SHORT REPORT

Clinical, neuropathological, and molecular study in two families with spinocerebellar ataxia type 6 (SCA6)

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Abstract

To clarify the clinical, neuropathological, and molecular characteristics of spinocerebellar ataxia type 6 (SCA6), two unrelated Japanese families with SCA6 were studied. A clinical feature of the two families was late onset "pure" cerebellar ataxia. Pathologically, three SCA6 brains consistently showed Purkinje cell dominant cortical cerebellar degeneration. Morphometric analysis showed that loss of the cerebellar granule cells and inferior olivary neurons were very mild compared with the severity of Purkinje cell loss. There was no obvious ubiquitin immunoreactive nuclear inclusions. All affected patients had identical expanded alleles, and the expansion was also homogeneously distributed throughout the brain without mosaicism. The present study showed that SCA6 is characterised by Purkinje cell dominant cortical cerebellar degeneration, highly stable transmission of the CAG repeat expansion, and lack of ubiquitin immunoreactive nuclear inclusions.

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Keywords: SCA6; purkinje cell; CAG repeat; neuropathology

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar ataxia that is strongly associated with the expansion of trinucleotide (CAG) repeat¹ in the α_{1A} voltage dependent calcium channel gene (CACNA1A).² We and others³⁻⁵ further analysed this mutation and found a significant inverse correlation between the number of CAG repeat units in the expanded allele and the age of onset, indicating that the CAG expansion is strongly associated with the pathogenic mechanism of SCA6. Pathology of SCA6 has been also described,^{6,7} although some variations are seen particularly in the degrees of degeneration of the cerebellar granule cells and inferior olivary neurons. In addition, it is not known whether ubiquitin immunoreactive nuclear inclusion, seen in

other diseases associated with CAG repeat expansion,⁸⁻¹¹ is also present in SCA6.

To define pathological and molecular characteristics as well as clinical features of SCA6, we examined two families, neither of which are the subset of families we described previously.³ Here we show that SCA6 has distinct clinical, neuropathological, and molecular features compared with other diseases associated with CAG repeat expansion.

Patients and methods

CLINICAL INVESTIGATION

Two unrelated Japanese families (UT1 and UT2) were studied. Neuropathological findings and alterations of neurotransmitter markers in one affected member (UT1-IV-5) have been previously described.^{12,13} During our subsequent follow up for 12 years, four members further developed ataxia and one patient (UT1-V-7) died, which allowed us to investigate this family more extensively. Clinical examination was performed as described¹⁴ in all available members (n=16) including at risk family members.

NEUROPATHOLOGICAL INVESTIGATION

Necropsy was performed in three patients: UT1-IV-5 (patient 1; the duration of illness: 28 years), UT1-V-7 (patient 2; 18 years) and UT2-III-1 (patient 3; 14 years). Formalin fixed, paraffin embedded sections were stained with haematoxylin and eosin, Klüver-Barrera, and modified Bielschowsky methods. Immunohistochemistry for ubiquitin (Dako; rabbit polyclonal; dilution 1:400) was also performed with the standard avidin-biotin-peroxidase complex method.

To clarify the degree of neuronal loss, a morphometric analysis was performed in the cerebellar cortex (the culmen, the simple lobule, and the tonsil) and the inferior olivary nucleus (midregion) according to the described methods.^{12,15} In brief, sagittal 6 μ m thick slices of the cerebellum and transverse 10 μ m thick slices through the midolivary region were chosen and the Purkinje cells, granule cells, and neurons of the principal inferior olivary nucleus were counted. For each area, the

Table 1 Morphometric data in the cerebellar cortex and inferior olivary nucleus

	Purkinje cells (cells/mm)			Granule cells ($\times 10^6$ cells/mm ³)			Inferior olivary neurons ($\times 10^2$ cells/mm ²)
	Superior vermis†	Superior hemisphere†	Tonsil	Superior vermis†	Superior hemisphere†	Tonsil	
Control (n=5)	5.64	5.88	5.93	2.24	2.21	2.16	150.83
Patient 1	0.42**	4.74*	4.52 *	1.67*	1.86*	2.12	135.47
Patient 2	0.37**	5.12	5.27	1.02*	2.03	2.15	129.95
Patient 3	0.31**	0.97**	2.53 **	0.77*	1.50*	1.84	76.26*

Patients 1 to 3 correspond to patients UT1-IV-5, UT1-V-7, and UT2-III-1, respectively (see text).

† The superior vermis and superior hemisphere designate the culmen and simple lobule, respectively.

The degree of neuronal loss was evaluated as follows; mild or minimal (no symbols): within 15% reduction compared with a normal control; moderate *: between 15 and 50% reduction; severe **: more than 50% reduction.

Note that the Purkinje cells are consistently and predominantly affected.

numbers of cells were counted in 10 different areas by using an image analyser (Videoplan, Zeiss, Germany), and finally averaged. Five age matched neurologically normal people served as controls.

MOLECULAR ANALYSIS

After obtaining informed consent, genomic DNA was extracted from peripheral blood lymphocytes in 10 people in UT1. For the three deceased members (patients 1–3), DNA was extracted from the following frozen tissues: the frontal cortex (Brodmann's area 6), occipital cortex (area 17), hippocampus, deep frontal white matter, caudate nucleus, thalamus, pontine base, and cerebellar hemispheric cortex. Except for the deep frontal white matter, CACNA1A is predicted to be expressed abundantly.¹⁶ The number of CAG repeat units was determined as described.^{3 14}

Results

CLINICAL FEATURES

The average age of onset was 52.1 (SD 7.3) years (n=10; range 37–65 years). The onset was slightly earlier in offspring than in parents (4.7 (SD 5.9) years), although this difference was not statistically significant. Clinically, our families showed slowly progressive ataxia without any remarkable extracerebellar signs

(“pure” cerebellar ataxia).^{14 17} Two members, both within 2 years after onset, showed subtle horizontal gaze nystagmus and mild gait ataxia evident only when performing “tandem gait”. None of the patients documented episodic ataxia.

Brain MRI (n=7) showed restricted atrophy of the cerebellum. Nerve conduction studies, EEG, and somatosensory evoked potentials were all normal (n=3).

NEUROPATHOLOGY

The brains weighed 1100 g (patient 1), 1200 g (patient 2), and 970 g (patient 3). Macroscopically, all patients showed marked atrophy of the cerebellum particularly in the superior vermis. By contrast, the brainstem and spinal cord were well preserved.

The cardinal histopathological changes were seen in the cerebellar cortex. Loss of Purkinje cells with Bergmann's gliosis was consistently severe in the superior vermis, whereas it became milder in the inferior vermis and cerebellar hemisphere (table 1). Loss of granule cells was always less severe than the Purkinje cell loss in the corresponding areas. The degree of degeneration was prominent and diffuse in patient 3, whereas both patients from UT1 (patients 1 and 2) had much milder degeneration. There was no apparent neuronal

Figure 1 Immunohistochemistry for ubiquitin in the cerebellum with SCA6. (A) There is no obvious ubiquitin immunoreactive nuclear inclusion in a Purkinje cell (arrow) of a patient with SCA6 (immunoperoxidase $\times 250$). (B) In a control brain affected with dentatorubral-pallidoluysian atrophy, a ubiquitin immunoreactive inclusion is seen in the centre of the nucleus in a cerebellar dentate neuron. The nucleolus is seen in the periphery of the nucleus (arrowhead; immunoperoxidase, originally $\times 250$).

loss in the dentate nucleus, although mild astrogliosis was seen. In the inferior olivary nucleus, neuronal loss was seen in its dorsomedial region in patient 3. However, the inferior olivary nucleus was preserved throughout the rostrocaudal extent in patients 1 and 2.

Other structures were fairly well preserved, except for large cerebral infarcts in patients 1 and 3, and mild neuronal loss with a few neurofibrillary tangles in the subthalamic nucleus, substantia nigra, and globus pallidus of patient 3. There was no obvious ubiquitin immunoreactive nuclear inclusions in the three brains examined (see fig 1).

MOLECULAR ANALYSIS

All affected members from the two families had expanded alleles with 22 CAG repeat units (fig 2). Normal alleles ranged from 11 to 16 repeats. One asymptomatic member aged 55 years at examination also harboured the same expansion. This member had no neurological signs except for a subtle horizontal gaze nystagmus. In the three SCA6 brains, the number of repeats in the expanded alleles was again identical in all regions examined (fig 2). Both normal and expanded alleles showed single distinct peaks demonstrating no somatic mosaicism.

Figure 2 Analysis of the SCA6 CAG repeats. All expanded alleles (EA) show the same 167 base pairs (bp) fragments (corresponding 22 CAG repeats; arrows), whereas normal alleles (NA) are seen in variable lengths, between 135 and 150 bp (11 to 16 CAG repeat) range. Note both EA and NA show distinct single peaks. Each lane shows fragments from the following samples: lanes 1, 12, and 31=external size markers (asterisk=150 bp); lanes 2–6=samples from different living members in UT1; lane 13=the frontal cortex of patient 1; lane 14=the frontal cortex of patient 2; lane 15=the occipital cortex of patient 2; lane 16=the hippocampus and parahippocampal gyrus of patient 2; lane 17=the deep frontal white matter of patient 2; lane 18=the caudate nucleus of patient 2; lane 19=the thalamus of patient 2; lane 20=the pontine base of patient 2; lane 21=the cerebellar cortex of patient 2; lane 23=the cerebellar cortex of patient 3.

Discussion

The number of CAG repeats in the expanded alleles in the two families was coincidentally the same 22 repeats, which corresponds to the most common expanded allele in the SCA6 families.^{1, 3–5, 18–21} Clinical features were summarised as “pure” cerebellar ataxia. Anticipation was not present, as in our previous set of families,³ which seems consistent with the fact that all affected members had expansions with identical size. Notably, three patients in a very early stage or in the presymptomatic stage showed only horizontal gaze nystagmus, suggesting that the nystagmus could be the earliest sign of ataxia as well as gait ataxia in SCA6. Although episodic ataxia type 2 (EA-2) and SCA6 clinically overlapped in some families,²² none of our affected members documented the episodic nature of ataxia, which fits to the idea that EA-2 and SCA6 have different pathogenic mechanisms.¹

The present study provided two new pathological aspects of SCA6. Firstly, the present study confirmed that the so called cerebello-olivary atrophy (as in patient 3) and the cortical cerebellar degeneration without obvious olivary involvement (as in patients 1 and 2) could be included under a single entity.²³ Notably, this pathological variation could not be simply explained by the differences in the length of expansion or the duration of illness. Our finding also indicates that degenerations of granule cells and inferior olivary neurons may occur secondarily to the loss of the Purkinje cells. However, the precise mechanism of degeneration needs to be elucidated, as CACNA1A is also expressed in both the granule cells and olivary neurons.¹⁶ Secondly, the fact that the ubiquitin immunoreactive nuclear inclusions are absent in SCA6 brains would suggest that the mechanism of neuronal death in SCA6 could be different from those in other CAG repeat diseases.^{8–11} However, recent studies showed that nuclear translocation of the mutant protein is essential, rather than the presence of ubiquitin positive nuclear inclusions, for the pathogenesis of polyglutamine diseases.^{24, 25} Therefore, it would be important to clarify whether the small CAG repeat expansion in CACNA1A leads to aggregation of the calcium channel protein in the nucleus.

The molecular study showed for the first time that the somatic mosaicism was not seen in various brain regions, where CACNA1A is predicted to be expressed,¹⁶ or in peripheral blood lymphocytes. Together with the stable transmission within affected family members, this fact would indicate that the transmission of CAG repeats in CACNA1A is very stable, which is by contrast with other “unstable” CAG expansions.^{26, 27}

In conclusion, SCA6 is characterised by the clinical feature of pure cerebellar ataxia, and predominant Purkinje cell degeneration. Highly stable transmission and lack of ubiquitin immunoreactive nuclear inclusion are features distinct from other diseases associated with CAG repeat expansions.

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