SYNE1 Mutations in Autosomal Recessive Cerebellar Ataxia

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In 2007, Gros-Louis et al described a new disease referred to as autosomal recessive cerebellar ataxia type I (ARCA-1) (OMIM 610743) or recessive ataxia of Beauce. Autosomal recessive cerebellar ataxia type I is an adult-onset, relatively pure cerebellar ataxia characterized by gait ataxia, dysarthria, dysmetria, mild oculomotor abnormalities, and diffuse cerebellar atrophy on brain imaging. Mutations in the synaptic nuclear envelope protein 1 (SYNE1) gene, located on chromosome 6p25, were first reported in patients who originated from a region known as “Beauce” in the province of Quebec, Canada.

Objective To better evaluate the prevalence of SYNE1 mutations in individuals with mild pure cerebellar ataxia and cerebellar atrophy, we screened the gene in additional French-Canadian (FC) families and individuals from other populations.

Design, Setting, and Participants Study participants were referred by their treating physician on the basis of core features of autosomal recessive cerebellar ataxia type I. After excluding individuals with known SYNE1 mutations, our cohort was composed mainly of 19 FCs and 21 individuals from other ethnic backgrounds.

Interventions Extraction of DNA from blood samples and complete resequencing of the SYNE1 gene.

Main Outcomes and Measures The involvement of SYNE1 mutations in individuals with ataxia worldwide by resequencing the SYNE1 gene.

Results Two novel truncating mutations were found among the FC participants, and 2 other novel mutations were found in a patient from France and a patient from Brazil (1 mutation each).

Conclusions and Relevance This is the second report, to our knowledge, of SYNE1 gene mutations in a population other than FCs. These data suggest that mutations in SYNE1 should be investigated in families with cerebellar ataxia who live outside the FC region.

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In 2007, Gros-Louis et al described a new disease referred to as autosomal recessive cerebellar ataxia type I (ARCA-1) (OMIM 610743) or recessive ataxia of Beauce. Autosomal recessive cerebellar ataxia type I is an adult-onset, relatively pure cerebellar ataxia characterized by gait ataxia, dysarthria, dysmetria, mild oculomotor abnormalities, and the presence of diffuse cerebellar atrophy on brain imaging. It is caused by mutations in the synaptic nuclear envelope protein 1 (SYNE1) gene (OMIM 608441), which is located on chromosome 6p25 and is one of the biggest genes in the human genome, with 147 exons encoding a 27 652–base pair messenger RNA that translates into an 8797–amino acid protein. Of the 7 mutations initially identified in SYNE1, 4 were truncating, 2 affected splice sites, and 1 was a deletion, all of which are predicted to lead to protein truncation. The most frequent truncating mutation was found in a homozygous state in approximately one-third of patients with ARCA-1. A genotype-phenotype correlation study segregating cases according to the most common genotypes and using variables such as age at onset or disease duration showed no statistically significant difference. Additionally, carriers of less common genotypes did not show atypical clinical features. Recently, Izumi et al reported the finding of 4 novel homozygous mutations in 3 Japanese patients with cerebellar ataxia.

The SYNE1 protein contains 2 N-terminal actin-binding regions that comprise tandem-paired calponin homology domains, a transmembrane domain, multiple spectrin repeats, and a

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C-terminal Klarsicht homology domain. Although SYNE1 is expressed in multiple tissues, it is particularly abundant in the cerebellum. In muscle, SYNE1 is involved in anchoring specialized myonuclei underneath the neuromuscular junctions.1,2 A muscle biopsy specimen from a patient with ARCA-1 showed that fewer myonuclei lie beneath the neuromuscular junction1; however, this sparsity of myonuclei does not appear to have clinical, electrophysiological, or ultrastructural consequences in humans.

Other than the 7 pathogenic mutations already identified, the origin of mild pure cerebellar ataxia with cerebellar atrophy among French-Canadian (FC) families was not further examined. Our objective was to identify new causative mutations in FC families who live outside the Beauce region in Quebec, Canada. We predicted that mutations in this gene would underlie a fraction of recessive or “sporadic” cases of pure cerebellar ataxia in individuals from other populations. This notion arises from the many different mutations already found in what has been documented to be a homogeneous founder population. SYNE1 is also a large gene and thus likely subject to many different mutations. Therefore, we also screened patients with ARCA-1 from other ethnic populations for mutations in SYNE1.

**Methods**

Study participants were referred by their treating physician because they exhibited core features of ARCA-1. All participants...
had ataxia and dysarthria associated with cerebellar atrophy. This recruitment protocol and genetic study were approved by the ethics committee of each recruiting institution. Each participant gave informed consent, and a blood sample was obtained from affected individuals and, if available, their unaffected siblings. Genomic DNA was extracted from the blood using a kit (Puregene; Gentra Systems, Inc), according to the manufacturer’s protocol. The first screening step, achieved by traditional Sanger sequencing, was performed to exclude participants harboring the 7 known causative SYNE1 mutations.

Our cohort was composed of 40 unrelated participants of different origins, specifically 19 FCs, 16 French individuals from France, and 5 others of different ethnic backgrounds (1 each from Brazil, Italy, Algeria, Tunisia, and the Netherlands). A control cohort of 190 healthy individuals free of any neurodegenerative disorder was used.

Polymerase Chain Reaction Amplification and Sequencing
The ExonPrimer program found in the genome browser of the University of California, Santa Cruz (http://genome.ucsc.edu/) was used to design 160 different sets of primers for
the amplification of the 147 exons and their respective flanking splice junctions. In the screening, we included additional exons reported in other isoforms. The complete list of primers used and the polymerase chain reaction conditions can be found in the Supplement (eTables 1 and 2). Amplification products were sequenced at the Genome Quebec Innovation Centre (Montréal, Quebec, Canada) using a DNA analyzer (3730XL DNAnalyzer; Applied Biosystems). Dedicated software (Mutation Surveyor, v.3.10; SoftGenetics) was used for mutation detection analysis, with NM_00371.3 as the reference sequence. Following the identification of a case with a definite mutation, DNA from relatives was amplified, when available, to determine whether the mutation segregated with the disease.

Results

The first FC family (family A) was composed of 3 affected siblings among a total of 10 siblings (Figure 1) arising from 2 healthy parents. The proband (II-10) developed dysarthria at 30 years of age, gait ataxia at 33 years of age, and abnormal eye movements with slow saccades and abnormal pursuit at 37 years of age. Deep tendon reflexes were slightly increased, and electromyographic and nerve conduction study results were normal. Magnetic resonance imaging showed marked diffuse cerebellar atrophy. No dementia, tremor, muscle cramps, fasciculation, or extrapyramidal features were noted. His affected sister (II-9) developed gait problems at 29 years of age and otherwise had a similar clinical progression. The whole family (8 other siblings and the mother) were seen by experts in ARCA-1 (J.-P.B., N.D., and G.A.R.), who confirmed the presence of another affected brother with the same phenotype (II-8) and determined that the remaining family members were unaffected. In this family, we identified 2 new truncating mutations in the SYNE1 gene showing perfect segregation with the disease (Figure 1). The first mutation is near the start of the SYNE1 protein (c.810C>T, p.R125X), and the second is more distal (c.10196G>A, p.W6620X). These 2 mutations were not found in any of the 196 healthy controls and also were not found in 2 public databases: the Single-Nucleotide Polymorphism database (dbSNP, Build 137) and the National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project database (EV5400).

The second FC family (family B) (Figure 2) had 2 affected sisters. The proband (III-1) reported onset of balance problems at age 14 years, particularly when trying to skate or ski. She underwent a brain scan at age 21 years, and marked diffuse cerebellar atrophy was found. She developed gait problems and dysarthria at approximately 25 years of age. Saccadic movements were slightly slow in all directions, and she had mild horizontal nystagmus. Her sister (III-2) was only mildly affected despite the presence of marked diffuse cerebellar atrophy on magnetic resonance imaging. Both had 1 known mutation, c.9153A>T, p.R2906X, which was transmitted by their mother. The second mutation, from their father and present again in both affected sisters, was c.21687C>T, p.R7084X, a new truncating mutation (Figure 2). Other unaffected family members were screened for the 2 mutations; none of them carried both. This new mutation was not found in 196 healthy controls or in dbSNP or ESV5400.

Screening the non-FC case patients with ataxia and cerebellar atrophy led to the identification of 2 individuals with SYNE1 mutations. Sporadic case A, an individual who originated from Brazil, had a homozygous protein truncating mutation: c.4335C>T, p.Q1300X (Figure 3). Sporadic case B, who originated from France, had a homozygous 5-nucleotide deletion: c.10753-10757delCCAAG. According to a prediction program (NNSPLICE, version 0.9; www.fruitfly.org/seq_tools/splice...
This deletion at the end of exon 62 does not affect the GT donor site but will cause a frameshift. This frameshift will lead to a premature stop codon 4 amino acids later, as shown in Figure 4. Together, these 2 mutations are the first SYNE1 mutations found in individuals with sporadic ARCA-1 who are not from the FC region.

Discussion

The objectives of this study were to identify new causative mutations in FC families and to determine whether SYNE1 mutations may underlie some cases of sporadic ataxia among affected individuals who live outside Quebec. Given the size of this gene, we were convinced that SYNE1 mutations would be responsible for ARCA-1 in populations other than those from the FC region. We identified 3 novel compound heterozygote truncating mutations in familial FC cases of ARCA-1 and 2 novel homozygous mutations predicted to be protein truncating in sporadic cases from Brazil and France. Therefore, we conclude that SYNE1 mutations may be responsible for sporadic or recessive cases of mild pure cerebellar ataxia with cerebellar atrophy.

One of the barriers to screening the SYNE1 gene is its size, which makes sequencing of the gene using classic methods slow and very expensive. With the arrival of new sequencing technologies, it is now possible to carry out exome sequencing and/or to custom design the process for SYNE1 targeted capture to screen for mutations in SYNE more rapidly and at a greatly reduced cost. In the years ahead, it should be possible to determine the prevalence of SYNE1 mutations in patients with ataxia from many different populations.
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REFERENCES