

Theoretically, biomarkers for neurodegenerative diseases should detect disease-specific neuropathology, non-specific neurodegenerative processes or biological events downstream of neuronal dysfunction. Ideal biomarkers are replicable, non-invasive, reliable, precise and inexpensive variables which are easy to measure. Currently studied biomarkers for polyglutamine diseases include: parameters of neuroimaging; biofluid analysis; physical assessment; gene expression profiles; proteomics; metabolomic analysis and histopathology, some of which have been investigated in preliminary clinical trials (Fig. (3)) [66].

Neuroimaging Markers

Because regional brain atrophy is a fundamental neuropathological finding in HD and SCAs, neuroimaging parameters have been the most popular biomarkers for polyglutamine diseases. In addition to assessment of brain structures, imaging techniques also provide important information on function, metabolites or perfusion. Recent advances in molecular imaging would enable the detection of causative protein accumulation and disease-specific molecular pathogenesis [67,68].

1. Structural Neuroimaging

Structural imaging studies using CT or MRI have delineated the correlation between brain morphology and cognitive functions in HD patients. Several neuro-radiological studies have shown that the earliest changes in HD appear in the striatum (caudate, putamen and globus pallidus), the degree of which has been intensively studied in quantitative studies using MRI. The caudate volumes have been reported to correlate with the Mini Mental State Examination (MMSE) and with performances on neurophysiological tests [69,70]. The volumes of the caudate and putamen are significantly decreased in preclinical HD carriers who are far from their estimated age of disease onset as calculated from CAG size, and the rate of caudate atrophy becomes significant approximately 11 years from the estimated age of onset [71]. In addition, more generalized brain atrophy has also been detected with MRI, suggesting that the rate of whole brain atrophy is also a potential biomarker [72,73]. Recently, computational neuroanatomic techniques have been applied to neuroimaging of HD. Voxel-based morphometry (VBM), a fully automated method that detects regional structural changes on a voxel-wise basis, delineates reduction in gray matter volume in the striatum of presymptomatic HD carriers [74]. Tensor-based morphometry, another automated technique, also demonstrates progressive regional gray matter atrophy within the striatum of pre-clinical at-risk HD carriers [75].

Regional atrophy has also been reported in polyglutamine-mediated SCAs. MRI volumetry using manual segmentation has also detected a significant atrophy of the cerebellum and brainstem in SCA1, SCA2, SCA3 and SCA7 patients [76-80]. The brainstem volume in MRI correlates with the Inherited Ataxia Clinical Rating Scales (ICARS) in SCA1 but not in SCA2 [80]. VBM

has also been applied to patients with SCAs, but it has yet to be defined whether parameters in this method can be used as biomarkers [81,82].

2. Functional Imaging of Resting State

Functional neuroimaging is intended to measure regional cerebral blood flow (rCBF), metabolites or neurotransmission activity as indicators of brain functions. There are two major classes of functional imaging methods: one examines the resting-state activity and the other measures the changes in brain activity during the performance of a task. In HD, neuroimaging studies of resting cerebral metabolism using positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have demonstrated metabolic abnormalities in the striatal and extra-striatal regions. Reduced rCBF in frontotemporal regions and in the caudate have been reported to correlate with cognitive functions [83,84]. Glucose hypometabolism has also been revealed in PET, the degree of which correlates with cognitive deficits in HD patients [85,86]. Reduction in glucose metabolism becomes more severe as the disease progresses [87]. Neural transplantation studies have demonstrated that increased metabolic activity in the striatum correlated with an improvement of cognitive functions in HD patients [88]. Receptor binding is another measurement that can be used to assess regional resting-state activity. Numerous studies have delineated a reduction in dopamine D2 receptor binding in the striatum, which correlates with cognitive dysfunction in HD individuals [89,90]. Extrastriatal reductions in the binding of this receptor have also been observed in HD [91]. PET studies in presymptomatic mutation carriers of HD have also demonstrated a reduction in striatal dopamine receptor binding, which is a more sensitive biomarker than glucose hypometabolism or MRI volumetry [92,93]. Another approach to visualize microglial activation in PET has also been tested in the brains of HD mice and patients [94].

Reduced rCBF has been reported in the cerebellum of SCA6 patients, and the degree of reduction correlates with the disease duration [95]. Regional glucose hypometabolism in PET has been reported in patients with SCA1, SCA2, SCA3, SCA6 and SCA17 [96,97]. PET studies on presymptomatic gene carriers of SCA2 and SCA3 have also demonstrated cerebellar glucose hypometabolism without clinical signs of spinocerebellar ataxia [98,99]. Topographic brain mapping indicates a correlation between hypometabolism in the frontal cortices and the ICARS scores in a group of patients with SCA2, SCA6 or sporadic ataxia [100]. Because extrapyramidal features are often seen in some forms of polyglutamine-mediated SCAs, striatal functional imaging has also been investigated. Reduction in dopamine receptor binding in the striatum has been described in patients with SCA2, SCA3 and SCA17 [101-103]. Dopamine transporter (DAT) density, an indicator of presynaptic dopaminergic neurotransmission, is also decreased in patients with SCA2, SCA3 and SCA17 [97,104-106]. In SCA3, a reduction in DAT density is observed in the patients with no detectable parkinso-

nism and also in asymptomatic carriers [107,108]. DAT loss correlates with the ICARS score in SCA2 patients, suggesting that functional neuroimaging may provide biomarkers of polyglutamine-mediated SCAs [109].

3. Functional Imaging of Activated State

Neuroimaging studies such as functional MRI (fMRI) have been used to assess cerebral activity during cognitive tasks. fMRI measures the blood oxygen level dependent (BOLD) hemodynamic responses in the brain, which indicates neural activities. BOLD responses in the striatum have been shown to be reduced in presymptomatic HD carriers having no volumetric or behavioral abnormalities [110]. In contrast, the responses in the cortices are increased in preclinical carriers far from their estimated disease onset age, probably reflecting compensation. Neuroimaging using activation has also been applied to SCAs [111].

4. MR Spectroscopy (MRS)

MRS is a non-invasive imaging technique used to measure the relative amounts of metabolites in selected brain regions. The variables assessed in MRS include: N-acetylaspartate (NAA, marker of neuronal number); lactate (marker of hypoxia); choline (marker of membrane turnover); creatine (marker of energy metabolism) and lipid (marker of necrosis). MRS studies on presymptomatic and manifest HD individuals have shown a decrease in NAA, creatinine and choline, as well as an increase in lactate, and have demonstrated the correlation between the amounts of metabolites and disease severity [112-114]. Other studies, however, have suggested the insensitivity of MRS parameters in the evaluation of the HD brain [115,116]. MRS studies on SCAs have also shown that NAA/creatine and choline/creatine levels are decreased in SCA1 and SCA2, and that the concentration of NAA in the pons correlates with the ICARS scores in SCA1 patients [80,117,118]. Although MRS may provide potential biomarkers for polyglutamine diseases, more investigations are needed for validation of the parameters.

5. Diffusion-Weighted Images

Diffusion-weighted MRI is an imaging modality to depict the local characteristics of water diffusion, which can be quantified by calculating the apparent diffusion coefficient (ADC), an indicator of water diffusion. An increased ADC in the affected regions has been reported in HD, SCA1, SCA2 and DRPLA [119-121]. A study using diffusion tensor imaging also showed that fractional anisotropy, a measure of the directional diffusion of water, was increased in the corpus callosum in early and preclinical HD individuals [122].

Clinical Measurements

Measurements from clinical assessment are also potential biomarkers, although the strategies using these parameters should overcome bias due to phenotypic variation in the patients and due to inter- and intra-rater variability. Quantitative parameters in cognitive functions have been shown to decline with disease pro-

gression, and thus have been used for the follow-up of symptomatic and presymptomatic individuals of HD [123]. Progressive impairments have been demonstrated in attention, inhibition, executive function and memory of HD individuals [124-126]. The extent of the reduction in visual processing speed and storage capacity has also been demonstrated to correlate with the disease duration in HD [127].

Eye movement is another target for identification of polyglutamine-related biomarkers. Quantitative measurements of eye movement have also shown that saccade variables, such as velocity and latency, are also potential biomarkers of disease progression in presymptomatic and early clinical stages of HD [128-130]. This approach is also likely to be feasible for clinical evaluation of SCA2 patients, in whom the saccade velocity correlates with the disease duration [131]. Given that neuropathy is often seen in patients with SCAs and those with SBMA, electrophysiological parameters are alternative potential biomarkers for these diseases [132,133].

Blood and Urine Markers

Because blood is one of the most accessible human samples, serum and plasma are desirable targets for biomarker identification. Given the implication of mitochondrial damage in the pathogenesis of HD and other neurodegenerative diseases, markers of oxidative stress are plausible parameters to monitor disease progression. The serum level of 8-hydroxydeoxyguanosine (8-OHdG), an indicator of oxidative damage to DNA, has been demonstrated to be increased in HD patients and to be reduced by creatine supplementation [134]. The level of leukocyte 8-OHdG has also been reported to be elevated in HD [135]. A decrease in the levels of mitochondrial DNA from leukocytes, another marker of oxidative stress, has been shown in the blood from patients with polyglutamine diseases [136]. There are also studies demonstrating a reduced function of the A2A adenosine receptor in peripheral blood cells of individuals with HD and those with polyglutamine-mediated SCAs, although no correlation was found between the measured variable, Bmax, and the disease severity in HD [137-139]. Another study suggested an increase in the level of neural specific enolase (NSE) in the serum of symptomatic patients of SCA3 [140]. Urine is another easily accessible sample to monitor metabolic and endocrinological abnormalities in polyglutamine diseases, although human data is lacking [141].

Cerebrospinal Fluid (CSF) Markers

Cocaine- and amphetamine-regulated transcript (CART) has been shown to be elevated in CSF samples from HD patients, probably reflecting the pathogenic lesions, especially those in the hypothalamus [142]. Although the loss of orexin neurons is another indicator of hypothalamic pathology, a reduction in the CSF level of orexin has not been demonstrated in HD patients

[143,144]. F₂-isoprostane, a marker of oxidative stress, is also increased in CSF from HD patients, although the level is not correlated with disease duration [145]. CSF level of homovanillic acid has been reported to be decreased in HD, SCA1, and SCA3, reflecting an altered dopamine metabolism [146,147]. Lactate/pyruvate ratio in CSF has been shown to be increased in HD and SCA3 [148,149]. In accord with the results of gene expression analysis, inflammatory activation has also been revealed by proteomic analysis of CSF and serum from presymptomatic and manifested HD individuals [150].

Histopathological Markers

Among polyglutamine diseases, SBMA is unique in that the accumulation of causative proteins, ARs, is dependent on serum levels of testosterone. Since human AR is widely expressed in various organs, the nuclear accumulation of the pathogenic AR protein is detected not only in the central nervous system, but also in non-neuronal tissues such as the scrotal skin of SBMA patients. The degree of AR accumulation in the scrotal skin epithelial cells tends to be correlated with that in the spinal motor neurons in autopsy specimens, and it is well correlated with CAG repeat length and inversely correlated with the motor functional scale [151]. These findings indicate that the degree of AR accumulation in the scrotal skin is a biomarker with which to monitor the pathogenic processes of SBMA.

Gene Expression Profiles

Altered gene expression is a unified pathogenic feature of polyglutamine diseases, which has been reported in both animal models and patients. The polyglutamine-induced alterations in gene expression may result from various pathogenic factors such as transcriptional dysregulation and microinflammation [152,153]. Intriguingly, some of these changes are shared by the animal models of HD and DRPLA, suggesting that gene expression profiles would provide common biomarkers for various polyglutamine diseases [154]. It has been shown that gene expression changes in skeletal muscle samples from HD mice are also present in muscle biopsied from patients, indicating the possibility that non-neuronal tissues could be the target of biomarker identification [155]. This view has been exemplified by a dose-finding study of an HDAC inhibitor, sodium butyrate, in HD patients [156]. In this large-scale analysis of mRNA from blood, a significantly altered gene expression was detected in symptomatic and preclinical HD individuals, and similar changes were also detected in the postmortem HD brain. The study also demonstrated that these changes in gene expression correlated with disease progression and response to HDAC inhibition. These intriguing results suggest that expression levels of certain genes in peripheral blood could be biomarkers for polyglutamine diseases, although the sensitivity of this approach is not likely high enough for small-scale clinical trials [157].

Metabonomics/Metabolomics

Metabonomics/metabolomics is an approach to quantify the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification, by measuring small molecular metabolites such as glucose and cholesterol. Given thermoregulatory and metabolic defects in HD mouse models, it appears legitimate to search for metabolic biomarkers for polyglutamine diseases [158,159]. Metabolite profiling by gas chromatography-time-of-flight-mass spectroscopy has demonstrated robust changes in various components of fatty acid metabolism and also those of several aliphatic amino acids in serum from HD mice and patients [160]. A decreased level of branched chain amino acids was also detected in another study on MRS analysis of the plasma from HD patients [161]. In addition, metabolite alterations have been seen in the MRS-based analysis on brain extract from SCA mice [162]. Taken together, metabolite profiling would provide important information on biomarker identification for polyglutamine diseases, although the changes in this modality may not reflect known hypotheses on the pathogenesis of neurodegeneration.

FUTURE PERSPECTIVE

Since the identification of a CAG repeat expansion within the AR gene as the cause of SBMA in 1991 [163], tremendous efforts have been made to understand and cure polyglutamine-mediated neurodegenerative disorders. It has proven to be more difficult than expected to apply disease-modifying therapies to patients, because polyglutamine diseases are relatively rare and progress slowly, and because presently available clinical parameters are not sensitive enough to detect the beneficial effects of disease-modifying therapies in interventional trials. Therefore, biomarkers reflecting the pathogenesis and severity of each disorder are needed to be identified and used as surrogate endpoints in clinical trials. In addition, biomarkers which sensitively detect presymptomatic biological changes in mutation carriers may also be applied to preventive trials, given that that early symptoms of polyglutamine diseases appear to reflect neuronal dysfunction rather than neuron loss.

Among the biomarkers so far studied, neuroimaging parameters appear to be the most reliable for symptomatic and preclinical individuals of HD. Alternative parameters, such as clinical measurements, blood markers and gene expression, are also potential biomarkers, which could be applied to one or more polyglutamine diseases, given that these markers have been identified on the basis of unified pathogenic mechanisms. It is foreseeable that a multimodal panel of biomarkers might be advantageous to define endpoints in clinical trials for polyglutamine diseases, because a combination of several markers would provide complementary information on the different aspects of the pathogenesis. Therefore, integrated approaches are fundamental for the identification of polyglutamine-related biomarkers. An increasing number of studies have

been providing new candidate biomarkers which depict polyglutamine-related molecular changes, such as an impaired ubiquitin-proteasome system [164]. The massive expansion of molecular imaging studies is also expected to aid in identifying novel molecular markers of polyglutamine diseases.

Reliance on biomarkers, however, can be misleading, because surrogate endpoints may not accurately predict the actual effects of therapeutic interventions. The limitation of biomarkers has been illuminated in various clinical trials [165]. In certain circumstances, a therapeutic intervention may improve clinical outcome measurements without any changes of biomarkers. Conversely, some therapeutics might affect surrogate endpoints with no amelioration of true clinical endpoints. These false-positive and false-negative possibilities underscore the cautious exploitation of biomarkers in therapy development. It is thus of critical importance to validate candidate biomarkers for polyglutamine diseases in both cross-sectional and longitudinal studies. Integrated approaches, such as long-term surveillance and phase 4 clinical trials, would reinforce the plausibility of biomarkers.

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Cognitive impairment in spinocerebellar ataxia type 6

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ABSTRACT

Objective: The aim of this study was to evaluate cognitive impairment in patients with spinocerebellar ataxia type 6 (SCA6) and to verify the role of cerebellar involvement in intellectual abilities.

Methods: Cognitive function was examined in 18 patients with genetically confirmed SCA6 and in 21 age and education matched controls using a test battery for attention, verbal and visuospatial memory, as well as executive function.

Results: Verbal fluency and immediate visual memory task were markedly impaired in SCA6 compared with the control group ($p = 0.007$, 0.004 and 0.014 , respectively). The results of the Rule Shift Cards Test was reduced in patients with SCA6, but the reduction was not significant. These cognitive dysfunctions did not correlated with CAG repeat length, age at onset, ataxic motor dysfunctional scale or depression.

Conclusions: Our results demonstrate that specific cognitive deficits occur in patients with SCA6, independent of ataxic motor dysfunction. These deficits may reflect disruption of cortico-cerebellar circuits.

Recently, reports have described increasing evidence of a non-motor role of the cerebellum.^{1,2} In addition, clinical reports of patients with cerebellar lesions have suggested a role for the cerebellum in cognitive functions.^{3,4} Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar atrophy caused by a CAG trinucleotide repeated expansion in the $\alpha 1A$ voltage dependent calcium channel subunit gene (CACNA1A gene) on chromosome 19p13. The CACNA1A gene is important for the function and survival of Purkinje cells.⁵ Neuropathologically, SCA6 is characterised by an almost exclusive cerebellar affect, particularly in the selective loss of the cerebellar Purkinje cells.⁶ Therefore, patients with SCA6 are an excellent model to reveal the contribution of the cerebellum to cognition. However, only a few studies have reported cognitive impairment in patients with SCA6⁷ and thus cognitive dysfunction in SCA6 patients has not been well elucidated. Furthermore, the mechanism of cerebellar involvement in cognitive dysfunction in patients with SCA6 has not been described.

In the present study, we sought (i) to determine the profile of cognitive impairment in patients with SCA6 and (ii) to clarify the relationship between the cerebellum and cortical association areas as a theoretical base of cognitive dysfunction in the cerebellar syndrome.

METHODS

Eighteen patients with genetically confirmed SCA6 and 21 control subjects were examined in this

study. Volunteers were recruited as control subjects. They were selected from a larger control subject pool to ensure that their ages and education were comparable with those of the patients with SCA6. Control subjects had no history of neuralgic or psychiatric disease that affected cognition. All of the patients and control subjects were native Japanese speakers. Written informed consent was received in advance from patients with SCA6 and control subjects. The study was approved by the Nagoya University Hospital Ethics Committee. The severity of ataxia in each subject was rated on the International Cooperative Ataxia Rating Scale (ICARS).⁸

Genomic DNA was extracted from peripheral blood of patients with SCA6 using a conventional technique. PCR amplification of the CAG repeat in the CACNA1A gene was performed using a fluorescein labelled forward primer (5'-AGC CCC CTC AAC ATC TGG TA-3') and a non-labelled reverse primer (5'-GAC CCG CCT CTC CAT CCT-3'). PCR conditions after a 3 min initial denaturation at 94°C were 35 cycles of 94°C for 1 min, annealing at 64°C for 1 min and elongation at 72°C for 1 min. 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) by comparison with co-electrophoresed PCR standards with known repeat sizes. The CAG repeat size of the PCR standard was determined by direct sequence using a sequence primer (5'-ACA TCT GGT ACC AGC ACT CC-3').

Each patient underwent a standard cognitive status assessment. All of the neuropsychological tests were performed on the same day. The neuropsychological tests were chosen for their minimal or absent reliance on motor performance in order to minimise any influence of dysarthria and appendice ataxia on test performance. The Mini-Mental State Examination was used as a screen.⁹ The Raven's Progressive Matrices tests, which require non-verbal ability, are the most popular.¹⁰ One of these, the Raven's Coloured Progressive Matrices,¹¹ was used. To evaluate working memory and attention, subjects underwent the digit span subtest of the Wechsler Adult Intelligence Scale-Revised, in which raw scores were used for statistical analysis.¹² Repeating numbers forward seems to be related mostly to attention while the backward task is likely to involve working memory and mental tracking.¹³ Visuospatial memory was examined using the visual paired associate subtest 1 and 2 of the Wechsler Memory Scale-Revised.¹⁴ Verbal memory

Table 1 Clinical characteristics of patients with SCA6 and controls*

Characteristic	Controls (n = 21)	SCA6 (n = 18)	p Value
Sex (M/F ratio)	7/14	9/9	
Age at onset (y)	–	52.1 (11.7) (30–75)	
Disease duration (y)	–	12.6 (8.2) (3–30)	
ICARS	–	36.4 (12.0) (14–58)	
CAG repeat length	–	23.1 (1.8) (21–27)	
Age at examination (y)	63.0 (9.4) (48–79)	64.1 (11.9) (37–81)	NS
Education (y)	12.3 (3.0) (8–16)	11.1 (2.3) (9–16)	NS
Mini-Mental State Examination	29.1 (1.0) (27–30)	27.9 (1.8) (24–30)	<0.05
Depression	4.6 (2.7) (0–9)	5.9 (3.5) (1–12)	NS
Anxiety	5.2 (3.6) (0–12)	2.9 (3.8) (0–14)	<0.05

*Data are given as mean (SD) (range) unless otherwise indicated.

ICARS, International Cooperative Ataxia Rating Scale; SCA6, spinocerebellar ataxia type 6.

was examined using word recall from the Alzheimer's Disease Assessment Scale-cognitive. We also assessed delayed recall of the Alzheimer's Disease Assessment Scale-cognitive 10 item word list after a delay of 5 min as part of memory testing.¹⁵ To evaluate visuospatial ability, the line orientation subtests of the Repeatable Battery for the Assessment of Neuropsychological Status¹⁶ was used. To evaluate verbal fluency, subjects were asked to name as many items as possible from a semantic category (animals) and from a phonemic category (Japanese nouns starting with the Japanese Kana character Ka) within 1 min. To evaluate executive function, the Stroop Interference Test¹⁷ and Rule Shift Cards Test were used.¹⁸ The Stroop Interference Test consists of a colour naming condition where subjects name the colour of coloured patches, and an interference condition where subjects are shown an array of colour names printed in different coloured inks. The interference measure was obtained by subtracting the time needed for the colour naming condition from the time needed for the interference condition.

The capacity for inhibition is widely regarded as a key component of executive functioning and has been strongly linked to frontal structures.¹⁹ The Rule Shift Cards Test was from the Behavioural Assessment of the Dysexecutive syndrome. In trial 1 of this test, the subject is asked to say "yes" to a red card and "no" to a black card, while 21 spiral bound non-court playing cards are turned over one by one. In trial 2, the subject is asked to say "yes" if the card is the same colour as the

previous one and to say "no" if the card is not the same colour. Finally, all subjects completed a self-reporting instrument concerning anxiety and depression (Hospital Anxiety and Depression Scale).²⁰

Statistical comparisons were made using the Mann-Whitney test. Differences were considered significant at $p < 0.05$. In order to achieve a global significance level of 5%, a conservative correction factor (Bonferroni) was used. For correlation studies, we used the Spearman correlation coefficient.

RESULTS

The clinical characteristics of patients with SCA6 and controls are shown in table 1. In all of the patients, imaging studies showed pure cortical cerebellar atrophy without involvement of the brainstem or other extracerebellar structures. None of the patients with SCA6 showed frontal symptoms. None of the patients with SCA6 in this study showed hemiplegic migraine or episodic ataxia. Four patients were able to ambulate without assistance, 11 used either a cane or an item of furniture to assist in walking and three patients were wheelchair bound. All subjects were right-handed.

Neuropsychological testing

Neuropsychological findings for the patients with SCA6 and controls are shown in table 2. The Raven's Coloured Progressive Matrices did not reveal any significant difference between patients with SCA6 and control subjects. The verbal memory tasks did not show significant group differences in immediate and delayed recall. The most prominent cognitive dysfunction was observed in visuospatial memory function and in naming nouns from phonemic and semantic categories (table 2). The visual paired associate task 1 was significantly reduced in patients with SCA6 compared with controls, even under Bonferroni's correction ($p = 0.01$) (table 2). The verbal fluency tasks in semantic and phonemic categories were remarkably impaired in patients with SCA6 for corrected significance ($p = 0.007, 0.004$) (table 2) compared with that of control subjects. Analysis of attention and working memory measures (digit span) did not reveal significant differences between patients with SCA6 and controls. Rule Shift Cards Test tasks were reduced in patients with SCA6 compared with controls, although the corrected p values did not reach significant levels.

Correlative studies

We analysed the relationships between the cognitive dysfunction results and their clinical characteristics, including age at onset, disease duration, degree of ataxia (ICARS), depression and CAG repeat length using the Spearman rank correlation

Table 2 Neuropsychological features in patients with SCA6 and controls*

Test	Control (n = 21)	SCA6 (n = 18)	p Value†
Raven's Coloured Progressive Matrices (/36)	30.3 (3.6)	27.8 (5.0)	NS
Immediate word recall	2.1 (1.2)	3.1 (1.4)	0.03 (0.33)
Delayed word recall	1.4 (0.9)	1.9 (1.7)	NS
Visual Paired Associate 1‡	11.4 (4.0)	6.7 (4.0)	0.0013 (0.014)
Visual Paired Associate 2‡	4.6 (1.7)	4.0 (2.1)	NS
Digit span‡	12.5 (2.8)	13.2 (4.0)	NS
Line orientation‡	16.8 (4.1)	16.0 (1.9)	NS
Verbal fluency (semantic)	15.9 (3.4)	12.3 (2.3)	0.0006 (0.007)
Verbal fluency (phonemic)	9.8 (2.7)	6.3 (2.8)	0.0004 (0.004)
Rule Shift Cards Test	3.8 (0.7)	2.8 (1.4)	0.0088 (0.097)
Stroop Interference Test (s)	12.0 (5.9)	18.4 (12.5)	NS

*Data are given as mean (SD) unless otherwise indicated.

†Mann-Whitney test. Statistical significance was set at $p < 0.05$. Corrected values by Bonferroni's correction are indicated in parentheses.

‡Raw scores.

SCA6, spinocerebellar ataxia type 6.

Research paper

Table 3 Correlation study*

Test	CAG repeat length	Age at onset	Disease duration	ICARS	Depression
Raven's Coloured Progressive Matrices	NS	NS	NS	NS	NS
Immediate word recall	NS	NS	NS	0.03 (0.52)	NS
Delayed word recall	NS	NS	NS	NS	NS
Visual Paired Associate 1	NS	NS	NS	NS	NS
Visual Paired Associate 2	NS	NS	NS	NS	NS
Digit SPAN	NS	NS	NS	NS	NS
Line orientation	NS	NS	NS	NS	NS
Verbal fluency (semantic)	NS	NS	0.04 (-0.50)	NS	NS
Verbal fluency (phonemic)	NS	NS	NS	NS	NS
Rule Shift Cards Test	NS	NS	NS	NS	NS
Stroop Interference Test	NS	NS	0.04 (0.50)	NS	NS

*Data are given as p values. Spearman rank correlation coefficients are shown in parentheses. ICARS, International Cooperative Ataxia Rating Scale.

coefficient (table 3). In particular, as speech disorder may influence the neuropsychological test performance, we analysed the correlation with the speech disturbance score in ICARS. Scores in the tasks showed marked impairment in patients with SCA6 in visuospatial memory and verbal fluency, but showed no significant correlation with CAG repeat length, age at onset, ICARS (speech disorder) or depression scores. Among the cognitive function tests, only the verbal fluency (semantic) task was significantly related to disease duration.

DISCUSSION

Neuropsychological studies of patients with SCA1,²¹ SCA2,²² and SCA3²³⁻²⁴ have shown that significant cognitive deficits, especially those affecting frontal lobe related executive functions, are present in these diseases. Extracerebellar involvement, including the frontal lobe, thalamus, brainstem or basal ganglia, is a characteristic feature in these types of SCA,²⁵⁻²⁶ and thus identifying the role of cerebellar involvement alone in developing cognitive deficits in these SCAs is difficult. However, in patients with SCA6, the disorder is characterised by degeneration that is greatly restricted to the cerebellum, with prominent loss of cerebellar Purkinje and granular cells, while cortical structures may be spared.²⁷ Therefore, SCA6 represents an excellent model for investigating the contribution of the cerebellum to cognition.

In the present study, we showed significant impairment in visual memory deficits and verbal fluency deficits in patients with SCA6, and mild deficits in the Rule Shift Cards Test. Previous studies demonstrated that frontal lobe function is associated with visual memory tasks.²⁸⁻²⁹ In addition, impairment in verbal fluency is considered to reflect frontal lobe damage,³⁰ suggesting that the cognitive deficits in SCA6 may be due to frontal lobe dysfunction. The Rule Shift Cards Test is a subtest of the Behavioural Assessment of the Dysexecutive Syndrome that is used for measuring executive dysfunction, including set-shifting and mental inhibition. Impairment of the Rule Shift Cards Tasks in SCA6 suggests frontal lobe dysfunction. Taken together, the cognitive dysfunction seen in patients with SCA6 suggest widespread frontal lobe involvement, although we need to address further whether patients with SCA6 had frontal lobe dysfunction using, for example, functional imaging. Nevertheless, preservation of the cerebral cortex has been demonstrated histopathologically in SCA6.⁶ Furthermore, an immunohistochemical study using antibodies specific for expanded polyglutamine failed to recognise aggregation in cortical neurons, including the frontal lobe.³¹

In this context, why do patients with SCA6 show cognitive dysfunction similar to those of frontal lobe origin when the cortical structures are well preserved. Schmahmann and Sherman³ have demonstrated that cerebellar lesions alone can affect cognitive function. Patients in their study with cerebellar damage only, such as cerebellar stroke or cerebellar tumour, showed a cognitive disturbance without neuropathological damage to the cerebral cortex. These results seem to suggest that the cognitive function of the cerebellum itself is independent of the cognitive function of the cerebral cortex. However, a number of studies have demonstrated connections between the cerebellum and cerebral cortex that are significant in cognition. Indeed, several authors postulated feedforward and feedback links between the cerebellum and cortical association areas as the theoretical base for cerebellar involvement in higher cognitive function.³² Moreover, functional imaging studies have shown activation of cerebellar and cortical areas during a memory task³³ and a verbal fluency task.³⁴ The cerebellar activation during performance of executive tasks was also shown to have a close functional relationship between the cerebellum and frontal cortex in an imaging study.³⁵ Based on these considerations, the cognitive deficits of SCA6 individuals may reflect dysfunctional cortico-cerebellar circuits.

Alternatively, the cognitive impairment seen in autosomal dominant cerebellar ataxias has been suggested to be due to ataxic motor dysfunction.³⁶ In patients with SCA6, most cognitive tasks were not related to the ICARS score (speech disorder). These results indicated that the low scores in cognitive tasks do not result from cerebellar ataxia in SCA6.

Cognitive dysfunction in patients with SCA6 showed no significant correlation with age at onset or depression. Age at onset and depression may not be influenced by the neuropsychological defect in SCA6. In the present study, CAG repeat length was not correlated with cognitive dysfunction in patients with SCA6. Several studies³⁷⁻³⁸ have suggested that the dosage of the CAG repeat expansion plays an important role in the phenotypic expression of SCA6, while another study suggested that factors other than gene dosage are also important in the phenotypic expression of SCA6.³⁹⁻⁴⁰ Our negative findings may be because of the small sample size and the narrow range of the expanded allele size. Indeed, the CAG repeat size of SCA6 alleles is smaller than that of other SCA genes, and there is a small difference in the CAG repeat expansion between healthy controls and patients with SCA6. A large scale study may provide sufficient information to clarify the relation between the neuropsychological defect and CAG repeat length in patients with SCA6.

In conclusion, our study demonstrated the presence of cognitive impairment, particularly in visuospatial memory and verbal fluency, in patients with SCA6. Moreover, we extended the view that cognitive dysfunction in a cerebellar disorder may result from disruption of cortico-cerebellar loops, in spite of the observation that the cortical structure is intact. We suggest that cognitive impairment in these patients is a manifestation of SCA6.

Competing interests: None.

Ethics approval: The study was approved by the Nagoya University Hospital Ethics Committee.

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

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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 (≥ 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, 2001a, 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, 2001c). 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, 2001d; 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 AGAATCTGTCCAGAGCGTGC-3') and a non-labelled reverse primer (5'-TGGCCTCGCTCAGGATGCTTTAAG-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 Fragly 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 (Dooyu *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 μ m 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, Olympus, 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 \pm 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 \pm SD	Range	n
Age at examination (years)	53.8 \pm 10.0	31–75	106
Duration from onset (years)	10.1 \pm 6.8	1–32	106
Age at onset (years)	43.7 \pm 10.4	25–68	106
CAG repeat size in AR gene (number)	47.8 \pm 3.1	41–57	97 ^a
Limb Norris score (normal score = 63)	53.9 \pm 7.3	34–63	99
Norris Bulbar score (normal score = 39)	33.0 \pm 4.3	20–39	99
ALSFRS-R (normal score = 48)	41.1 \pm 4.3	22–48	99

^aThe 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.

^aWe 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.

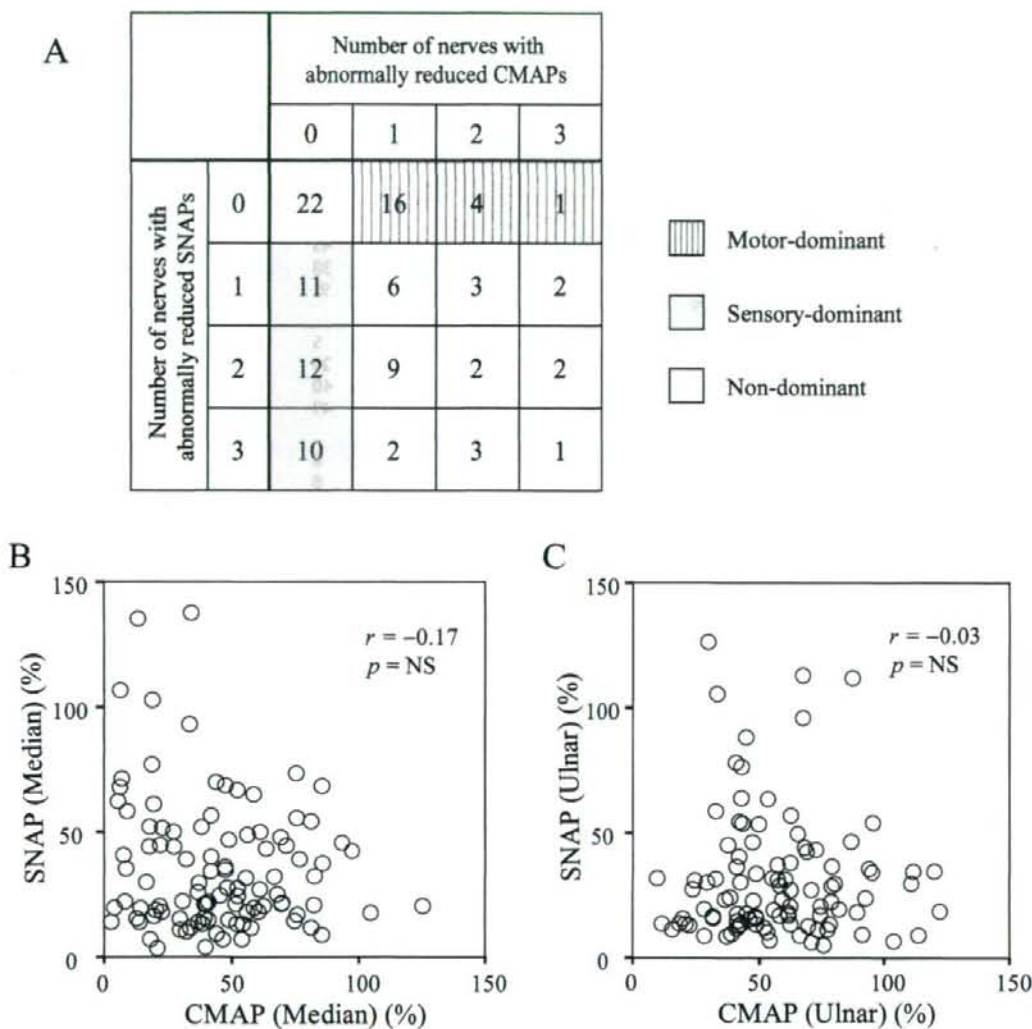


Fig. 1 Electrophysiological discrepancies in motor and sensory nerve involvement in SBMA patients. **(A)** The cross tabulation of the number of motor and sensory nerves showing an abnormally decreased action potential. The vertical stripe area corresponds to the motor-dominant phenotype and the gray 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; CMAP = 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 immunohistochemically 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 (Ikeuchi *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 (Ikeuchi *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

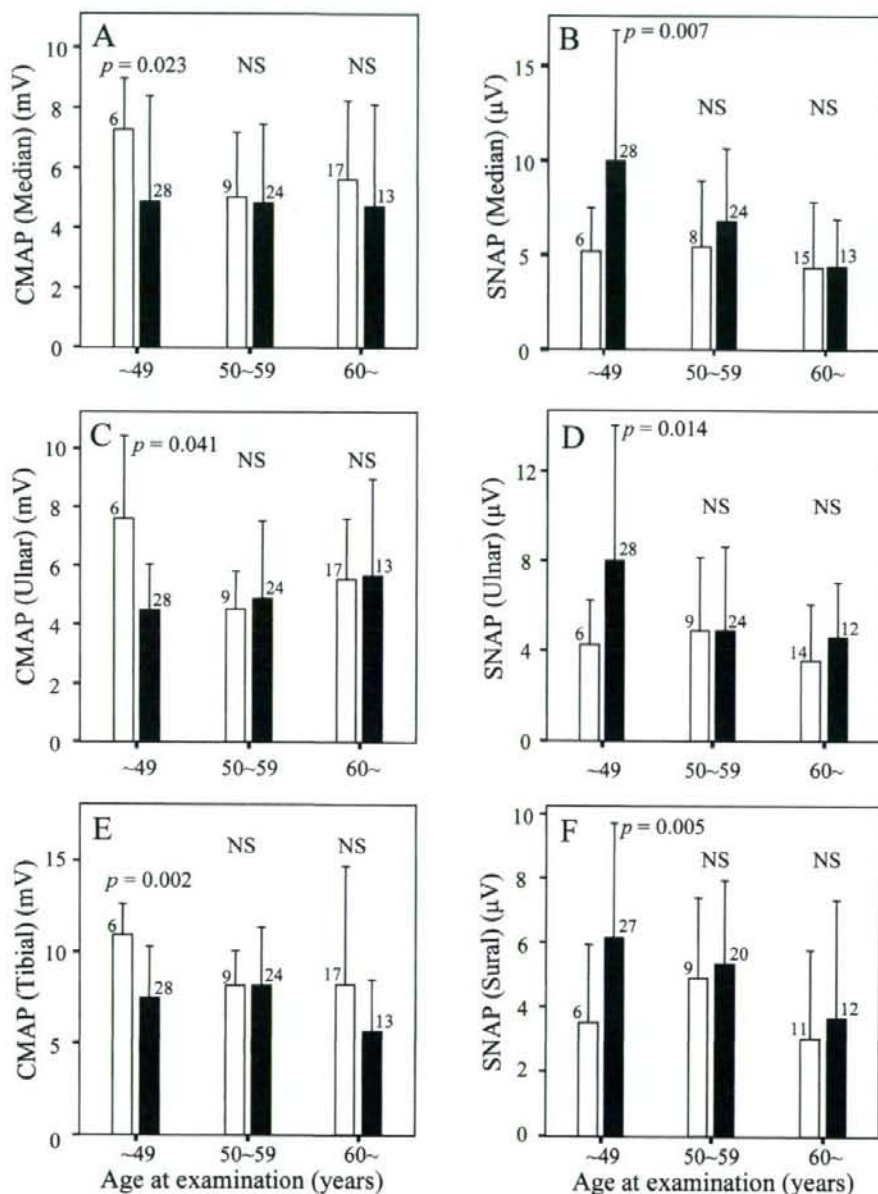


Fig. 2 (A–F) Age- and CAG-dependent changes in motor and sensory amplitudes in SBMA. CMAPs and SNAPs in the median (A and B), ulnar (C and D), tibial (E) and sural (F) nerves in different age groups are shown. The white columns are the mean values of the patients with a shorter CAG repeat (<47), while 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

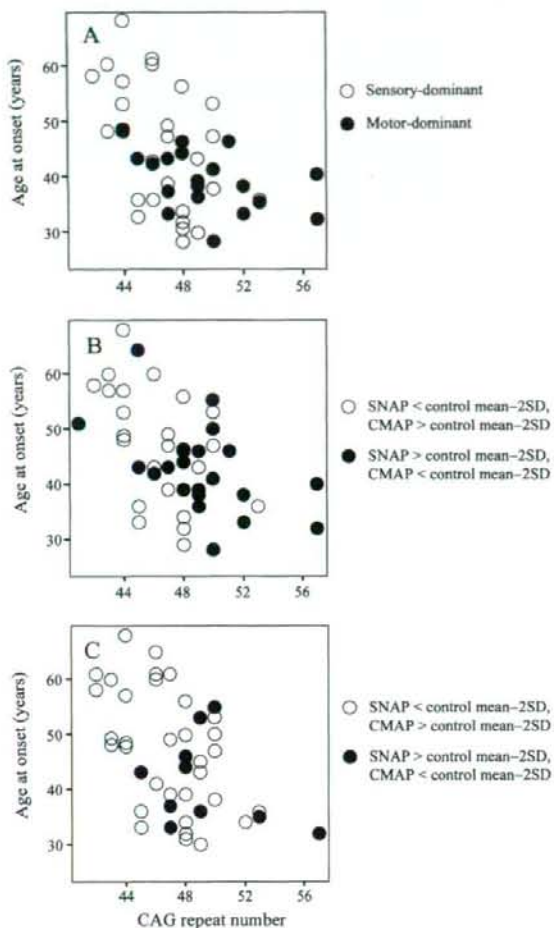


Fig. 3 CAG repeat size determines the age at onset in SBMA. (A) Relation between the CAG repeat size and the age at onset according to the phenotypes determined by CMAPs and SNAPs. A longer CAG repeat was closely linked to the motor-dominant phenotype, and a shorter CAG repeat was closely linked to the 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.