Case Report
Clinical and molecular effect on offspring of a marriage of consanguineous spinocerebellar ataxia type 7 mutation carriers: a family case report

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Abstract: Spinocerebellar ataxia type 7 (SCA7) is a genetic disorder characterized by degeneration of the cerebellum, brainstem, and retina that is caused by abnormal expansion of a CAG repeat located in the *ATXN7* gene encoding sequence on chromosome 3p21.1. Although SCA7 is an uncommon autosomal dominant ataxia, we previously found increased prevalence of the disease in a Southeastern Mexican population. In this study, we described to our knowledge for the first time a marriage of consanguineous SCA7 mutation carriers and their offspring effect. We characterized a severely affected infantile-onset female patient whose parents and two siblings exhibited no symptoms of the disease at time of diagnosis. A comprehensive clinical analysis of the proband showed a progressive cerebellar syndrome, including gait ataxia, movement disorders, and saccadic movements, as well as hyperreflexia, visual deterioration, urinary and cardiovascular dysfunction, and impaired nerve conduction. The SCA7 mutation was detected in the proband patient. Subsequently, genetic examination using four *ATXN7* gene-linked markers (three centromeric microsatellite markers [D3S1228, D3S1287, and D3S3635] and an intragenic Single Nucleotide Polymorphism [SNP-3145G/A]) revealed that the proband descends from a couple of consanguineous SCA7 mutation carriers. Genotyping analysis demonstrated that all offspring inherited only one mutant allele, and that the severe infantile-onset phenotype is caused by germinal expansion (from 37 to 72 CAG repeats) of the paternal mutant allele. Interestingly, the couple also referred a miscarriage. Finally, we found no CAA interruptions in the *ATXN7* gene CAG repeats tract in this family, which might explain, at least in part, the triplet instability in the proband.

Keywords: CAG repeats, CAA interruptions, consanguineous marriage, electrophysiological findings, infantile-onset phenotype, intergenerational transmission, spinocerebellar ataxia type 7
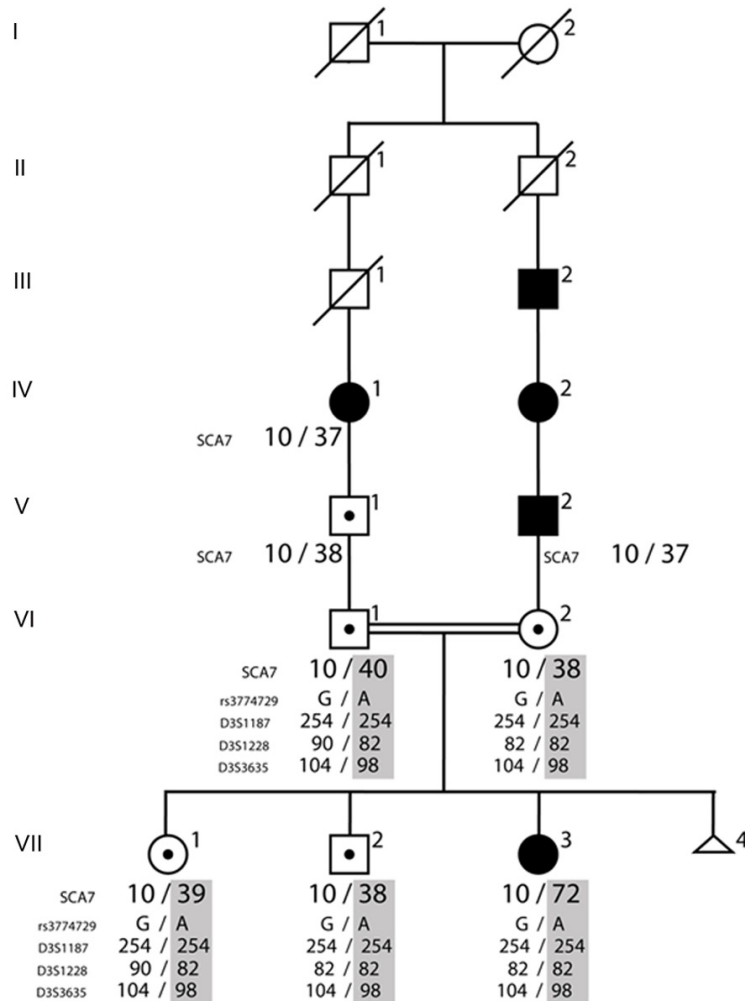
Introduction

Autosomal dominant spinocerebellar ataxias (SCA) conform a group of rare and heterogeneous hereditary disorders with distinctive clinical characteristics, including gait ataxia, cerebellar dysarthria, dysmetria, adiadochokinesia, and postural tremor [1]. To date, at least 36 subtypes of SCA are known, and in eight of these (SCA1, 2, 3, 6, 7, 12, and 17, and Dentato-rubro-pallido-Luysian atrophy), the abnormal expansion of Cytosine-Adenine-Guanine (CAG) trinucleotide repeats were located in the coding region of their respective genes underlying the disease [2, 3]. Expanded CAG repeats result in an elongated polyglutamine tract that interferes with normal protein function, as well as with other cellular processes.

In SCA7, one of the rarest forms of SCA [4, 5], the polymorphic tract of CAG repeats, ranges from 4-18 repeats in normal population, and from 36 up to 460 in affected individuals [6]. Larger alleles generally correlate with increased disease severity and earlier age-at-onset in successive generations of a given SCA genealogy.

I

II

III

IV

SCA7   10 / 37

V

SCA7   10 / 38   SCA7   10 / 37

VI

	SCA7	10 / 40	10 / 38
	rs3774729	G / A	G / A
	D3S1187	254 / 254	254 / 254
	D3S1228	90 / 82	82 / 82
	D3S3635	104 / 98	104 / 98

VII

	SCA7	10 / 39	10 / 38	10 / 72
	rs3774729	G / A	G / A	G / A
	D3S1187	254 / 254	254 / 254	254 / 254
	D3S1228	90 / 82	82 / 82	82 / 82
	D3S3635	104 / 98	104 / 98	104 / 98

Figure 1. Pedigree of the SCA7 proband's family. Numbers below symbols indicate repeat number at the SCA locus. Filled symbols indicate clinically affected individuals and symbols with a central point mark indicate asymptomatic carriers. Genotyping analysis using four SCA7 gene-linked markers revealed the parental transmission on offspring of a marriage of consanguineous SCA7 mutation carriers. The analysis showed that in the proband (VII-3) and in her brother (VII-2), the D3S1228 marker was linked with their mutant allele from the father.

In our previous study, we reported a large series of patients with SCA7 in a Mexican population, which suggests a founder mutation effect in this particular group [7, 8]. In this study, we identify a severely affected infantile-onset female patient in this population whose parents and siblings exhibited no symptoms of the disease. We perform a detailed clinical and genetic analysis of this family and determined that the proband descends from consanguineous parents who are both carriers of the SCA7 mutation. Although all siblings inherited the mutation, the infantile-onset phenotype of the proband occurs on paternal transmission of the mutation through large expansion of the CAG repeat, while the patient's two asymptomatic siblings inherited the mutant allele with no further expansion.

Material and methods

Clinical evaluation

From a sample of 200 subjects recruited from the Tlaltetela community in the Mexican state of Veracruz, a population with high prevalence of SCA7 [7], we identified the proband's family. Neurological examination was carried out following Mayo Clinic procedures [9]. Ataxia-associated symptoms were assessed using the Scale and Rating of Ataxia (SARA) [10], while extracerebellar features were evaluated by the Inventory of Non-Ataxia Symptoms (INAS) [11]. Autonomic nervous system function was examined using the Scales for Outcomes in PD-Autonomic (SCOPA-AUT) [12]. Ophthalmological examination of patients included ocular fundoscopic and color vision tests; the latter was carried out employing the Ishihara pseudo-isochromatic plate. Signed informed consent was obtained from all studied subjects and the research protocol was approved by the National Institute of Rehabilitation (INR) Ethics and Investigation Committee.

Electrophysiological recording

Electrophysiological evaluation was performed as previously noted [13], including peripheral motor and sensitive nerve conduction studies, multimodal evoked potentials, and conventional electro-encephalographic recording.

Genotyping and sequencing of ATXN7 gene CAG repeats

Genomic DNA was extracted from peripheral blood leukocytes utilizing the Gentra Puregene blood kit (Qiagen, Hilden, NRW, Germany) and then amplified by fluorescent multiplex PCR [7, 14] on an Applied Biosystems thermal cycler

(Applied Biosystems, Foster City, CA, USA), using specific oligonucleotides for amplification of the polymorphic CAG repeat tract on the *ATXN7* gene [7] and the Taq DNA polymerase enzyme (Roche Diagnostics GmbH, Mannheim, BW, Germany). Aliquots of PCR reactions were mixed with deionized formamide and the internal size standard (ABI GeneScan-500 TAMRA), heated, cooled on ice, and then electrophoresed at 15 kV through capillaries on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA, USA). Homozygous alleles were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems), and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Heterozygous alleles were analyzed by PCR-based amplification, and then PCR products were purified with the QIAquick PCR purification Kit (Qiagen) and sequenced as mentioned previously.

Identification of parental transmission

Four *ATXN7* gene-linked markers (three centromeric microsatellite markers [D3S1228, D3S-1287, and D3S3635] and an intragenic Single Nucleotide Polymorphism [SNP-3145G/A]) [8, 15] were used for parental transmission analysis. Microsatellite markers were amplified by PCR and analyzed on the ABI PRISM 310 sequencer (Applied Biosystems). The SNP marker was analyzed using the 5'exonuclease-based real-time PCR assay on the StepOne™ thermal cycler (Applied Biosystems).

Results

Family history and clinical features

The proband was an 11 year-old-female (**Figure 1**, VII-3) who began with visual impairment at the age of 6 years, and with gait instability and incoordination in the upper limbs some months later. Progression of clinical manifestations confined the patient to a wheelchair at 9 years of age. Neurological examination demonstrated progressive cerebellar syndrome characterized by severe dysmetria and bilateral dysdiadochokinesis; the patient's poor coordination impeded application of the heel-to-shin test. In addition, the proband had truncal ataxia, postural and intention tremor, hypotonia, and her speech was unintelligible the majority of the time, while SARA score was 39, implying cerebellar

damage. Furthermore, the patient showed severe muscular atrophy, Babinsky signs, and prominent hyperreflexia in her upper and lower limbs, including crossed-supraclavicular, pectoral, and hip adductor. Other clinical features include significant cognitive disturbances, choreiform movements, as well as myokymia with an INAS score of 9. Sleep disturbances were frequent, with complaints that included insomnia, muscle cramps, and Restless legs syndrome (RLS). Ophthalmological examination revealed null visual acuity (no light perception), which impeded the patient from performing the Ishihara color blindness test. Fundoscopy revealed bilateral optic disk pallor and pigmentary changes in macular and peripheral retinal regions. The patient also exhibited oculomotor defects, including slowing saccadic movements and limited ocular movements (upward, horizontally, and downward). The Scales for Outcomes in Parkinson's disease-Autonomic (SCOPA-AUT) score demonstrated urinary and cardiovascular dysfunction as well as constipation and hypohidrosis. Neurophysiological assessment revealed significant reduction of distal and proximal motor amplitudes and prolonged latency for median and peroneal nerves. Sensory nerve conduction analysis showed no response for median and sural nerves, while Brainstem auditory evoked potential (BSAEP) examination revealed abnormal morphology and reproducibility of the potentials, which rendered difficult detection of the different components, while Visual evoked potentials (VEP) detected no bioelectric responses. Likewise, Somatosensory evoked potentials for the Tibial nerves (Tn-SSEP) were absent. Electroencephalogram analysis revealed a significant slow diffuse background with decreased occipital dominant rhythm. Finally, brain Magnetic resonance imaging (MRI) showed severe atrophy in cerebellum and brainstem, and mild cerebral atrophy.

With respect to the proband's siblings, her 12-year-old brother and 16-year old sister (**Figure 1**, VII-2 and VII-1, respectively) they did not exhibit any disease symptom, nor neurological signs of cerebellar dysfunction or ophthalmological defects, which was consistent with normal scores for SARA and INAS. Similarly, the proband's parents referred no disease-associated symptoms. Neurological examination of the proband's 36-year-old father (**Figure 1**, VI-1) revealed solely hyperreflexia, and a 20/25 visual acuity with no injury in macula and reti-
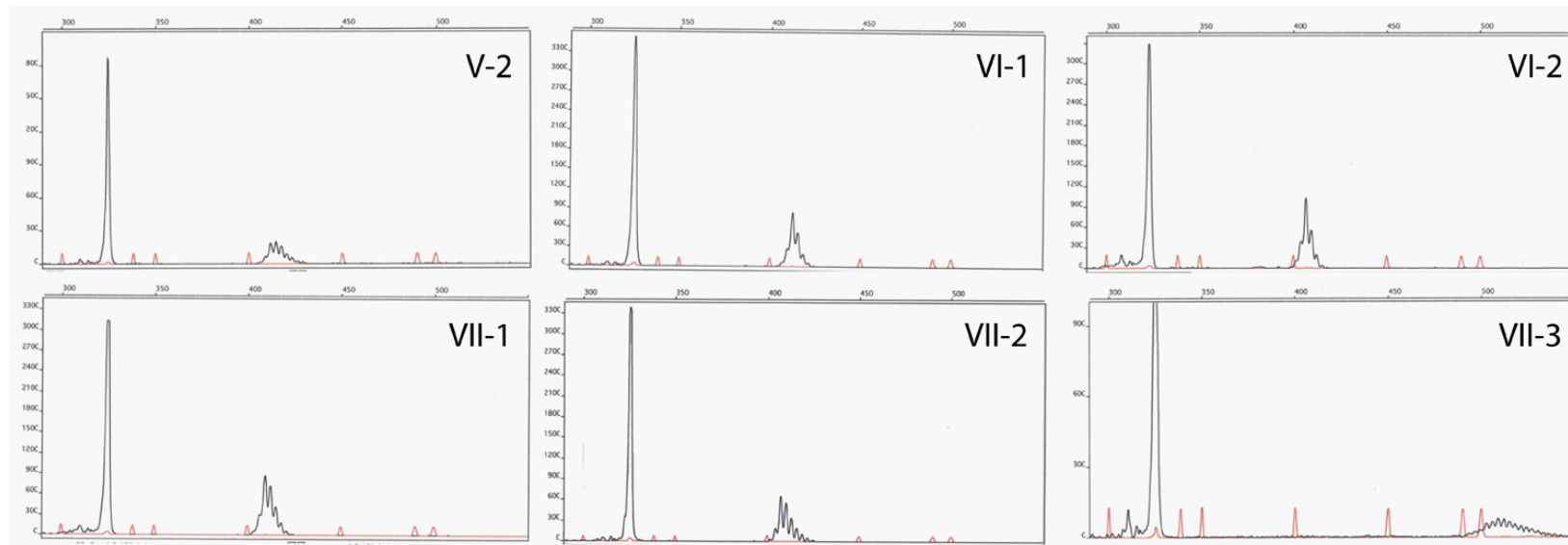
Figure 2. Genotyping of CAG repeats at the *ATXN7* gene by fluorescent PCR. Analysis of CAG repeats was carried by conventional PCR and the amplified products were separated by capillary electrophoresis. Electropherograms of SCA7 heterozygous patients carrying a normal allele of 10 CAG repeats and a mutant allele of diverse CAG repeats are shown (V-2: 37 CAG repeats; VI-1: 40 CAG repeats; VI-2: 38 CAG repeats; VII-1: 39 CAG repeats; VII-2: 38 CAG repeats; and VII-3: 72 CAG repeats).

na. However, he exhibited early partial color blindness (> 3 errors) in response to the Ishihara test; analysis of paternal family history identified two members with confirmed diagnosis of SCA7 (**Figure 2**, V-1 and IV-1). The 35-year-old proband's mother (**Figure 1**, VI-2) exhibited no visual or motor symptoms; visual acuity and integrity of macula and retina were normal. Furthermore, the proband's mother's scores for SARA and INAS were normal. She referred a miscarriage in her last pregnancy (**Figure 1**, VII-4). The maternal family history revealed that her father (**Figure 1**, V-2) initiated with visual impairment and gait ataxia at age 54 years and developed the majority of SCA7-associated symptoms over time. Currently, the proband's maternal grandfather is 64 years of age and his symptomatology includes dysarthria, dysmetria of the extremities, dysdiadochokinesia, postural and intention tremor, hyperreflexia (crossed-supraclavicular, pectoral, and hip adductor and slowing saccadic movements), ophthalmoparesis, and ophthalmoplexia. Furthermore, ophthalmologic appraisal revealed decreased visual acuity (20/100) and granular maculopathy, while the Ishihara test demonstrated total color blindness. SARA (9) and SCOPA-AUT (20) scores implied mild cerebellar involvement and altered autonomic signs, respectively. While INAS score (2) did not show a greater involvement. In addition, this subject's numerous siblings presented characteristics compatible with SCA7.

Molecular diagnosis and analysis of parental transmission

Molecular diagnosis performed on the SCA7 genealogy established that all members carry one mutant allele. **Figure 2** depicts electropherograms of each family member. The proband bears a normal allele with 10 CAG repeats and an expanded allele with 72 CAG repeats (**Figure 1**, VII-3). This mutant allele, which represents the largest expansion detected to date in this population, resulted in infantile-onset phenotype. The proband's father carries a 40-CAG repeat mutant allele, while her mother bears a 38-repeat allele (**Figure 1**, VI-1 and VI-2, respectively). The proband's siblings, VII-1 and VII-2, carry expanded alleles of 39 and 38 CAG repeats, respectively, while the proband's maternal grandfather (V-2) carries a 37-repeats mutant allele. Therefore, all asymptomatic members of the family are indeed carriers of the mutation.

Although the proband's parents denied that they were blood relatives, the fact that both carry the SCA7 mutation strongly suggests a consanguineous relationship between them. To approach this hypothesis, we performed a comprehensive familiar history evaluation and a linkage analysis using four *ATXN7* gene-linked markers: rs3774729; D3S1187; D3S1228, and D3S3635 [15]. **Figure 1** depicts haplotypes for each family member; alleles were named as previously noted [8]. We established that the proband's parents are indeed blood relatives and share a common ancestor five generation back (**Figure 1**). *ATXN7* gene-linked markers were also employed to analyze parent-offspring transmission of mutant alleles. We found that D3S1228 was the sole informative marker in this genealogy (**Figure 1**). In the proband and in her brother (VII-2), the D3S1228-82 allele, which is linked with the wild type *ATXN7* allele, were inhered from the mother; thus, their mutant allele must come from the father. Contrariwise, in the proband's sister (VII-1) the D3S1228-90 allele, which is linked with the wild-type *ATXN7* allele, comes from the father; therefore the mutant allele must be inherited from the mother. Overall, our result showed that paternal transmission caused expansion of the mutant allele and ultimately, early-onset and severe disease symptomatology exclusively in the proband, implying the existence of SCA7-modulating factors in this family.

Because the presence of CAA interruptions within the CAG repeats tract could modulate triplet stability in various polyglutamine diseases [16], we searched for such interruptions in the *ATXN7* gene of five family members with SCA7 and 30 normal subjects from Mexican general population. None of the normal or mutant *ATXN7* alleles contained CAA interruptions.

Discussion

In this study, we described an infantile-onset female patient with severe SCA7 symptomatology, including, gait ataxia, cerebellar dysartria, severe visual impairment, hyperreflexia, muscular atrophy, oculomotor defects, and movement disorders (chorea and myokymia). Electrophysiological and imaging findings were consistent with impaired nerve conduction and extensive and severe degeneration of the majority of the structures of the peripheral and central nervous system, including the autonomic nervous system.

SCA7 is generally detected in adult patients with a family history consistent with autosomal dominant inheritance [17]. However, this patient with infantile disease onset had no familial history related with the disease at time of diagnosis, and detailed clinical evaluation uncovered no ataxic symptoms in the family's relatives, with the exception of hyperreflexia in her father. Genotyping in the SCA7 family solved the enigma; we identified an uncommonly large mutant allele (72 CAG repeats) in the infantile-onset patient and unexpectedly, the presence of one mutant allele in all family members. The low worldwide prevalence of SCA7 (< 1:100,000) renders the random occurrence of the *ATXN7* gene mutation in both parents practically impossible, raising the possibility of a consanguineous relationship between them. Genealogy and linkage analysis utilizing different *ATXN7* gene-linked polymorphic markers confirmed the notion that the offspring was the result of an unaware consanguineous marriage of a couple of SCA7 mutation carriers, with the identification of a common ancestor five generation back by family history.

It is noteworthy that the proband suffered a dramatic expansion of the mutant allele, from 40 to 72 repeats. Such germinal instability generally results in earlier age-at-onset and more severe symptomatology in the offspring of affected parents [18, 19], with alleles bearing 55-460 repeats giving rise to the infantile-onset phenotype and a rapid and fatal course of the disease [20, 21]. This background is consistent with the severe clinical presentation of the proband, as described above. It is thought that the extent of germinal expansions is influenced by the gender of transmitting parent, with larger expansions occurring through paternal transmission [22-25]. Intriguingly, although the proband and her brother inherited the paternal mutant allele from the father, the extent of the CAG repeats expansion and consequently, the SCA7 symptomatology, were markedly different in each one from each other. This implies that in addition to gender, there are other unknown factors that determine the germinal stability/expansion of CAG triplets in SCA7 (i.e., triplet stability-modifying genes, epigenetic factors, or somatic mosaicism). In various polyglutamine diseases, the presence of CAA interruptions could improve the stability of the CAG repeat tract [16]. However, we found no CAA interruptions in the *ATXN7*-gene CAG repeat tract in any of the normal and mutant alleles analyzed, which is consistent with a previous report [24]. Finally, the couple of SCA7 carriers referred a miscarriage (**Figure 1**, VII-4) which might be caused by the presence of a homozygous mutation. Many authors have postulated that a homozygous mutation could be incompatible with life. Nevertheless, some homozygous cases have been reported in polyglutamine diseases [26, 27]. With only few reports of homozygotes worldwide, the controversy of gene dosage effect cannot be concluded. Other rational explanation for the miscarriage could be the presence of a largest abnormal CAG expantion; Monckton et al, revealed extraordinarily CAG repeat instability in male germline, and suggested that largest expansions might be associated with embryonic lethality or dysfunctional sperm [25].

This study reflects the importance of the molecular diagnostic. In many late onset neurodegenerative diseases it is frequent that the couples have already satisfied their reproductive needs by the time when the diagnosis of the condition is made. Therefore, reproductive options like prenatal testing or preimplantation genetic diagnosis cannot be offered to them. It highlights the importance of developing programs for presymptomatic diagnosis of these diseases, particularly in populations with a founder effect and an unusually elevated frequency of presymptomatic carriers. Genetic counseling is a valuable tool not only for couples wishing to have healthy descendents, but also for the rest of the family whom may be unaware of their own risk.

In summary, we presented a detailed clinical and genetic characterization of an infantile-onset patient with SCA7. We determined that the offspring is the result of a consanguineous marriage of a couple of *SCA7* gene-mutation carriers and that the severe infantile-onset phenotype is caused by a germinal expansion of the paternal mutant allele.

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patients and members of SCA-affected families.

Disclosure of conflict of interest

None.

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