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Spinocerebellar Ataxia Type 2 in Seven Korean Families: CAG Trinucleotide Expansion and Clinical Characteristics

Studies on spinocerebellar ataxias (SCA) have been hampered by a lack of disease markers. Clinical and pathological heterogeneity also made the classification unreliable. Linkage studies established that there are multiple subtypes of SCA. Five types are found to have unstable CAG expansion; the diagnosis can be established by molecular genetic study. Therefore, we systemically screened degenerative ataxia patients for these five SCA types, and identified eight patients with SCA2 (seven from six families and one sporadic case). This paper presents the clinical information on the seven patients, whose clinical information was available in detail. CAG repeat expansion in the patients ranged from 38 to 47 (normal control, 19 to 27). The onset ages ranged from 16 to 41 with 27.1 years as the mean, which correlated inversely with repeat lengths. All patients presented dysarthria and gait ataxia. Upper limb dysmetria or dysdiadochokinesia appeared later but progressed, causing severe disability. Slow saccade (4 patients in 7) and decreased DTR (4 in 7) were common. MRIs showed severe atrophy of the brainstem and cerebellum in all patients. We conclude that SCA2 is the most frequent type in Korea and carries rather pure cerebellar syndrome, slow saccade, and hyporeflexia.

Key Words: *Spinocerebellar degeneration; Cerebellar ataxia; Cerebral angiography*

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INTRODUCTION

Autosomal dominant cerebellar ataxia (ADCA) represents a clinically and pathologically heterogeneous group of neurodegenerative diseases. Previously, it was divided into three types according to clinical manifestation (1): ADCA type I, progressive cerebellar ataxia with ophthalmoplegia, pyramidal and extrapyramidal signs, amyotrophy, sensory impairment and dementia; ADCA type II, cerebellar ataxia and a progressive macular degeneration; and ADCA type III, a pure cerebellar syndrome. Linkage studies established that there are at least seven different genetic loci causing ADCA: spinocerebellar ataxia type 1 (SCA1) to chromosome 6p22-23 (2), SCA2 to 12q23-24.1 (3-5), SCA3/Machado-Joseph disease to 14q32.1 (6, 7), SCA4 to 16q24-ter (8), SCA5 to the centromeric region of chromosome 11 (9, 10), SCA6 to 19p13 (11), and SCA7 to 3p14-2 (12-14). These subtypes have both phenotypic variability within the same genotype and overlapping phenotypes with other genotypes. Therefore, clinical information alone is inadequate in making a diagnosis. Unstable CAG expansion has been found in five of the seven subtypes of SCA. Using this information, it became possible to make the confirmative diagnosis of these subtypes by simple PCR technique.

The authors systematically screened degenerative ataxia patients and identified eight SCA2 patients. We present the methods used in the gene study and clinical information of these patients.

MATERIALS AND METHODS

Subjects

We studied 22 families with ADCA, referred from all over the country. In this study, the diagnosis of ADCA was made according to: 1) a family history consistent with autosomal dominant inheritance, 2) slowly progressive cerebellar ataxia with or without additional neurologic signs or symptoms, and 3) cerebellar atrophy on brain computed tomography and/or magnetic resonance imaging studies. All families were not related. In addition, we analyzed 21 patients who have the clinical fea-

tures of cerebellar ataxia without a family history. These patients were considered to be the sporadic cases of ADCA.

Clinical information was obtained including the age of onset, cognitive impairment, dysarthria, dysphagia, limb and truncal ataxia, ocular movement and retina, deep tendon reflex, extensor plantar response, sensory function, amyotrophy, tremor, dystonia, myoclonus, rigidity, spasticity, parkinsonism and sphincter disturbances.

Molecular genetic analysis

Peripheral blood was drawn in an EDTA tube with informed consent. High-molecular-weight genomic DNA was extracted from lymphocytes, following the standard procedure (15). The SCA2 gene containing CAG repeats was amplified by polymerase chain reaction (PCR) using the primers SCA 2-A and SCA 2-B (3). Reaction mixtures contained the following components: 200 ng of genomic DNA, 20 pmol of each primer, 1.25 unit of *Taq* DNA polymerase, 2 μL of 2.5 mM dNTPs, 4 μL of 50% glycerol and 8 μL of distilled water in 10×PCR buffer, to reach a final volume of 20 μL. Initial DNA denaturation at 95℃ for 5 min was followed by 35 cycles of denaturation at 95℃ for 90 seconds, annealing at 63 ℃ for 30 seconds, and extension at 72℃ for 90 seconds. The final extension step lasted for 7 min. To determine the CAG repeat length in the SCA2 gene, the aliquots of PCR products were run on a 6% polyacrylamide/7 M urea gel. Band was visualized by silver staining.

Statistical analysis

The correlation between onset age and CAG repeat length was examined by linear regression.

RESULTS

Frequency of SCA2 repeat expansion

Of 22 families and 21 sporadic cases, seven patients from six families and one sporadic case were found to have SCA2 (38.9%). Five patients from two families had SCA1 (11.1%), eight patients from five families and one sporadic case had SCA3/MJD (33.3%), 13 patients from two families had SCA6 (11.1%), and one sporadic case had SCA7 (5.6%).

Distribution of CAG repeat length in the SCA2 gene

Fig. 1 shows the distribution of the repeat length in normal and expanded alleles of the SCA2 gene. The range of CAG repeats in the SCA2 locus in 84 individuals without neurological diseases was from 19 to 27; we examined a total of 168 chromosomes and found that 89.9% had 22 repeats (mean±standard deviation: 22.0 ±0.79). The repeat length in expanded alleles of SCA2 patients ranged from 38 to 47 repeats (Fig. 2). There was no overlap between normal and pathologic alleles. In one family, the proband and his sister were confirmed

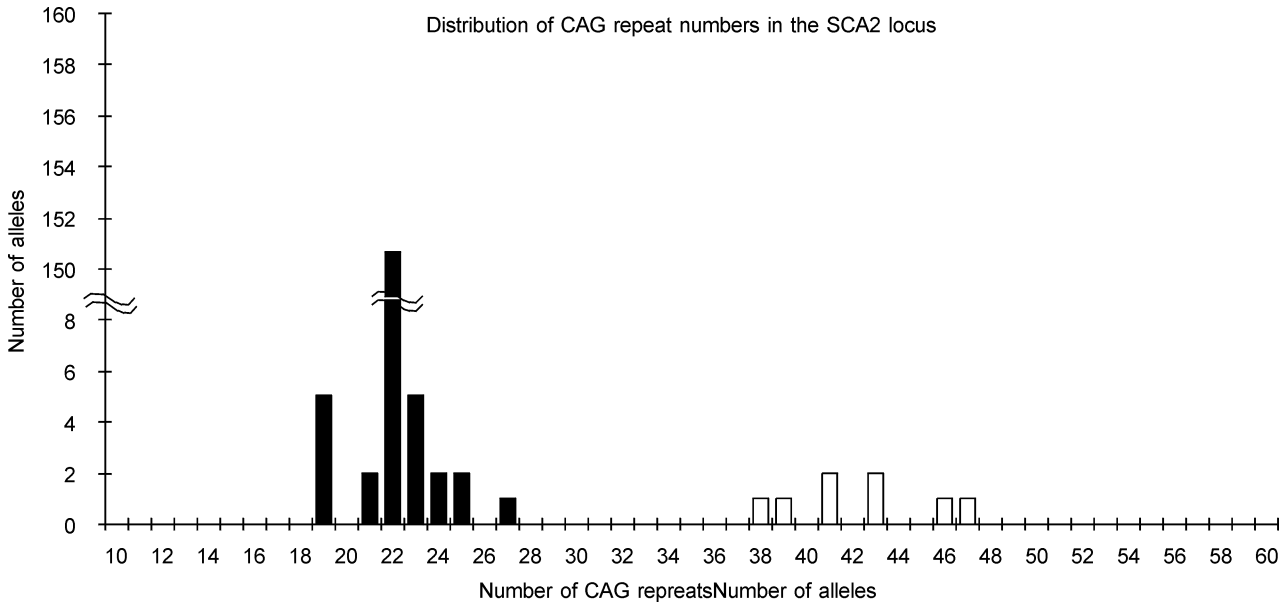

Fig. 1. Distribution of the CAG repeat length in normal and expanded alleles of the SCA2 gene. Black bars represent normal alleles; empty bars, expanded alleles.

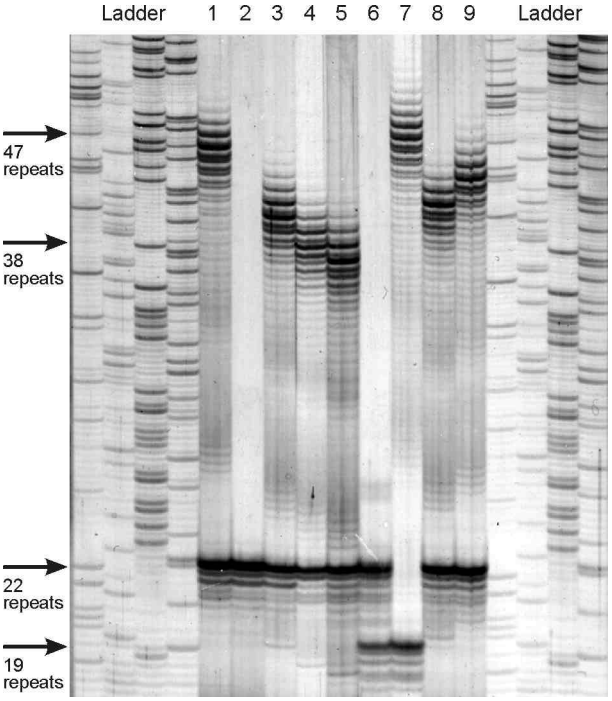

Fig. 2. CAG repeat analysis of SCA2 locus, showing seven patients in six families. Lane 1: Case 1, CAG repeats 46/22; Lane 2: Brother of Case 1, 23/22; Lane 3: Case 2, 41/22; Lane 4: Case 3, 39/22; Lane 5: Case 4, 38/22; Lane 6: Mother of Case 5, 22/19; Lane 7: Case 5, 47/19; Lane 8: Sister of Case 6, 41/22; Lane 9: Case 6, 43/22; Ladder: beta-globin sequences.

to have the expanded allele with CAG repeat numbers of 44 and 42, respectively. In one sporadic case without a family history, the expansion number was 46. All patients were heterozygous for SCA2 repeat expansion.

Age of onset and CAG repeat length

The mean onset age of seven SCA2 patients, whose detailed clinical information was available, was 27.1±9.7 years with a range of 16 to 41. Fig. 3 shows the relationship between the age of onset and the repeat length in SCA2. There was an inverse correlation between these two factors with a r^2 of 0.7 ($p<0.05$).

Clinical features

Clinical characteristics of the seven patients, whose clinical information is available in detail, are summarized in Table 1. There were three men and four women. Disease duration was 7.9±3.4 years. All patients presented dysarthria and gait or limb ataxia. Upper limb dysmetria or dysdiadochokinesia tended to appear later, but progressed to cause severe disability in all patients. The length of CAG repeat did not seem to influence the severity of cerebellar dysfunction. No patients showed

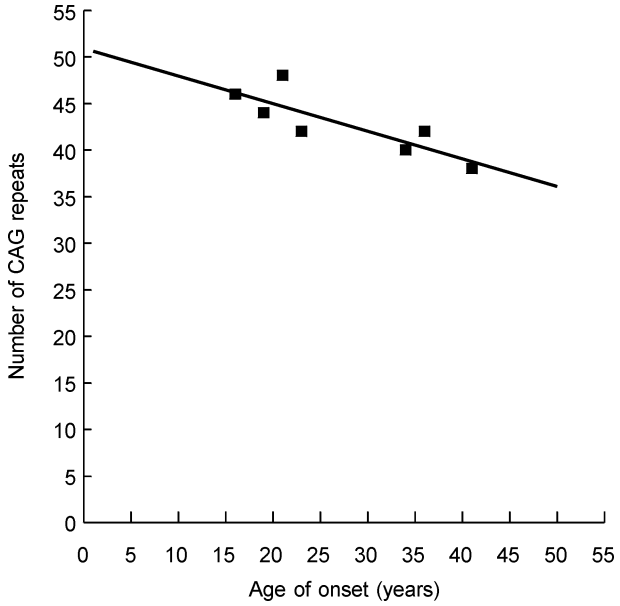

Fig. 3. Age of onset and CAG repeat length. The significant inverse correlation between the onset age and the repeat length was found ($r^2=0.7$, $p<0.05$).

Table 1. Clinical characteristics of seven spinocerebellar ataxia type 2 patients

Dementia or cognitive dysfunction	0
Gaze limitation	2
Gaze nystagmus	0
Impaired smooth pursuit	5
Dysarthria	7
Dysphagia	1
Weakness	0
Muscle tone	
Rigidity	0
Spasticity	0
Hypotonia	0
Muscle stretch reflex	
Hyperactive	2
Reduced	7
Extensor plantar response	1
Sensory disturbance	
Superficial sensation (pain, touch)	0
Deep sensation (position, vibration)	0
Truncal and gait ataxia	7
Limb ataxia	7
Sphincter disturbances	0
Head titubation	1
Myoclonus	1
Bradykinesia, resting tremor, dystonia	0

cognitive impairment. Slow saccade was common, which was found in five patients. But ophthalmoparesis was observed in two patients. No patients had optic atrophy or pigmentary retinal degeneration. Dysphagia was a less

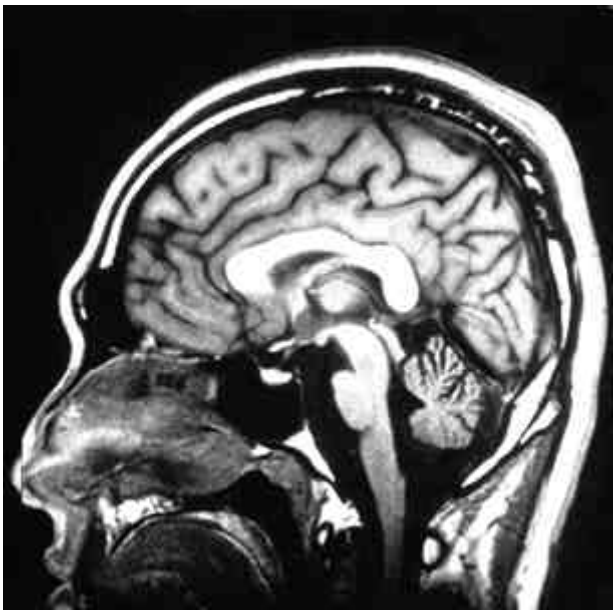

Fig. 4. T1-weighted sagittal MR image of a patient. Cerebellar vermis is atrophic and the fourth ventricle is enlarged.

frequent symptom. Decreased deep tendon reflex in more than one limb was common, and increased deep tendon reflex was detected in two patients at the time of the first neurological examination. Sensory examination was normal in all patients. Extensor plantar response was detected in one patient. None had amyotrophy and sphincter disturbances. One patient showed head titubation; and another had myoclonus. None showed rigidity, spasticity, or parkinsonism.

Fig. 4 shows a representative example of T1-weighted sagittal brain MR images. Brain CT or MR imaging studies were done in six patients, and all showed severe atrophy of the cerebellar vermis, cerebellar hemisheres, middle cerebellar peduncle, pontine base, and medulla oblongata with enlargement of the fourth ventricle.

Nerve conduction studies were performed in three patients, two of whom had axonal neuropathy.

DISCUSSION

In this study, we identified seven patients from six families and one sporadic case with SCA2. We found that SCA2 was the most frequent type (38.9%) among SCAs in Korea. The SCA1 mutation was positive in two families; the SCA3/MJD mutation in five families and one sporadic case; the SCA6 mutation in two families; the SCA7 mutation in one sporadic case (5.6%). The results of this study show different distribution of SCA types from that of other countries (16-20). However, in a recent report by Giunti et al., SCA2 was the most frequent genotype among ADCA I patients (21). These discrepancies may reflect differences in genetic frequencies among countries. The haplotype analysis was not performed in this series. A further study will be required to identify the founder effect.

The range of CAG repeats in the SCA2 locus in normal alleles was 19-27; SCA2 alleles in patients contained moderately expanded CAG repeats of 38 to 47 units, which were comparable to sizes previously reported (3, 22-24). In contrast with Huntington's disease (25), there was no overlap between normal and expanded alleles; therefore, a molecular diagnosis of SCA2 gene was readily done (Fig. 1). Conversely, all patients with expanded CAG repeats in SCA2 gene had progressive cerebellar ataxia and other neurologic deficits.

We found that two sibling patients carried different-sized CAG repeats. This finding reflects the fact that expanded CAG repeats are unstable, and usually change in repeat length during transmission from parents to children (2, 4, 26, 27).

The onset age in SCA2 patients in our series was similar to that in other reports (22, 28). As other diseases associated with CAG repeat expansion, an inverse correlation between onset age and repeat length was found (Fig. 3). This may suggest direct involvement of CAG repeat expansion in the pathogenesis of the disease. In four SCA2 families, who showed cerebellar ataxia in two or more successive generations, blood samples from the parents and related family members were not available because of prior death. Therefore, the degree of anticipation and the existence of paternal imprinting were not analyzed.

We identified one sporadic case with SCA2. The number of CAG repeats and the severity of clinical manifestations were not different from those of other patients with SCA2. The onset age was the lowest in this series, and her parents were healthy. It is not certain whether the parents had relatively small expansion or the sporadic case had a new mutation, because we could not obtain blood samples from the parents. The presence of a sporadic case suggests that we should consider the diagnosis of SCA2 in patients with cerebellar ataxia and without a family history.

Previous clinical descriptions of SCA2 have mentioned a wide variation of phenotypes (18, 22, 29). In this study, all patients had cerebellar dysfunction. Most patients began with dysarthria and gait ataxia. As the disease progressed, limb ataxia became pronounced. Additional non-cerebellar symptoms occurred in all patients. Pyramidal tract signs including increased deep tendon reflex and Babinski sign were detected in a small proportion of patients. These findings are different from those of Burk et al. (16). One of our patients had decreased deep ten-

don reflex and negative Babinski sign initially, and later developed hyperreflexia and positive Babinski sign at a three-year follow-up. Even in this patient, the muscle tone was normal. Pyramidal signs might change with disease duration.

Slow saccadic movement has been observed in several ADCA families (16). In a report which measured saccadic velocities with electrooculography, the velocities in 56% of SCA1 patients, 100% of SCA2 patients, and 30% of SCA3/MJD patients fell below the range of the control group (16). Saccadic velocities were greatly reduced in SCA2, and there was a relatively clear distinction from SCA1 or SCA3/MJD. In our series, slow saccades were found in 71% of SCA2 patients. Although it is not specific for SCA2, it may be a helpful diagnostic clue (20, 22, 23, 29, 30).

At initial examination, all patients with SCA2 showed decreased deep tendon reflex at least in one limb. However, sensory examination including deep sensory modalities revealed no definite abnormalities in all patients. Nerve conduction studies performed in three patients demonstrated sensory polyneuropathy in two patients. Decreased deep tendon reflex has been reported in various genotypes of ADCA (16, 31), however, it may be one of the central features of SCA2 (20, 22, 23, 29).

Parkinsonism, dystonia, fasciculation-like movement or sphincter disturbance were not found in our series in contrast to reports by Burk et al. and Giunti et al. (16, 20). Further long-term observation might reveal these features in our patients.

Although the lack of rigidity or spasticity and the presence of slow saccades and hyporeflexia have been noted in various genotypes of ADCA, the coexistence of these features may point toward SCA2 (16, 17, 20-23, 29).

In six patients, brain MR images were available. There was prominent atrophy of the brain stem and cerebellar structures in all patients. We could not find any definite correlation between the CAG repeat number or the onset age and the degree of atrophy in MR images, which was estimated by visual inspection.

In conclusion, we were able to document the presence of multiple types of SCA in our population, with SCA2 as the most frequent. This result shows that the distribution of SCA types differ from that of other countries, and may reflect the founder effect. Slow saccades and hyporeflexia may help differentiate SCA2 from other SCAs.

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