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ARTICLE

Germ-line CAG repeat instability causes extreme CAG repeat expansion with infantile-onset spinocerebellar ataxia type 2

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The spinocerebellar ataxias (SCA) are a genetically and clinically heterogeneous group of diseases, characterized by dominant inheritance, progressive cerebellar ataxia and diverse extracerebellar symptoms. A subgroup of the ataxias is caused by unstable CAG-repeat expansions in their respective genes leading to pathogenic expansions of polyglutamine stretches in the encoded proteins. In general, unstable CAG repeats have an uninterrupted CAG repeat, whereas stable CAG repeats are either short or interrupted by CAA codons, which – like CAG codons – code for glutamine. Here we report on an infantile SCA2 patient who, due to germ-line CAG repeat instability in her father, inherited an extremely expanded CAG repeat in the SCA2 locus. Surprisingly, the expanded allele of the father was an interrupted CAG repeat sequence. Furthermore, analyses of single spermatozoa showed a high frequency of paternal germ-line repeat sequence instability of the expanded SCA2 locus.
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INTRODUCTION

The spinocerebellar ataxias (SCA) are a genetically and clinically heterogeneous group of diseases, characterized by dominant inheritance, progressive cerebellar ataxia and a variety of extracerebellar symptoms. Mutations in 32 different genes have so far been associated with dominantly inherited autosomal SCA.[1] SCA 1, 2, 3, 6, 7, 12, 17 and dentatorubral pallidoluysian atrophy (DRLPA) are all caused by unstable CAG-repeat expansions in their respective genes leading to pathogenic expansions of polyglutamine stretches in the encoded proteins, hence the name polyQ diseases.[1] The CAG-repeat disorders also share a tendency of anticipation, which is more likely to occur when the mutation is transmitted through the paternal germ line. In general, unstable CAG repeats have uninterrupted CAG motifs, whereas stable CAG repeats are either short or interrupted by CAA codons also coding for glutamine.[1–3] SCA 2, 7, Huntington's disease and DRLPA can present with extreme expansions in the CAG repeats leading to infantile/juvenile onset of the disease, often displaying different phenotypes.[4,5]

Adult-onset SCA2 is characterized by progressive ataxia, extremely slow saccades, dysarthria, supranuclear ophthalmoplegia, cognitive impairment, peripheral neuropathy, as well as action or postural tremor, and the mean age of symptom onset is 30–35 years. The CAG repeat is located on chromosome 12 in the Ataxin-2-gene (*ATXN2*), and normal alleles have 31 or fewer CAG repeats.[1]

Only a few infantile SCA2 patients with onset between 2 and 12 months have been reported. They were heterozygous for CAG repeats between 92 and 750 and presented with developmental motor delay, hypotonia, mental retardation and ocular involvement.[4–6]

Here we report on an infantile SCA2 patient, who inherited an extremely expanded CAG repeat in *ATXN2* from her father due to paternal germ-line CAG repeat instability. Contrary to the general assumption, the expanded allele of the father was an interrupted CAG repeat sequence. Furthermore, analyses of single spermatozoa revealed a high frequency of paternal germ-line repeat sequence instability of the *ATXN2* locus.

MATERIALS AND METHODS

Clinical examination

The proband was examined neurologically, and magnetic resonance imaging (MRI) scan and neuropsychological tests were conducted. His daughter was examined clinically and blood samples were obtained and analyzed for metabolic, mitochondrial and lysosomal abnormalities. Furthermore, electroencephalography (EEG) and brain MRI were conducted.

Molecular analysis

DNA was isolated from blood by standard methods. Genotyping of the *ATXN2* repeat was performed by fluorescent-labeled primer PCR and the presence of the extremely elongated CAG repeats was tested by triplet repeat primed PCR (TP-PCR). Analyses of the CAG repeat in single cells were conducted using TP-PCR, after isolation of single spermatozoa.[7] Several blank controls were included in each run. All fragment-length determinations were performed with capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Nærum, Denmark).

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To identify the exact sequence of the CAG/CAA repeats, PCR products containing the *ATXN2* CAG/CAA repeats were amplified from genomic DNA, cloned into the PCR2.1 vector (Invitrogen, Nærum, Denmark) and sequenced. In order to exclude the possibility that the sequenced CAG/CAA patterns were caused by an artifact of the PCR amplification, each allele was cloned twice from two different PCR reactions.

Primer information, PCR conditions and so on are available as Supplementary Information.

RESULTS

Clinical report

The proband, a 49-year-old man (II-3, Figure 1), was referred because of gait disturbances and imbalance, difficulties in speaking and slightly decreased tactile sensation of the fingertips. The symptoms had progressed slowly during the last 8 to 10 years.

Neurological examination revealed bulging eyes, difficulties in initiating saccades, slow saccades, fasciculations in the face and on the medial side of the thighs, dysarthria and limb ataxia. The gait was wide-based, and he was unable to perform tandem walking (12 on the scale for the assessment and rating of ataxia[8]). Neuropsychological test revealed problems with concentration and speed, as well as mild executive dysfunction. A brain MRI showed discrete cerebellar atrophy and an enlarged cerebello-medullary cistern (Figure 2). Taken together, this was suggestive of SCA and the patient was subsequently tested positive for SCA2 with CAG/CAA repeat lengths of 22 and 45 in *ATXN2*.

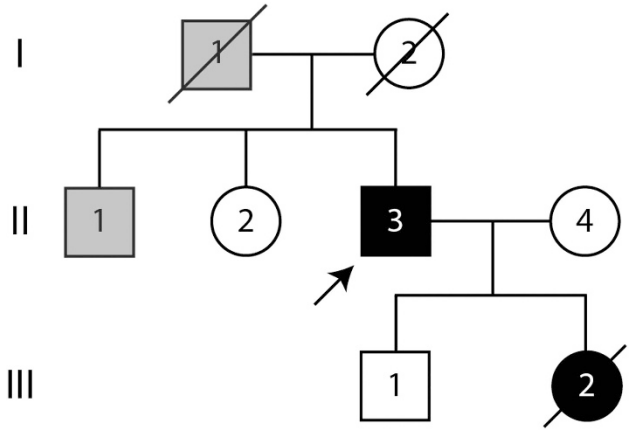

Figure 1 Pedigree of the SCA2 family. Individuals marked with solid black have a molecular diagnosis of SCA2 accompanied by clinical manifestations. Individuals marked with gray shading have a clinical diagnosis of a neurological disorder that has not been confirmed at the molecular level. The proband is marked by an arrow.

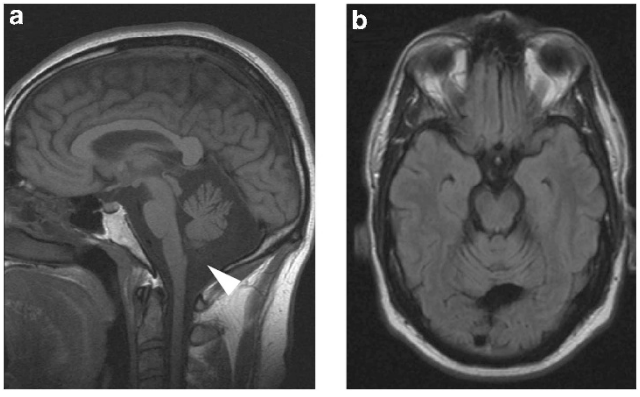

Figure 2 T1-weighted brain MRI scans of the proband. Sagittal (**a**) and axial views (**b**) of the proband showing marked cerebellar and brain stem atrophy and an enlarged cerebello-medullary cistern (arrowhead).

The family history revealed that the proband's father (I-1) developed increasing balance and gait problems at the age of 40 and died 30 years later of what at that time was thought to be Alzheimer's disease. His brother (II-1) was reported to have similar gait disturbances, and his daughter (III-2) had died from multi-organ failure at the age of 19 months.

A detailed review of hospital records revealed that the daughter (III-2), 13 years before the diagnosis of the proband, developed myoclonic jerks, delayed motor milestones and signs of visual impairment at the age of 3 months. She was born at term after a normal pregnancy. At the age of 6 months, she was referred to a local hospital, where she was described as having no or very sparse eye contact, uncoordinated eye movement with parallel eye axes, lack of head control and hypotonia in the upper extremities and the trunk. Furthermore, generalized myoclonic jerks and athetoid movements were noticed. Muscle tone and reflexes were normal. EEG showed bilateral spike foci in the frontal and parietal regions. At the age of 9 months, brain MRI was normal except for a relatively large cerebello-medullary cistern. Metabolic screening showed no sign of inborn errors of metabolism, mitochondrial or lysosomal abnormalities. Antiepileptic therapy and physiotherapy were instituted. At the age of 13 months, she had motor improvement, but still had many dyskinetic movements while awake. Ophthalmologic examination showed delayed visual development, pallor of the optic nerves and hyperpigmentation of dystrophic retinae. A tentative diagnosis of dyskinetic cerebral palsy was assigned. At the age of 17 months, she developed generalized edema and proteinuria, and minimal change glomerulonephritis was diagnosed based on a kidney biopsy. She died of sepsis and multi-organ failure 2 months later despite intensive care treatment. The parents objected to an autopsy, but DNA from peripheral leukocytes was saved for future analyses.

Mutation analyses

The diagnosis of SCA2 in the proband prompted testing of genomic DNA from his daughter. Initial fragment analysis showed apparent homozygosity for a 22-CAG repeat in *ATXN2*. However, TP-PCR analysis revealed the presence of an expanded allele of at least 124 repeats (Figure 3), and it was concluded that the girl had suffered from infantile SCA2. Cloning and direct sequencing of the proband's alleles revealed the following CAG/CAA patterns:

'22': $(CAG)_8CAA(CAG)_4CAA(CAG)_8$
'45': $(CAG)_{36}CAA(CAG)_8$.

Cloning of the girl's short allele showed a CAG/CAA pattern of $(CAG)_8CAA(CAG)_4CAA(CAG)_8$. Cloning of the long allele was unsuccessful.

Single-cell sperm analyses

To investigate whether the transmission of the extremely elongated allele was due to a single rare event during meiosis, we analyzed the length of the CAG repeat in *ATXN2* in single spermatozoa from the proband by TP-PCR. In 33 single spermatozoa we found CAG repeats ranging from 22 to at least 116 CAGs (Table 1 and Figure 3), and in a healthy control we found alleles with CAG repeat lengths of only 22 and 23 in accordance with results obtained by fragment analyses of genomic DNA from peripheral leukocytes; thus, no signs of meiotic instability were present in the healthy control (Table 1). Finally, single

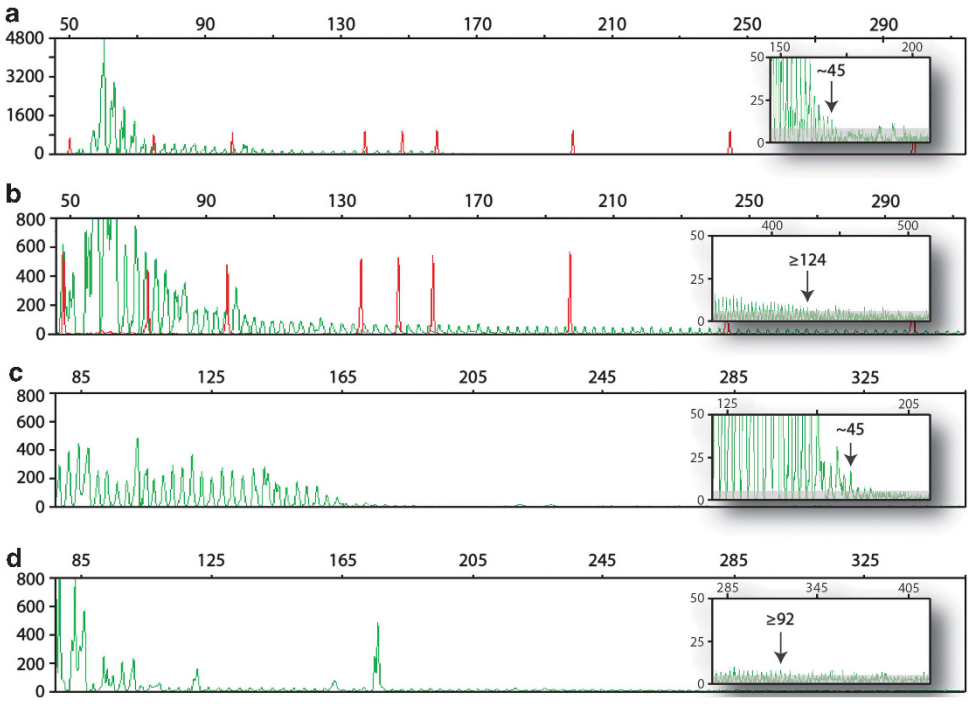

Figure 3 Estimation of CAG repeat length in *ATXN2* by triplet repeat primed PCR (TP-PCR). The TP-PCR peak profiles (green) show a gradually descending array of peaks. The length of the CAG repeat is estimated from the length of the longest TP-PCR product distinguishable from signal noise (gray-shaded area in the insets). Insets show zooms of the corresponding profiles in order to more precisely estimate the minimal CAG repeat length. Numerical values on the x-axis denote the product size, whereas the y-axis denotes arbitrary units of fluorescence intensity. Size standards are shown in red. (**a**) Profile from TP-PCR on genomic DNA of the proband. The size of the longest allele of the proband was estimated to be 45 CAG repeats. (**b**) Profile from TP-PCR on genomic DNA of the proband's daughter. The size of the longest allele was estimated to be at least 124 CAG repeats. (**c**, **d**) Representative results from two isolated single cells of the proband show repeat lengths of about 45 (c) and at least 92 (d).

Table 1 Length of *ATXN2* CAG repeats detected in single-cell spermatozoa

Length of CAG repeat	*Proband*	*Unrelated patient*	*Healthy control*
22	15	24	16
23	0	0	12
38	0	21	0
45	14	0	0
50	1	0	0
58	1	0	0
≥70	0	1	0
≥80	0	1	0
≥92	1	1	0
≥100	0	1	0
≥116	1	0	0
Total[a]	**33** (80)	**49** (63)	**28** (50)

[a]Numbers in parentheses denote the total number of single spermatozoa on which analysis was attempted. The single copy of DNA in combination with the TP-PCR method and the high risk of contamination lowered the number of successfully genotyped single spermatozoa to the numbers indicated in bold.

spermatozoa of an unrelated SCA2 patient with repeat lengths of 22 (repeat structure: $(CAG)_8CAA(CAG)_4CAA(CAG)_8$) and 38 (no CAA interruptions) showed a similar degree of meiotic instability (Table 1), as 4 in 25 cells originating from cells carrying the patient's 38 allele had long CAG repeats (≥70) in *ATXN2*.

DISCUSSION

Here we report on a family in which infantile-onset SCA2 was diagnosed several years post-mortem after disease manifestation in her father. Molecular genetic analysis revealed that the infant had a CAG repeat length in *ATXN2* of at least 124, expanded from her father's allele of only 45 CAG repeats. CAG repeat instability has previously been described in a number of polyQ diseases as germ-line instability or intergenerational variations in repeat size.[4,5,7,9,10] However, to our knowledge, analyses of germ-line instability have not been investigated in SCA2. Out of 18 single spermatozoa with the expanded *ATXN2* allele, 4 (22%) showed an expansion beyond the 45 CAG repeats detected in somatic cells. Two spermatozoa harbored CAG repeat lengths of at least 92 and 116, respectively. Ninety-two CAG repeats is the shortest repeat known to have caused infantile-onset SCA2.[5] A high degree of germ-line instability is also found in SCA1, SCA3, SCA7 and DRPLA. However, in SCA1 and SCA3 the longest gains reported were by 12 and 24 CAG repeats,[7,11,12] respectively, whereas much longer gains have been reported for SCA7 and DRPLA.[9,10] Exactly why CAG repeats in some genes are gaining more repeats than others remains obscure, but it seems that within a given disease longer repeats tend to be more unstable, as do pure CAG repeats, compared with repeats interrupted by CAA codons.[2,3] The extreme expansion observed in the girl was surprising, given the fact that her father's pathogenic allele of 45 glutamine codons was interrupted by a CAA codon at position 37. Furthermore, although SCA2 primarily has been associated with pure repeats of more than 35 CAGs[13–15] and interrupted repeats with lengths between 34 and 49 glutamine codons with parkinsonism,[16,17] the proband in our case had a classic SCA2 phenotype. The observation that the CAG expansion in the proband's interrupted allele has happened prior to the interruption is consistent with previous results showing that the 5′-tract of interrupted repeats is much more prone to expansions than the 3′-CAG tract.[3]

Upon analyses of sperm from two unrelated SCA2 patients, we found high frequencies of extremely long CAG repeats suggesting that expansion to extreme lengths is a fairly common event. This suggests that such meiotic instability is a general feature of SCA2, although analyses of sperm from more patients are needed to draw firm conclusions in this regard. However, few cases of infantile-onset SCA2 have been reported worldwide, and it is possible that those spermatozoa with very long repeats are dysfunctional or have impaired survival, or that very long repeats are associated with reduced embryonic survival.

In conclusion, we report on an infantile SCA2 patient diagnosed post-mortem after manifestation of the disease in her father. This highlights the importance of considering rare genetic disorders during diagnosis of infants and children even without known neurodegenerative disorders in the family. Furthermore, we show a high degree of germ-line instability in the *ATXN2* locus of her father in spite of a CAA interruption in his CAG repeat as well as in an unrelated patient with a pure CAG repeat. Our observations question the general assumption that instability of *ATXN2* is primarily limited to pure CAG repeats and that extreme expansion of the CAG repeat is very rare events.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Consent and ethics statement: Written informed consent for publication of this report was obtained from all patients alive. The study was approved by the Ethics Committee for the Copenhagen Regional Area, journal no. H-KF-328548.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)