Expanding the Clinical Phenotype Associated With ELOVL4 Mutation
Study of a Large French-Canadian Family With Autosomal Dominant Spinocerebellar Ataxia and Erythrokeratodermia

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IMPORTANCE The autosomal dominant spinocerebellar ataxias (SCAs) are a complex group of neurodegenerative disorders with significant genetic heterogeneity. Despite the identification of 20 SCA genes, the cause of the disorder in a significant proportion of families with SCA remains unexplained. In 1972, a French-Canadian family segregating a combination of SCA and erythrokeratodermia variabilis (EKV) in an autosomal dominant fashion was described.

OBJECTIVE To map and identify the causative gene in this large family with SCA and EKV using a combination of linkage analysis and whole-exome sequencing.

DESIGN, SETTING, AND PARTICIPANTS A total of 32 individuals from the family have undergone complete neurologic and dermatologic examinations.

MAIN OUTCOMES AND MEASURES Mutations in ELOVL4 have been reported in families with macular degeneration. Recently, homozygous mutations were found in patients with ichthyosis, spastic paraplegia, and severe neurodevelopmental defects. In the present study, we report on a heterozygote mutation in ELOVL4 in affected individuals from the family with SCA and EKV. The mutation segregates with a milder phenotype consisting of early-onset patches of erythema and hyperkeratosis, as well as SCA manifesting in the fourth or fifth decade of life.

RESULTS We describe the mapping and the identification of a c.504G>C transversion in ELOVL4 resulting in the p.L168F substitution. We also provide clinical characterization of the phenotypes in 19 mutation carriers.

CONCLUSIONS AND RELEVANCE We report, to our knowledge, the first mutation in ELOVL4 that is associated with SCA and EKV. This gene encodes a member of the elongase family, which is responsible for the elongation of very long-chain fatty acids (at least 26 carbons). These fatty acids participate in a wide variety of physiological functions, including skin barrier formation and peroxisome β-oxidation. Overall, these results provide additional insight into the pathogenesis of these complex neurodegenerative disorders.

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Clinical Phenotype Associated With ELOVL4 Mutation

T
he autosomal dominant spinocerebellar ataxies (SCAs) are a complex group of neurodegenerative disorders characterized by progressive cerebellar ataxia of gait and the extremities variably associated with other neurologic signs. The prevalence of inherited ataxias is approximately 3 to 6 individuals per 100,000 people in the general population. At least 32 distinct loci have been identified for SCAs and, of these, 20 SCA genes have so far been documented. Nucleotide repeat expansions, genomic deletions and duplications, and point mutations have been associated with the disease, suggesting genetic heterogeneity of the pathogenic mechanisms. To date, a significant proportion of SCA in families remains genetically unexplained.

In 1972, a unique French-Canadian (FC) family segregating a combination of ataxia and skin lesions in an autosomal dominant fashion (SCA34, OMIM 133190) was reported by Giroux and Barbeau. The skin lesions were typical of erythrokeratodermia variabilis (EKV, OMIM 133200), a heterogeneous group of diseases characterized by erythematous lesions and hyperkeratosis. The pure form of EKV has been associated with mutations in 2 connexin genes, GJB3 and GJB4, on chromosome 1p35.1, but additional genes causing EKV remain to be identified. There is growing evidence that mutations in genes causing keratodermias also predispose people to various neurologic disorders, including deafness, peripheral neuropathy, and mental retardation. Interestingly, a mutation in the gene GJB1 has recently been associated with X-linked ataxia with peripheral nervous system features. However, to our knowledge, neither skin lesions nor keratodermia have been reported in families with SCA except for the FC family.

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Preliminary studies with several affected individuals from this family have suggested linkage at the EKV1/EKV2 locus, but no mutation has been identified. We describe in the present study the mapping of the gene for this unique syndrome on chromosome 6p12.3-q16.1, as well as the identification of a p.L168F (c.504G→C) mutation in the gene ELOVL4 (RefSeq NM_022726) by using whole-exome sequencing. The p.L168F mutation segregates well with the disease, and we provide detailed clinical characterization of 19 mutation-positive individuals from this family.

Methods

Participants

We investigated an FC kindred presenting a combination of erythrokeratodermia with ataxia. The description of clinical phenotypes of the early generations of the family has been previously described. We collected genomic DNA from 32 individuals of this family. These individuals underwent complete neurologic and dermatologic examinations. Measurement of long-chain fatty acids (C22, C24, and C26) was performed on 2 affected individuals (V-3 and V-24 [Figure 1A]) (Centre Hospitalier Universitaire de Sherbrooke). An additional cohort of 95 patients with unexplained SCA was used for gene screening. After participants provided written informed consent, genomic DNA was extracted from blood samples using standard protocols. This project was approved by the research ethics committee of the Centre Hospitalier de l’Université de Montréal. The participants received no financial compensation.

Genotyping and Linkage Analysis

A genome-wide scan of the FC family was performed at deCODE genetics using 524 evenly distributed microsatellite markers covering the whole genome at intervals of approximately 8 centimorgans. Microsatellite markers used to refine the disease locus were genotyped by polymerase chain reaction incorporating primers with fluorescently labeled M13mp18 sequence tails (primer and polymerase chain reaction details available on request), as described elsewhere. We calculated pairwise logarithm of odds scores (MLINK program, version 5.1; LINKAGE; http://www.jurgott.org/linkage/LinkagePC.html) under the assumption of an autosomal dominant mode of inheritance, a disease frequency of 1:10,000, 90% penetrance, 1/1000 of phenocopy, and allele isofrequency. We also performed a multipoint linkage analysis using the same variables with a different program (SimWalk, version 2.91; University of California, Los Angeles, Human Genetics; http://www.genetics.ucla.edu/software/).

Copy Number Variation

A fine-tiling array comparative genomic hybridization, covering the entire chromosome 6, was performed on one affected individual from the large FC family (participant IV-16). We used 3 different arrays with a probe density of 1/4000 base pair (bp), 1/8000 bp, and 1/20,000 bp (NimbleGen). We also performed a whole-genome genotyping and copy number variation analysis (human 610-Quad BeadChip; Illumina).

Exome Sequencing and Validation of Variants

Three affected individuals (V-3, V-5, and V-24) from distinct branches of the family (Figure 1A) were selected for exome sequencing. Genomic DNA (5 μg) was fragmented and enriched (SureSelect Human All Exon V4 kit; Agilent Technologies). The prepared library was sequenced with paired-end reads (HiSeq 2000; Illumina). The reads were mapped against the human genome (hg19) (Burrows-Wheeler Aligner; http://bio-bwa.sourceforge.net). Reads with a unique mapping location were kept for downstream analysis. Gene Analysis Tool Kit (Broad Institute; http://www.broadinstitute.org/gatk/) and ANNOVAR (http://www.openbioinformatics.org/annovar/) were used for the calling of single-nucleotide variations, small insertions, and small deletions (indels). Called variants were filtered against dbSNP132 as well as individuals from our in-house exome database. For the validation of next-generation sequencing variants, primers were designed with the software Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) to amplify the coding region encompassing the putative variation.

Sequencing of ELOVL4 in Patients With SCA

We sequenced all of the coding exons as well as 20 bp of the exon-intron boundaries of the gene ELOVL4 in 95 patients with SCA. Polymerase chain reaction primers were designed using Primer 3. The primer sequences and polymerase chain...
reaction conditions are available on request. The amplicons were then sequenced (ABI 3730xl DNA Analyzer; Life Technologies) and analyzed (Mutation Surveyor, version 3.0; Softgenetics).

Results

Linkage Analysis and Exome Sequencing
We first excluded linkage at the EKV1/EKV2 and MEDNIK loci in the FC family (data not shown). Genome-wide linkage analysis revealed 3 positive chromosomal regions with a logarithm of odds score superior to 1.5 on chromosomes 1, 6, and 7 (data not shown). Further analysis with additional markers and additional affected individuals confirmed linkage to chromosome 6, with a maximum logarithm of odds score of $Z = 5.33$ at $\theta = 0$ found for marker D6S452. Haplotype analysis allowed the identification of a candidate locus of 46.8 megabases between markers D6S459 and D6S417 at chromosome 6p12.3-q16.1 (Supplement [eFigure]). We also performed comparative genomic hybridization analysis that did not reveal significant copy number variations in this candidate gene region.
In turn, whole-exome sequencing allowed us to identify a p.L168F (c.504G>C) mutation in ELOVL4 (Figure 1B) (RefSeq NM_022726), a gene located in the candidate interval (Supplement [eTable 1]). This genetic variation was found in the 3 affected individuals (V-3, V-5, and V-24). Sanger sequencing confirmed that this genetic variation segregated with the disease haplotype in 19 individuals. This mutation was not found in 189 additional control individuals, nor was it found in public databases, such as 1000 Genomes (http://browser.1000genomes.org) and Exome Variant Server (http://evs.gs.washington.edu/EVS). Screening of 95 additional patients with SCA failed to identify additional mutations in ELOVL4.

Clinical Manifestations of Mutation Carriers

The summary of the detailed clinical examination performed on 19 mutation carriers from the FC family is reported in the Supplement (eTable 2). Erythrokeratodermia variabilis (n = 3), ataxia (n = 1), or a combination of both phenotypes (n = 11) was found in the mutation carriers; the others were unaffected. As previously described, 2 types of skin lesions can be distinguished. First, erythematous and hyperkeratotic plaques are most commonly observed over the dorsal aspects of the hands and feet, the elbows, the ankles, and the external ears. The hyperkeratosis usually is more prominent than the erythema, and a fine scaling is observed over those persistent plaques (Figure 2A). Second, transient, figurate, and erythematous patches are observed over the legs, thighs, and buttocks (Figure 2B). Both types of lesions are usually symmetrically distributed and vary in severity among patients, becoming worse in winter and improving with the use of emollients. The onset is in early infancy and, in most cases, the lesions disappear after the patient is 25 years of age. In all patients, hair, nails, and scalp were normal. No hearing impairment was detected, and cognition appeared normal. The onset of gait ataxia usually occurred in the fourth or fifth decade of life (mean, 51 years), but it could occur as early as the third decade in some patients. The progression of gait disturbance is very slow. Patients may need to use a cane after 10 years of disease progression. The affected individuals in the present study usually required a walker in their mid-60s or 70s, although other medical conditions may have contributed to the gait disturbance (eg, aortic aneurysm, metastatic breast cancer, chronic alcohol consumption, and severe emphysema) (Supplement [eTable 2]). Magnetic resonance imaging conducted in 7 individuals with mild or moderate ataxia showed cerebellar and pontine atrophy (Figure 2C). In these affected individuals, the atrophy was more severe in the vermis than in the cerebellar hemispheres. In turn, 2 individuals with EKV lesions without ataxia had normal magnetic resonance imaging findings. Individual V-24, who had EKV lesions and very mild ataxia, also exhibited cerebellar hypometabolism on 18F-fluorodeoxyglucose positron emission tomography (Figure 2D). An electromyogram obtained in 8 affected family members, including 1 study participant (13%) with EKV lesions only, disclosed mild axo-
nal peripheral neuropathy in 4 of the 8 individuals (50%) (Supplement [eTable 2]). The mutation was also found in 4 individuals (V-16, VI-4, VI-6, and VI-11; 21%) (Figure 1A) without any neurologic manifestations or skin lesions. Lipid analysis from whole-blood samples of 2 affected individuals (V-3 and V-24) did not reveal abnormal levels of long-chain fatty acids (C22, C24, and C26). However, the linoleic acid level was slightly above the reference range.

**Discussion**

We evaluated a p.L168F mutation in ELOVL4 segregating with SCA and EKV in a large FC family by using a combination of genome scan and whole-exome sequencing. Based on the strong linkage found on chromosome 6p12.3-q16.1, we filtered for genetic variants and found the ELOVL4 mutation as the only coding genetic variation in 3 affected individuals who were tested. The mutation segregates well with the disease phenotype and was not found in control individuals or in public databases. Moreover, ELOVL4 is a very good candidate gene because mutations, in both human and animal models, have been associated with skin defects and neurologic impairments. Taken together, our data are consistent with the identification of ELOVL4 as the causative gene for the EKV and SCA in this FC family.

The ELOVL4 gene encodes for a member of the elongase family and is involved in the elongation of very long-chain fatty acids. The Elovl4 protein is expressed in the retina, brain, testes, and skin. As a 314–amino acid protein, Elovl4 fatty acids. The Elovl4 protein is expressed in the retina, family and is involved in the elongation of very long-chain fatty acids. Interestingly, knockout mice for Elovl4 die because mutations, in both human and animal models, have been associated with skin defects and neurologic impairments. Particularly, 2 additional homozygous mutations were found in patients with ichthyosis and severe neurologic disturbances, such as spastic paraplegia and neurodevelopmental defects. The p.L168F missense mutation described in the present study is located within the third transmembrane domain (Figure 1C) and is predicted to be pathogenic by Mutation Taster (http://www.mutationtaster.org) and PROVEAN, and possibly damaging by PolyPhen2. Further characterization of the mutation will be necessary to understand the role of the mutation in the disease. It is possible that the localization of the mutation in the FC family in a different domain of the protein could be responsible for the phenotypic differences observed in patients with Stargardt disease type 3.

In the present study, a total of 19 ELOVL4 p.L168F mutation carriers were identified for whom we obtained detailed neurologic and physical examination results (Supplement [eTable 2]). Most (11 [58%]) individuals bearing the p.L168F mutation exhibited a combination of EKV and ataxia. Other mutation carriers (3 [16%]) showed only EKV, most likely because they were younger (aged 36, 33, and 44 years). One (5%) mutation carrier showed cerebellar ataxia at 55 years, without apparent skin lesions. The EKV lesions are usually apparent during childhood, may be minimized by hydrating lotions, and in some instances disappear over time. Therefore, we cannot exclude that EKV has been overlooked in this individual despite careful assessment. Alternatively, incomplete penetrance for the skin phenotype may exist. Finally, we have identified asymptomatic mutation carriers (4 [21%]), suggesting incomplete penetrance for EKV (Supplement [eTable 2]). Because of their relatively young age and considering the late-onset disease course of the ataxic features, it is possible that these carriers may eventually become symptomatic.

The heterogeneity of clinical manifestations associated with mutations in ELOVL4 in humans is intriguing. Although originally described in macular degeneration (Stargardt disease type 3) without skin lesions, homozygous mutations have been associated with ichthyosis, encephalopathy, and epilepsy, without macular degeneration. In our FC family, EKV lesions were reminiscent of ichthyosis, but we found no clinical evidence of macular degeneration. In Elovl4 knockout mice, skin lesions appear to be a common feature, but depletion of very long-chain polyunsaturated fatty acids in the retina does not seem to affect visual function. These observations are consistent with other EKV and ichthyosis genes that are associated with heterogeneous neurologic manifestations.

**Conclusions**

To our knowledge, we have described, for the first time, a missense mutation in the gene ELOVL4 associated with SCA as well as EKV. Finding additional mutations in ELOVL4 in other SCA families, as well as functional characterization of these mutations, should provide additional insights on disease pathogenesis.
Clinical Phenotype Associated With ELOVL4 Mutation

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REFERENCES

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