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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 ($\geq$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

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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.

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.

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, 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.

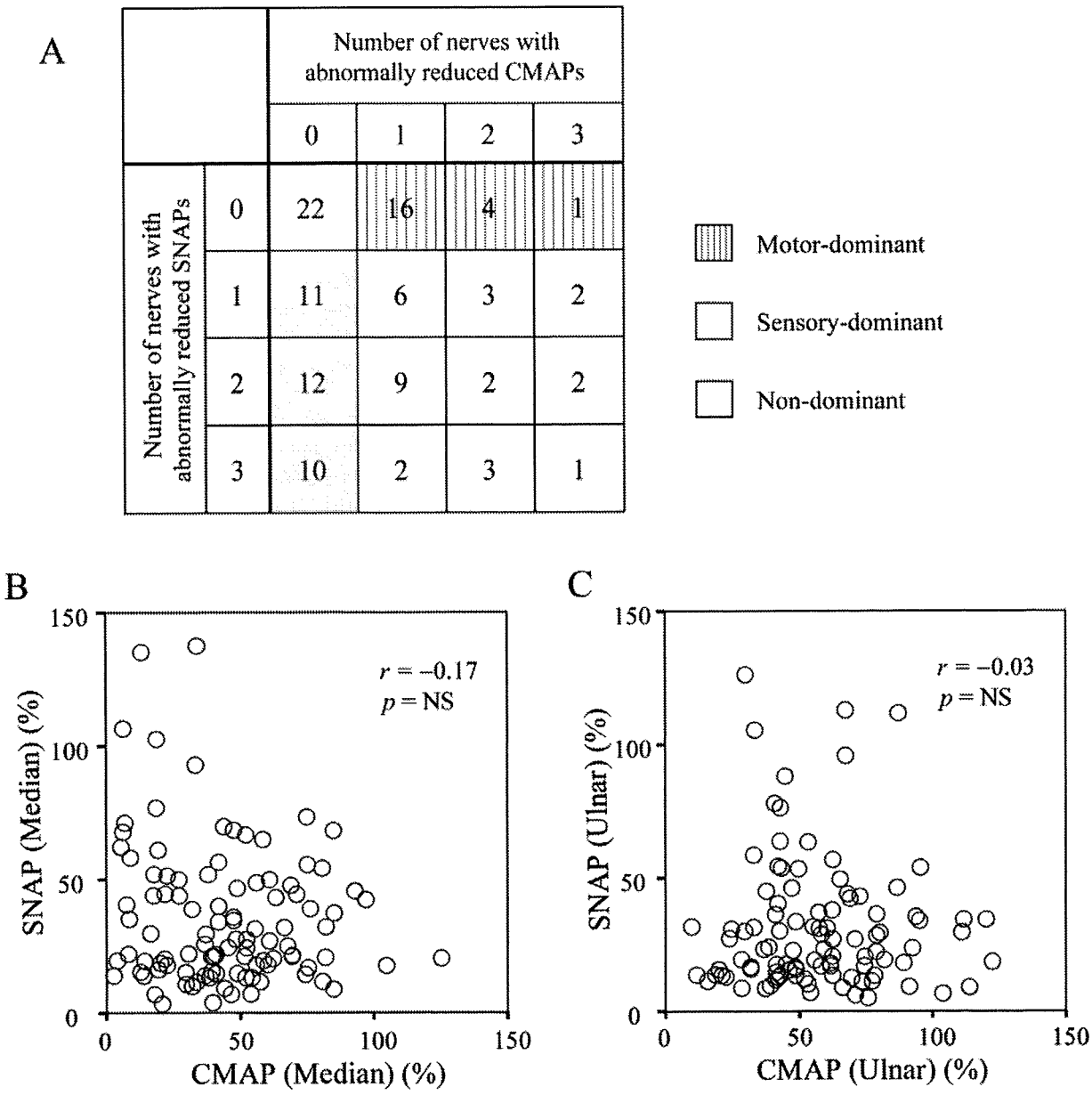

Fig. 1 Electrophysiological discrepancies in motor and sensory nerve involvement in SBMA patients. (**A**) The cross tabulation of the number of motor and sensory nerves showing an abnormally decreased action potential. The vertical stripe area corresponds to the motor-dominant phenotype and the gray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

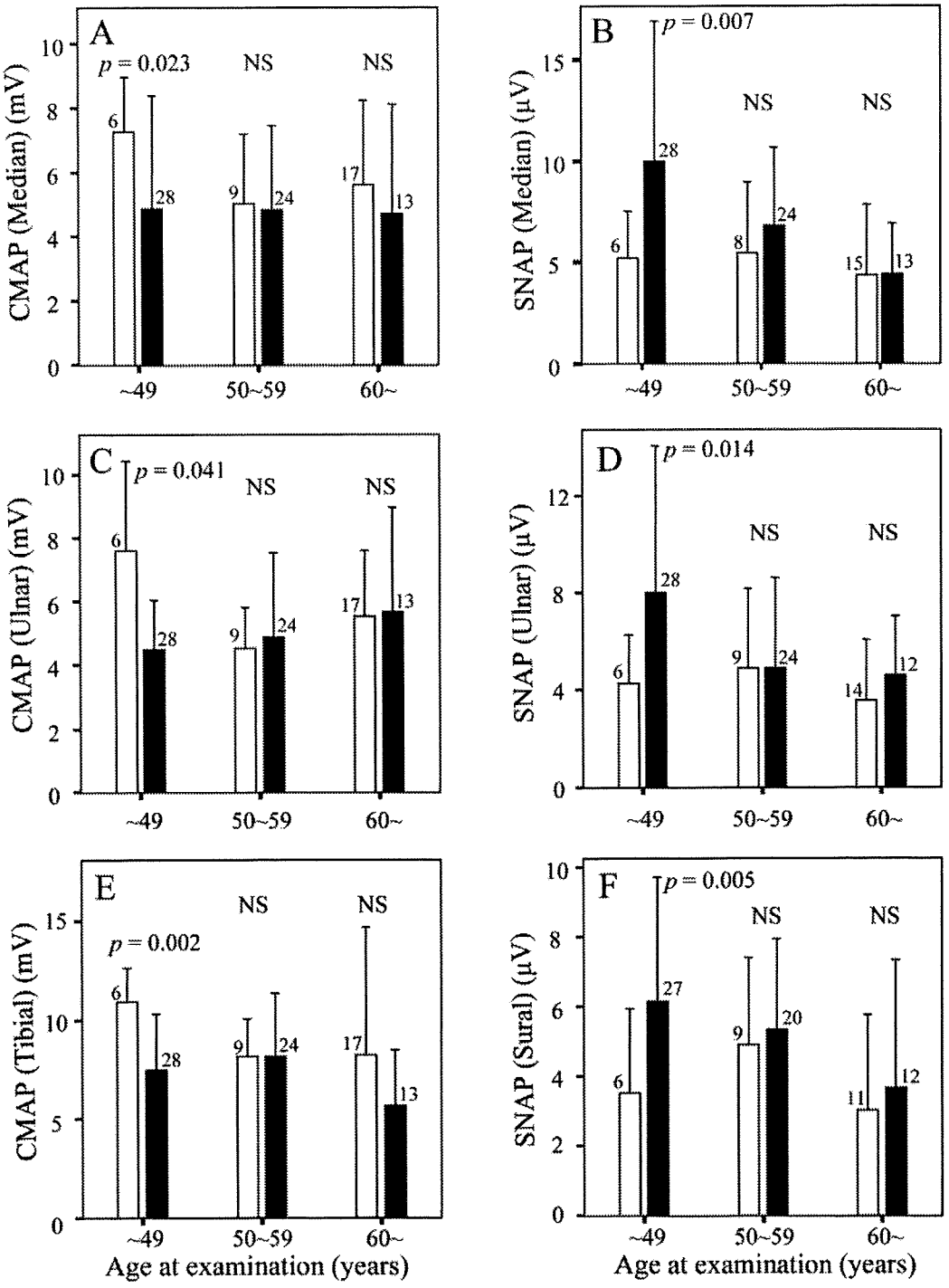

Fig. 2 **(A–F)** Age- and CAG-dependent changes in motor and sensory amplitudes in SBMA. CMAPs and SNAPs in the median (A and B), ulnar (C and D), tibial (E) and sural (F) nerves in different age groups are shown. The white columns are the mean values of the patients with a shorter CAG repeat (<47),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

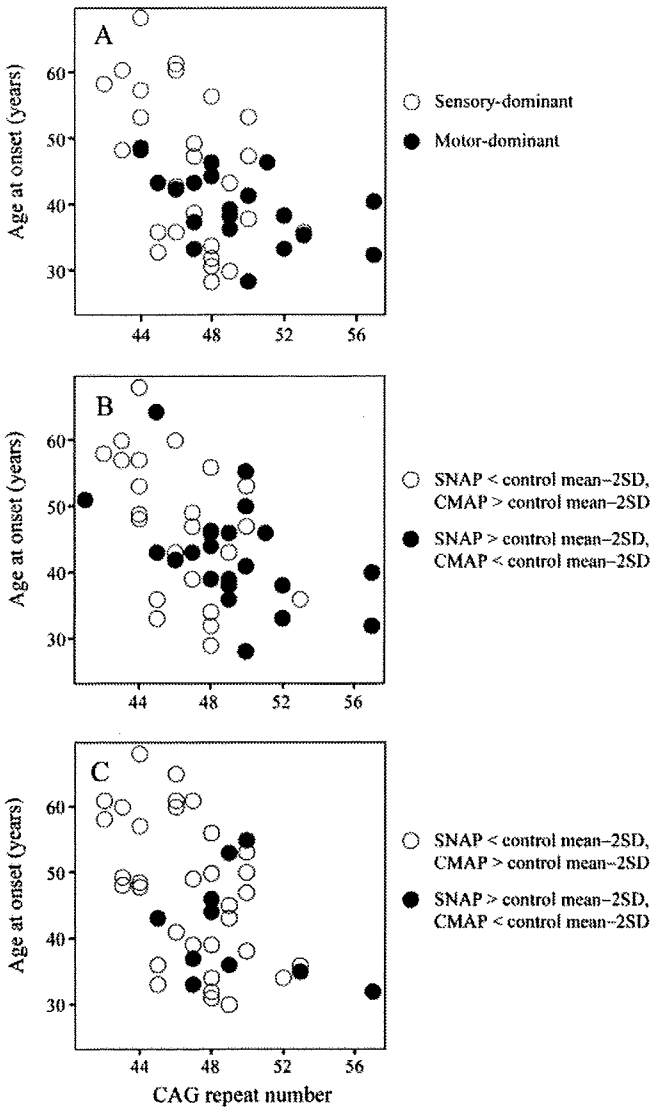

Fig. 3 CAG repeat size determines the age at onset in SBMA. **(A)** Relation between the CAG repeat size and the age at onset according to the phenotypes determined by CMAPs and SNAPs. A longer CAG repeat was closely linked to the motor-dominant phenotype, and a shorter CAG repeat was closely linked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

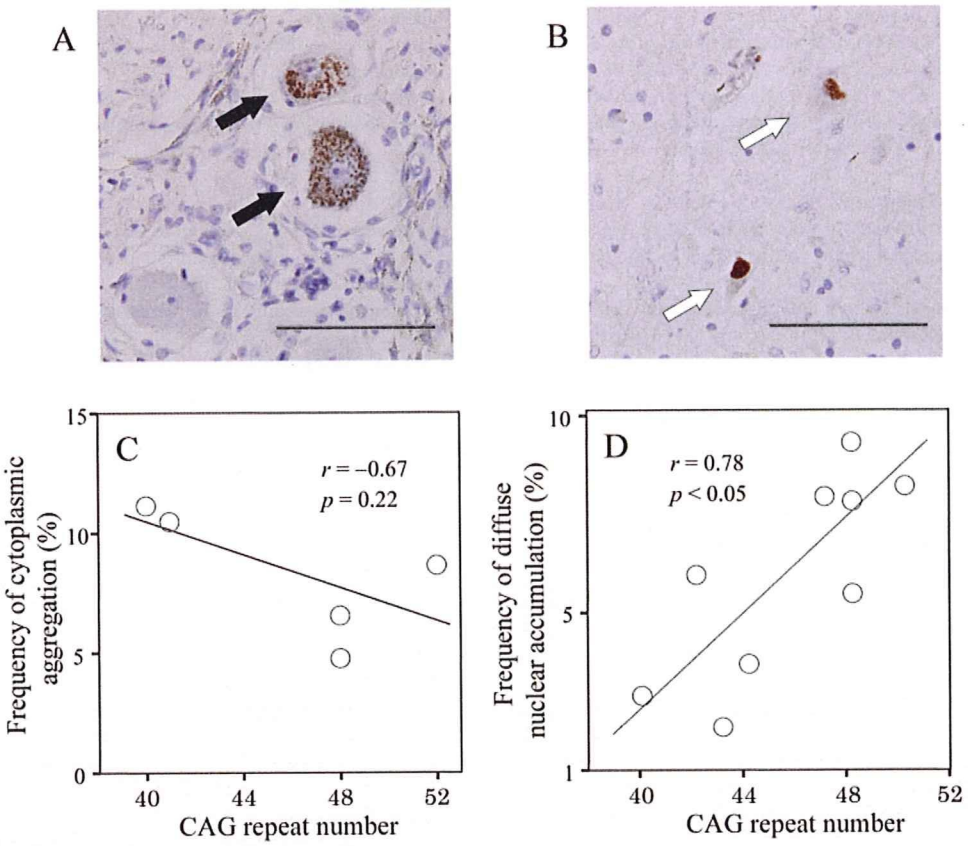

Fig. 4 Immunohistochemical analyses of mutant androgen receptor (AR) accumulation in the dorsal root ganglion (DRG) and that in the spinal anterior horn of SBMA patients. (**A**) Aggregates of mutant AR in the cytoplasm of DRG neurons (black arrows). Scale bar = 100 μm. (**B**) Mutant AR accumulates 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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References

Adachi H, Katsuno M, Minamiyama M, Waza M, Sang C, Nakagomi Y, et al. Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. Brain 2005; 128: 659–70.

Andrew SE, Goldberg YP, Hayden MR. Rethinking genotype and phenotype correlations in polyglutamine expansion disorders. Hum Mol Genet 1997; 6: 2005–10.

Antonini G, Gragnani F, Romaniello A, Pennisi EM, Morino S, Ceschin V, et al. Sensory involvement in spinal-bulbar muscular atrophy (Kennedy's disease). Muscle Nerve 2000; 23: 252–8.

Atsuta N, Watanabe H, Ito M, Banno H, Suzuki K, Katsuno M, et al. Natural history of spinal and bulbar muscular atrophy (SBMA): a study of 223 Japanese patients. Brain 2006; 129: 1446–55.

Banno H, Adachi H, Katsuno M, Suzuki K, Atsuta N, Watanabe H, et al. Mutant androgen receptor accumulation in spinal and bulbar muscular atrophy scrotal skin: a pathogenic marker. Ann Neurol 2006; 59: 520–6.

Chevalier-Larsen ES, O'Brien CJ, Wang H, Jenkins SC, Holder L, Lieberman AP, et al. Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy. J Neurosci 2004; 24: 4778–86.

Doyu M, Sobue G, Mukai E, Kachi T, Yasuda T, Mitsuma T, et al. Severity of X-linked recessive bulbospinal neuronopathy correlates with size of the tandem CAG repeat in androgen receptor gene. Ann Neurol 1992; 32: 707–10.

Ferrante MA, Wilbourn AJ. The characteristic electrodiagnostic features of Kennedy's disease. Muscle Nerve 1997; 20: 323–9.

Fischbeck KH. Kennedy disease. [Review]. J Inherit Metab Dis 1997; 20: 152–8.

Gatchel JR, Zoghbi HY. Diseases of unstable repeat expansion: mechanisms and common principles. Nat Rev Genet 2005; 6: 743–55.

Guidetti D, Vescovini E, Motti L, Ghidoni E, Gemignani F, Marbini A, et al. X-linked bulbar and spinal muscular atrophy, or Kennedy disease: clinical, neurophysiological, neuropathological, neuropsychological and molecular study of a large family. J Neurol Sci 1996; 135: 140–8.

Harding AE, Thomas PK, Baraitser M, Bradbury PG, Morgan-Hughes JA, Ponsford JR. X-linked recessive bulbospinal neuronopathy: a report of ten cases. J Neurol Neurosurg Psychiatry 1982; 45: 1012–9.

Ikeuchi T, Koide R, Tanaka H, Onodera O, Igarashi S, Takahashi H, et al. Dentatorubral-pallidoluysian atrophy: clinical features are closely related to unstable expansions of trinucleotide (CAG) repeat. Ann Neurol 1995; 37: 769–75.

Johansson J, Forsgren L, Sandgren O, Brice A, Holmgren G, Holmberg M. Expanded CAG repeats in Swedish spinocerebellar ataxia type 7 (SCA7) patients: effect of CAG repeat length on the clinical manifestation. Hum Mol Genet 1998; 7: 171–6.

Kachi T, Sobue G, Sobue I. Central motor and sensory conduction in X-linked recessive bulbospinal neuronopathy. J Neurol Neurosurg Psychiatry 1992; 55: 394–7.

Katsuno M, Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, et al. Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 2002; 35: 843–54.

Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, et al. Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med 2003; 9: 768–73.

Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, et al. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci USA 2005; 102: 16801–6.

Katsuno M, Adachi H, Minamiyama M, Waza M, Tokui K, Banno H, et al. Pathogenesis, animal models and therapeutics in spinal and bulbar muscular atrophy (SBMA) [Review]. Exp Neurol 2006; 200: 8–18.

Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. Neurology 1968; 18: 671–680.

Kimura J. Principles and valiations of nerve conduction studies. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd edn. New York: Oxford University Press; 2001a. p. 91–129.

Kimura J. Assessment of individual nerves. In: Kimura J, editor. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd edn. New York: Oxford University Press; 2001b. p. 130–77.

Kimura J. The F wave and the A wave. In: Kimura J, editor. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd edn. New York: Oxford University Press; 2001c. p. 439–65.

Kimura J. The F wave and the A wave. In: Kimura J, editor. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd edn. New York: Oxford University Press; 2001d. p. 307–38.

Koike H, Mori K, Misu K, Hattori N, Ito H, Hirayama M, et al. Painful alcoholic polyneuropathy with predominant small-fiber loss and normal thiamine status. Neurology 2001; 56: 1727–32.

Koike H, Iijima M, Sugiura M, Mori K, Hattori N, Ito H, et al. Alcoholic neuropathy is clinicopathologically distinct from thiamine-deficiency neuropathy. Ann Neurol 2003; 54: 19–29.

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991; 352: 77–9.

Li M, Sobue G, Doyu M, Mukai E, Hashizume Y, Mitsuma T. Primary sensory neurons in X-linked recessive bulbospinal neuropathy: histopathology and androgen receptor gene expression. Muscle Nerve 1995; 18: 301–8.

Li M, Miwa S, Kobayashi Y, Merry DE, Yamamoto M, Tanaka F, et al. Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. Ann Neurol 1998; 44: 249–54.

Mahant N, McCusker EA, Byth K, Graham S; Huntington Study Group. Huntington's disease: clinical correlates of disability and progression. Neurology 2003; 61: 1085–92.

Minamiyama M, Katsuno M, Adachi H, Waza M, Sang C, Kobayashi Y, et al. Sodium butyrate ameliorates phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 2004; 13: 1183–92.

Mori K, Iijima M, Koike H, Hattori N, Tanaka F, Watanabe H, et al. The wide spectrum of clinical manifestations in Sjogren's syndrome-associated neuropathy. Brain 2005; 128: 2518–34.

Norris FH, Calanchini PR, Fallat RJ, Panchari S, Jewett B. The administration of guanidine in amyotrophic lateral sclerosis. Neurology 1974; 24: 721–8.

Olney RK, Aminoff MJ, So YT. Clinical and electrodiagnostic features of X-linked recessive bulbospinal neuronopathy. Neurology 1991; 41: 823–8.

Perutz MF, Pope BJ, Owen D, Wanker EE, Scherzinger E. Aggregation of proteins with expanded glutamine and alanine repeats of the glutamine-rich and asparagine-rich domains of Sup35 and of the amyloid beta-peptide of amyloid plaques. Proc Natl Acad Sci USA 2002; 99: 5596–600.

Polo A, Teatini F, D'Anna S, Manganotti P, Salviati A, Dallapiccola B, et al. Sensory involvement in X-linked spino-bulbar muscular atrophy (Kennedy's syndrome): an electrophysiological study. J Neurol 1996; 243: 388–92.

Sakahira H, Breuer P, Hayer-Hartl MK, Hartl FU. Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. [Review]. Proc Natl Acad Sci USA 2002; 99 (Suppl 4): 16412–8.

Sobue G, Hashizume Y, Mukai E, Hirayama M, Mitsuma T, Takahashi A. X-linked recessive bulbospinal neuronopathy. A clinicopathological study. Brain 1989; 112: 209–32.

Sobue G, Doyu M, Kachi T, Yasuda T, Mukai E, Kumagai T, et al. Subclinical phenotypic expressions in heterozygous females of X-linked recessive bulbospinal neuronopathy. J Neurol Sci 1993; 117: 74–8.

Sone J, Hishikawa N, Koike H, Hattori N, Hirayama M, Nagamatsu M, et al. Neuronal intranuclear hyaline inclusion disease showing motor-sensory and autonomic neuropathy. Neurology 2005; 65: 1538–43.

Sperfeld AD, Karitzky J, Brummer D, Schreiber H, Haussler J, Ludolph AC, et al. X-linked bulbospinal neuronopathy: Kennedy disease. Arch Neurol 2002; 59: 1921–6.

Takeyama K, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, et al. Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. Neuron 2002; 35: 855–64.

Tanaka F, Doyu M, Ito Y, Matsumoto M, Mitsuma T, Abe K, et al. Founder effect in spinal and bulbar muscular atrophy (SBMA). Hum Mol Genet 1996; 5: 1253–7.

Tanaka F, Reeves MF, Ito Y, Matsumoto M, Li M, Miwa S, et al. Tissue-specific somatic mosaicism in spinal and bulbar muscular atrophy is dependent on CAG-repeat length and androgen receptor-gene expression level. Am J Hum Genet 1999; 65: 966–73.

The ALS CNTF Treatment Study (ACTS) Phase I-II Study Group. The amyotrophic lateral sclerosis functional rating scale: Assessment of activities of daily in patients with ALS. Arch Neurol 1996; 53: 141–7.

Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, et al. 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 2005; 11: 1088–95.

Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration [Review]. Annu Rev Neurosci 2000; 23: 217–47.

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RESEARCH ARTICLE

Pupillary supersensitivity and visual disturbance in Parkinson's disease

Norio Hori
Motoko Takamori
Masaaki Hirayama
Hirohisa Watanabe
Tomohiko Nakamura
Fumitada Yamashita
Hiroki Ito
Naoki Mabuchi
Gen Sobue

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N. Hori · M. Hirayama · H. Watanabe
T. Nakamura · F. Yamashita
G. Sobue (✉)
Dept. of Neurology
Nagoya University Graduate School of Medicine
Nagoya 466-8550, Japan
Tel.: +81-52/744-2385
Fax: +81-52/744-2384
E-Mail: sobueg@med.nagoya-u.ac.jp

M. Takamori
Dept. of Neurology
Shizuoka Saiseikai General Hospital
Shizuoka, Japan

H. Ito
Dept. of Neurology
Ichinomiya Municipal Hospital
Aichi, Japan

N. Mabuchi
Dept. of Neurology
Okazaki Municipal Hospital
Aichi, Japan

Abstract This study evaluated pupillary postganglionic autonomic dysfunction and its relationship to visual disturbance in idiopathic Parkinson's disease (PD). Pupillary sensitivity was examined in relation to a parasympathomimetic agent [0.05% pilocarpine hydrochloride (PL)] and to a sympathomimetic agent [0.02% dipivefrine hydrochloride (DPE)] using infrared pupillography in 40 PD patients and 17 age-matched controls. Visual disturbances were evaluated as well, including blurring, photophobia, night blindness and involuntary eyelid closure in response to light. Pupillary supersensitivity to PL and DPE and their relation to visual disturbances were found to be significantly greater in PD patients than in controls (22.3 ± 15.1 vs. $10.4 \pm 11.4\%$, $P < 0.005$, and 14.5 ± 14.5 vs. $4.9 \pm 8.7\%$, $P < 0.01$, respectively). In addition, pupillary sympathetic supersensitivity did not correlate with a reduction of ^{123}I-metaiodobenzylguanidine (MIBG) cardiac accumulation. Patients with PD reported more blurred vision ($P < 0.001$) and involuntary eyelid closure in response to light ($P < 0.05$) than controls. Patients with supersensitivity to both PL and DPE complained more often of blurred vision than patients without supersensitivity ($P < 0.05$). Pupillary sensitivity to PL correlated significantly with a summed score for visual disturbance ($P < 0.05$, $r = 0.417$), but DPE sensitivity did not. PD patients have both parasympathetic and sympathetic postganglionic impairments affecting the pupil. Our findings demonstrate that parasympathetic dysfunction contributes significantly to visual disturbance in PD.

Key words Parkinson's disease (PD) · eye-drop test · postganglionic autonomic dysfunction · pupillary response · visual disturbance

Introduction

Patients with Parkinson's disease (PD) often complain of visual disturbances such as photophobia or blurred vision, although a general ophthalmologic examination usually detects no abnormality. Moreover, visual disturbance has not been recognized as a principal PD symptom; as a symptom, visual disturbance is overshadowed by motor impairment. However, visual disturbance influences the quality of daily living and can be a serious problem [13], especially for PD patients.

Autonomic dysfunction is recognized as a common disease manifestation in PD patients and can affect a

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wide range of organs including those comprising the cardiovascular, gastrointestinal, sudomotor, urinary and genital systems [17]. In the cardiovascular system, peripheral autonomic dysfunction has been demonstrated by denervation supersensitivity to norepinephrine [18] and a reduction of ^{123}I-metaiodobenzylguanidine (MIBG) cardiac accumulation. This latter sign is now used widely as a sensitive marker in the differential diagnosis of PD [10, 9, 19].

In previous studies, peripheral pupillary impairment in PD has been controversial [14, 20, 23]. Drugs are given often through eye drops to evaluate pupillary autonomic disorders of a peripheral nature. A pupil deprived of its innervation becomes more sensitive to the neurotransmitter-mimetic drugs [22]. While a substantially diluted solution containing an agonist does not show any remarkable effect on the normal pupil, the same solution elicits hyper-responsiveness in the centrally denervated pupil. Diluted solutions of pilocarpine hydrochloride (PL), a parasympathomimetic agent causing miosis, and dipivefrine hydrochloride (DPE), a sympathomimetic agent causing mydriasis, are used often in eye-drop tests [22]. However, the optimal concentrations of such eye drops as well as the time interval required to adequately assess supersensitivity of the pupil in PD have not been evaluated fully. In addition, the association between pupillary supersensitivity and the degree of visual disturbance has not been investigated. This study determined the optimal procedures for these eye-drop tests and assessed the relationship of the severity of pupillary autonomic involvement to visual symptoms such as blurred vision, photophobia, night blindness and involuntary eyelid closure in response to light in PD patients.

Subjects and methods

To assess the relationship between visual disturbance and pupillary sensitivity, 40 PD patients and 17 healthy gender and age-matched controls were examined (Table 1). PD was diagnosed according to the criteria of the United Kingdom Brain Bank [5]. PD severity was assessed according to Hoehn and Yahr (H–Y) stage and was at Stage I in 5 patients, Stage II in 2 patients, Stage III in 27 patients, Stage IV in 5 patients and Stage V in 1 patient. L-dopa was being taken by 37 patients, a dopamine agonist was being taken by 21 patients and an anticholinergic drug was used by 9 patients. Patients with ocular disease (e.g., cataract surgery, glaucoma), diabetes mellitus or peripheral neuropathy were excluded. Pupillary dynamics were measured with an infrared video camera (Binocular IRISCORDER Model C-2514, Hamamatsu Photonics Co. Ltd., Shizuoka, Japan). This device is an electronic pupillometer and projects a light stimulus of 1 second and calculates several parameters, including pupillary area before the light stimulus (mm^2, D1), minimum pupillary area caused by the light stimulus (mm^2, D2), and contraction rate (CR) calculated as (D1 – D2)/D1. CR was considered sensitive to each drug. Patients who could not keep their eyes open for at least 5 seconds were excluded, because the infrared video camera required 5 seconds to measure pupil diameter. The baseline pupillary diameters were measured in both eyes using the infrared Iriscorder after 5 minutes of dark adaptation. The daily dose of antiparkinsonian drug was continued and it is described in the discussion.

Questionnaires concerning visual disturbance were administered to both PD patients and controls. The questionnaire dealt with four items: blurred vision, photophobia, night blindness and involuntary eyelid closure in response to light. The frequency of occurrence and the degree were noted by patients and scored as follows (see also "Appendix"): 0 (never), 1 (sometimes), 2 (often) and 3 (always).

Of the 40 PD patients, 32 were examined for ^{123}I-MIBG uptake. The ratio of uptake in regions of interest in the heart to those in the mediastinum (H/M ratio) was calculated from delayed images according methods described previously [10].

To determine the optimal concentrations of the drugs and appropriate times for the eye-drop tests, eight PD patients with the following characteristics were examined: four men and four women, mean age ± standard deviations (SD) (59.8 ± 10.2 years), and mean disease duration (12.9 ± 5.5 years). H–Y stages were Stage III in six patients and Stage IV in two patients. L-dopa and dopamine agonists were used by all eight patients and anticholinergic drugs were used by one. As a control group, eight age-matched healthy

Table 1 Patient characteristics, pupillary sensitivity, and scores for visual disturbance in control subjects and patients with Parkinson's disease

	PD (n = 40) Mean ± SD	Controls (n = 17) Mean ± SD	P value
Age (years)	63.2 ± 10.2	60.9 ± 19.5	NS
Gender (M:F)	23:17	11:06	NS
Disease duration (years)	8.3 ± 6.9	NA	
Delayed-phase H/M ratio	1.4 ± 0.3	NA	
Pupil diameter at baseline (mm)			
Right	5.0 ± 0.9	5.1 ± 0.9	NS
Left	5.0 ± 1.0	5.1 ± 1.0	NS
Pupillary sensitivity (%)			
To 0.05% PL	22.3 ± 15.1	10.4 ± 11.4	<0.005
To 0.02% DPE	14.5 ± 14.5	4.9 ± 8.7	<0.01
Scores for visual disturbance			
Blurred vision	1.4 ± 1.0	0.1 ± 0.3	<0.001
Photophobia	0.6 ± 0.8	0.5 ± 0.7	NS
Night blindness	0.6 ± 0.8	0.4 ± 0.7	NS
Involuntary eyelid closure in response to light	0.3 ± 0.5	0 ± 0	<0.05

PD Parkinson's disease, *SD* standard deviation, *NA* not available, *NS* not significant, *H/M* ^{123}I-metaiodobenzylguanidine (MIBG) uptake in regions of interest in the heart to those in the mediastinum, *PL* pilocarpine hydrochloride, *DPE* dipivefrine hydrochloride

controls (4 men and 4 women with a mean age of 58.1 ± 19.5 years) were evaluated. A percentage change in pupillary diameter exceeding the mean value + 2 (SD) in the control group was defined as indicating supersensitivity. The Mann–Whitney *U* test was used to assess differences between PD patients and controls, as well as to assess the differences between PD patients with supersensitivity and without supersensitivity. Pearson's correlation coefficient was used to analyze relationships between pupillary sensitivities to 0.05% PL or 0.02% DPE and age at onset, age at examination, disease duration, dose of L-dopa or H/M ratio in delayed images. Relationships between pupillary sensitivities to 0.05% PL or 0.02% DPE and H–Y stage or the summed score for visual disturbances were analyzed using Spearman's correlation coefficient. Calculations were performed using the StatView statistical software package (version 5.0; Abacus Concepts, Berkeley, CA, USA). Statistical significance was defined by $P < 0.05$. Values are presented as the means ± SD. The Ethics Committee of Nagoya University School of Medicine approved all aspects of this study. All subjects gave informed consent for participation.

The baseline pupillary diameters in both eyes were measured using the infrared Iriscorder after 5 minutes of dark adaptation. Three different drug concentrations (PL, 0.025, 0.05 and 0.10%; DPE, 0.02, 0.04 and 0.10%) were administered after an interval of at least 72 hours. Two drops of PL were applied to the right eye and two drops of DPE were applied to the left eye. Pupillary diameter was assessed at 15, 30, 60 and 120 minutes after drug administration. Sensitivity to each drug was calculated as percent change (%) in pupil diameter.

Based on the results from the previous methods, in remaining portions of the study two drops of 0.05% PL were applied to the right eye and two drops of 0.02% DPE to the left eye. Change in pupillary diameter was recorded at 60 minutes after PL administration and 120 minutes after DPE administration. A change exceeding the mean value in the control group + 2 SD (i.e., 28% pupillary change at 60 minutes after 0.05% PL administration or 14% pupillary change at 120 minutes after 0.02% DPE administration) was defined as supersensitivity.

Results

Optimal PL and DPE concentrations and times for assessment in eye-drop tests

Pupil diameters at baseline in PD patients were 5.0 ± 0.8 mm on the right and 5.1 ± 0.7 mm on the left, were slightly, but not significantly, smaller than diameters observed in controls (5.5 ± 1.0 mm on the right and 5.4 ± 1.1 mm on the left). Percent change over time in pupil diameter after the administration of PE and DPE is shown in Fig. 1.

Parkinson's disease patients showed a maximum pupillary change at 60 minutes after PL administration and pupils started to recover at 120 minutes with all concentrations. Pupillary change at 60 minutes after 0.05% PL administration in PD patients was significantly greater than in controls (36 ± 10 vs. 8 ± 10%, $P < 0.005$). The change after 0.10% PL administration in PD patients was also significantly greater than in controls (40 ± 18 vs. 20 ± 15%, $P < 0.05$). Pupillary diameter after DPE administration continued to increase even at 120 minutes, with dilation at 120 minutes after 0.02 and 0.04% DPE administration being significantly greater in PD patients than of that in controls (0.02, 19 ± 15 vs. 2 ± 6%, $P < 0.05$; 0.04, 21 ± 15 vs. 8 ± 7%, $P < 0.05$).

Pupillary response to 0.05% PL and 0.02% DPE

Pupil diameter at baseline did not differ significantly between PD patients and controls. Of the 40 PD patients, 12 (30%) showed supersensitivity to PL, 19 (48%) showed supersensitivity to DPE and 7 (18%) showed supersensitivity to both PL and DPE. Pupillary sensitivity to 0.05% PL in PD patients was significantly greater than in controls (22.3 ± 15.1 vs. 10.4 ± 11.4%, $p < 0.005$) and sensitivity to 0.02% DPE in PD patients was also significantly greater than in controls (14.5 ± 14.5 vs. 4.9 ± 8.7%, $P < 0.01$; Table 1). Pupillary sensitivity to 0.05% PL and 0.02% DPE did not correlate with age at PD onset, daily dose of L-dopa, H–Y stage or delayed-phase H/M ratio in ^{123}I-MIBG scintigraphy. Pupillary sensitivity to 0.02% DPE correlated weakly, but significantly with age at examination ($r = 0.350$, $P < 0.05$; Fig. 2a) and with disease duration ($r = 0.334$, $P < 0.05$; Figure 2b). Sensitivity to 0.05% PL did not show such correlations. No significant difference was found in pupillary sensitivity to 0.05% PL between patients who took an anticholinergic drug and patients who did not (23 ± 15 vs. 22 ± 15%). In addition, no significant difference in pupillary sensitivity to 0.02% DPE was evident between these two groups (21 ± 22 vs. 13 ± 11%).

Visual disturbance and eye-drop tests

Patients with PD noted blurred vision ($P < 0.001$) and involuntary eyelid closure in response to light ($P < 0.05$) more frequently than did controls. PD patients complained of photophobia and night blindness slightly more frequently than controls, but the difference was not significant (Table 1).

No score concerning visual disturbance differed between groups with supersensitivity to PL or DPE and the group without supersensitivity, although the score for blurred vision in the group with supersensitivity to both PL and DPE was significantly greater than in the group without supersensitivity (Table 2).

Pupillary sensitivities to 0.05% PL correlated significantly with the summed score for all visual disturbances ($r = 0.417$, $P < 0.05$; Fig. 3), while sensitivity to 0.02% DPE did not.

Discussion

Pharmacologic pupillary function tests are useful methods for evaluating denervation supersensitivity

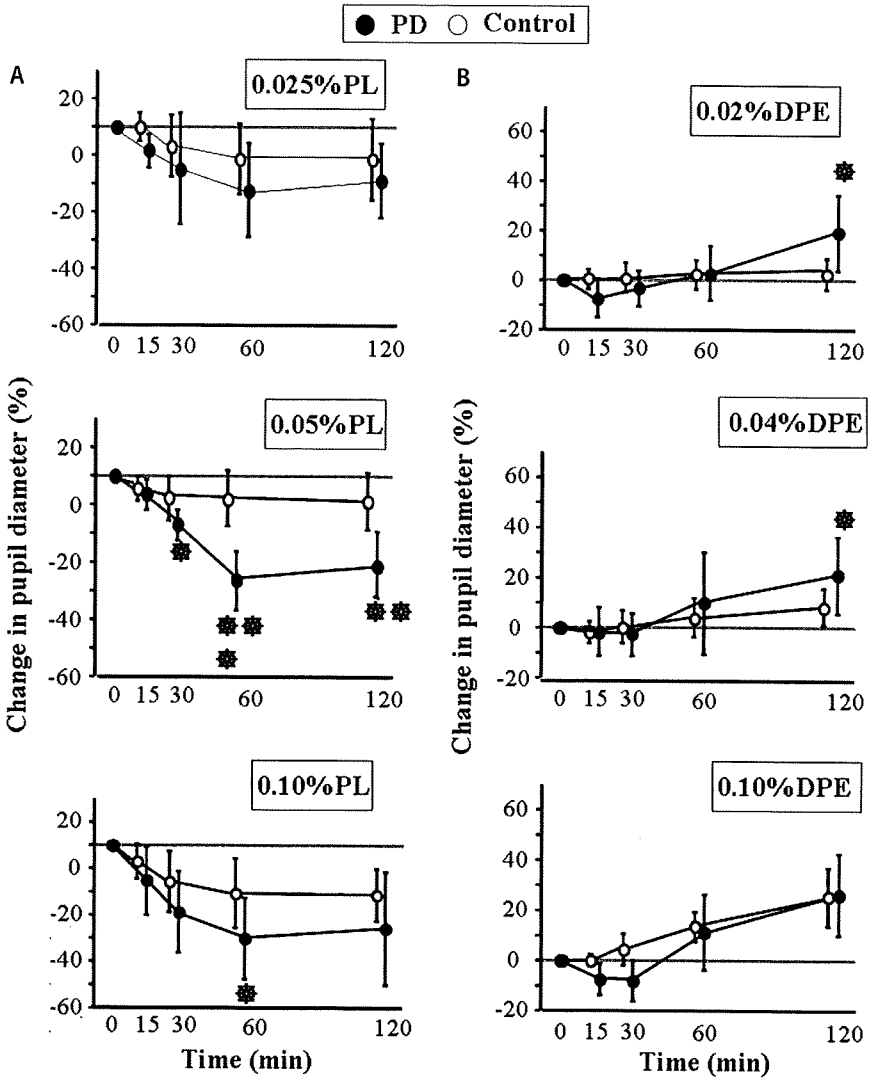

Fig. 1 Response of pupils in terms of time and concentration after application of PL and DPE Patients with Parkinson's disease (PD) showed significant differences from controls in pupillary sensitivity to 0.05% or 0.10% PL (**A**) and to 0.02% or 0.04% DPE (**B**) with the lower concentration best eliciting such a difference at 60 and 120 min, respectively. Values are means, with error bars indicating standard deviation (SD). PL, pilocarpine hydrochloride; DPE, dipivefrine hydrochloride. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$ vs. controls

in patients with peripheral impairment, especially postganglionic impairment, of the pupillary autonomic system [22]. PL, a parasympathomimetic agent, and DPE, a sympathomimetic agent, are used often in eye-drop tests, but the optimal drug concentrations and time points for evaluation are not well defined [4, 23, 26]. In the present study, PD patients showed maximum pupillary change at 60 minutes after PL administration, while pupils started to recover at 120 minutes at all concentrations administered. The degree of miosis at 60 minutes after 0.05 and 0.1% PL administration, and also of mydriasis at 120 minutes after 0.02 and 0.04% DPE administration, were significantly greater in PD patients than in controls. Indeed, the administration of 0.05% PL or 0.02% DPE did not change pupil diameter in controls significantly. Thus, pupillary measurement at 60 minutes after 0.05% PL and at 120 minutes after 0.02% DPE administration represents an optimal method for detecting cholinergic and adrenergic supersensitivity in PD patients, given that the smallest concentration of drug permitting adequate detection should be used.

Pupillary abnormalities with central parasympathetic involvement has been widely recognized in PD [12, 17]. Lewy bodies, neurofibrillary degeneration and neuronal loss have been reported in the Edinger–Westphal nucleus in PD patients [6, 11]. In addition, abnormally slow pupillary responses to light and pain have been described in these patients [16]. Neuropathology in the Edinger–Westphal nucleus is reflected in the impairment of pupillary constriction; furthermore, the peak constriction amplitude of the pupillary light reflex, reduced in PD patients compared with that in controls, can be attributed to this impairment [8]. However, only a few studies have assessed the peripheral parasympathetic system [15]. While no reports have examined pathologic involvement of the

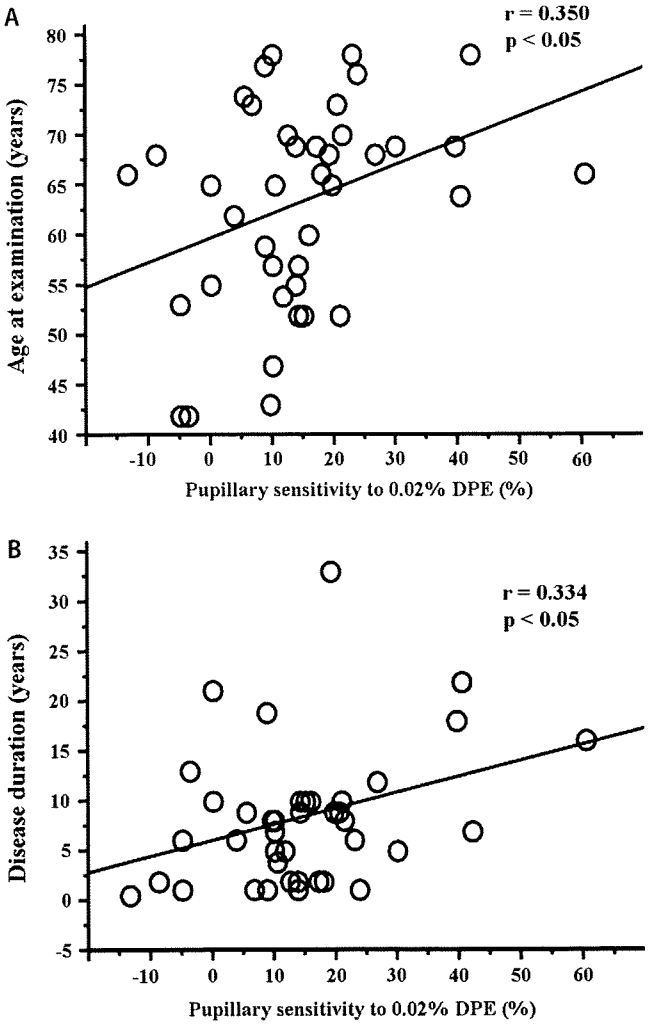

Fig. 2 Correlation between pupillary sensitivity for 0.05% DPE and disease duration and agent examination. Significant positive correlations of pupillary sensitivity to DPE were evident with Parkinson's disease patients' age (**A**) and with disease duration (**B**). DPE, dipivefrine hydrochloride

ciliary ganglion in PD patients, Lewy bodies have been identified in submandibular ganglion neurons [24], suggesting widespread pathologic involvement of the peripheral parasympathetic system in PD. Sugiyama et al. [23] reported that pupillary supersensitivity to cholinergic agonists was prominent even in early-stage PD patients. Our findings support these previous results.

Compared to results concerning parasympathetic impairment of the pupil in PD patients, available data are more limited with respect to sympathetic impairment. However, pupillary constriction time was found to be delayed in PD patients, suggesting impairment at sympathetic ganglia [14]. In a previous study, sympathetic supersensitivity was reported to be rare in early PD stages (H–Y I and II), usually becoming detectable only in Stage III patients by eye-drop testing using DPE [23]. In the present study, patients with PD manifested significant sympathetic supersensitivity compared to controls. Most of our PD patients (34/40, or 85%) were at a relatively advanced stage representing disease at least as severe as in H–Y Stage III. This might contribute to differences between previous and present reports. In addition, pupillary sensitivity to 0.02% DPE correlated with age at examination as well as disease duration, suggesting that pupillary sympathetic impairment worsened with PD progression.

It has been suggested that a measure of papillary sympathetic innervation could be found in the difference between phenylephrine- and cocaine-induced mydriasis, Sawada et al. [20]. Longer and thinner axons have been suggested to be relatively vulnerable in PD [2]. Postganglionic sympathetic nerve fibers are characteristically thin and unmyelinated. The pupillary sympathetic system is derived from the superior cervical ganglion and follows a longer course than parasympathetic fibers derived from the ciliary ganglion. In general, cocaine blocks norepinephrine uptake and cocaine-induced mydriasis is dependent on the sympathetic nerve terminal density. In contrast, phenylephrine acts directly on adrenergic receptors to cause mydriasis. This relationship correlated significantly with a reduction of ^{123}I-MIBG cardiac accumulation in patients with PD. Considering that α-synuclein inclusions have been recognized in sympathetic ganglion cells [21], the result would seem

Table 2 Scores for visual disturbance in Parkinson's disease patients with and without supersensitivity to eye drops

	To PL		To DPE		To both PL and DPE	
	Supersensitivity +(n = 12)	Supersensitivity −(n = 28)	Supersensitivity +(n = 19)	Supersensitivity −(n = 21)	Supersensitivity +(n = 7)	Supersensitivity −(n = 33)
Blurred vision	1.7 ± 1.1	1.3 ± 1.0	1.7 ± 1.0	1.2 ± 1.0	2.3 ± 0.5*	1.2 ± 1.0
Photophobia	0.8 ± 1.0	0.5 ± 0.8	0.6 ± 0.8	0.6 ± 0.9	0.7 ± 0.8	0.5 ± 0.9
Night blindness	0.8 ± 0.8	0.5 ± 0.8	0.6 ± 0.9	0.5 ± 0.8	1 ± 0.8	0.5 ± 0.8
Involuntary eyelid closure in response to light	0.3 ± 0.2	0.3 ± 0.3	0.4 ± 0.7	0.1 ± 0.3	0.3 ± 0.5	0.2 ± 0.6

PL pilocarpine hydrochloride, *DPE* dipivefrine hydrochloride
* $P < 0.05$ versus without supersensitivity

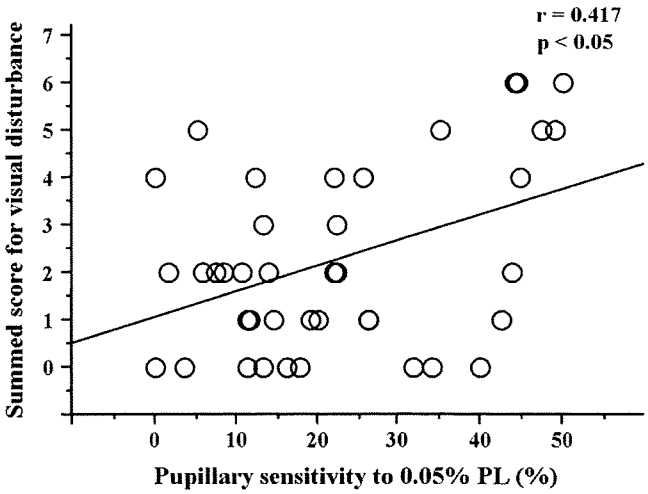

Fig. 3 Correlation between pupillary sensitivity for 0.05% PL and summed score of visual disturbance. In patients with Parkinson's disease, pupillary sensitivity to 0.05% PL correlated significantly and positively, with a summed score for visual subjective disturbance. PL, pilocarpine hydrochloride

reasonable since the dilator muscle of the pupil receives innervation from the cervical sympathetic ganglia, similar to cardiac muscle. However, pupillary sympathetic supersensitivity did not correlate with a reduction of ^{123}I-MIBG cardiac accumulation in this study. The different pharmacological mechanisms of DPE from phenylephrine and cocaine may contribute to these different results. DPE has a relatively high corneal permeability [22]. In the ophthalmologic field, DPE is often used. Furthermore, phenylephrine, cocaine and DPE are all sympathomimetic agents; however, these three drugs have different pharmacological mechanisms on α and β sympathetic receptors [22]. Nevertheless, further investigation is necessary to determine whether the eye-drop test could substitute for myocardial ^{123}I-MIBG scintigraphy in the diagnosis of PD.

The results of the present study showed that the frequency of blurred vision was greater in PD patients than in controls and that the extent of sensitivity to PL, but not DPE, correlated with a summed score concerning visual subjective disturbance. Thus, while both parasympathetic and sympathetic pupillary supersensitivity were recognized in PD patients, only parasympathetic sensitivity correlated with the summed score for visual disturbance. The pupil functions by regulating the amount of light transmitted to the retina, which influences retinal image quality [3]. In general, parasympathetic innervation is of primary importance in focusing the image, while sympathetic innervation is limited to a supporting role in this function [27]. In addition, postganglionic fibers in short ciliary nerves derived from the Edinger–Westphal nucleus supply both the pupillary sphincter and the ciliary muscle, which changes the thickness of the lens [1]. Based upon these observations, cholinergic disturbance in the ocular system appears chiefly responsible for visual disturbance in patients with PD. However, patients with supersensitivity to both PL and DPE noted significantly more blurring of vision than those with only PL supersensitivity. This observation suggests that sympathetic involvement also appears to contribute to some extent to blurred vision in patients with PD.

A limitation of this study is that we did not assess the effect of corneal permeability, which influences the pharmacologic reactivity of the pupil. Tamer et al. [25] reported that PD patients showed abnormal lachrymal function and a decreased blink rate compared to an age-matched control group. Both of these factors can influence corneal permeability. No past reports have directly examined corneal permeability in PD patients, therefore, further studies are necessary.

In the present study, the daily dose of antiparkinsonian drug was continued. Gottlob et al. [7] demonstrated in their study that levodopa influenced dark adaption thresholds, but did not alter in pupil size. In our study, we measured pupillary dynamics using an infrared Iriscorder after 5 minutes of dark adaptation. In addition, in our study, pupillary sensitivity to PL and DPE was not influenced by L-dopa dose or the use of an anticholinergic drug, in agreement with the results of a previous report by Sugiyama and Utsumi [23] showing that antiparkinsonian medication had no statistically significant influence on pupillary characteristics. Thus, orally administrated levodopa was regarded as not affecting pupillary dynamics.

In conclusion, eye-drop tests are an inexpensive way to assess peripheral autonomic dysfunction affecting the pupil. It is recommended that PD patients who complain of visual disturbance should be given such tests, when routine ophthalmologic examinations do not identify a cause. This study identified the optimal concentrations of drugs in the eye-drop test for PD patients and controls and the time interval of this test. By means of this method, we evaluated pupillary change in response to drugs in PD patients and controls. In this study, we found (1) pupillary sympathetic supersensitivity did not correlate with a reduction of ^{123}I-MIBG cardiac accumulation, (2) pupillary sensitivity to 0.02% DPE correlated significantly with age at examination and with disease duration, although sensitivity to 0.05% PL did not show such correlations, and (3) parasympathetic dysfunction contributes to visual disturbance in PD.

Appendix

Visual subjective disturbance scale in this study

Please read the following questions and try to answer on the basis of how you feel in daily life.

A. Blurred vision: during the day, how often do you feel blurred vision?

0 Never
1 Sometimes, but it does not bother me
2 Often, so I have trouble seeing
3 Always

B. Photophobia: how much do you feel photophobia?

0 Never
1 Sometimes, but it does not bother me
2 Often, so I have trouble seeing
3 Always

C. Night blindness: in darkness, do you have no trouble seeing?

0 No, I have any trouble seeing
1 Yes, I sometimes have trouble seeing in darkness, but it does not bother me
2 Yes, I often have trouble seeing in darkness
3 Yes, I cannot seeing in darkness

D. Involuntary eyelid closure in response to light: how often do you have involuntary eyelid closure in response to light?

0 Never
1 Sometimes, but it does not bother me
2 Often, so I have difficulty in some of daily performance
3 Always, so I have difficulty in almost all of daily performance

References

1. Appenzeller O, Oribe E (1997) The pupil. In: Appenzeller O, Oribe E (ed) The autonomic nervous system. An introduction to basic and clinical concepts, 5th edn. Elsevier, Amsterdam, pp 443–466.
2. Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318:121–134.
3. Buehren T, Collins MJ (2006) Accommodation stimulus-response function and retinal image quality. Vision Res 46:1633–1645.
4. Cahill M, Eustace P, de Jesus V (2001) Pupillary autonomic denervation with increasing duration of diabetes mellitus. Br J Ophthalmol 85:1225–1230.
5. Daniel SE, Lees AJ (1993) The clinical features of Parkinson's disease in 100 histologically proven cases. Adv Neurol 60:595–599.
6. Forno LS (1996) Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 55:259–272.
7. Gottlob I, Strenn K, Schneider B (1994) Effect of levodopa on human dark adaption threshold. Grafes Arch Clin Exp Ophthalmol 232:584–588.
8. Granholm E, Morris S, Galasko D, Shults C, Rogers E, Vukov B (2003) Tropicamide effects on pupil size and pupillary light reflexes in Alzheimer's and Parkinson's disease. Int J Psychophysiol 47:95–115.
9. Hakusui S, Yasuda T, Yanagi T, Tohyama J, Hasegawa Y, Koike Y, Hirayama M, Takahashi A (1994) A radiological analysis of heart sympathetic functions with meta-[123I]iodobenzylguanidine in neurological patients with autonomic failure. J Auton Nerv Syst 49:81–84.
10. Hamada K, Hirayama M, Watanabe H, Kobayashi R, Ito H, Ieda T, Koike Y, Sobue G (2003) Onset age and severity of motor impairment are associated with reduction of myocardial 123I-MIBG uptake in Parkinson's disease. J Neurol Neurosurg Psychiatry 74:423–426.
11. Hunter S (1985) The rostral mesencephalon in Parkinson's disease and Alzheimer's disease. Acta Neuropathol 68:53–58.
12. Jellinger KA (1999) Post mortem studies in Parkinson's disease—is it possible to detect brain areas for specific symptoms? J Neural Transm Suppl 56:1–29.
13. Kesler A, Korczyn A (2006) Visual disturbances in Parkinson's disease. Pract Neurol 6:28–33.
14. Korczyn AD (1990) Autonomic nervous system disturbances in Parkinson's disease. Adv Neurol 53:463–468.
15. Matthews MR (1999) Autonomic ganglia and preganglionic neurones in autonomic failure. In: Bannister R, Mathias CJ (eds) Autonomic failure. A textbook of clinical disorders of the autonomic nervous system, 4th edn. Oxford University Press, pp 329–339.
16. Micieli G, Tassorelli C, Martignoni E, Pacchetti C, Bruggi P, Magri M, Nappi G (1991) Disordered pupil reactivity in Parkinson's disease. Clin Auton Res 1:55–58.
17. Micieli G, Tosi P, Marcheselli S, Cavallini A (2003) Autonomic dysfunction in Parkinson's disease. Neurol Sci 24 (suppl 1):S32–S34.
18. Niimi Y, Ieda T, Hirayama M, Koike Y, Sobue G, Hasegawa Y, Takahashi A (1999) Clinical and physiologic characteristics of autonomic failure with Parkinson's disease. Clin Auton Res 9:139–144.
19. Orimo S, Oka T, Miura H, Tsuchiya K, Mori F, Wakabayashi K, Nagao T, Yokochi M (2002) Sympathetic cardiac denervation in Parkinson's disease and pure autonomic failure but not in multiple system atrophy. J Neurol Neurosurg Psychiatry 73:776–777.
20. Sawada H, Yamakawa K, Yamakado H, Hosokawa R, Ohba M, Miyamoto K, Kawamura T, Shimohama S (2005) Cocaine and phenylephrine eye drop test for Parkinson disease. JAMA 293:932–934.
21. Schlossmacher MG, Frosch MP, Gai WP, Medina M, Sharma N, Forno L, Ochiishi T, Shimura H, Sharon R, Hattori N, Langston JW, Mizuno Y, Hyman BT, Selkoe DJ, Kosik KS (2002) Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. Am J Pathol 160:1655–1667.

22. Smith SA, Smith SE (1999) Investigation of autonomic disorders. In: Bannister R, Mathias CJ (eds) Autonomic failure. A textbook of clinical disorders of the autonomic nervous system, 4th edn. Oxford University Press, pp 245–253.
23. Sugiyama T, Utsumi T (1990) Pupillary dynamics in Parkinson's disease. Neuroophthalmology 10:1–7.
24. Takeda S, Yamazaki K, Miyakawa T, Arai H (1993) Parkinson's disease with involvement of the parasympathetic ganglia. Acta Neuropathol 86:397–398.
25. Tamer C, Melek IM, Duman T, Oksuz H (2005) Tear film tests in Parkinson's disease patients. Ophthalmology 112:1795.
26. Utsumi T (2000) Pharmacological eye drop test. In: Japan Society of Neurovegetative Research (ed) Autonomic function test, 3rd edn. Bunkodo, pp 272–275.
27. Walshe, Hoyt W (1984) The Pupil: Embryology, anatomy, innervation and normal function. In: Miller (ed) Clinical neuro-ophthalmology, 4th edn. Williams & Wilkins, Baltimore, pp 400–441.

of cocontraction such as in Case 2. However, it has also been suggested that clonus arises from the action of a central oscillator within the spinal cord, which would rhythmically activate the α-motoneuron pathways.[2] Dimitrijevic noted that clonus was only present when the lateral corticospinal tract was lesioned.[4] Therefore, we could also suggest that the cervical myelopathy and the capsular thalamic stroke presented by our cases could be responsible for a deprivation of supraspinal control of the stretch reflex loops.

The 2 cases we report are very similar to the early description of "provoked trepidation" or clonus as part of the upper motor neuron syndrome by Charcot, in the 19th century.[10–12] He had observed that the ankle clonus or "trepidation in hemiplegic patients possessing some slight power of movement" might "also manifest itself in consequence of a voluntary movement" and that "an analogous phenomenon" was "occasionally produced when the hand of a hemiplegic patient was suddenly lifted up by the tips of the fingers" or when "patients raised on their paralyzed arm." The "trembling was similar to that which occurred in the lower limb" but was "much more uncommon than the corresponding effect which was called the foot-phenomenon." At that time, Charcot already considered that the "phenomenon in question was reflex" and "belonged to the semeiotics of spinal affections." Even though action tremor is usually observed in cerebellar, task-specific, dystonic, or Holmes tremor, and has been already described as a possible manifestation of a partial lesion of the descending motor pathways, these 2 videotaped cases show that action-induced clonus, particularly on the UL, may be misdiagnosed in clinical practice.[12,13] Both symptoms may be very similar and the differential diagnosis should be considered by clinicians, especially in patients with moderate spasticity.

LEGENDS TO THE VIDEO

Segment 1. Postural and kinetic tremor of Patient 1.

Segment 2. Clinical features of the pyramidal syndrome of Patient 1.

Segment 3. Isometric tremor of Patient 2.

Segment 4. Clinical features of the pyramidal syndrome of Patient 2.

REFERENCES

1. Deuschl G, Raethjen J, Lindemann M, Krack P. The pathophysiology of tremor. Muscle Nerve 2001;24:716–735.
2. Dimitrijevic MR, Sherwood A, Nathan PW. Clonus: peripheral and central mechanisms. In: Desmedt JE, editor. Progress in clinical neurophysiology. Basel: Karger; 1978. p 173–182.
3. Deuschl G, Koster B, Lucking CH, Scheidt C. Diagnostic and pathophysiological aspects of psychogenic tremors. Mov Disord 1998;13:294–302.
4. Dimitrijevic MR, Nathan PW, Sherwood AM. Clonus: the role of central mechanisms. J Neurol Neurosurg Psychiatry 1980;43:321–332.
5. Dimitrijevic MR, Nathan PW. Studies of spasticity in man. II. Analysis of stretch reflexes in spasticity. Brain 1967;90:333–358.
6. Dimitrijevic MR, Nathan PW. Studies of spasticity in man. I. Some features of spasticity Brain 1967;90:1–30.
7. Young RR. Spasticity: a review. Neurology 1994;44(11, Suppl 9):S12–S20.
8. Rack PM, Ross HF, Thilmann AF. The ankle stretch reflexes in normal and spastic subjects. The response to sinusoidal movement. Brain 1984;107 (Part 2):637–654.
9. Rothwell J. Investigating reflex pathways and their functions. In: Rothwell J, editor. Control of human voluntary movement. London: Chapman & Hall; 1994.p 153–216.
10. Charcot J. Onzième leçon: sémiologie générale des dégénérations secondaires du faisceau pyramidal. In: Médical LP, editor. Leçons sur les localisations dans les maladies du cerveau. Paris: Adrien Delahaye éditeurs; 1876. p 287–297.
11. Corcos DM, Gottlieb GL, Penn RD, Myklebust B, Agarwal GC. Movement deficits caused by hyperexcitable stretch reflexes in spastic humans. Brain 1986;109 (Part 5):1043–1058.
12. Pearce JM. Charcot on "provoked trepidation", or clonus. J Neurol Neurosurg Psychiatry 2003;74:1277.
13. Deuschl G, Bergman H. Pathophysiology of nonparkinsonian tremors. Mov Disord 2002;17 (Suppl 3):S41–S48.

The Video Images of Sleep Attacks in Parkinson's Disease

Masaaki Hirayama, MD, PhD,*
Tomohiko Nakamura, MD, PhD, Norio Hori, MD,
Yasuo Koike, MD, PhD, and Gen Sobue, MD, PhD

Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

Video 

Abstract: **We describe a sleep attack, which was induced by taking excessive levodopa and pergolide, in a 73-year-old woman with Parkinson's disease. At the onset of the sleep attack, her head suddenly sagged and sometimes hit the table, but she did not notice these symptoms. Her family noticed that this sleep attack occurred when she began to speak slowly. Her family recorded this attack with a video camera. This sleep attack resolved with control of her medication. This is the first report of video images of a sleep attack due to excessive levodopa and a dopamine agonist.**

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This article includes supplementary video clips, available online at http://www.interscience.wiley.com/jpages/0885-3185/suppmat.

*Correspondence to: Masaaki Hirayama, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan. E-mail: hirasan@med.nagoya-u.ac.jp

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