

Hiroki Takahashi · Kinya Ishikawa · Takeshi Tsutsumi
Hirotoshi Fujigasaki · Akihiro Kawata · Ryoichi Okiyama
Tsuneo Fujita · Kazuo Yoshizawa · Shigeki Yamaguchi
Hitoshi Tomiyasu · Fumihito Yoshii · Kazuko Mitani
Natsue Shimizu · Mineo Yamazaki · Tomoyuki Miyamoto
Tomoyuki Orimo · Shin'ichi Shoji · Ken Kitamura
Hidehiro Mizusawa

A clinical and genetic study in a large cohort of patients with spinocerebellar ataxia type 6

Received: 4 December 2003 / Accepted: 25 February 2004 / Published online: 8 April 2004
© The Japan Society of Human Genetics and Springer-Verlag 2004

Abstract In order to clarify the clinical and genetic features of SCA6, we retrospectively analyzed 140 patients. We observed an inverse correlation between the age of onset and the length of the expanded allele, and also between the age of onset and the sum of CAG repeats in the normal and the expanded alleles. The ages of onset of four homozygous patients correlated better with the sum of CAG repeats in both alleles rather than with the expanded allele calculated from heterozygous SCA6 subjects. Clinically, unsteadiness of gait was the main initial symptom, followed by vertigo and oscillopsia, and cerebellar signs were detected in nearly 100% of the patients. In contrast, extracerebellar signs were relatively mild and infrequent. The results of neuro-otological

examination performed in 22 patients suggested the purely cerebellar abnormalities of ocular movements in nature. There was a close relationship between downbeat positioning nystagmus (DPN) and positioning vertigo, which became more common in the later stage. We conclude that total number of CAG repeat-units in both alleles is a good parameter for assessment of age of onset in SCA6 including homozygous patients. In addition, clinical and neuro-otological examinations suggested that SCA6 is a disease with predominantly cerebellar dysfunction.

Keywords SCA6 · Spinocerebellar degeneration · Ataxia · Downbeat positioning nystagmus · Vertigo

H. Takahashi · K. Ishikawa · H. Fujigasaki · H. Mizusawa (✉)
Department of Neurology and Neurological Science, Tokyo
Medical and Dental University Graduate School of Medicine,
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
E-mail: h-mizusawa.nuro@med.tmd.ac.jp
Tel.: +81-3-58035233
Fax: +81-3-58030134

T. Tsutsumi · K. Kitamura
The Department of Audio-Vestibular Neuroscience,
Tokyo Medical and Dental University, Tokyo, Japan

A. Kawata · R. Okiyama
The Department of Neurology,
Tokyo Metropolitan Neurological Hospital, Tokyo, Japan

T. Fujita
The Department of Neurology, Hitachi General Hospital,
Ibaraki, Japan

K. Yoshizawa
The Department of Neurology, Mito National Hospital,
Mito, Japan

S. Yamaguchi
The Department of Neurology,
Yokohama City University Medical Center,
Yokohama, Japan

H. Tomiyasu · F. Yoshii
The Department of Neurology,
Tokai University School of Medicine, Isehara, Japan

K. Mitani
The Department of Neurology,
Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan

N. Shimizu
The Department of Neurology,
Teikyo University School of Medicine,
Ichihara Hospital, Chiba, Japan

M. Yamazaki
The Second Department of Internal Medicine,
Nihon Medical School, Tokyo, Japan

T. Miyamoto
The Department of Neurology, Dokkyo Medical School,
Tochigi, Japan

T. Orimo
The Department of Neurology, Kanto Chuo Hospital,
Tokyo, Japan

S. Shoji
The Department of Neurology, Institute of Clinical Medicine,
University of Tsukuba, Tsukuba, Japan

Introduction

Spinocerebellar ataxia type 6 (SCA6) is one of the autosomal dominant cerebellar ataxias (ADCAs), and its main clinical characteristic is slowly progressive ataxia consistent with ADCA type III (Harding 1982). SCA6 is caused by the expansion of CAG repeats in the gene for the α_{1A} (P/Q type) subunit of the voltage-gated calcium channel, which is located on the short arm of chromosome 19 (Zhuchenko et al. 1997). The specific feature of SCA6 is the small expansion in CAG repeats, which is usually within normal range in other polyglutamine diseases. Since the length of CAG repeat in the normal allele is quite close to that of the expanded allele, the normal allele may have an additional effect on the pathogenesis in SCA6, as has been suggested by a *in vitro* study (Kubodera et al. 2003).

In Japan, SCA6 seems to be either the most common or second most common subtype of the SCAs (Matsumura et al. 1997; Watanabe et al. 1998; Sasaki et al. 2000; Maruyama et al. 2002), but its prevalence is lower in Western Europe and North America (Geschwind et al. 1997; Schöls et al. 1997, 1998; Stevanin et al. 1997). In contrast, other dominantly inherited pure cerebellar ataxias seem to be infrequent in Japan, including SCA5 (Holmberg et al. 1995), SCA10 (Zu et al. 1999), SCA11 (Worth et al. 1999), SCA14 (Yamashita et al. 2000), SCA15 (Knight et al. 2003), SCA16 (Miyoshi et al. 2001), and SCA22 (Chung et al. 2003). There is also another SCA that has the clinical presentation of ADCA type III linked to chromosome 16q (Takashima et al. 2001; Li et al. 2003).

The main clinical feature of SCA6 is slowly progressive cerebellar ataxia, but extracerebellar symptoms, such as pyramidal tract signs, abnormal involuntary movements, parkinsonism, hyporeflexia, intellectual impairment, and urinary incontinence, have also been reported (Geschwind et al. 1997; Gomez et al. 1997; Ikeuchi et al. 1997; Matsumura et al. 1997; Schöls et al. 1997; Stevanin et al. 1997; Watanabe et al. 1998; Yabe et al. 1998). The induction of vertigo and oscillopsia by changes of the head position can also occur in SCA6 patients. However, these extracerebellar features have been reported with different frequencies (Geschwind et al. 1997; Gomez et al. 1997; Harada et al. 1998; Sinke et al. 2001; Durig et al. 2002; Yabe et al. 2003).

Therefore, the present study was performed to clarify the genetic, clinical and neuro-otological characteristics of SCA6 on a large cohort of patients molecularly diagnosed in our department from 1997 to 2002. Although this is a retrospective study, we set out this study firstly by defining terms and obtaining clinical information by board-certified neurologists through a standard questionnaire. Then, the data were collected and the frequencies of various symptoms were calculated. Detailed neuro-otological tests were also performed in our institution.

Materials and methods

Genetic testing for SCA6

Genomic DNA was extracted from peripheral blood leukocytes after obtaining informed consent. The CAG repeat in the α_{1A} subunit of the voltage-dependent calcium channel (α_{1A} Ca-channel subunit) gene was amplified by the polymerase chain reaction using S5 primers (Zhuchenko et al. 1997) and the reaction products were analyzed using an automated DNA sequencer (Pharmacia Biotech, Sweden) (Ishikawa et al. 1997).

We analyzed the relationship between age at onset and the number of CAG repeats in both the expanded allele alone and the total repeats number of the normal plus expanded alleles. The reason for this analysis is that the effect of normal allele may not be neglected because of the shortness of the CAG repeat length even in the expanded allele. Additionally, both SCA6 patients and normal individuals were reported to have the intermediate CAG repeats number of 19–20 (Ishikawa et al. 1997; Katayama et al. 2000; Mariotti et al. 2001), and these findings cannot be explained only by the effect of the expanded allele.

Evaluation of clinical and genetic features

The clinical and genetic features of the SCA6 patients diagnosed at our department were retrospectively analyzed. For each patient, the age at onset, duration of disease, symptoms (including the presenting symptom), present status, and existence of specific features were evaluated. The specific features included cerebellar signs (truncal ataxia, limb ataxia, hypotonia, and cerebellar dysarthria), vertigo (typically rotating or sometimes dizzy feeling), oscillopsia (sense of oscillating vision), diplopia (double vision), dysphagia, episodic symptoms (abrupt onset or fluctuation of specific symptoms and migraine, e.g. ataxia or headache), abnormality in tendon reflex, pathological reflexes, abnormal involuntary movements, parkinsonism, intellectual impairment, and urinary incontinence. Questionnaires for these features were sent to board-certified neurologists who had been seeing patients diagnosed as SCA6 in our department from 1997 to 2002. The detailed clinical information was available for 140 patients from 110 families. The results were then analyzed statistically. The results of magnetic resonance imaging (MRI) of the brain were reviewed, and complications that could influence the neurological findings (such as diabetes mellitus, cerebrovascular disease and cervical spondylosis) were assessed in each patient.

Neuro-otological testing

Neuro-otological examination was performed in 22 patients at the Department of Audio-Vestibular

Neuroscience of Tokyo Medical and Dental University. Eye movements were recorded by electronystagmography and the subjects were assessed for tracking, gaze-evoked nystagmus, positional and positioning nystagmus, and optokinetic nystagmus (OKN). The caloric test was also performed. Abnormal findings were defined as a decreased velocity of saccadic movements, ocular dysmetria, saccadic pursuit, gaze-evoked nystagmus, rebound nystagmus, positional nystagmus, a poor OKN response, and positioning vertical or positioning nystagmus evoked by changes of head position, as well as poor caloric response and impaired visual suppression of the caloric nystagmus. Positioning nystagmus was assessed by moving the subject from the sitting position to the supine position with the head hanging down. Each of the findings was compared between patients with and without positioning vertigo, i.e. vertigo induced by changing of head position (Tsutsumi et al. 2001).

Statistical analysis

The relationship between the age at onset and the number of CAG repeats in the α_{1A} Ca-channel subunit gene was assessed by Pearson's correlation coefficient analysis. The frequency of each neuro-otological finding in patients with and without positioning vertigo was compared using Fisher's exact test.

The subjects were divided into two groups according to the disease duration from the onset to the time of examination, which were group A, with a disease duration of less than 5 years, and group B, with a disease duration of longer than 5 years. Then the frequency of each neuro-otological finding was compared between patients with a short and long duration of illness using Fisher's exact test. The disease duration was also compared between groups of subjects who were positive and negative for each neuro-otological feature using the Wilcoxon rank-sum test.

Results

Correlation between age of onset and number of CAG repeats in the α_{1A} Ca-channel subunit gene

One hundred and forty SCA6 patients had expansion of CAG repeats in the α_{1A} (P/Q type) Ca-channel subunit gene, ranging from 20 to 28 repeats. The number of CAG repeats in the normal allele ranged from 10 to 19. Information about the age of onset was considered reliable in 79 heterozygous and four homozygous subjects, in whom the age at onset ranged from 20 to 73 years (mean: 48.9 ± 11.6 years) (Fig. 1). For heterozygous patients, there was a strong inverse correlation between the age at onset and the number of CAG repeats in the expanded allele ($r = -0.635$; $P < 0.0001$), as has been described elsewhere. When the relationship

between the age at onset and the total number of CAG repeats of the normal and expanded alleles was analyzed, a significant inverse correlation was also observed. ($r = -0.479$; $P < 0.0001$) (Fig. 1). The two correlation coefficients (r) were not statistically different.

Four patients were observed homozygous for the expansion of CAG repeats in this gene [patient A (21 repeats/21 repeats), patient B (21 repeats/22 repeats), patient C (21 repeats/21 repeats) and patient D (21 repeats/21 repeats)]. Patient A showed a slightly earlier age of onset (37 years) than the 95% confidence interval, ranging between 38.7 and 75.8 years, calculated from the 79 heterozygous patients. However, the remaining three patients, namely, patient B (age of onset at 45 years), patient C (age of onset at 58 years), and patient D (age of onset at 52 years), were within the 95% confidence interval. In contrast, all homozygous patients were within the 95% confidence interval of age calculated with the sum of CAG repeats of both alleles (Fig. 1).

Clinical characteristics

The most frequent initial symptom was unsteadiness of gait (78%). Vertigo or oscillopsia were often difficult to separate based on the history obtained from the patients through questionnaires. When both were combined, vertigo/oscillopsia became the second most frequent initial symptom (12%). The clinical characteristics of the 140 patients in the present study were compared with those of patients from eight other studies in Table 1.

Ataxia of gait and stance was present in 99% of the patients, while limb ataxia and dysarthria were present in 93.5 and 94.1%, respectively. Nystagmus was present in 66.6% of the patients and vertical nystagmus was observed in 72.7% of the patients with nystagmus (48% of all patients). Dysphagia occurred in 8% of the patients. Vertigo, positional vertigo, and oscillopsia were reported in 8.8, 4.3, and 4.3%, respectively. Episodic features were infrequent, with episodic ataxia and migraine only being seen in 0.7 and 1.4% of the patients, respectively. A minority of the patients had symptoms and signs other than cerebellar features. Among pyramidal tract signs, hyperreflexia was present in 6.0% and extensor plantar response (Babinski's sign) was seen in 1.4% of the patients. Hyporeflexia was observed in 15.2% of the patients. Abnormal involuntary movements were relatively infrequent, with tremor occurring in 8.6% and myoclonus in 0.7%. Urinary incontinence was noted in 2.1% of the patients and intellectual impairment was found in 0.7%.

The mean age at examination of the subjects with dysphagia and hyporeflexia was older than the mean age for all of the patients. Hyperreflexia was observed in all of the patients who had dysphagia, but in only 7.7% of those without it. Nerve conduction studies showed normal findings even in patients with hyporeflexia, except for three who also had diabetes mellitus. The only exception we experienced was a female patient with the longest CAG expansion in our cohort (13/28) who had

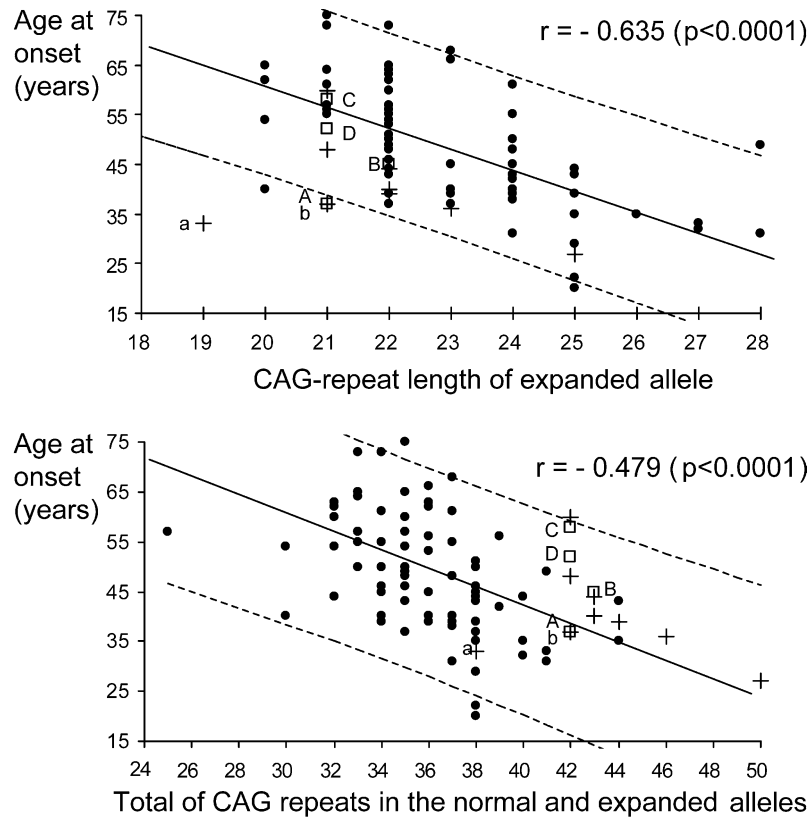


Fig. 1 Relationship between age at onset and the number of CAG repeats in both the expanded allele alone and the total repeats number of the normal plus expanded alleles. There were strong inverse correlations between the age at onset and the number of CAG repeats in both the expanded allele alone ($r = -0.635$, $P < 0.0001$, $n = 79$) and the total number of CAG repeats of the normal plus expanded alleles ($r = -0.479$, $P < 0.0001$, $n = 79$), and the difference between these r was not significant. Only one (A: 21/21) of the four homozygous patients in this study (B, C, D) and two

homozygous patients in the past reports (a: 19/19, b: 21/21) showed an earlier age of onset than the 95% confidence limit for heterozygous patients with the same number of CAG repeats in the expanded allele. The age at onset for no homozygous patients was earlier than the 95% confidence interval for heterozygous patients with the same total number of repeats in the normal and expanded alleles. *Open square* homozygous cases in this study, *plus sign* homozygous cases in the past reports, *filled circle* heterozygous cases, *dotted line* 95% confidence interval

an evidence for sensory axonal polyneuropathy with no obvious etiology. Detailed clinical information of this patient will be described separately.

Among the homozygous patients, patient A had severe clinical manifestations, including an earlier onset and frequent episodes of positioning vertigo, blurred vision, dysphagia, dysarthria, and gait disturbance.

There were 14 families with more than two family members. The largest family had 15 affected members. Some differences among families seemed to be in the mean age at onset and in the mean CAG repeat numbers of the expanded alleles, but these differences were not significant. In addition, some particular symptoms seemed to be present in some families, such as tremor or hyporeflexia.

Neuro-otological findings

Table 2 summarizes the results of neuro-otological examinations. Because the number of patients performed differed from test to test, the denominator

number of each finding varied. None of the nine patients tested showed a reduced velocity of saccades, but 13 out of 14 showed dysmetria. Saccadic pursuit was observed in all 18 subjects tested. Horizontal gaze-evoked nystagmus was present in 19 out of 22 patients tested and vertical gaze-evoked nystagmus was present in 14 out of 22 patients. Rebound nystagmus was found in eight out of 11 subjects tested. Positional nystagmus was found in 12 out of 15 subjects tested. Positioning vertical nystagmus, mainly downbeat positioning nystagmus (DPN) (Fig. 2), was elicited in 11 out of 16 patients tested and positioning horizontal nystagmus was elicited in eight of the 16 patients. Spontaneous downbeat nystagmus was found in two out of 11 subjects tested. The OKN response was impaired in all 16 subjects examined. In the caloric test, none of the 12 subjects tested showed weak caloric response, but visual suppression of caloric nystagmus was impaired in nine out of 12 subjects. Positioning vertigo was detected in seven out of 18 tested subjects and oscillopsia was found in four out of 15 subjects.

When neuro-otological findings were compared between the patients with and without positioning

Table 1 Clinical characteristics of SCA6

	Our data	Schöls	Geschwind	Stevanin	Gomez	Ikeuchi	Matsumura	Yabe	Watanabe
No. of patients	140	27	12	12	34	48	35	43	7
Age at onset	48.9	30–71		24–67	24–61	35–67	28–73	19–69	
No. of CAG repeats	20–28		22–25	22–28	22–26	21–26	21–29	21–33	
<i>Symptoms (%)</i>									
Cerebellar dysfunction									
Ataxia of gait and stance	99.0	100	100	100	100	100	94	100	100
Limb ataxia	93.5	100	100				100	100	100
Dysarthria	94.1	100	92	58.3	88	96	80	100	100
Nystagmus	66.6	96	83		96.7	90	> 71	88.3	86
Vertigo	8.8				62				
Positioning vertigo	4.3		42						
Oscillopsia	4.3								
Episodic ataxia	0.7		33		17				
Headache	1.4				25				
Ocular disorders									
Ophthalmoplegia	2.8	24		8.3			29		
Diplopia	2.8	24			44				
Dysphagia	8.0	53		33.3	65		26		
Pyramidal signs									
Hyperreflexia	6.0	30	33		50	21	17		29
Spasticity	3.8	35					3		
Babinski's sign	1.4	0			41.7	6	3	7	43
Parkinsonism									
Akinesia	0.0								
Rigidity	0.7						6		
Abnormal involuntary movements									
Tremor	8.6	4				23			43
Choreiform hyperkinesia	0.0								
Dystonia	0.7	0				15			
Myoclonus	0.7								14
Neuropathy		57	16						
Hyporeflexia	15.2	22		25	14.7	25	54	9.3	57
Amyotrophy	0.0								
Impaired vibration sense	4.3	57	58			8	43	18.6	29
Impaired thermal sense	1.4								
Intellectual impairment	0.7	0			5.9	8			
Urinary incontinence	2.1	6				6	6		

Table 2 Neuro-otological findings in the SCA6 patients. Because the number of patients performed each finding differed, the denominator number of findings varied

	No.(+)/no. tested (%)
Saccades	
Reduced velocity	0/9 (0)
Dysmetria	13/14 (92.8)
Saccadic pursuit	18/18 (100)
Gaze-evoked nystagmus	
Horizontal	19/22 (86.3)
Vertical	14/22 (63.6)
Rebound	8/11 (72.7)
Positional nystagmus	12/15 (80)
Positioning nystagmus	
Horizontal	8/16 (50)
Vertical (DPN)	11/16 (68.8)
Spontaneous downbeat nystagmus	2/11 (18.1)
OKN abnormality	16/16 (100)
Caloric test	
Impaired visual suppression	9/12 (66.7)
Caloric weakness	0/12 (0)
Positioning vertigo	7/18 (38.9)
Oscillopsia	4/15 (26.6)

vertigo, the seven patients with positioning vertigo showed a significantly higher prevalence of DPN than the nine patients without such vertigo (7/7 versus 4/9, $P < 0.05$) (Table 3).

Group B, with a longer duration of disease, also had a significantly higher prevalence of positioning vertigo ($P < 0.001$) than group A with a shorter duration (Table 4). When the patients who were positive and negative for each neuro-otological feature were compared, the mean duration of disease was significantly longer in patients with positioning vertigo ($P < 0.01$), positional nystagmus ($P < 0.01$) or horizontal gaze-evoked nystagmus ($P < 0.05$), than in patients without these features (Table 4). The mean age at onset and the mean age at examination of group A with a disease duration of less than 5 years were 50.5 and 51.5, and those of group B, with a disease duration of longer than 5 years, were 52.1 and 57.1, respectively. The differences in the mean ages at onset or the mean ages at examination between group A and B were not significant.

Fig. 2 Downbeat positioning nystagmus was induced by moving the patient from the sitting to the supine position with the head hanging down

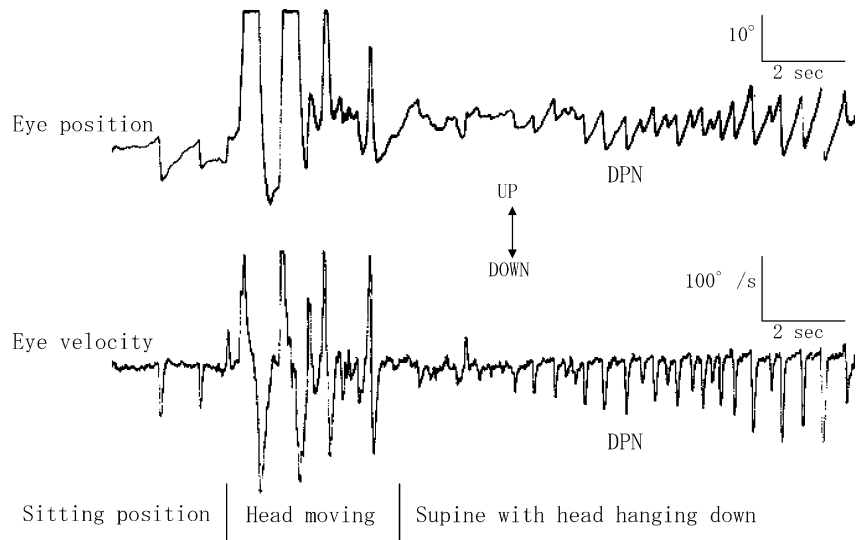


Table 3 Neuro-otological findings compared between SCA6 patients with and without positioning vertigo

Positioning vertigo	Vertigo (+)/ tested (%) (n = 7)	Vertigo (-)/ tested (%) (n = 12)
Saccades		
Dysmetria	5/6 (83)	4/4 (100)
Gaze-evoked nystagmus (GEN)		
Horizontal GEN	7/7 (100)	7/11 (64)
Vertical GEN	5/7 (71)	3/11 (27)
Rebound nystagmus	5/6 (83)	3/6 (50)
Positional nystagmus	7/7 (100)	5/8 (63)
Positioning nystagmus		
Horizontal	5/7 (71)	3/9 (33)
Vertical (DPN)	7/7* (100)	4/9 (44)
Spontaneous downbeat nystagmus	1/7 (14)	1/5 (20)
Caloric test		
Impaired visual suppression	5/7 (71)	4/5 (80)
Caloric weakness	0/7 (0)	0/5 (0)
Oscillopsia	1/6 (17)	2/6 (33)

* $P < 0.05$, vs vertigo (-) group

Discussion

This was a clinical and genetic analysis on a large number of patients with SCA6. The aim of this study was to define the genetic, clinical and neuro-otological features of SCA6.

Genetic aspects

The present study showed that not only the length of expanded CAG repeat but also the sum of CAG repeat-units in normal and the expanded alleles inversely correlate with the age of onset of SCA6 patients. This is, to our knowledge, the first time that the relationship between age of onset and the sum of repeat numbers on both chromosomes, among polyglutamine diseases, has been examined.

The 19 CAG repeat-units have been reported as the lower limit of expansion causing SCA6 (Katayama et al. 2000; Mariotti et al. 2001), although this repeat has been observed in normal alleles, including ours (Ishikawa et al. 1997). When the age of onset versus repeat length in the expanded allele is applied, the analysis of age of onset with the total number of CAG repeats in both chromosomes may be important. For instance, the lower limit of expansion causing SCA6 became obscure: the 19 repeat-units in α_{1A} (P/Q type) subunit gene was previously reported "normal" (Ishikawa et al. 1997), while patients with 19 repeats showing progressive ataxia have recently been reported (Katayama et al. 2000; Mariotti et al. 2001). This difference may also be explained by the difference in the total repeat number. Three asymptomatic cases with 19 repeats harbored 13 repeats in the other normal chromosome. Therefore, these subjects had 32 repeats in total. In contrast, Mariotti et al. reported a homozygous patient with 19/19 repeats (total repeat number 38), and three other cases with 19/11, 19/11 and 19/13 repeats (total repeat number 30, 30 and 32), respectively. Among these individuals, ataxia was seen in only one case with homozygous 19/19 repeats. This report suggests the presence of the total repeat number effect because of the higher total repeat number in the patient than in the normal cases. Katayama et al. reported a 19 repeats patient showing progressive ataxia whose repeat number in the normal allele was 7, yielding the total repeat number as 26. Since, the patient had atypical signs for SCA6 such as autonomic signs or pyramidal signs, further confirmations would be needed to show that this patient was indeed affected with SCA6. Nevertheless, it is obvious that there are other factors that would influence the age of onset of individuals: we have also experienced one individual homozygous for 19 repeats with no symptoms at the age of 70 years.

The total repeat number in analysis of age of onset may be more suitable for homozygous patients. Gene dosage effect, i.e. earlier age of onset in homozygous patients, was

Table 4 Neuro-otological findings in short and long duration groups, and duration in groups positive and negative for each feature

	Findings (%) in short and long duration groups		Duration (years) in groups positive and negative for each feature	
	Group A (0–5 years) (n = 11)	Group B (> 5 years) (n = 7)	Positive, mean ± SD	Negative, mean ± SD
Gaze-evoked nystagmus				
Horizontal	72.7	100	8.47 ± 8.09	2 ± 1.73*
Vertical	36.4	71.4	9.56 ± 9.38	5.22 ± 5.47
Rebound	50	83.3	10.3 ± 9.53	6.5 ± 9.11
Positional nystagmus	60	100	11 ± 8.96	2 ± 1.41**
Positioning nystagmus				
Horizontal	50	71.4	8.75 ± 6.76	9.6 ± 11.9
Vertical (DPN)	50	100	10.8 ± 9.18	3.33 ± 0.58
Spontaneous downbeat nystagmus	25	14.3	11.5 ± 10.6	10 ± 9.11
Caloric test				
Impaired visual suppression	75	71.4	11.8 ± 10.2	5.33 ± 2.08
Positioning vertigo	0	100***	14 ± 9.2	3 ± 1.4**
Oscillopsia	25	16.7	3.33 ± 2.52	9.46 ± 9.41

* $P < 0.05$, ** $P < 0.01$ vs positive group, *** $P < 0.001$ vs group A

seen in previous reports in SCA6 (Ikeuchi et al. 1997; Matsumura et al. 1997), although in some other groups this was not evident (Matsuyama et al. 1997; Takiyama et al. 1998). In the present series, only one of four homozygous patients showed a significantly earlier onset compared with heterozygous patients who had the same number of CAG repeats in the expanded allele. When their ages of onset were plotted against the total number of CAG repeat, no patients were proven to have earlier onset than the range of onset for heterozygous patients with the same total number of CAG repeats. This was also true for previous reports. The two homozygous patients in previous reports (Ikeuchi et al. 1997; Matsumura et al. 1997) showed earlier ages of onset than our 95% confidence interval for the expanded allele alone, but were within the 95% confidence interval for total repeat numbers.

From these observations, it is possible that the total number of repeat-units in both alleles is a good parameter to predict the age of onset in SCA6. Since this is the first observation among polyglutamine diseases, it would be important to clarify whether the effect of normal allele is present in other polyglutamine diseases as well. In addition, we have also observed that there is a substantial variation in the age of onset with the given number of repeat, both for expanded allele alone and for total repeat number. For example, the ages of onset varied for 36 years in patients with 22 CAG repeats in the expanded allele, or it varied for 34 years in patients with a total of 34 repeats in both alleles. Therefore, it is also important to elucidate additional factors influencing the age of onset.

Clinical aspects

Unsteadiness of gait was the most frequent initial symptom in the present series. We defined unsteadiness as a sense of instability, especially while walking. Therefore, the symptom would mainly reflect gait ataxia,

which was also the most frequent initial symptom according to many previous reports (Geschwind et al. 1997; Gomez et al. 1997; Ikeuchi et al. 1997; Stevanin et al. 1997). The second most frequent initial symptom was vertigo or oscillopsia. Yabe et al. (2003) reported that vertigo or oscillopsia were experienced by 68% of their patients, while only 12% of patients had these symptoms in our cohort. The reason for this significant difference in the frequency of vertigo is not clear. One possibility is that this difference could be due to the confusion of vertigo with unsteadiness. Frequencies of vertigo or oscillopsia need to be evaluated in further studies, preferably in a prospective manner.

Vertigo is one of the particular clinical features of SCA6 among various SCAs (Gomez et al. 1997). Symptomatic vertigo was present in only 8.8% of the subjects, but neuro-otological testing revealed positioning vertigo in 38.9% of the subjects assessed. This difference suggests that daily activities may not cause stimulation strong enough to induce vertigo related to a change of head position or that patients may avoid those situations inducing vertigo unconsciously. It is also possible that patients may confuse ataxia with vertigo, and complain of “unsteadiness” instead of vertigo. Various episodic features, including vertigo and episodic ataxia, were reported in previous studies of SCA6 (Geschwind et al. 1997; Gomez et al. 1997; Yabe et al. 2003), but only a small number of our subjects had episodic symptoms as in others (Matsumura et al. 1997). Episodic symptoms were frequently reported in foreign reports, and this variability might be due to the difference in modifying gene effect among ethnicities.

Neuro-otological aspect

This is the largest number of neuro-otological examination reports in SCA6 patients. Our neuro-otological

findings suggest, for the first time, the close relationship between positioning vertigo and DPN, and the appearance of positioning vertigo in later stage of SCA6.

Both our report and previous reports indicated a high prevalence of DPN in SCA6 (Jen et al. 1998; Tsutsumi et al. 2001; Yabe et al. 2003), while DPN was uncommon or variable in non-SCA6 autosomal dominant cerebellar atrophy (Yabe et al. 2003), multiple system atrophy (Tsutsumi et al. 2001; Bertholon et al. 2002; Yabe et al. 2003) and other ADCAs, particularly SCA3 (Tsutsumi et al. 2001; Yabe et al. 2003). In the present study, patients with positioning vertigo showed a significantly higher prevalence of DPN than patients without vertigo, suggesting that there was a close relationship between positioning vertigo and DPN. Previous reports have indicated that DPN were related to impaired cancellation of the vestibulo-ocular reflex, i.e. the inhibitory effect of Purkinje cells in the flocculus and nodulus on the vestibular nuclei is impaired, on the basis of a hyperactive VOR (Takemori 1975; Halmagyi et al. 1983; Thurston et al. 1987). There has been one report of extensive Purkinje cell loss affecting most of the cerebellar cortex, including the flocculus, in SCA6 patients (Gomez et al. 1997). Our neuro-otological findings suggested lesions in the vermis, flocculus, paraflocculus or nodulus with sparing of the paramedian pontine reticular formation, and these findings were consistent with the purely cerebellar disorders of eye movements in previous reports (Zee et al. 1981; Henn et al. 1984; Fetter et al. 1994; Moschner et al. 1994; Buttner and Grundei 1995; Buttner et al. 1998; Burk et al. 1999; Lin and Young 1999). Further studies will be necessary to confirm the relationship between anatomical lesions and the neuro-otological features of SCA6, especially the relationship between DPN and lesions of the flocculus or nodulus.

When we compared the prevalence of neuro-otological findings in patients with different disease durations, the longer duration group (> 5 years) showed a significantly higher prevalence of positioning vertigo than in those with shorter duration (within 5 years). A significantly longer disease duration was observed in patients with positioning vertigo, positional nystagmus and horizontal gaze-evoked nystagmus than in the groups negative for each of these features. Such findings suggest that positioning vertigo may be less frequent in the earlier stage of SCA6 and may appear in the later stage.

Conclusions

The total number of CAG repeats on both chromosomes may be valuable for analyzing the age of onset in SCA6. However, there are substantial variations in the age of onset with a given number of repeats, indicating the importance of other factors influencing the age of onset. The most frequent presenting symptom of SCA6 seems to be "unsteadiness" or gait ataxia. Extracerebellar

symptoms are not frequent and may not be due to SCA6 itself.

The present neuro-otological study demonstrated purely cerebellar eye movement disorders, and suggested a close relationship between positioning vertigo and DPN. Positioning vertigo may be less frequent in the earlier stage of SCA6, but tends to appear in the later stage.

Acknowledgements We thank Professor H. Tanaka for statistical analysis and Professor Y. Shinoda for helpful discussion.

References

- Bertholon P, Bronstein AM, Davies RA, Rudge P, Thilo KV (2002) Positional down beating nystagmus in 50 patients: cerebellar disorders and possible anterior semicircular canalithiasis. *J Neurol Neurosurg Psychiatry* 72:366–372
- Burk K, Fetter M, Abele M, Laccone F, Brice A, Dichgans J, Klockgether T (1999) Autosomal dominant cerebellar ataxia type I: oculomotor abnormalities in families with SCA1, SCA2, and SCA3. *J Neurol* 246:789–797
- Buttner U, Grundei T (1995) Gaze-evoked nystagmus and smooth pursuit deficits: their relationship studied in 52 patients. *J Neurol* 242:384–389
- Buttner N, Geschwind D, Jen JC, Perlman S, Pulst SM, Baloh RW (1998) Oculomotor phenotypes in autosomal dominant ataxias. *Arch Neurol* 55:1353–1357
- Chung MY, Lu YC, Cheng NC, Soong BW (2003) A novel autosomal dominant spinocerebellar ataxia (SCA22) linked to chromosome 1p21–q23. *Brain* 126:1293–1299
- Durig JS, Jen JC, Demer JL (2002) Ocular motility in genetically defined autosomal dominant cerebellar ataxia. *Am J Ophthalmol* 133:718–721
- Fetter M, Klockgether T, Schulz JB, Faiss J, Koenig E, Dichgans J (1994) Oculomotor abnormalities and MRI findings in idiopathic cerebellar ataxia. *J Neurol* 241:234–241
- Geschwind DH, Perlman S, Figueroa KP, Karrim J, Baloh RW, Pulst SM (1997) Spinocerebellar ataxia type 6. Frequency of the mutation and genotype–phenotype correlations. *Neurology* 49:1247–1251
- Gomez CM, Thompson RM, Gammack JT, Perlman SL, Dobyns WB, Truwit CL, Zee DS, Clark HB, Anderson JH (1997) Spinocerebellar ataxia type 6: gaze-evoked and vertical nystagmus, Purkinje cell degeneration, and variable age of onset. *Ann Neurol* 42:933–950
- Halmagyi GM, Rudge P, Gresty MA, Sanders MD (1983) Downbeating nystagmus. A review of 62 cases. *Arch Neurol* 40:777–784
- Harada H, Tamaoka A, Watanabe M, Ishikawa K, Shoji S (1998) Downbeat nystagmus in two siblings with spinocerebellar ataxia type 6 (SCA 6). *J Neurol Sci* 160:161–163
- Harding AE (1982) The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of "Drew family of Walworth". *Brain* 105:1–28
- Henn V, Lang W, Hepp K, Reisine H (1984) Experimental gaze palsies in monkeys and their relation to human pathology. *Brain* 107:619–636
- Holmberg M, Johansson J, Forsgren L, Heijbel J, Sandgren O, Holmgren G (1995) Localization of autosomal dominant cerebellar ataxia associated with retinal degeneration and anticipation to chromosome 3p12–p21.1. *Hum Mol Genet* 4:1441–1445
- Ikeuchi T, Takano H, Koide R, Horikawa Y, Honma Y, Onishi Y, Igarashi S, Tanaka H, Nakao N, Sahashi K, Tsukagoshi H, Inoue K, Takahashi H, Tsuji S (1997) Spinocerebellar ataxia

- type 6: CAG repeat expansion in α_{1A} voltage-dependent calcium channel gene and clinical variations in Japanese population. *Ann Neurol* 42:879–884
- Ishikawa K, Tanaka H, Saito M, Ohkoshi N, Fujita T, Yoshizawa K, Ikeuchi T, Watanabe M, Hayashi A, Takiyama Y, Nishizawa M, Nakano I, Matsubayashi K, Miwa M, Shoji S (1997) Japanese families with autosomal dominant pure cerebellar ataxia map to chromosome 19p13.1–p13.2 and are strongly associated with mild CAG expansions in the spinocerebellar ataxia type 6 gene in chromosome 19p13.1. *Am J Hum Genet* 61:336–346
- Jen JC, Yue Q, Karrim J, Nelson SF, Baloh RW (1998) Spinocerebellar ataxia type 6 with positional vertigo and acetazolamide responsive episodic ataxia. *J Neurol Neurosurg Psychiatry* 65:565–568
- Katayama T, Ogura Y, Aizawa H, Kuroda H, Suzuki Y, Kuroda K, Kikuchi K (2000) Nineteen CAG repeats of the SCA6 gene in a Japanese patient presenting with ataxia. *J Neurol* 247:711–712
- Knight MA, Kennerson ML, Anney RJ, Matsuura T, Nicholson GA, Salimi-Tari P, Gardner RJ, Storey E, Forrest SM (2003) Spinocerebellar ataxia type 15 (SCA15) maps to 3p24.2–3pter: exclusion of the ITPR1 gene, the human orthologue of an ataxic mouse mutant. *Neurobiol Dis* 13:147–157
- Kubodera T, Yokota T, Ohwada K, Ishikawa K, Miura H, Matsuoka T, Mizusawa H (2003) Proteolytic cleavage and cellular toxicity of the human α_{1A} calcium channel in spinocerebellar ataxia type 6. *Neurosci Lett* 341:74–78
- Li M, Ishikawa K, Toru S, Tomimitsu H, Takashima M, Goto J, Takiyama Y, Sasaki H, Imoto I, Inazawa J, Toda T, Kanazawa I, Mizusawa H (2003) Physical map and haplotype analysis of 16q-linked autosomal dominant cerebellar ataxia (ADCA) type III in Japan. *J Hum Genet* 48:111–118
- Lin CY, Young YH (1999) Clinical significance of rebound nystagmus. *Laryngoscope* 109:1803–1805
- Mariotti C, Gellera C, Grisoli M, Mineri R, Castucci A, Di Donato S (2001) Pathogenic effect of an intermediate-size SCA-6 allele (CAG)19 in a homozygous patient. *Neurology* 57:1502–1504
- Maruyama H, Izumi Y, Morino H, Oda M, Toji H, Nakamura S, Kawakami H (2002) Difference in disease-free survival curve and regional distribution according to subtype of spinocerebellar ataxia: a study of 1,286 Japanese patients. *Am J Med Genet* 114:578–583
- Matsumura R, Futamura N, Fujimoto Y, Yanagimoto S, Horikawa H, Suzumura A, Takayanagi T (1997) Spinocerebellar ataxia type 6. Molecular and clinical features of 35 Japanese patients including one homozygous for the CAG repeat expansion. *Neurology* 49:1238–1243
- Matsuyama Z, Kawakami H, Maruyama H, Izumi Y, Komure O, Uda F, Kameyama M, Nishio T, Kuroda Y, Nishimura M, Nakamura S (1997) Molecular features of the CAG repeats of spinocerebellar ataxia 6 (SCA6). *Hum Mol Genet* 6:1283–1287
- Miyoshi Y, Yamada T, Tanimura M, Taniwaki T, Arakawa K, Ohyagi Y, Furuya H, Yamamoto K, Sakai K, Sasazuki T, Kira J (2001) A novel autosomal dominant spinocerebellar ataxia (SCA16) linked to chromosome 8q22.1–24.1. *Neurology* 57:96–100
- Moschner C, Perlman S, Baloh RW (1994) Comparison of oculomotor findings in the progressive ataxia syndromes. *Brain* 117:15–25
- Sasaki H, Yabe I, Yamashita I, Tashiro K (2000) Prevalence of triplet repeat expansion in ataxia patients from Hokkaido, the northernmost island of Japan. *J Neurol Sci* 175:45–51
- Schöls L, Amoiridis G, Buttner T, Przuntek H, Epplen JT, Riess O (1997) Autosomal dominant cerebellar ataxia: phenotypic differences in genetically defined subtypes? *Ann Neurol* 42:924–932
- Schöls L, Kruger R, Amoiridis G, Przuntek H, Epplen JT, Riess O (1998) Spinocerebellar ataxia type 6: genotype and phenotype in German kindreds. *J Neurol Neurosurg Psychiatry* 64:67–73
- Sinke RJ, Ippel EF, Diepstraten CM, Beemer FA, Wokke JH, van Hilten BJ, Knoers NV, van Amstel HK, Kremer HP (2001) Clinical and molecular correlations in spinocerebellar ataxia type 6: a study of 24 Dutch families. *Arch Neurol* 58:1839–1844
- Stevanin G, Durr A, David G, Didierjean O, Cancel G, Rivaud S, Tourbah A, Warter JM, Agid Y, Brice A (1997) Clinical and molecular features of spinocerebellar ataxia type 6. *Neurology* 49:1243–1246
- Takashima M, Ishikawa K, Nagaoka U, Shoji S, Mizusawa H (2001) A linkage disequilibrium at the candidate gene locus for 16q-linked autosomal dominant cerebellar ataxia type III in Japan. *J Hum Genet* 46:167–171
- Takemori S (1975) Visual suppression of vestibular nystagmus after cerebellar lesions. *Ann Otol Rhinol Laryngol* 84:318–326
- Takiyama Y, Sakoe K, Namekawa M, Soutome M, Esumi E, Ogawa T, Ishikawa K, Mizusawa H, Nakano I, Nishizawa M (1998) A Japanese family with spinocerebellar ataxia type 6, which includes three individuals homozygous for an expanded CAG repeat in the SCA6/CACNL1A4 gene. *J Neurol Sci* 158:141–147
- Thurston SE, Leigh RJ, Abel LA, Dell’Osso LF (1987) Hyperactive vestibulo-ocular reflex in cerebellar degeneration: pathogenesis and treatment. *Neurology* 37:53–57
- Tsutsumi T, Kitamura K, Tsunoda A, Noguchi Y, Mitsushashi M (2001) Electronystagmographic findings in patients with cerebellar degenerative disease. *Acta Otolaryngol* 545:136–139
- Watanabe H, Tanaka F, Matsumoto M, Doyu M, Ando T, Mitsuuma T, Sobue G (1998) Frequency analysis of autosomal dominant cerebellar ataxias in Japanese patients and clinical characterization of spinocerebellar ataxia type 6. *Clin Genet* 53:13–19
- Worth PF, Giunti P, Gardner-Thorpe C, Dixon PH, Davis MB, Wood NW (1999) Autosomal dominant cerebellar ataxia type III: linkage in a large British family to a 7.6-cM region on chromosome 15q14–21.3. *Am J Hum Genet* 65:420–426
- Yabe I, Sasaki H, Matsuura T, Takada A, Wakisaka A, Suzuki Y, Fukazawa T, Hamada T, Oda T, Ohnishi A, Tashiro K (1998) SCA6 mutation analysis in a large cohort of the Japanese patients with late-onset pure cerebellar ataxia. *J Neurol Sci* 156:89–95
- Yabe I, Sasaki H, Takeichi N, Takei A, Hamada T, Fukushima K, Tashiro K (2003) Positional vertigo and macroscopic downbeat positioning nystagmus in spinocerebellar ataxia type 6 (SCA6). *J Neurol* 250:440–443
- Yamashita I, Sasaki H, Yabe I, Fukazawa T, Nogoshi S, Komeichi K, Takada A, Shiraishi K, Takiyama Y, Nishizawa M, Kaneko J, Tanaka H, Tsuji S, Tashiro K (2000) A novel locus for dominant cerebellar ataxia (SCA14) maps to a 10.2-cM interval flanked by D19S206 and D19S605 on chromosome 19q13.4–qter. *Ann Neurol* 48:156–163
- Zee DS, Yamazaki A, Butler PH, Gucer G (1981) Effects of ablation of flocculus and paraflocculus of eye movements in primate. *J Neurophysiol* 46:878–899
- Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A} voltage-dependent calcium channel. *Nat Genet* 15:62–69
- Zu L, Figueroa K, Grewal R, Pulst S (1999) Mapping of a new autosomal dominant spinocerebellar ataxia to chromosome 22. *Am J Hum Genet* 64:594–599