Massive CAG Repeat Expansion and Somatic Instability in Maternally Transmitted Infantile Spinocerebellar Ataxia Type 7

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Spinocerebellar ataxia type 7 (SCA7) is a neurodegenerative disorder. It is one of at least 40 genetic conditions, including several spinocerebellar ataxias (types 1-3, 6-8, 10, 12, 17, 31, and 36), caused by expansion of repeated nucleotide sequences. Clinically, SCA7 is characterized by cerebellar ataxia and progressive retinal degeneration as well as other clinical features, including dementia, hypoacusia, and auditory hallucinations. It is caused by a CAG repeat expansion in the \( ATXN7 \) gene (OMIM 164500), which encodes for a polyglutamine tract in ataxin-7. At the messenger RNA level, ataxin-7 is most highly expressed in central nervous system (CNS) tissues and to a lesser degree in non-CNS tissues, including kidney and muscles.

In SCA7, unaffected individuals typically have 4 to 18 CAG repeats in the \( ATXN7 \) gene, while pathogenic alleles have between 36 and 38 repeats and as many as 460 repeats. Symptoms usually manifest in the third to fourth decades of life, with repeat sizes in the range of the mid-30s to 40. Individuals with 55 to 460 repeats develop juvenile or infantile forms of SCA7 as the repeat number increases. Juvenile SCA7 occurs via maternal and paternal transmission of the mutation, whereas the infantile form has only been observed via paternal transmission. Previously reported maternal transmission expansions are in the range of 5 to 10 gained repeats.

Somatic CAG instability has been reported in a SCA7 transgenic mouse model, but intertissue CAG instability has not been reported in human SCA7 tissues. We report the first maternally transmitted infantile case of SCA7 to date, with marked intertissue repeat instability and renal involvement.

Report of a Case

A 38-year-old woman of Peruvian descent was seen with progressive ataxia, muscle weakness, and visual disturbance. She had first developed leg weakness and fatigue in her early 20s and difficulties with balance at age 28 years. She subsequently developed bulbar symptoms and memory deficits during the year before clinical evaluation. Her early medical and developmental history was normal. Clinical examination at age 38 years revealed decreased visual acuity, mild generalized proximal weakness, dysarthria, dysmetria, dysdiadochokinesthesia, brisk deep tendon reflexes, and gait ataxia. Visual evoked potentials showed bilateral delayed central conduction, and brain magnetic resonance imaging revealed cerebellar and pontine atrophy (Figure 1A). The family history revealed that her father had ataxia (with gait disturbance beginning in his 30s) and poor vision. She had a son who had died at age 3 years of an undiagnosed progressive neurological disease, who also had focal segmental glomerulosclerosis. Commercial genetic analy-
sis of the proband revealed 42 and 12 CAG repeats in the \textit{ATXN7} gene, confirming a diagnosis of SCA7.

The proband’s son had been born following a pregnancy complicated by maternal hypertension. Delivery was induced at 36 weeks’ gestation, and the birth weight was 3400 g. The neonatal history was normal. He achieved head control at age 3 months, rolling at age 7 months, sitting at age 11 months, and cruising and crawling at age 1 year. He never gained the ability to walk independently and never developed speech. At age 16 months, he had regression of motor and cognitive skills. He subsequently lost the ability to cruise and crawl and maintain head control, and he developed ataxia and tremor. At age 2 years 5 months, brain magnetic resonance imaging revealed marked atrophy of both cerebellar hemispheres and vermis (Figure 1B). At age 2 years 6 months, he developed proteinuria, and a renal biopsy specimen revealed focal segmental glomerulosclerosis and abnormal podocytes with cytoplasmic inclusions but no nuclear inclusions (Figure 2). A muscle biopsy specimen revealed a mild increase in glycogen content with scattered atrophic type II fibers (Figure 3). Echocardiography showed an asymmetric hypertrophic left ventricle. On examination at age 3 years, he had alternating esotropia, severe axial and peripheral hypotonia with significant head lag, and absent deep tendon reflexes. Plantar responses were extensor. He died at age 3 years 2 months.

The proband’s son underwent many investigations before any genetic testing in the proband. These tests included a karyotype, chromosomal breakage studies, routine metabolic inves-

![Figure 1. Brain Magnetic Resonance Images of the Proband and Her Son](image1)

A, Sagittal T1-weighted image of the proband (age 38 years). B, A T2-weighted image of her son (age 2½ years). While the proband and her son have atrophy involving both cerebellar hemispheres and the vermis, this is much more pronounced in the son.

![Figure 2. Renal Biopsy Specimen of the Proband’s Son](image2)

A-C. By light microscopy, podocytes (arrowheads) are enlarged with cytoplasmic vacuolation (B and C) and inclusions that are positive by silver staining (C). There is associated collapse and sclerosis (\textit{S}) of the basement membranes, indicative of focal segmental glomerulosclerosis. D-F. By electron microscopy, podocytes show membrane-bound cytoplasmic vacuoles, with contents that vary from rarified (D) to particulate (E) to solid (F). Hematoxylin-phloxine-safranin, original magnification \times400 (A); periodic acid-Schiff, original magnification \times400 (B); and periodic acid-ammonical silver, original magnification \times400 (C), \times15 000 (D), \times30 000 (E), and \times10 000 (F).
Investigations, very-long-chain fatty acids, testing for congenital disorders of glycosylation, hexosaminidase B levels, testing for genetic causes of congenital nephrotic syndrome, full mitochondrial sequencing, and mitochondrial and metabolic microarray analysis (MitoMetPlus aCGH Analysis; Baylor College of Medicine). He also underwent genetic testing for SCA types 1, 2, 3, 6, 8, 10, 13, 14, and 27 and the SCA31-related gene BEAN (OMIM 117210). All test results were negative. Initial test results for SCA7 were also negative, demonstrating homozygous CAG repeat sizes of 12. Given the proband’s subsequent diagnosis of SCA7, an infantile form of SCA7 was strongly suspected in her son. Repeat testing was requested at 2 independent commercial clinical laboratories with the maternal history clearly indicated; however, both test results were negative for SCA7.

Molecular Methods

This study was approved by the Research Ethics Board of the Hospital for Sick Children. After written informed consent was obtained, DNA had been extracted from blood samples of the proband and her son, as well as from the left quadriceps and kidney of the child. The DNA was isolated by standard protocols.12 The SCA7 CAG repeat was amplified by polymerase chain reaction (PCR) as previously described12 using PCR primers SCA7-A and SCA7-BR in 20-μL reactions containing 2.0 μL of 10× buffer, 1.0 μL of 25mM magnesium chloride, 0.15 μL of 10mM of each primer, 6.0 μL of betaine hydrochloride, 0.4 μL of 10mM deoxynucleotide triphosphates, 0.1 μCi of α-32P-dATP, 1.5 U of PCR polymerase (AmpliTaq; Applied Biosystems), and 20 ng of genomic DNA. After a predenaturation step at 94°C for 5 minutes, 32 cycles of 94°C for 45 seconds, 60°C for 1 minute, and 72°C for 1 minute were performed, with a final extension at 72°C for 10 minutes. The PCR-amplified DNAs and a size marker were resolved on 4% polyacrylamide gels and exposed to autoradiographic film (BioMax MR film; Kodak).

Results

Analyses of the SCA7 repeat revealed high levels of somatic CAG instability, with smears and multiple products having distinct expansion sizes (Figure 4). A blood sample from the mother (II-2) showed an expanded allele of (CAG)45 (confirmed by sequencing) and a nonexpanded allele of (CAG)12. The son (III-2) had incurred a large CAG expansion in addition
to a nonexpanded allele. The blood sample from the son showed a broad range of large expansions, with a major peak at (CAG)140, a minor peak at (CAG)92, and a smear extending up to lengths as large as (CAG)250. The kidney and skeletal muscle (left quadriceps) also contained large expansions of (CAG)123 and (CAG)117, respectively. As a positive control, we amplified DNA from a transgenic SCA7 mouse model with (CAG)90 previously described.12

Discussion

Infantile SCA7 is rapidly progressive and fatal, with death occurring in infancy or childhood. The initial features of our patient are in keeping with previous descriptions of infantile SCA7.2-3,8-10 Proteinuria, nonspecific glomerular and tubular changes, and cardiomegaly were observed in an infant with approximately 240 repeats who died at age 11 months.9 Nuclear aggregation of ataxin-7 was widespread in the nervous system, including spinal cord and retina, and in non-CNS tissues, including skeletal and cardiac muscle, kidney, stomach and intestine, pancreas, and pituitary and adrenal glands, in a child with 180 CAG repeats at the time of autopsy (age 2 years 4 months).9

The genetically confirmed diagnosis of SCA7 in our proband made apparent the need to reinvestigate the initial negative SCA7 genetic test results in her son. Clinical laboratories use standard molecular methods (PCR and capillary electrophoresis fragment analysis) for SCA7 testing, which have an upper limit of detecting CAG repeats of approximately 80. This likely is the reason for the apparently normal 12 CAG repeats in the homzygous state on repeat testing of the proband’s deceased son. Subsequent testing by PCR across the SCA7 repeat tract revealed the massively expanded CAG repeat sizes, confirming a diagnosis of maternally transmitted infantile SCA7. This finding illustrates the need for careful evaluation of the family history and clinical assessment of family members, particularly when an infantile or childhood presentation of a more common adult-onset disorder is suspected. Although rare, infantile forms of SCAs and other repeat expansion disorders must be considered in infants and children with neurodegeneration, and adults with these conditions should also be informed about the potential for a child to be born with an infantile presentation owing to anticipation. This case also highlights the limitations of current testing technologies used in clinical laboratories for repeat expansion disorders. For families interested in prenatal genetic testing, the limitations of molecular methods should be considered, and testing that can detect massively expanded alleles should be prioritized where possible. A tiered approach may be appropriate such that standard testing is followed by triplet repeat primed PCR or Southern blot of PCR products if homozygosity for a normal SCA7 allele is detected.13

To our knowledge, this is the first maternally transmitted case of infantile SCA7 reported in the literature; all others cases were paternally inherited.2-3,8,9 Large repeat expansions (including repeats in the infant-onset range) in the germ line of men with SCA7 seem to be more common than in other SCAs (including SCA2).7,14 Severe forms of other repeat diseases show a parental transmission bias. Congenital myotonic dystrophy type 1, the most severe form of myotonic dystrophy, is predominantly seen in the offspring of affected mothers and is only rarely transmitted by fathers.15 While the cause of the severe pathogenesis of infantile and congenital disease forms is unknown, the large expansions are thought to contribute. Huntington disease and SCA1 frequently show contractions of expanded CAG tracts during parent-to-offspring transmission.16

Our patient had variable CAG repeat sizes of more than 100 repeats in 3 different tissues. This is the first evidence to date of intertissue somatic instability of the SCA7 CAG tract in human tissues. Unfortunately, CNS tissues were unavailable for analysis. Intertissue repeat instability is evident in a mouse model of SCA7 with 92 repeats.12 The genomic and epigenetic context contributes to intertissue repeat instability in mice, and these factors likely are contributors to human disease pathology.

Renal involvement in SCA7 is not well documented, even for SCA7 animal models. However, evidence from case reports of infantile SCA7 with large CAG expansions suggests that renal complications may be common and contribute to the morbidity and mortality associated with this condition.8,9 These authors reported nuclear aggregation of ataxin-7 in CNS and non-CNS tissues, including the kidney, detected by immunohistochemistry. No nuclear inclusions were seen in our patient by light or electron microscopy, but immunohistochemistry for ataxin-7 was unavailable. In our patient, podocytes were atypical and increased in size with cytoplasmic vacuolations that corresponded to membrane-bound inclusions, with contents ranging from rarified to particulate to solid. Focal segmental glomerulosclerosis was attributed to the podocyte abnormalities. The source of renal involvement is unknown. The possibility that CAG expansions in the kidney may lead to renal defects deserves further study. Our observation of CAG repeat instability in the kidney is the first reported to date for SCA7. The most pronounced CAG somatic instability in an SCA7 mouse model was present in the kidney.12

Conclusions

We report the first maternal transmission to date of a massively expanded CAG repeat leading to infant-onset SCA7. Current molecular tests used by clinical genetic testing laboratories may not detect large expanded repeat alleles, and careful consideration of testing methods is recommended for potential infantile cases and prenatal testing. Infant-onset cases of SCA7 are rare but are important for understanding disease pathogenesis and mechanism. To date, we document the first somatic instability of the CAG repeat size in SCA7 human tissues and report the first maternally transmitted infantile form of SCA7.
Massive CAG Repeat Expansion in Infantile SCA7

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