Clinical and Molecular Correlations in Spinocerebellar Ataxia Type 6

A Study of 24 Dutch Families

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Background: Autosomal dominant cerebellar ataxias (ADCAs), or spinocerebellar ataxias (SCAs), are a heterogeneous group of neurodegenerative disorders. Mild CAG repeat expansions in the \( \alpha_{1A} \) voltage–dependent calcium channel gene are associated with SCA type 6 (SCA6).

Objective: To obtain further insight into the contribution of SCA6 mutations to the phenotypic variability in Dutch patients with ataxia.

Design: Survey and case series.

Setting: Hospitalized care, referral center.

Patients and Methods: The SCA6 locus was analyzed for CAG repeat expansions in a referred sample of 220 Dutch families with progressive cerebellar ataxia. Clinical characteristics of patients with SCA6 were investigated and correlated with molecular findings.

Results: The diagnosis SCA6 was confirmed in 24 families comprising 30 familial and 4 sporadic cases. Mean±SD age at onset was 50.1±11.1 years. Expanded CAG repeats with sizes 22, 23, and 25 were found. These sizes correlated inversely with age at onset. No intergenerational changes in CAG repeat size were detected. Despite this, 2 families showed clinical anticipation.

Conclusions: This study provides the first detailed description of Dutch patients with SCA6. Clinical analysis identifies SCA6 as a late-onset ataxia in which eye movement abnormalities are prominent and consistent early manifestations. No single clinical sign can be considered specific for SCA6. Some patients have ataxia combined with episodic headaches or nausea, suggesting an overlap among SCA6, episodic ataxia type 2, and familial hemiplegic migraine. Spinocerebellar ataxia type 6 accounts for approximately 11% of all Dutch families with ADCA. Analysis of SCA6 contributes further to the genetic classification of patients with ADCA, including patients without a clear family history of the disease.

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PATIENTS, MATERIALS, AND METHODS

PATIENTS

Patients were identified through their clinical symptoms or family history of the disease. For genetic classification, DNA samples from approximately 60% of the population of the Netherlands, including 3 of the 8 Dutch university hospitals (Amsterdam, Leiden, Maastricht, Nijmegen, and Utrecht), which often serve as tertiary referral facilities for general hospitals, were referred to the Department of Medical Genetics, University Medical Center Utrecht, Utrecht. Samples were collected over 6 years and represented 220 families with progressive cerebellar ataxia, not all of them necessarily representing autosomal dominant disease, and a few had been initially referred as possibly having ADCA or Friedreich ataxia. After identification of a sample as harboring an SCA6 mutation, clinical information was collected retrospectively from patient medical records. The sample comprised 34 affected and 3 presymptomatic cases, identified through an affected relative. Proband who had positive findings for Friedreich ataxia were excluded from our analysis.

MOLECULAR ANALYSIS

High-molecular-weight genomic DNA samples were isolated from peripheral blood lymphocytes according to standard procedures.

The SCA6 locus was analyzed for CAG repeat expansions using polymerase chain reactions and S-5-F1 and S-5-R1 primers. These reactions were carried out in a PE9600 GenAmp PCR System (Applied Biosystems, Foster City, Calif). Typically, each reaction contained 100 ng of genomic DNA, 200 ng of forward (5'-fluorescein isothiocyanate labeled), and reverse primers in a final volume of 50 µL containing 1.25 U of Taq polymerase (PerkinElmer, Inc, Wellesley, Mass), 1.5 mM deoxy nucleotide triphosphate, 10% dimethylsulfoxide, 0.15-mg/mL bovine serum albumin, 67mM Tris-hydrochloride (pH 8.8), 6.7mM magnesium chloride, 10mM β-mercaptoethanol, 6.7µM disodium EDTA, and 16.6mM diammonium sulphate. The samples were denatured at 94°C for 4 minutes, followed by 33 cycles of denaturation (94°C for 1 minute), annealing (55°C for 1 minute), and extension (72°C for 2 minutes). Then, the polymerase chain reaction products were analyzed on 6% polyacrylamide/7M urea gels on an ABI377 semi-automated DNA Sequencer (Applied Biosystems). The respective allele sizes were determined using internal size standards.

GENEALOGICAL STUDIES

To detect possible founder effects, the origin of the SCA6 families was traced back as far as possible to identify common ancestry by using birth, marriage, and church registries.

drew et al21 and Sinden22). In addition, SCA6 CAG repeats are remarkably stable during intergenerational transmission, whereas all other expanded SCA alleles have been shown to be unstable to varying degrees. Despite the observed intergenerational stability of the repeat, clinical anticipation is reported, based on intrafamilial increased severity of symptoms and earlier age at onset. The molecular basis of this phenomenon still remains to be elucidated.

Clinically, the phenotype caused by SCA6 mutations presents relatively mild symptoms compared with the other autosomal dominant ataxias. Spinocerebellar ataxia type 6 is characterized predominantly by adult-onset ataxia, dysarthria, and early ocular motor disturbances. However, overlap with the other ADCAs is considerable, and patients with SCA6 may present with variable clinical symptoms, ranging from the classic, rather severe ADCA type I (cerebellar ataxia, dysarthria, pyramidal and extrapyramidal signs, optic atrophy, ophthalmoplegia, and deep sensory loss or dementia) to ADCA type III (the late-onset, purely cerebellar phenotype). In general, age at onset seems to be somewhat later than for other SCAs, and disease progression is often less severe. Also, in contrast to most other ADCA variants, especially SCA1, SCA2, and SCA3, a clear family history is not always apparent. Several authors27,30,31 describe sporadic patients who carry a CAG repeat expansion at the SCA6 locus.

To obtain further insight into the contribution of SCA6 mutations to the phenotypic variability of this subset of patients with ADCA, we report the clinical and molecular analysis of patients with SCA6 from 24 families that were identified, and consecutively analyzed, in a large panel of Dutch families with progressive cerebellar ataxia.

RESULTS

MUTATION ANALYSIS

From our DNA bank we identified 34 affected individuals with SCA6 expansions; 30 presented with a clear family history of the disease and 3 seemed to be the only affected individual in their respective families. The remaining proband claimed to be sporadic, but insufficient family history data did not allow confirmation. In addition, 3 individuals who submitted DNA for presymptomatic testing were found to carry an expanded CAG repeat.

These 34 affected individuals represented 24 separate Dutch families of the total of 220 tested families (11%). Spinocerebellar ataxia type 6 accounted for 31% of SCA-positive (ie, SCA1, SCA2, SCA3, SCA6, or SCA7) families in our cohort (data not shown).

Of these 24 families, 20 index patients had a clear family history of cerebellar ataxia on initial clinical presentation. Four index patients were diagnosed initially as being sporadic patients. However, on identification of the SCA6 mutation, 1 patient had a positive family history after all. Family investigation did not reveal additional affected family members of the remaining 3 patients. Furthermore, the clinical diagnosis was confirmed in 10 additional affected individuals from 8 families.

CLINICAL CHARACTERISTICS OF PATIENTS WITH SCA6

Symptoms at Onset

Information about symptoms at onset was available for 33 of the 34 affected patients (16 men and 17 women).
The following were first noticed by the patients themselves or by their relatives as initial symptoms: unsteadiness of gait or problems with cycling (n = 20); vertigo or dizziness that was commonly evoked by head movements (n = 14); speech difficulties (n = 7); diplopia or oscilloscopia (n = 2); nausea (n = 2); and manual clumsiness (n = 1). Often, multiple symptoms manifested at more or less the same age, particularly vertigo, unsteadiness, and speech impairment.

Manual clumsiness, indicative of upper limb ataxia, was always milder than gait ataxia. In the 6 patients in whom the onset of clumsiness could be estimated, it followed gait ataxia in 5 patients by 2 to 5 years (mean, 3.4 years). In the sixth patient, manual clumsiness was associated with gait problems as a presenting symptom.

A 35-year-old woman, with an affected mother and an affected sister, complained only of episodic nausea and showed subtle eye tracking difficulties; on neurological examination, no other abnormalities were found. These episodic manifestations may therefore represent either a first or a sole disease manifestation.

**Age at Onset and Duration**

Spinocerebellar ataxia type 6 starts at a relatively late age (Table 2). Taking the age of first appearance of any of these symptoms as the age at onset of the disease, the mean ± SD age at onset is 50.1 ± 11.1 years (n = 31) (range, 16-72 years). The patient who claimed to experience gait difficulties from age 16 years onward was first examined at age 34 years, when his gait ataxia was still mild, so in this case the indicated age at onset may have been estimated to be too young. However, 2 well-documented cases had onset at ages 34 and 35 years, respectively, whereas 1 patient who mentioned onset at age 72 years had only slight gait ataxia without dysarthria at age 74 years. Therefore, these onset ages, 34 vs 72 years, may represent the normal range of onset (Table 2). Apart from a late onset, SCA6 runs a protracted course. The mean ± SD duration of disease, ie, the time from estimated onset until most recent examination, was 12.4 ± 9.1 years (n = 29). Of these 29 patients, 8 had disease durations of 0 to 5 years; 4, of 6 to 10 years; 12, of 11 to 20 years; 3, of 21 to 30 years; and 2, of 31 years. For 7 patients, information was available on the number of disease years after which a wheelchair was required (range, 5-23 years; mean ± SD, 14.4 ± 7.1 years). Thus, the disease may last more than 3 decades.

**Clinical Spectrum of SCA6**

The full-blown clinical picture generally consisted of the following: (1) oculomotor abnormalities, (2) gait ataxia, (3) mild upper limb ataxia, and (4) dysarthria. Findings from at least 1 detailed neurological examination could be extracted from the medical records for 27 affected patients.

**Oculomotor Abnormalities.** In 26 patients, findings from an oculomotor examination were reported at least once during the course of the disease. The absence of eye movement abnormalities was specifically mentioned in only 1 of these patients, although gait and limb ataxia and dysarthria were present. The following were noted in these patients: saccadic intrusions during smooth pursuit in...
the disease were inconsistently noted in medical records, logical abnormalities were present. 21 persons and to be absent in 5, although other neurological abnormalities were present. Dysarthria. Dysarthria was considered to be present in 15 patients, was deemed absent in 2, and was considered ambiguous in 5. No notes on upper limb ataxia were found for 2 patients.

Mild Upper Limb Ataxia. Mild to severe upper limb ataxia in the course of the disease was noted in 18 patients, was deemed absent in 2, and was considered ambiguous in 5. No notes on upper limb ataxia were found for 2 patients.

Dysarthria. Dysarthria was considered to be present in 21 persons and to be absent in 5, although other neurological abnormalities were present. Additional neurological features during the course of the disease were inconsistently noted in medical records, but the following summary gives an impression of the evolving clinical picture. In 10 patients, hyperreflexia was documented in the course of the disease; in 4 patients, this occurred within 5 years of onset. In 2 of these 10 patients, amyotrophy was also documented. Polyneuropathy, not further specified, was noted in 1 person. In 1 instance, bilateral hand dystonia was noted, whereas in 1 patient with amyotrophy, a staring face was prominent, resulting in a clinical picture similar to that of Machado-Joseph disease. Headache episodes were recorded in 5 patients, and in 3 these were definitely absent.

**GENOTYPE/PHENOTYPE CORRELATION AND ANTICIPATION**

The range of the expanded alleles represented is limited, with all but 2 patients having either 22 or 23 repeats and the remaining 2 having 25 repeats (Figure). Normal alleles are represented by sizes 7 to 14. There is no overlap between normal and disease alleles.

We used an unpaired, 1-tailed t test to compare the mean±SD onset age in patients with CAG size 22 (n = 22) vs CAG size 23 (n = 8). A significant difference between the onset age of the allele size 22 group (53.5±8.9 years) and the allele size 23 group (47.1±7.3 years) was noted (P = .03, 1-sided), illustrating the inverse correlation between CAG repeat size and onset age.

We did not detect any intergenerational change in CAG repeat size. In 9 families, more than 1 individual with an expanded CAG repeat is identified, but no intergenerational changes in allele size have been detected (Table 3). Clinical anticipation, defined as 5 or more years’ difference in disease onset, is present in 2 of 4 families in whom onset data on a parent and at least 1 affected child were obtained. The onset of cerebellar ataxia in the affected parent of this last family, however, was assessed retrospectively only, after many years of illness.

Genealogical studies enabled linking of 2 families to 2 other families in this sample. Two independently referred probands (from families 8 and 11) seemed to be second cousins, whereas 2 other probands (from families 12 and 13) are related 4 generations back. In this last extended family, 2 affected individuals are at 8 meioses distance from one another, yet the CAG size is 22 for both.

**COMMENT**

Identification of the SCA6 mutation by Zhuchenko et al allows further genetic classification of patients with ADCA. In their ethnically diverse clinical sample, Zhuchenko et al identified 6% of the index cases as having SCA6. In our cohort, which consists of an unselected clinical sample of mainly Dutch ancestry, the relative prevalence of SCA6 is 11%. Within Europe, this frequency is comparable to the frequency of SCA6 in the western part of Germany (10%) but higher than in the United Kingdom (5%), France (2%), and Spain (1%).35-34 Similar regional differences are reported in Japan, where the SCA6 frequency ranges from 6% to 31% depending on the population studied.35,36

The mean±SD age at onset of SCA6 in our patients (50.1±11.1 years) falls within ranges described by other
researchers,\textsuperscript{26,34,35,37} which vary from 43 ± 13 years to 52 ± 13 years. Therefore, SCA6 may be considered to be a relatively late-onset, autosomal dominant, purely cerebellar ataxia (ADCA type III in the Harding classification\textsuperscript{23}).

Early eye movement abnormalities are among the earliest and most consistently detected presenting clinical features. Together with a history of dizziness, vertigo, or nausea, these manifestations seem to be more specific for early SCA6 than do gait ataxia or dystarthis, which are almost always the earliest signs in the other ADCAs. No single clinical sign can be considered to be specific for SCA6.

The different SCA6 alleles in Dutch patients contained expanded CAG repeats of 22 to 25 units, whereas the normal alleles contained 7 to 14 units, which are comparable to the sizes reported elsewhere.\textsuperscript{8,26-28,31,34-37} Despite the relatively small sample size, an inverse correlation between onset age and SCA6 repeat size was noted. We did not observe any change in the SCA6 repeat length during transmission, as reported in most other studies except 2.\textsuperscript{30,38} The stability of the SCA6 CAG repeat is even more apparent by the detection of SCA6 alleles with identical repeat numbers in probands of independently referred families linked through genealogical studies and separated by 6 and 8 meioses. In addition, preliminary haplotype analysis with polymorphic markers from the SCA6 region (results not shown) suggests a shared SCA6 haplotype for several families with identical CAG repeat sizes, indicating SCA6 CAG repeat stability at even more distant relationships.

Despite the stability of the expanded SCA6 repeat alleles, of the 4 families in whom reliable data were available, 2 show a marked decrease in age at onset. Taken together with the 3 patients without a clear family history of the disease, these results suggest clinical anticipation. Other researchers\textsuperscript{27,31,35-37} have also reported clinical anticipation for SCA6, mostly on the basis of age at onset. However, to our knowledge, the underlying molecular defect, ie, a further expansion of the SCA6 CAG repeat, has not been identified to date. Therefore, the observed anticipation in families with SCA6 may well represent an ascertainment bias caused by the significant variability of the disease’s onset. In a late-onset disorder such as SCA6, an earlier onset in later generations will accordingly be noted, whereas an onset at an even later age than the patients’ parents will not be detected in clinical surveys. In the former situation, the possible effect of additional genetic (modifier genes) or environmental factors must be considered.

Twenty of the 24 index patients had a positive family history of the disease. For one of the probands, a positive family history could be retrospectively elicited after identification of the disease, typically by a detailed family history. In 3 patients, the disease manifested as sporadic ataxia, suggesting that SCA6 should be considered in the diagnostic workup of patients with sporadic late-onset cerebellar ataxia.

A limited number of patients with sporadic SCA6 are reported in most studies, and herein we identified 4 patients with SCA6 without a known family history of the disease. To our knowledge, there is no report of non-penetrance in SCA6. Thus, the lack of further family analysis of these patients with sporadic SCA6 still leaves open the possibility that they indeed represent true sporadic patients carrying de novo SCA6 mutations. Alternatively, the SCA6 diagnosis for the parents of these patients may have been missed because the onset may have been late in life, very mild, or episodic. Episodic features such as headache and nausea, mentioned by a few of our patients, suggest that the SCA6-mutated calcium channel subunit may give rise to episodic clinical complaints, similar to the manifestations of EA-2 and familial hemiplegic migraine. Similar findings were described by Geschwind et al.\textsuperscript{29} Jodice et al\textsuperscript{30} described a family with EA-2 in which the ataxia phenotype resulted from a CAG repeat expansion instead of from point mutations, as reported by Ophoff et al.\textsuperscript{31} Patients with a CAG\textsubscript{23} allele presented with severe progressive ataxia, whereas those with a CAG\textsubscript{49} allele presented with an EA-2 phenotype. This is the second study, to our knowledge, of an intergenerational increase in SCA6 allele size (see also Matsuyama et al\textsuperscript{32}), and it demonstrates the overlap between EA-2 and SCA6. This may provide new clues for the apparently true sporadic cases. In addition, Yue et al\textsuperscript{33} report point mutations in the CACNA1A gene to be the cause of a severe progressive cerebellar ataxia syndrome with episodic features. In conclusion, it seems that point mutations and CAG repeat expansions in the CACNA1A gene result in clinically overlapping syndromes. Additional family and functional studies will be required to resolve the overlap between these clinically different disorders.

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