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Autosomal dominant cerebellar ataxia (SCA6): clinical, genetic and neuropathological study in a family

Received: 27 May 1997 / Revised, accepted: 27 October 1997

Abstract We describe a family with dominantly inherited ataxia of late adult onset. Expansion of a CAG repeat in the gene encoding the α_{1A} voltage-dependent calcium channel was identified at autopsy in one patient, a 65-year-old woman with a disease duration of 11 years. In this patient, pathological changes were confined to the cerebellar cortex and inferior olivary complex. The cerebellar cortex showed severe loss of Purkinje cells with proliferation of Bergmann's glia, being more pronounced in the superior parts of the vermis and hemispheres. In the inferior olivary complex, a reduced neuronal cell population, which could be interpreted as a change secondary to the cerebellar cortical lesion, was evident. We conclude that the pathological phenotype of this newly classified autosomal dominant cerebellar ataxia, SCA6, is cerebello-olivary atrophy, or more strictly cerebellar cortical atrophy.

Key words Ataxia · Autosomal dominance · Cerebello-olivary atrophy · CAG repeat expansion · SCA6

Introduction

The rapid progress made in the genetic investigation of dominantly inherited spinocerebellar ataxias (SCAs) has made it possible to provide a genotype classification, in which several disorders have already been listed with

clarity [10, 20]. Recently, Sanpei et al. [22] found that novel expansion of a CAG repeat is the causative mutation responsible for spinocerebellar ataxia type 2 (SCA2). Shortly after this, Zhuchenko et al. [26] reported that expansion of a CAG repeat in the gene encoding the α_{1A} voltage-dependent Ca^{2+} channel was the cause of late-onset dominantly inherited ataxia, and this became a new addition to the above classification as an autosomal dominant spinocerebellar ataxia, SCA6. At present, however, the neuropathological features of this new genetically defined disease have been described for only one family [23]. Here we report another family with SCA6, of which one affected member was examined at autopsy.

Materials and methods

Clinical study

In this family, clinical examinations of two affected members, patients 1 (IV-1) and 2 (IV-6), were performed. An autopsy was later performed on patient 1. From accounts given by the family members, including patient 1, three other individuals, patients 3 (III-2), 4 (II-1) and 5 (I-1), were said to have been affected by an almost identical disorder. Three siblings of patient 1 were said to have died in infancy, but the causes of death were unknown.

Genetic analysis

High-molecular-weight genomic DNA was extracted from the frozen brain tissue of patient 1 [21]. The CAG repeat size in the α_{1A} voltage-dependent Ca^{2+} channel gene was determined by the polymerase chain reaction (PCR) using oligonucleotide primers (CACNL1AF: 5'-TCAACATCTGGTACCAGCACTCC-3', CACNL1AR: 5'-TACCTCCGAGGGCCGCTGGTG-3') in 25 μ l of solution containing 200 μ M each of dATP, dGTP, and TTP, 20 μ M dCTP, 1 μ M of each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM TRIS-HCl, pH 8, 200 ng of genomic DNA, 0.15 μ l of [^{32}P]dCTP (111 TBq/mmol), and 1 unit of *Taq* polymerase (Takara, Japan). The thermal controller for amplification was programmed for an initial 2-min denaturation at 96°C, followed by 30 cycles of 1 min at 96°C, 1 min at 62°C and 1 min at 72°C, and a final 10-min extension at 72°C. Aliquots of the PCR products were run through 6% denaturing polyacrylamide gels in 1 \times TRIS-borate (TBE) buffer, and autoradiographed on Fuji RX film at -70°C using an intensifying screen.

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Neuropathological methods

Histological examination of patient 1 was performed on formalin-fixed, paraffin-embedded sections using several stains, including hematoxylin and eosin (H&E), Klüver-Barrera (Luxol fast blue/cresyl violet), Holzer, Bodian's method and methenamine silver. Selected sections were also stained by the modified Gallyas-Braak method [5, 24], or immunostained with a mouse monoclonal antibody against tau-2 (Dakopatts, Glostrup, Denmark; diluted 1:2000), using the avidin-biotin-peroxidase method.

Results

Case reports

Patient 1 (proband, IV-1, Fig. 1)

The patient had been healthy until the age of 54 years, when slurred speech and unsteady gait appeared. On neurological examination, instability when standing on one

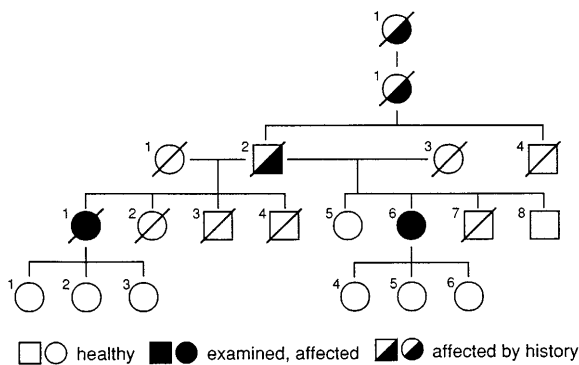


Fig. 1 Family pedigree



Fig. 2 A computed tomography scan of patient 2 showing severe cerebellar cortical atrophy and dilatation of the fourth ventricle

leg was observed. The Achilles' tendon reflex was absent on the left side, whereas the other deep tendon reflexes were exaggerated. Thereafter, gait ataxia and dysarthria progressed slowly, but steadily. At the age of 56, ataxia was also apparent in the upper limbs. At the age of 59, the patient became unable to walk without assistance. At the age of 65 years, she became bedridden, and exhibited gaze-evoked nystagmus and intention tremor, and died about 11 years after onset of the disease. The direct cause of death was colon cancer, which was found and removed surgically 7 months before death, but metastasis to the liver was already present at that time. During the course of her illness, there was no evidence of dementia, and no sensory or autonomic disorder, or muscle weakness developed. Results of laboratory tests, including cerebrospinal fluid and blood, were unremarkable.

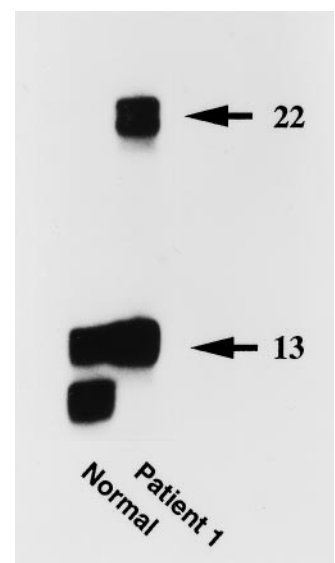
Patient 2 (IV-6)

- I This female patient had been healthy until the age of 48 years, when slurred speech and unsteadiness of gait appeared. These symptoms progressed slowly. At the age of 57, gaze-evoked nystagmus became apparent. At age 59, the patient became unable to walk without assistance. At age 63, intention tremor and ataxia in the upper limbs became apparent. At the time of writing, she is 67 years old and virtually chair bound. Computed tomography revealed atrophy of the cerebellum (Fig. 2). All deep tendon reflexes were exaggerated. No pathological reflexes were elicited. There was no evidence of dementia, and no sensory or autonomic disorder, or muscle weakness has developed so far.
- II
- III
- IV
- V

Patients 3 (III-2) and 4 (II-1)

Patient 3 had been healthy until the age of 50 years, when speech and gait difficulties appeared. At the age of 60, he

Fig. 3 The expanded allele with 22 CAG repeats was shown in patient 1 at autopsy



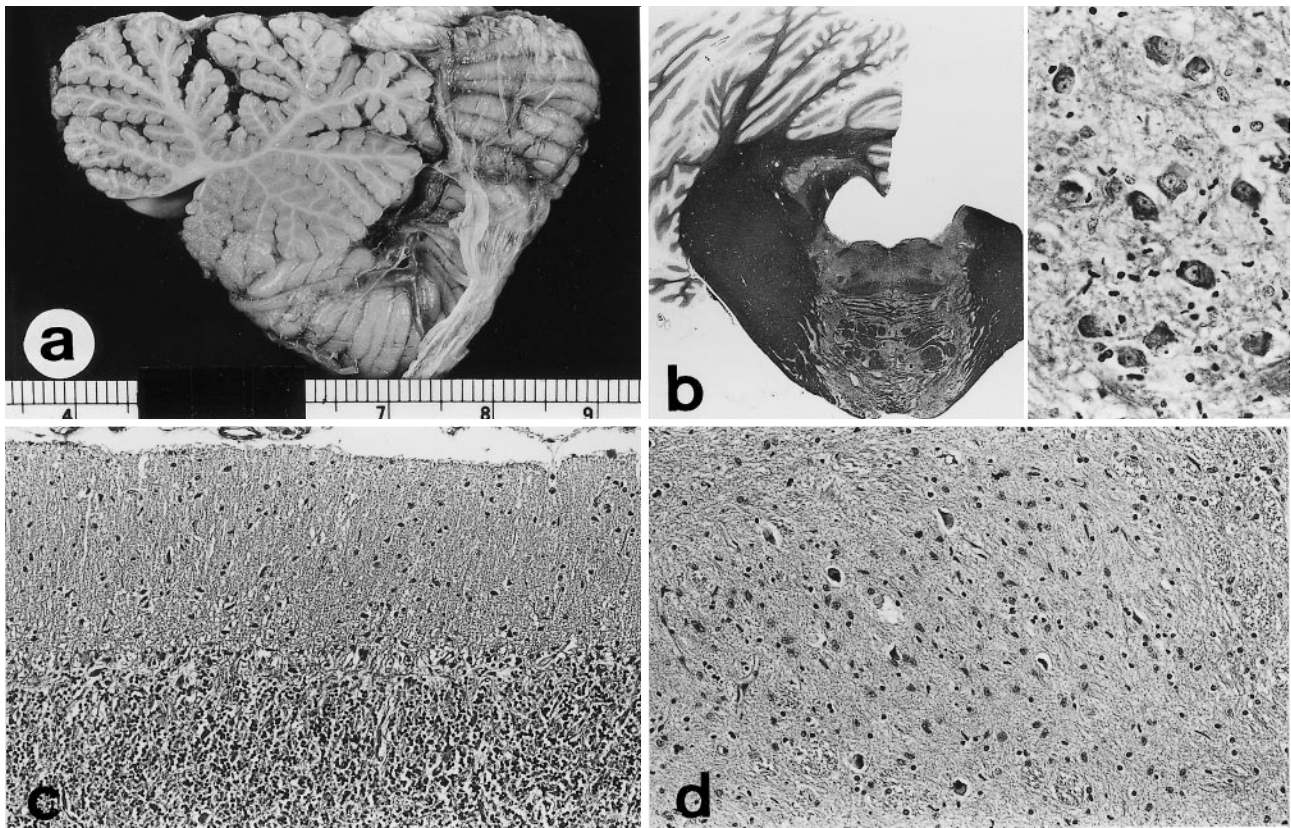


Fig. 4 **a** Mid-sagittal section through the cerebellar vermis showing folial atrophy, being more marked in the superior part. **b** No pathological changes are evident in the pons, including the middle cerebellar peduncle (*left*). Neurons in the pontine nuclei are well preserved (*right*). **c** The superior part of the vermis showing almost complete loss of Purkinje cells. **d** The rostral part of the principal inferior olivary nucleus showing obvious neuronal loss and gliosis. **b** Klüver-Barrera, *left* $\times 1.2$, *right* $\times 200$; **c, d** H&E, **c** $\times 100$, **d** $\times 125$

committed suicide because of the distress caused by the disease. Patient 4 died at the age of 80 years.

Genetic analysis

The numbers of CAG repeats in patient 1 were 22 and 13 (Fig. 3).

Neuropathological findings

Autopsy of patient 1 was performed 11 h after death. The investigation was limited to the brain and the spinal cord, together with the anterior and posterior nerve roots, the cervical dorsal root ganglion, and the tibial nerve in the popliteal region.

The brain weighed 1310 g before fixation. The cerebellum appeared very small and atrophic in comparison with the cerebrum and brain stem, which were fairly well proportioned in relation to each other. The spinal cord was unremarkable. In sections, the cerebellum showed atrophy

of the folia with open fissures, being more marked in the superior parts of the vermis and hemispheres (Fig. 4a). The cerebrum and brain stem were of normal appearance. Histologically, the significant changes were confined to the cerebellar cortex and inferior olivary complex. No apparent changes were found elsewhere in the CNS, including the spinal cord, particularly the pons (Fig. 4b) and basal ganglia, which were of normal histological appearance. The lumbar anterior and posterior nerve roots, cervical dorsal root ganglion and tibial nerve were also intact.

The cerebellar cortex revealed severe loss of Purkinje cells with proliferation of Bergmann's glia (Fig. 4c). Severe loss of granule cells was also noted. The molecular layer was reduced in width with an increase of astrocytic nuclei. These changes were more pronounced in the superior parts of the vermis and hemispheres. The folial white matter showed myelin pallor and fibrillary gliosis, the degree of which appeared to correspond to that of the cortical degeneration. The deep white matter and cerebellar peduncles were of normal histological appearance. The dentate nucleus was well preserved with abundant neurons, although gliosis was evident there, and also in the surrounding white matter (amiculum).

In the inferior olivary complex, which was examined at three different levels, mild to moderate loss of neurons with gliosis was observed, being more pronounced in the rostral part of the principal inferior olivary nucleus (Fig. 4d) as well as in the caudal part of the medial accessory olivary nucleus. In the principal inferior olivary nucleus

itself, such changes were more marked in the dorsal band than in the ventral band.

No argyrophilic or tau-positive structures could be found in the cerebellum, pons or medulla oblongata, as far as could be examined. In the cerebrum, only a small number of neurofibrillary tangles were observed in the hippocampus and parahippocampal gyrus.

Discussion

In this family, it appears almost certain that through five successive generations, at least five patients developed very similar late-onset cerebellar ataxia and that the disease was inherited as an autosomal dominant trait. In the two patients examined, the cardinal symptoms were slowly, but steadily progressive gait and limb ataxia, and dysarthria. Nystagmus was also noted, but other oculomotor findings were negative. None of the patients had dementia, sensory or autonomic disorder, or muscle weakness. The clinical features appeared to be those of "pure" autosomal dominant cerebellar ataxia of late onset (ADCA III) [10–12].

Our genetic study revealed that patient 1 had a disease resulting from the SCA6 mutation with 22 CAG repeats, i.e., the expansion of a CAG repeat in the gene encoding the α_{1A} voltage-dependent Ca^{2+} channel. Zhuchenko et al. [26] reported that the patients they examined had alleles with larger repeat numbers (21–27) compared to the number of repeats (4–16) in control subjects. It is noteworthy that other neurological diseases, including familial hemiplegic migraine and episodic ataxia type-2, have been reported to be caused by different mutations in the same gene encoding the α_{1A} voltage-dependent Ca^{2+} channel [16].

In our patient 1, the neuropathological changes were confined to the cerebellar cortex and inferior olivary complex. The neuropathology of SCA6 has so far been described for only one family; Zhuchenko et al. [26] simultaneously disclosed that one family with dominantly inherited ataxia with late onset, which had been described originally by Subramony et al. [23], was an example of SCA6, showing mutations with 22 CAG repeats in one living affected member and one autopsied one. Subramony et al. [23] performed neuropathological examinations of two affected members, and reported that the features, which were concordant between the two, were consistent with cerebello-olivary atrophy [6]; there was severe loss of Purkinje cells with less severe loss of granule cells and thinning of the molecular layer. Mild to moderate loss of neurons in the inferior olives and dentate nucleus was also evident. The spinal cord was examined in one affected member, showing no pathological changes.

Several families with dominantly inherited ataxias and cerebello-olivary atrophies have been described [1–4, 8, 9, 13, 19, 25]. It is not certain whether these represent a genetically homogeneous entity, and they may represent spinocerebellar ataxia type 5 (SCA5), whose clinical phenotype is very similar to that of SCA6 [10, 18, 20]. However, reviewing the reported pathological findings appears

to be of importance for a better understanding of SCA6 neuropathology. Akelatis [1] reported two patients from one family on whom autopsies were performed, who had died within 2 years after onset. Concordance of neuropathological findings was documented in the two; the grossly normal cerebella showed loss of Purkinje cells, being more marked in the superior vermis, but the molecular and granular layers as well as the white matter were of normal histological appearance, suggesting that the initial and primary change occurs in the cerebellar Purkinje cells, starting from the superior vermis. With regard to loss of the granule cells in the cases of SCA6 examined by Subramony et al. [23] and our group, the possibility that it is a change secondary to loss of Purkinje cells can be considered.

In our patient 1, the distribution of neuronal loss in the inferior olivary complex appeared to correspond anatomically to that of Purkinje cell loss in the cerebellar cortex. Similar observations were made by previous investigators [1, 2, 8, 9, 13, 19, 25]. This type of neuronal loss in the inferior olivary complex has been interpreted as a change representing retrograde transsynaptic degeneration secondary to the cerebellar cortical lesion [14]. Indeed, the extent of the inferior olivary complex degeneration never surpasses that of cerebellar cortical degeneration in cerebello-olivary atrophy.

The dentate nucleus may show neuronal loss [6]. Hoffman et al. [13] stated that the dentate nucleus was involved, with loss of approximately 10% of its neurons in the case they studied. However, in several other cases, neuronal loss was not evident in this nucleus [1, 8, 9, 25]. Richter [19] emphasized that there were local differences in the severity of neuronal degeneration in the dentate nucleus, and that there was obvious fibrillary gliosis in the dentate nucleus itself as well as in the surrounding white matter. The cases of SCA6 described by Subramony et al. [23] were reported to show mild to moderate neuronal loss in the dentate nucleus. In our patient 1, neurons in the dentate nucleus were well preserved, although myelin pallor and gliosis were evident in the background and surrounding white matter (amiculum). It is considered that in cerebello-olivary atrophy, the dentate nucleus is usually unremarkable, and even if neuronal loss is evident, it is a change representing anterograde transsynaptic degeneration secondary to loss of the cerebellar Purkinje cells [19], and presumably, also of the inferior olivary complex neurons.

It is interesting to note that Gilman et al. [7] have recently reported a family with the SCA1 mutation, of which at autopsy one patient showed argyrophilic, tau-positive glial cytoplasmic inclusions in several regions in the CNS, including the cerebellum. These glial cytoplasmic inclusions were very similar, if not identical, to those first described in multiple system atrophy [15, 17]. In our patient 1, however, no such inclusions could be found. Subramony et al. [23] did not mention this feature.

In conclusion, the three autopsy cases from two different families with genetically identified SCA6 described so far strongly suggest that the pathological phenotype of

this disease is cerebello-olivary atrophy [6]. Taken together with the clinicopathologically similar previous cases, we believe that in a strict sense, the disease is a form of cerebellar cortical atrophy [19], and that glial cytoplasmic inclusions could not be a feature.

Acknowledgements We wish to express our appreciation to Mr. S. Egawa, Mr. T. Hasegawa, Ms. C. Tanda, and Ms. Y. Ohta for their technical assistance, and to Ms. M. Machida and Ms. K. Abe for their help in preparing the manuscript.

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