutamine diseases. In SBMA patients, there is an inverse correlation between the number of CAGs and the age at onset (Doyu *et al.*, 1992; Atsuta *et al.*, 2006). The histopathological hallmarks of this disease are an extensive loss of lower motor neurons in the spinal cord and brain stem, together with degeneration of the dorsal root ganglions (DRG) (Sobue *et al.*, 1989; Adachi *et al.*, 2005). Intranuclear accumulations of mutant AR protein in the residual motor neurons are another hallmark (Li *et al.*, 1998; Adachi *et al.*, 2005). The molecular basis for motor neuron degeneration is thought to be testosterone-dependent nuclear accumulation of the mutant AR, and androgen deprivation rescues neuronal dysfunction in animal models of SBMA (Katsuno *et al.*, 2002, 2003; Takeyama *et al.*, 2002; Chevalier-Larsen *et al.*, 2004). Androgen deprivation with a luteinizing hormone-releasing hormone (LHRH) analog also suppresses nuclear accumulation of mutant AR in the scrotal skin of SBMA patients (Banno *et al.*, 2006). Other candidates for potent therapeutics such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) or geranylgeranylacetone (GGA), enhancers of molecular chaperone expression and function, and a histone deacetylase (HDAC) inhibitor have also emerged from studies of animal models of SBMA (Minamiyama *et al.*, 2004; Katsuno *et al.*, 2005; Waza *et al.*, 2005).

The main symptoms of SBMA are weakness and atrophy of the bulbar, facial and limb muscles (Katsuno *et al.*, 2006). The onset of weakness is usually between 30 and 60 years of age. Postural tremor of the fingers is often observed prior to weakness. The symptoms are slowly progressive in SBMA, and the susceptibility for aspiration pneumonia increases as bulbar paralysis develops (Atsuta *et al.*, 2006). The most common cause of death is pneumonia. Many patients also have hypertension, hyperlipidemia, liver dysfunction and glucose intolerance. Serum creatine kinase is increased in the majority of patients.

In addition to motor symptoms, sensory impairment such as vibratory sensory disorder is often observed, and electrophysiological involvement has also been described in sensory nerves of SBMA patients (Harding *et al.*, 1982; Olney *et al.*, 1991; Li *et al.*, 1995; Guidetti *et al.*, 1996; Polo *et al.*, 1996; Ferrante *et al.*, 1997; Antonini *et al.*, 2000; Sperfeld *et al.*, 2002). In addition, sensory nerve axon loss, particularly of the central and peripheral rami of primary sensory neurons, has been documented to be profound (Harding *et al.*, 1982; Sobue *et al.*, 1989; Li *et al.*, 1995). Spinal dorsal column involvement and loss of axons in the sural nerve are common pathological features (Sobue *et al.*, 1989; Li *et al.*, 1995), and abnormalities in sensory nerve conduction and sensory evoked potentials are well known features of SBMA (Kachi *et al.*, 1992). Since the sensory symptoms are not generally severe in most patients, sensory nerve involvement has not been given much attention, particularly when compared to motor symptoms. However, the involvement of primary sensory neurons is one of the major phenotypic manifestations in SBMA (Sobue *et al.*, 1989).

The age at onset and the severity of motor symptoms are variable among SBMA patients (Kennedy *et al.*, 1968; Sperfeld *et al.*, 2002). One of the major factors determining clinical features is the CAG repeat size in the AR gene (Doyu *et al.*, 1992; Atsuta *et al.*, 2006). However, the age at onset and severity are also variable even among the patients with the same CAG repeat size (Doyu *et al.*, 1992; Atsuta *et al.*, 2006), indicating that some unknown genetic or environmental factors may influence the development of clinical heterogeneity (Atsuta *et al.*, 2006). In sensory impairments, there is also a variable degree of severity. Some patients show profound sensory symptoms and sensory nerve electrophysiological abnormalities, while other patients appear almost normal (Olney *et al.*, 1991; Li *et al.*, 1995; Guidetti *et al.*, 1996; Antonini *et al.*, 2000). In contrast to motor symptoms, the age at onset for sensory symptoms is rather difficult to determine, and the role of CAG repeat size in the severity of symptoms and the onset of sensory symptoms is unknown.

In order to clarify motor and sensory nerve involvement in SBMA, we examined nerve conduction properties including F-waves in 106 patients with genetically confirmed SBMA and 85 control subjects. We further analysed the influence of the CAG repeat size within the AR gene on the electrophysiological motor- and sensory-dominancy, as well as the histopathological background underlying the phenotypic diversity in nerve conduction of SBMA patients.

Subjects and Methods

Patients

A total of 106 male patients with the diagnosis of SBMA confirmed by genetic analysis and 85 male normal control subjects were included in this study. The data of SBMA patients were collected between May 2003 and May 2007. We analysed various electrophysiological examinations, motor function, sensory disturbance, disease duration and CAG repeat size in the AR gene in these patients. We defined the onset of disease as when the muscular weakness began, but not when tremor of the fingers appeared. As a functional assessment, we applied the Limb Norris score, Norris Bulbar score and ALS functional rating scale-revised (ALSFRS-R), which are aimed at motor function evaluations of patients with amyotrophic lateral sclerosis (ALS) (Norris *et al.*, 1974; The ALS CNTF Treatment Study (ACTS) Phase I-II Study Group, 1996).

All studies conformed to the ethics guideline for human genome/gene analysis research and the ethics guideline for epidemiological studies endorsed by the Japanese government. The ethics committee of Nagoya University Graduate School of Medicine approved the study, and all SBMA patients and normal subjects gave their written informed consent to the investigation.

Electrophysiological assessments

Motor and sensory nerve conduction studies were performed in the median, ulnar, tibial and sural nerves in 106 patients during their initial clinical assessment at Nagoya University Hospital using a standard method with surface electrodes for stimulation and recording as described previously (Sobue *et al.*, 1989; Kimura, 2001*a*, *b*; Koike *et al.*, 2003; Mori *et al.*, 2005). Motor conduction was investigated in the median, ulnar and tibial nerves, recording from the abductor pollicis brevis, abductor digiti minimi and abductor hallucis brevis, respectively. The following nerve segments were used for calculating motor conduction velocities (MCV): wrist to elbow for the median nerve, wrist to distally at the elbow for the ulnar nerve, and ankle to popliteal fossa for the tibial nerve. Sensory conduction was investigated in the median, ulnar and sural nerves, using antidromic recording from ring electrodes at the second and fifth digit for the median and ulnar nerves, respectively, and bar electrodes at the ankle for the sural nerve. Sensory conduction velocities (SCV) were calculated for the distal segment. Amplitudes of compound muscle action potentials (CMAP) and those of sensory nerve action potentials (SNAP) were measured from the baseline to the first negative peak. Control values were obtained in 56–85 age-matched normal volunteers (31–75 years) (Koike *et al.*, 2001; Mori *et al.*, 2005).

F-waves were also examined in the median and tibial nerves at the same time as the nerve conduction studies using a standard method as described previously (Kimura, 2001*c*). Sixteen consecutive supramaximal stimuli with frequency of 1 Hz were delivered to the median and tibial nerves, while recording from the same muscles as the normal nerve conduction studies. The following variables were estimated: occurrence, minimum latency and maximum F-wave conduction velocity (FWCV). FWCV was calculated using the formula $2D/(F-M-1)$, where D is the surface distance measured from the stimulus point to the C7 spinous process in the median nerves or to the T12 spinous process in the tibial nerves, F is the latency of the F-wave and M is the latency of the CMAP. Control values were obtained in 28–47 age-matched normal volunteers (31–75 years). All nerve conduction studies and F-wave studies were carried out on the right side of the body.

We defined the nerve conduction, CMAPs and SNAPs as abnormal, when these values were less than the mean -2 SD of normal controls on the examined nerves. We also expressed the CMAP and SNAP values as the percentage of the mean values of normal controls, when we need the standardized expression of the degree of CMAP and SNAP involvement as compared to normal controls.

Standard needle electromyography (EMG) was performed using concentric needle electrodes in 93 SBMA patients, with the muscles at rest and during weak and maximal efforts (Sobue *et al.*, 1993; Kimura, 2001*d*; Sone *et al.*, 2005).

Genetic analysis

Genomic DNA was extracted from peripheral blood of SBMA patients using conventional techniques (Tanaka *et al.*, 1996). PCR amplification of the CAG repeat in the AR gene was performed using a fluorescein-labelled forward primer (5′-TCC AGAATCTGTTCCAGAGCGTGC-3′) and a non-labelled reverse primer (5′-TGGCCTCGCTCAGGATGTCTTTAAG-3′). Detailed PCR conditions were described previously (Tanaka *et al.*, 1996, 1999). Aliquots of PCR products were combined with loading dye and separated by electrophoresis with an autoread sequencer SQ-5500 (Hitachi Electronics Engineering, Tokyo, Japan). The size of the CAG repeat was analysed on Fraglys software version 2.2 (Hitachi Electronics Engineering) by comparison to co-electrophoresed PCR standards with known repeat sizes. The CAG repeat size of the PCR standard was determined by direct sequence as described previously (Doyu *et al.*, 1992).

Immunohistochemistry for mutant AR in the sensory and motor neurons

For immunohistochemistry of primary sensory and spinal motor neurons, autopsy specimens of lumbar DRG and spinal cord from five genetically diagnosed SBMA patients (70.4 ± 11.0 years old) were used. The lumbar DRG and spinal cord were excised at autopsy and immediately fixed in 10% buffered formalin solution. The collection of tissues and their use for this study were approved by the Ethics Committee of Nagoya University Graduate School of Medicine. Lumbar DRG and spinal cord sections of 6 μm were deparaffinized, treated with 98% formic acid at room temperature for 3 min and with microwave oven heating for 10 min in 10 mM citrate buffer at pH 6.0, and incubated with an anti-polyglutamine antibody (1C2, 1:20 000; Chemicon, Temecula, CA). Subsequent staining procedures are performed using the Envision+ kit (Dako, Glostrup, Denmark).

For quantification of primary sensory neurons in which mutant AR accumulates, we prepared at least 100 transverse sections from the lumbar DRG, and performed 1C2 immunohistochemistry as described above. The frequency of 1C2-positive and -negative cells within the DRG was assessed by counting all the neurons with 1C2-positive cytoplasmic inclusions against total neuronal cells with obvious nuclei on every 10th section under the light microscope (BX51N-34, Olympas, Tokyo, Japan). The results were expressed as frequency of 1C2-positive cells in the 10 sections of the DRG. As for quantification of spinal motor neurons, the detailed procedure has been described previously (Adachi *et al.*, 2005). We have also examined five control autopsied specimens from patients died from non-neurological diseases, and found that there were no 1C2-positive cytoplasmic or nuclear staining.

Data analysis

Quantitative data was presented as means ± SD. Statistical comparisons were performed using the Student's *t*-test. Correlations among the parameters were analysed using Pearson's correlation coefficient. P values less than 0.05 and correlation coefficients (r) greater than 0.4 were considered to indicate significance. Calculations were performed using the statistical software package SPSS 14.0J (SPSS Japan Inc., Tokyo, Japan).

Results

Clinical and genetic backgrounds of SBMA patients

The clinical background of the SBMA patients is described in Table 1. All of the patients examined were of Japanese nationality. The duration from onset assessed from the first notice of motor impairment (Atsuta *et al.*, 2006) ranged from 1 to 32 years. There was no significant difference between the median CAG repeat size in the present study

Table 1 Clinical and genetic features of SBMA patients

Clinical and genetic features	Mean ± SD	Range	n
Age at examination (years)	53.8 ± 10.0	31–75	106
Duration from onset (years)	10.1 ± 6.8	1–32	106
Age at onset (years)	43.7 ± 10.4	25–68	106
CAG repeat size in AR gene (number)	47.8 ± 3.1	41–57	97[a]
Limb Norris score (normal score = 63)	53.9 ± 7.3	34–63	99
Norris Bulbar score (normal score = 39)	33.0 ± 4.3	20–39	99
ALSFRS-R (normal score = 48)	41.1 ± 4.3	22–48	99

[a]The abnormal elongation of the CAG repeat was confirmed by gene analysis using agarose gel electrophoresis without determining the repeat number in the remaining nine patients. AR = androgen receptor; ALSFRS-R = ALS functional rating scale-revised

and those reported previously in SBMA patients (La Spada *et al.*, 1991; Tanaka *et al.*, 1996; Andrew *et al.*, 1997).

All patients were ambulatory with or without aid, and none were bed-ridden. The mean Limb Norris score, Norris Bulbar score and ALSFRS-R also suggested that the ADL of patients in this study was not severely impaired. Vibratory sensation disturbance was detected in 78.2% of the SBMA patients. Touch and pain sensation abnormalities were found in 10.9 and 9.1% of the patients, respectively. Joint position sensation was intact in all of the patients examined.

In EMG, all the examined patients showed high amplitude potentials, reduced interference and polyphasic potentials, suggesting neurogenic changes in SBMA.

Nerve conduction and F-wave studies indicate CMAP and SNAP reduction as a profound feature of SBMA

MCV, CMAP, SCV and SNAP were significantly decreased in all the nerves examined in the SBMA patients when compared with those of the normal controls (Table 2). Sensory nerve activity could not be evoked in some cases, whereas activity in the motor nerves was elicited in all patients examined. The most profound finding in the nerve conduction studies was the reduction in the amplitude of the evoked potentials in both motor and sensory nerves. The mean values of CMAPs were reduced to 47–76%, and SNAPs were reduced to 31–47% of the normal mean values. The decrease in conduction velocity was relatively mild, but definitely present in both motor and sensory nerves. The conduction velocity was reduced to 94–96% in MCV and 87–91% in SCV of the normal mean values. The F-wave latencies were also mildly, but significantly prolonged in the median and tibial nerves of SBMA patients. The mean occurrence rate of F-waves in the median nerve was significantly less in SBMA patients, and they were absent in 30 cases (28.3%) (Table 2).

When we compared the CMAP and MCV values of the individual patients in the median, ulnar and tibial nerves, MCV was decreased only in the patients with a severely decreased CMAP (Supplementary Fig. 1). In addition, SCV reduction was observed only in the patients with severely decreased SNAP (Supplementary Fig. 1). These observations strongly suggest that the most profound impairment of the SBMA patients is a reduction of the amplitude of evoked potentials, possibly due to axonal loss (Sobue *et al.*, 1989; Li *et al.*, 1995).

As for the spatial distribution of electrophysiological involvements, the frequency of abnormal values of CMAP was most remarkable in the median nerve followed by the ulnar and tibial nerves (Table 3). The decrease in SNAP was also remarkable in the median and ulnar nerves when compared with those in the sural nerve (Table 3). The absence of F-waves was more frequent in the median nerve than in the tibial nerve (Table 3). These findings indicate that more significant abnormalities in nerve conduction and F-waves are observed in the nerves of the upper limbs than in those of the lower limbs.

Electrophysiologically defined motor and sensory phenotypes

When we analysed the relationship between the degree of motor and sensory nerve involvement by assessing the number of nerves showing abnormally reduced amplitudes (less than control mean − 2 SD) in the sensory (median, ulnar and sural nerves) and motor (median, ulnar and tibial nerves) nerves, we found that the patients could be distinguished by either a motor-dominant, sensory-dominant or non-dominant phenotype (Fig. 1A). It should be noted that there were patients showing only abnormally reduced SNAPs, while the CMAPs were well preserved (Fig. 1A). Alternatively, patients demonstrating CMAPs abnormalities with well preserved SNAPs were also seen (Fig. 1A).

When we analysed the relationship between CMAPs and SNAPs on a standardized scale of percentage of the mean values of normal controls in the median and ulnar nerves (Fig. 1B and C), we found that there were patients with different electrophysiological phenotypes. Some patients showed well preserved CMAPs, being 50% or more of the mean value in the controls, while showing profoundly reduced SNAPs of less than 50% of the mean value in the controls. In contrast, other patients showed well-preserved SNAPs and significantly reduced CMAPs (Fig. 1B and C). Finally, some patients showed a similar involvement of CMAPs and SNAPs. These observations suggest that a subset of SBMA patients shows predominantly motor impairments, while another subset shows predominantly sensory impairments.

The CAG repeat size correlates to electrophysiologically defined motor and sensory phenotypes

Since the CAG repeat size is a key factor dictating clinical presentation in polyglutamine diseases (Zoghbi *et al.*, 2000),

Table 2 Nerve conduction studies and F-wave examinations

	SBMA		Normal		P
	(Mean ± SD)	n	(Mean ± SD)	n	
Median nerve					
MCV (m/s)	54.3 ± 6.5	106	57.9 ± 3.6	79	<0.001
Distal latency (m/s)	4.3 ± 1.0	106	3.4 ± 0.4	79	<0.001
CMAP (mV)	5.1 ± 2.9	106	10.8 ± 3.3	79	<0.001
SCV (m/s)	52.3 ± 6.1	103	57.4 ± 4.4	85	<0.001
SNAP (μV)	7.0 ± 5.2	103	20.0 ± 7.9	85	<0.001
Not evoked	Three cases (2.8%)		None		
F-wave minimum latency (ms)	28.2 ± 3.0	76	22.3 ± 1.9	46	<0.001
FWCV maximum (m/s)	58.7 ± 10.5	74	66.4 ± 8.6	41	<0.001
F-wave occurrence (%)	24.5 ± 22.5	106	67.6 ± 20.3	47	<0.001
Absent	30 cases (28.3%)		None		
Ulnar nerve					
MCV (m/s)	55.9 ± 5.2	106	58.2 ± 4.7	71	0.003
Distal latency (ms)	3.2 ± 0.6	106	2.7 ± 0.3	71	<0.001
CMAP (mV)	5.1 ± 2.4	106	8.4 ± 2.4	71	<0.001
SCV (m/s)	48.1 ± 7.5	102	55.0 ± 3.8	74	<0.001
SNAP (μV)	5.6 ± 4.6	102	18.3 ± 7.4	74	<0.001
Not evoked	Four cases (3.8%)		None		
Tibial nerve					
MCV (m/s)	44.5 ± 3.8	106	47.2 ± 3.7	56	<0.001
Distal latency (ms)	5.0 ± 1.0	106	4.5 ± 0.8	56	0.003
CMAP (mV)	7.8 ± 3.7	106	10.3 ± 3.4	56	<0.001
F-wave minimum latency (ms)	48.3 ± 4.1	106	41.4 ± 3.0	31	<0.001
FWCV maximum (ms)	43.9 ± 5.6	105	47.4 ± 3.3	28	<0.001
F-wave occurrence (%)	94.3 ± 11.6	106	96.3 ± 12.5	31	NS
Absent	None		None		
Sural nerve					
SCV (m/s)	44.1 ± 5.7	94	50.8 ± 5.1	68	<0.001
SNAP (μV)	5.1 ± 3.5	94	10.8 ± 4.6	68	<0.001
Not evoked	12 cases (11.3%)		None		

MCV = motor nerve conduction velocity; CMAP = compound muscle action potential; SCV = sensory nerve conduction velocity; SNAP = sensory nerve action potential; FWCV = F-wave conduction velocity; NS = not significant.

we compared the phenotypes based on present symptoms and the electrophysiological phenotypes between patients with a CAG repeat size <47 and those with 47 or more CAGs, according to the previous report on clinical features of SBMA (Atsuta *et al.*, 2006) (Table 4). The age at onset and the age at examination were higher in patients with a shorter CAG repeat than in those with a longer repeat ($P < 0.001$). Disease duration and functional scale, including the Limb Norris score, Norris Bulbar score and ALSFRS-R,

Table 3 Frequency of patients with abnormal values in nerve conduction studies and F-wave examinations

	Number of patients with abnormal values[a]	n	Frequency (%)
Median nerve			
MCV	20	106	18.9
CMAP	43	106	40.6
SCV	23	106	21.7
SNAP	45	106	42.5
FWCV maximum	38	104	36.5
F-wave occurrence	91	106	85.8
Ulnar nerve			
MCV	5	106	4.7
CMAP	24	106	22.6
SCV	40	106	37.7
SNAP	49	106	46.2
Tibial nerve			
MCV	6	106	5.7
CMAP	8	106	7.5
FWCV maximum	17	105	16.2
F-wave occurrence	7	106	6.6
Sural nerve			
SCV	36	106	34.0
SNAP	26	106	24.5

MCV = motor nerve conduction velocity; CMAP = compound muscle action potential; SCV = sensory nerve conduction velocity; SNAP = sensory nerve action potential; FWCV = F-wave conduction velocity.
[a]We defined the abnormal values as those values that were either less than the mean − 2 SD of normal controls on the examined nerves or not evoked.

were similar between these groups. The CMAP values in the median, ulnar and tibial nerves were not significantly different, but showed a tendency to be decreased in the patients with a longer CAG repeat in all three nerves (Table 4). SNAPs in the median, ulnar and sural nerves were all significantly decreased in the patients with a shorter CAG repeat (Table 4). These observations suggest that a shorter CAG repeat is linked to a more significant SNAP decrease, while a longer CAG repeat is linked to a more profound CMAP decrease.

Furthermore, considering the possibility that action potentials are influenced by the age at examination, we compared the CMAPs and SNAPs in the patient subsets with a longer CAG repeat and those with a shorter CAG repeat between different age groups (Fig. 2). Patients <49 years old showed a significant difference in CMAPs and SNAPs ($P = 0.041–0.002$). The patients <49 years old and with a longer CAG repeat showed a more significant decrease in CMAPs, while those with a shorter CAG repeat showed a more significant decrease in SNAPs.

We selected patients with the sensory-dominant phenotype and those with the motor-dominant phenotype to further analyse the implication of CAG repeat size on the age at onset and electrophysiological phenotypes of SBMA.

A

		Number of nerves with abnormally reduced CMAPs			
		0	1	2	3
Number of nerves with abnormally reduced SNAPs	0	22	16	4	1
	1	11	6	3	2
	2	12	9	2	2
	3	10	2	3	1

Motor-dominant

Sensory-dominant

Non-dominant

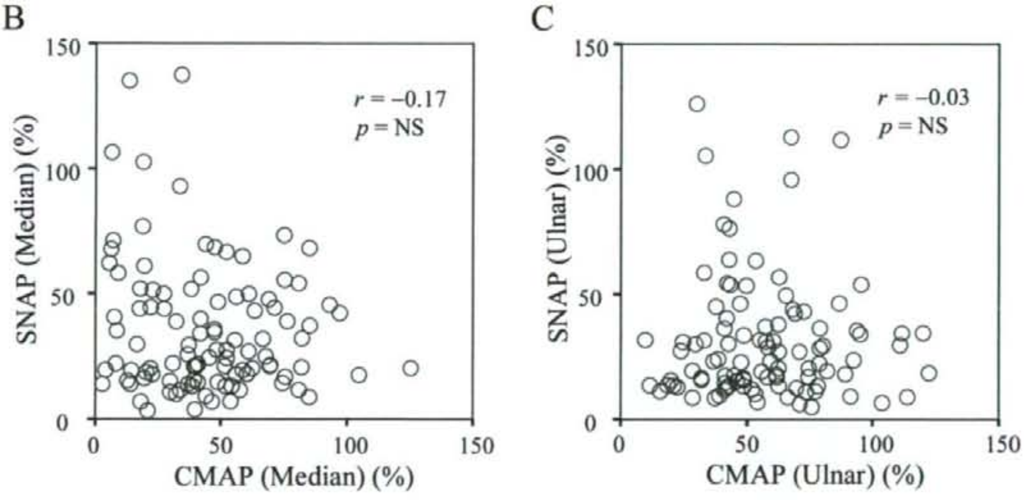

Fig. 1 Electrophysiological discrepancies in motor and sensory nerve involvement in SBMA patients. (**A**) The cross tabulation of the number of motor and sensory nerves showing an abnormally decreased action potential. The vertical stripe area corresponds to the motor-dominant phenotype and the gray area denotes the sensory-dominant phenotype. The white area represents the non-dominant phenotype. (**B** and **C**). Relation between CMAP and SNAP in the median and ulnar nerves on a standardized scale of percentage to normal control mean values. Some patients have only decreases of CMAP with preserved SNAP, while other patients show declines of SNAP with conserved CMAP.

As shown in Fig. 1A, the sensory-dominant phenotype was determined if patients show a reduced SNAP (less than control mean −2 SD) in at least one nerve without any decrease in CMAPs, whereas the motor-dominant phenotype denotes patients showing a reduced CMAP (less than control mean −2 SD) in at least one nerve without any decrease in SNAPs. We examined the relationship between CAG repeat number and the age at onset in these patients ($n = 54$) (Fig. 3A). We found that the mean CAG repeat number and the age at onset were significantly different between patients with motor- and sensory-dominant phenotypes ($P < 0.001$, Fig. 3A), indicating that a longer CAG repeat is more closely linked to the motor-dominant phenotype, and a shorter CAG repeat is more closely linked to sensory-dominant phenotype. Similar findings were observed when we classified patients based on abnormally reduced action potentials (less than control mean −2 SD) in the median nerve or the ulnar nerve (Fig. 3B and C).

Table 4 Clinical and electrophysiological features in terms of CAG repeat size in AR gene

	CAG repeat <47		CAG repeat ⩾47		P
	(Mean ± SD)	n	(Mean ± SD)	n	
Age at examination	58.9 ± 10.2	32	51.7 ± 8.9	65	0.001
Duration from onset	9.6 ± 7.4	32	10.7 ± 6.6	65	NS
Age at onset	49.3 ± 11.5	32	41.0 ± 8.9	65	0.002
Limb Norris score	54.2 ± 8.3	28	53.9 ± 7.0	63	NS
Norris Bulbar score	32.4 ± 5.1	28	33.4 ± 3.9	63	NS
ALSFRS-R	41.1 ± 4.1	28	41.2 ± 4.5	63	NS
CMAP (mV)					
Median	5.7 ± 2.4	32	4.8 ± 3.1	65	NS
Ulnar	5.6 ± 2.2	32	4.9 ± 2.4	65	NS
Tibial	8.7 ± 4.9	32	7.4 ± 3.1	65	NS
SNAP (μV)					
Median	4.8 ± 3.3	29	7.7 ± 5.6	65	0.011
Ulnar	4.1 ± 2.6	29	6.2 ± 5.0	64	0.037
Sural	3.8 ± 2.6	26	5.4 ± 3.4	59	0.022

AR = androgen receptor; ALSFRS-R = ALS functional rating scale-revised; CAMP = compound muscle action potential; SNAP = sensory nerve action potential; NS = not significant.

The CAG repeat size correlates directly with the frequency of nuclear accumulation in the motor neurons and inversely with that of cytoplasmic aggregation in the DRG

In order to investigate the relationship between CAG repeat size and the degree of motor and sensory nerve involvement, we performed immunohistochemistry using anti-polyglutamine antibody (1C2) on autopsied spinal cord and DRG specimens from SBMA patients, and quantified the primary sensory neurons in which mutant AR accumulated. In primary sensory neurons within the DRG, mutant AR was detected immunochistochemically as punctuate aggregates in the cytoplasm (Fig. 4A). On the other hand, diffuse nuclear accumulation of mutant AR was detected in motor neurons of the spinal anterior horn (Fig. 4B). The size of CAG repeat in the AR gene tended to be inversely correlated with the number of primary sensory neurons bearing cytoplasmic aggregates (Fig. 4C). This result is in contrast with the previously reported correlation between the frequency of mutant AR accumulation in spinal motor neuron and the CAG repeat size (Adachi *et al.*, 2005) (Fig. 4D).

Discussion

The present study demonstrated extensive abnormalities in both motor and sensory nerve conduction in SBMA patients, reflecting principal pathological lesions in the lower motor neurons and in the DRG. Previous studies on nerve conduction in SBMA patients showed a characteristic decrease in SNAP compared with normal controls, whereas SCV and MCV were variably reported as either normal or decreased, and CMAP decreased to variable extents (Harding *et al.*, 1982; Olney *et al.*, 1991; Li *et al.*, 1995; Guidetti *et al.*, 1996; Polo *et al.*, 1996; Ferrante *et al.*, 1997; Antonini *et al.*, 2000; Sperfeld *et al.*, 2002). In the present study, the reductions in both CMAP and SNAP were remarkable, in agreement with previous reports. This suggests that axonal degeneration is the principal peripheral nerve damage in SBMA patients. In addition, MCVs and SCVs were significantly decreased in the SBMA patients, and distal latencies were also significantly increased.

Several reports have examined the F-wave in SBMA patients. Those studies showed that the latency is almost normal or slightly extended (Olney *et al.*, 1991; Guidetti *et al.*, 1996). In the present study, the minimum F-wave latency was significantly longer and the maximum FWCV was significantly decreased in SBMA patients compared to that in normal controls. The occurrence of F-waves in SBMA patients was significantly less in the upper limb, but not in the lower limb compared with that of controls.

As for the spatial distribution of involvement, we demonstrated that nerves of the upper limbs are more severely disturbed than those of the lower limbs in SBMA patients. These observations suggest that nerve involvement does not reflect a length-dependent process of primary neuropathy, but a neuronopathy process, which is consistent with our results from histopathological studies (Sobue *et al.*, 1989; Li *et al.*, 1995).

The most striking observations in the present study are that motor and sensory nerves are differentially affected in SBMA patients, that electrophysiologically defined motor-dominant and sensory-dominant phenotypes are present, especially in young patients, and that the CAG repeat size in the AR gene is a factor determining these electrophysiologically defined motor and sensory phenotypes. Previous studies have reported that the number of CAGs determine not only the age at onset, but also the clinical phenotype in polyglutamine diseases (Ikeutchi *et al.*, 1995; Johansson *et al.*, 1998; Mahant *et al.*, 2003). For example, DRPLA patients with a longer CAG repeat (earlier age at onset) showed a progressive myoclonus epilepsy phenotype, whereas patients with a shorter CAG repeat (later age at onset) showed a non-progressive myoclonus epilepsy phenotype, but high frequencies of choreoathetosis and psychiatric symptoms (Ikeutchi *et al.*, 1995). Moreover, in spinocerebellar ataxia type-7 (SCA7) patients with ⩾59 CAGs, visual impairment was the most common initial symptom observed, while ataxia predominated in patients with <59 CAGs (Johansson *et al.*, 1998). Additionally, in HD patients, younger age at onset was associated with less chorea and more dystonia (Mahant *et al.*, 2003). In SBMA, only the relationship between CAG repeat and the age at onset or the severity of motor

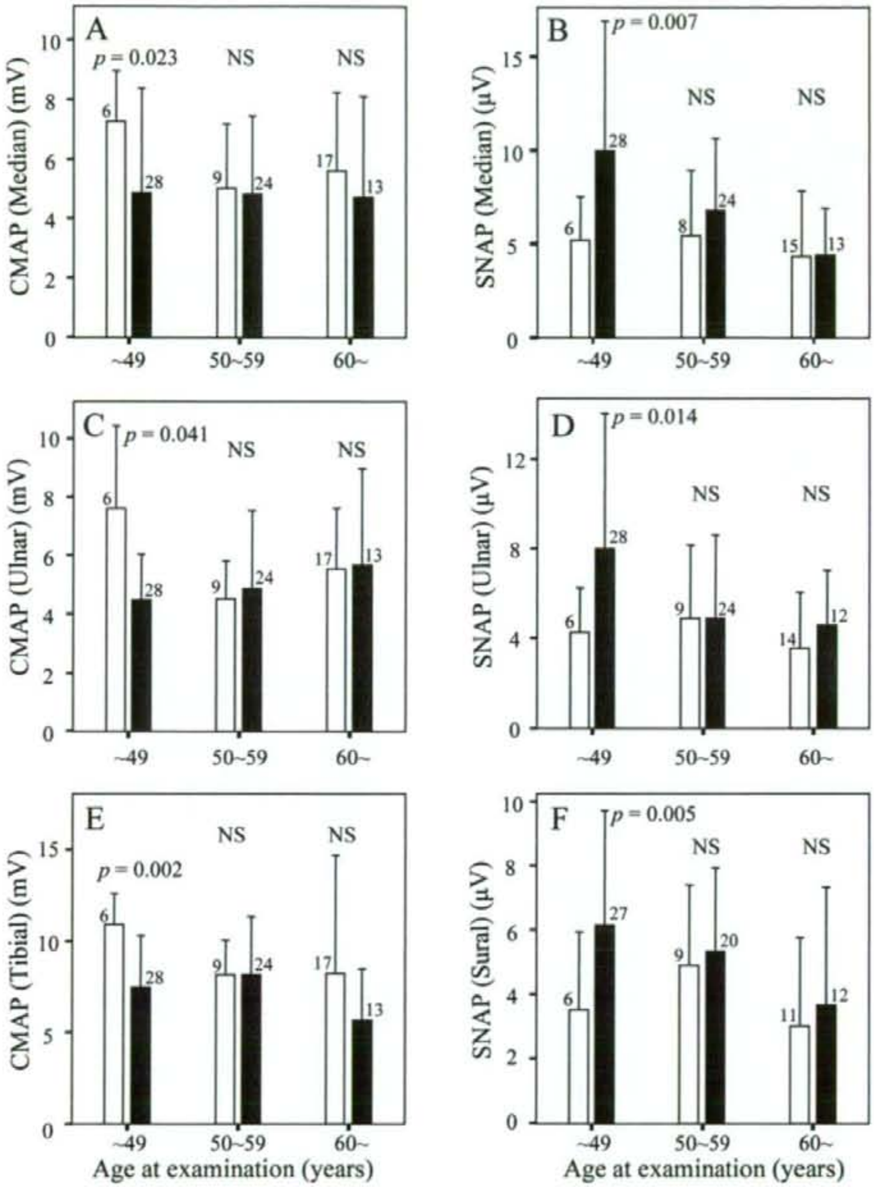

Fig. 2 (**A–F**) Age- and CAG-dependent changes in motor and sensory amplitudes in SBMA. CMAPs and SNAPs in the median (A and B), ulnar (C and D), tibial (E) and sural (F) nerves in different age groups are shown. The white columns are the mean values of the patients with a shorter CAG repeat (<47), while the black columns are the mean values of the patients with a longer CAG repeat (≥47). The error bars are SD. The number of patients examined is shown above each column. The young patients with a longer CAG repeat showed significantly low values of CMAPs compared to those with a shorter CAG repeat. Conversely, young patients with a shorter CAG repeat showed significantly lower values of SNAPs than those with a longer CAG repeat. Patients more than 49 years old did not show a significant difference between shorter and longer CAG repeat.

function has been reported (Doyu *et al.*, 1992; Atsuta *et al.*, 2006), but a CAG size-dependent clinical phenotype has not been described. This may be because the expansion of CAG repeat in the AR gene is shorter than that in the causative genes for DRPLA, SCA7 or HD. Alternatively, as compared to outstanding motor dysfunction, the clinical manifestations of sensory nerve impairment are less severe in SBMA patients, which may result in overlooking the motor and

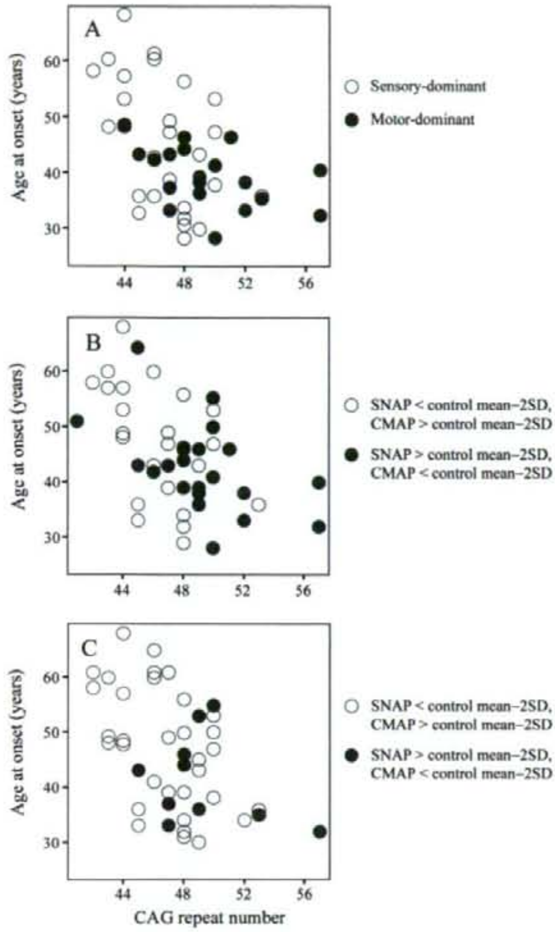

Fig. 3 CAG repeat size determines the age at onset in SBMA. (**A**) Relation between the CAG repeat size and the age at onset according to the phenotypes determined by CMAPs and SNAPs. A longer CAG repeat was closely linked to the motor-dominant phenotype, and a shorter CAG repeat was closely linked to the sensory-dominant phenotype. Motor- and sensory-phenotypes were determined as shown in Fig. 1A. (**B**) Relation between the CAG repeat size and the age at onset according to the phenotype determined by using CMAPs and SNAPs in the median nerve. (**C**) Relation between the CAG repeat size and the age at onset according to the phenotype determined by using CMAPs and SNAPs in the ulnar nerve.

sensory discrepancy. Our present findings in SBMA patients strongly suggest that the phenotypic diversity determined by CAG repeat size is a common feature shared by various polyglutamine diseases.

Although the pathological mechanism by which CAG repeat size influences clinical phenotype is unknown, a common molecular basis appears to underlie the heterogeneity of clinical presentations in polyglutamine diseases. The polyglutamine tract encoded by an expanded CAG repeat forms a β-sheet structure, leading to conformational changes and the eventual accumulation of causative proteins (Perutz *et al.*, 2002; Sakahira *et al.*, 2002). Since the propensity of aggregation is dependent on CAG repeat size, the different length of polyglutamine tract may result in a CAG repeat size-dependent pathology.

The observations that a longer CAG repeat results in the motor-dominant phenotype, while a shorter CAG leads to the sensory-dominant presentation, are further reinforced by results of previous studies on the cell-specific histopathological changes in SBMA. A diffuse loss and atrophy of anterior horn cells accompanied by a mild gliosis is characteristic of SBMA (Kennedy *et al.*, 1968; Sobue *et al.*, 1989), suggesting that the pathology of spinal motor neurons is neuronopathy. On the other hand, no substantial neuronal loss in the DRG despite severe axonal loss in the central and peripheral rami suggests that the pathology of sensory neurons is distally accentuated axonopathy, although the primary pathological process may be present in the perikarya of sensory neurons (Sobue *et al.*, 1989; Li *et al.*, 1995). Moreover, the accumulation of mutant AR, a pivotal feature of SBMA pathology, is also different in motor and sensory neurons (Adachi *et al.*, 2005). Mutant AR accumulates diffusely in the nucleus of spinal motor neurons, but cytoplasmic aggregation is predominant in sensory neurons within the DRG (Adachi *et al.*, 2005). The extent of diffuse nuclear accumulation of mutant AR in motor neurons is closely related to CAG repeat size, providing a molecular basis for the present observations that patients with a longer CAG repeat show a greater decrease in CMAPs. On the other hand, the results of anti-polyglutamine immunohistochemistry in this study indicate that cytoplasmic aggregation of mutant AR is more frequent in the patients with a shorter CAG repeat. Taken together, the differential accumulation pattern of mutant AR between motor and sensory neurons, and their differential correlation to CAG repeat size may be the pathophysiological background for the development of motor- and sensory-dominant phenotypes.

In conclusion, the results of the present study are unequivocal electrophysiological phenotypes, motor-dominant, sensory-dominant and non-dominant, especially in young patients of SBMA. These features are dependent on the CAG repeat size within the AR gene, with a longer CAG repeat size is more closely related to the motor-dominant phenotype and a shorter CAG repeat size related to the sensory-dominant phenotype. Our observations shed light on new roles of CAG repeat size in the clinical presentation of SBMA.

Supplementary materials

Supplementary materials are available at *Brain* online.

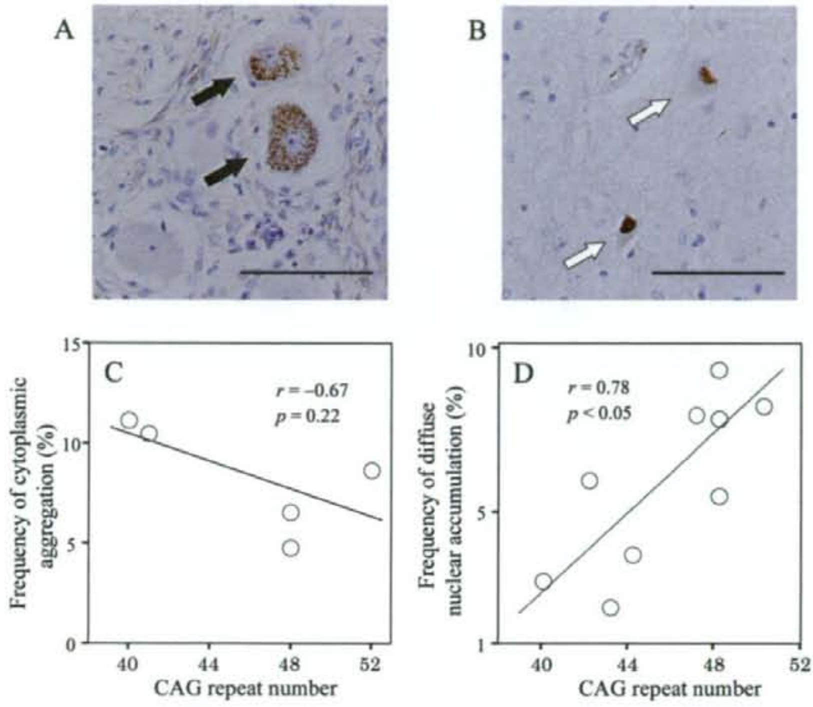

Fig. 4 Immunohistochemical analyses of mutant androgen receptor (AR) accumulation in the dorsal root ganglion (DRG) and that in the spinal anterior horn of SBMA patients. (**A**) Aggregates of mutant AR in the cytoplasm of DRG neurons (black arrows). Scale bar = 100 µm. (**B**) Mutant AR accumulates in the motor neuron nuclei (white arrows). Scale bar = 100 µm. (**C**) Relation between the CAG repeat size and cytoplasmic aggregations in the primary sensory neuron. Cytoplasmic aggregation tended to be more frequent in the patients with a shorter CAG repeat. (**D**) Relation between the CAG repeat size and diffuse nuclear accumulation of mutant AR in the spinal motor neuron. Panel D is reconstructed from the previous report (Adachi et al., 2005).

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