

A family with spinocerebellar ataxia and retinitis pigmentosa attributed to an *ELOVL4* mutation

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Neurol Genet 2019;5:e357. doi:10.1212/NXG.0000000000000357

Abstract

Objective

To identify the genetic cause of autosomal dominant spinocerebellar ataxia and retinitis pigmentosa in a large extended pedigree.

Methods

Clinical studies were done at 4 referral centers. Ten individuals in the same extended family participated in at least a portion of the study. Records were obtained from an 11th, deceased, individual. Neurologic and dermatological examinations were performed. Ophthalmologic evaluation including funduscopic examination and in some cases ocular coherence tomography were used to identify the presence of retinal disease. Whole exome sequencing (WES), in conjunction with Sanger sequencing and segregation analysis, was used to identify potential genetic mutation.

Results

Affected individuals reported slowly progressive cerebellar ataxia with age at onset between 38 and 57. Imaging demonstrated cerebellar atrophy (3/3). WES identified a novel heterozygous mutation in the elongation of very long chain fatty acids 4 (*ELOVL4*) gene (c.512T>C, p.Ile171Thr) that segregated with ataxia in 7 members tested. Four of 8 members who underwent ophthalmologic evaluation were found to have retinitis pigmentosa. No skin findings were identified or reported. Ocular movement abnormalities and pyramidal tract signs were also present with incomplete penetrance.

Conclusions

We report a family with both spinocerebellar ataxia and retinal dystrophy associated with an *ELOVL4* mutation. In addition, to supporting prior reports that *ELOVL4* mutations can cause spinocerebellar ataxia, our findings further broaden the spectrum of clinical presentations associated with spinocerebellar ataxia 34.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

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Glossary

EKV = erythrokeratoderma variabilis; ELOVL4 = elongation of very long chain fatty acids 4; PUFA = poly-unsaturated; SCA = spinocerebellar ataxias; SFA = saturated; WES = whole exome sequencing.

The spinocerebellar ataxias (SCAs) are a group of autosomal dominant disorders characterized by progressive cerebellar ataxia. Some subtypes of SCA are associated with other neurologic dysfunction including ocular movement abnormalities, peripheral neuropathy, pyramidal or extrapyramidal signs, and in some cases non-neurological signs. To date, over 40 causative genes have been described.¹

Mutations in the elongation of very long chain fatty acids 4 (ELOVL4) gene have been associated with spinocerebellar ataxia SCA34 (OMIM 133190) in 3 families and 2 other individuals. One French Canadian family and 2 individuals were reported to have spinocerebellar ataxia with erythrokeratoderma variabilis (EKV) while 2 Japanese families have a pure SCA with no skin findings.²⁻⁵ Although ELOVL4 mutations are also known to cause autosomal dominant Stargardt-like macular dystrophy, none of the previous reports identified ophthalmologic abnormalities.

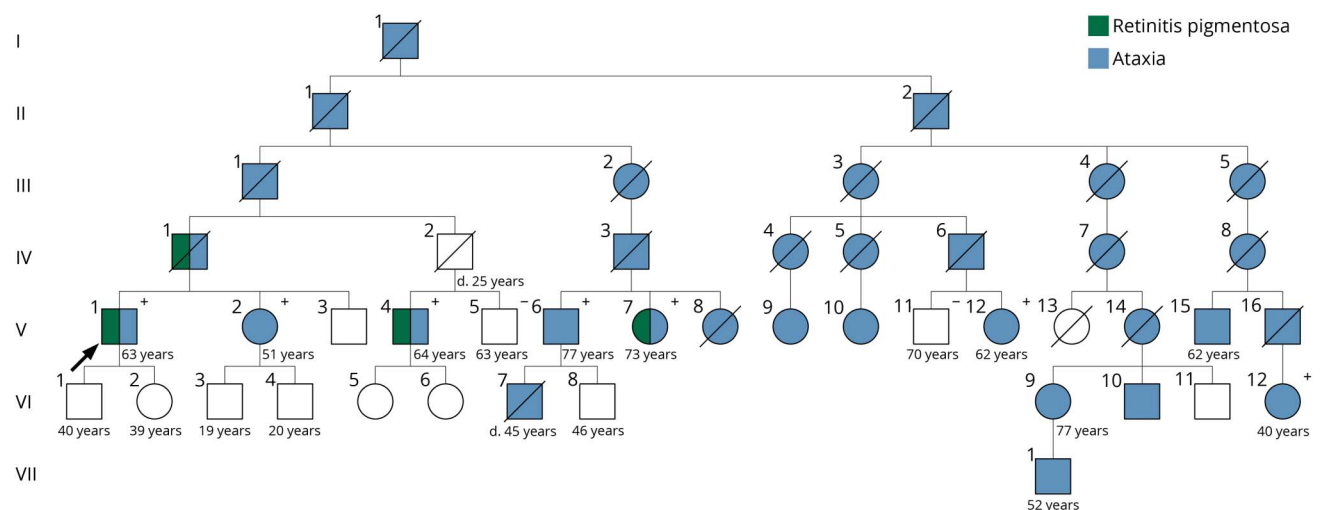
We report an American family with a late onset SCA in which many affected individuals also have retinitis pigmentosa on ophthalmologic evaluation. Whole exome sequencing (WES) with confirmative Sanger sequencing identified a novel mutation in the ELOVL4 gene (c512.T>C, pIle171Thr) that segregated with the disease in this family.

Methods

Ten individuals with ataxia from the same extended family located in 8 different cities participated in the elements of the study based on logistical availability. The pedigree can be found in figure 1. DNA was collected from 7 affected and 2 unaffected individuals. Clinical studies were done at 4 referral centers. Participants were referred for neurologic, dermatologic, and ophthalmic evaluations. Participants who were unable to reach a referral center were interviewed remotely, and clinical records were obtained when possible. Six individuals participated in neurologic and dermatologic examination. On neurologic evaluation, both Scale for the Assessment and Rating of Ataxia and Inventory of Non-Ataxia Signs scales were recorded. Seven individuals participated in ophthalmic examination. Clinical records of one deceased individual (IV-1) were also obtained.

Genetic analysis was done independently at 3 Clinical Laboratory Improvement Amendments-certified centers. Two individuals (V-1 and V-112) were evaluated for ataxia via targeted analysis of WES. Details of sequencing and bioinformatics used are previously published. See supplemental materials NGS Pipeline and Bioinformatics (links.lww.com/NXG/A182) for details. In conjunction, Sanger sequencing and segregation analysis were used to confirm and support the genetic mutation. Another individual (IV-1), now deceased, underwent allele-specific prescreen followed by targeted analysis of WES at a third center for familial retinitis pigmentosa.

Figure 1 Pedigree of an American Family with a Novel Mutation in ELOVL4



Pedigree of a large extended family including all members of our study. Individuals who participated in genetic testing for p.I171T variant denoted in + for presence and - for absence.

Standard protocol approvals, registrations, and patient consents

Elements of this study involving human subjects were approved by the institutional review board of participating institutions (University of Iowa ID# 200202022 and University of Chicago 14707A-CR004). Other studies were standard of care. All participants provided informed written consents prior to initiating research and data collection.

Data availability

The authors confirm that the deidentified data supporting the findings of this study are available within the article and supplementary materials (links.lww.com/NXG/A181).

Results

A summary of clinical findings can be found in the table. The age at ataxia onset was in early 40s for most individuals, though this ranged from 38 to 57. All 10 affected individuals reported slowly progressive gait instability, 6 of 10 complained of dysarthria, and 8 of 10 noted handwriting changes. Three individuals have progressed to using a wheelchair (average 18.3 years after onset). One of 10 complained of diplopia, 7 of 10 wear corrective lenses. Two of 10 complained of nighttime vision loss corresponding to onset of ataxia. All persons denied bowel/bladder issues as well as history of dermatologic disease. Disease progression was extremely slow. Of the 10 persons interviewed, one required a cane and 2 required wheelchairs at the time of interview.

Table Comparison of clinical characteristics among patients with spinocerebellar ataxia 34

	American family (present study)	Japanese family (Ozaki et al.)	French Canadian family (Giroux et al.; Cardieux-Deion et al.)	Case report (Bourassa et al.)	Case report (Bourque et al.)
Mutation	ELOVL4 (c.512T>C, p.I171T); heterozygous	ELOVL4 (c.736T>G, p.W246G); heterozygous	ELOVL4 (c.504G>C, p.L168F); heterozygous	ELOVL4 (c.539A>C, p.Q180P); heterozygous	ELOVL4 (c.698C>T, p.T233M); heterozygous
Approximate mean age at symptom onset	42.6	33.9	51	20s	15
Progression	Slow	Slow	Slow	Unclear	Slow
Ataxia	Gait ataxia (10 of 10), limb ataxia (8 of 10), dysarthria (6 of 10)	Gait ataxia, limb ataxia, and dysarthria (9 of 9)	Gait ataxia (12 of 19), limb ataxia and dysarthria observed	Gait ataxia, limb ataxia, dysarthria	Gait
Oculomotor signs	Horizontal nystagmus (5 of 6), vertical nystagmus (4 of 6), vertical gaze palsy (1 of 6), slow saccades (5 of 6), impaired smooth pursuits (5 of 6)	Horizontal gaze evoked nystagmus (7 of 9), supranuclear gaze palsy (3 of 9), impaired smooth pursuit (5 of 9)	Nystagmus (7 of 19), slow saccade and slow pursuit observed	Nystagmus, ophthalmoplegia	Saccadic pursuit, square wave jerks, and periodic alternating skew deviation
Pyramidal tract signs	Increased DTRs (6 of 6)	Increased DTRs or positive Babinski (8 of 9)	Decreased DTRs	None	Decreased DTRs
Autonomic disturbance	None	Bladder disturbance (4 of 9), constipation (2 of 9)	None	Not reported	Not reported
Ophthalmologic findings	Retinitis pigmentosa (4 of 8)	None	None	Not reported	Not reported
Skin findings	None	None	Erythrokeratoderma variabilis (14 of 19)	Erythrokeratoderma variabilis	Erythrokeratoderma variabilis
Radiographic findings	Pontine and cerebellar atrophy (2 of 3), mild cerebellar atrophy (1 of 3)	Pontine and cerebellar atrophy (8 of 8), hot cross bun sign (4 of 6), pontine midline linear hyperintensity (2 of 6), hyperintensity of middle cerebellar peduncles on FLAIR (1 of 6)	Pontine, cerebellar, cerebral atrophy (4 of 9), pontine and cerebellar atrophy (1 of 9), mild cerebellar atrophy (1 of 9), normal MRI (3 of 9)	Pontine and cerebellar atrophy	Mild cerebellar and pontine basal atrophy
Other findings	Decreased vibration (2 of 6), decreased olfaction (3 of 6)	None	Mild peripheral axonal neuropathy (4 of 8)	None	None

Abbreviation: DTR = deep tendon reflexes; ELOVL4 = elongation of very long chain fatty acids 4; FLAIR = fluid-attenuated inversion recovery sequence on MRI.

Genetic testing

Data from WES on 2 individuals with ataxia (VI and VI12) were analyzed independently by different labs and resulted in identification of a variant in *ELOVL4* (c.512T>C, p.Ile171Thr). The variant was deemed likely pathogenic based on American College of Medical Genetics Standards and Guidelines. DNA from 5 more affected and 2 unaffected members of this family was analyzed for the presence of this mutation by Sanger sequencing. All affected family members carried the mutation, while the 2 unaffected members lacked the mutation. A third lab independently identified the same mutation in *ELOVL4* in a now deceased individual (IV1) during evaluation for retinal pigmentary changes; this individual was reported to have ataxia. A representation of the novel variant can be found in figure 2; its relation to previously described variants is detailed in figure 3.

Neurologic evaluation

On neurologic examination, affected individuals had gait ataxia (6/6), limb ataxia (5 of 6), dysarthria (3 of 6) along with ocular movement abnormalities (5 of 6), and pyramidal tract signs (6 of 6).

MRI of the brain was done in 3 affected individuals, all showing cerebellar and pontine atrophy that correlated with their disease duration. Representative images can be found in figure 4.

[F3]

Dermatological workup

None of the 10 affected individuals had a history of skin findings that could be attributed to a form of EKV. A complete skin examination done by a dermatologist found no evidence of EKV or unusual skin lesions in 4 of 4 participants.

Ophthalmologic evaluation

Clinical images can be found in figure 5. Three of 7 affected individuals had peripheral bone-spicule-like pigmentary changes on funduscopic examination consistent with retinitis pigmentosa. Records obtained from an eighth, now deceased, individual (IV1) also showed peripheral bone-spicule-like pigmentary changes consistent with retinitis pigmentosa. Electroretinogram results on this individual showed rod-cone photoreceptor degeneration (figure 6).

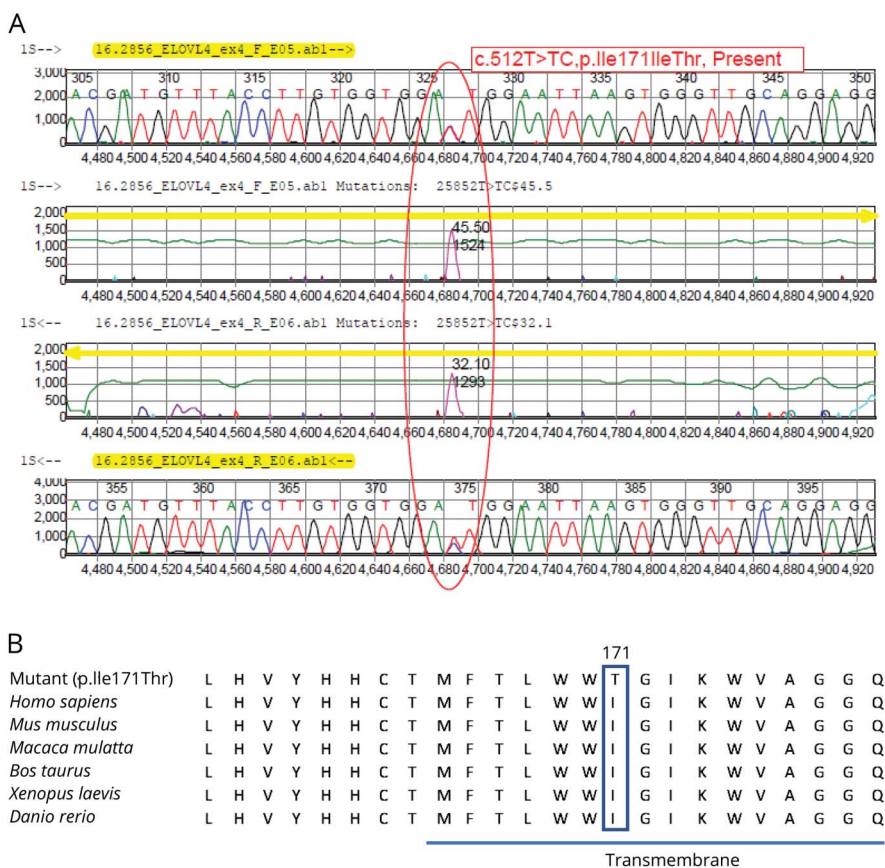
[F5]

[F6]

Discussion

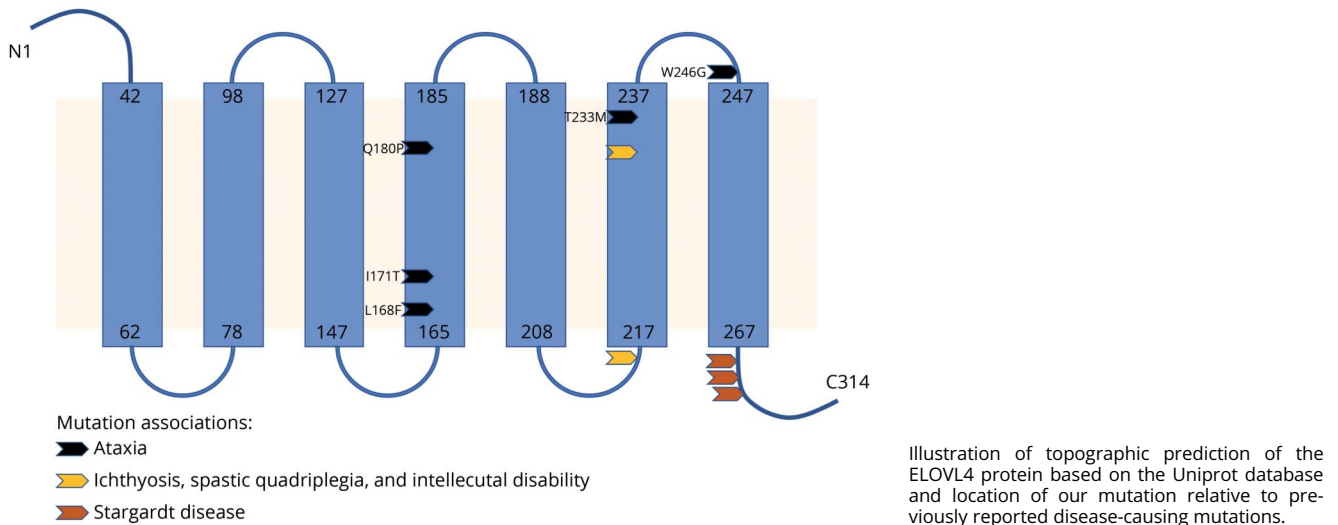
We identified a novel missense variant in *ELOVL4* in a large American family with SCA. This variant is in a highly conserved amino acid residue, is predicted to be likely pathogenic by American College of Medical Genetics

Figure 2 Novel Mutation in *ELOVL4*



(A) Whole-exome sequencing identified a novel heterozygous mutation in the elongation of very long chain fatty acids 4 (*ELOVL4*) gene (c.512T>C, p.Ile171Thr) that segregated with symptoms in 9 individuals tested. (B) The nucleic acid change is highly conserved across species and is predicted to be likely pathogenic.

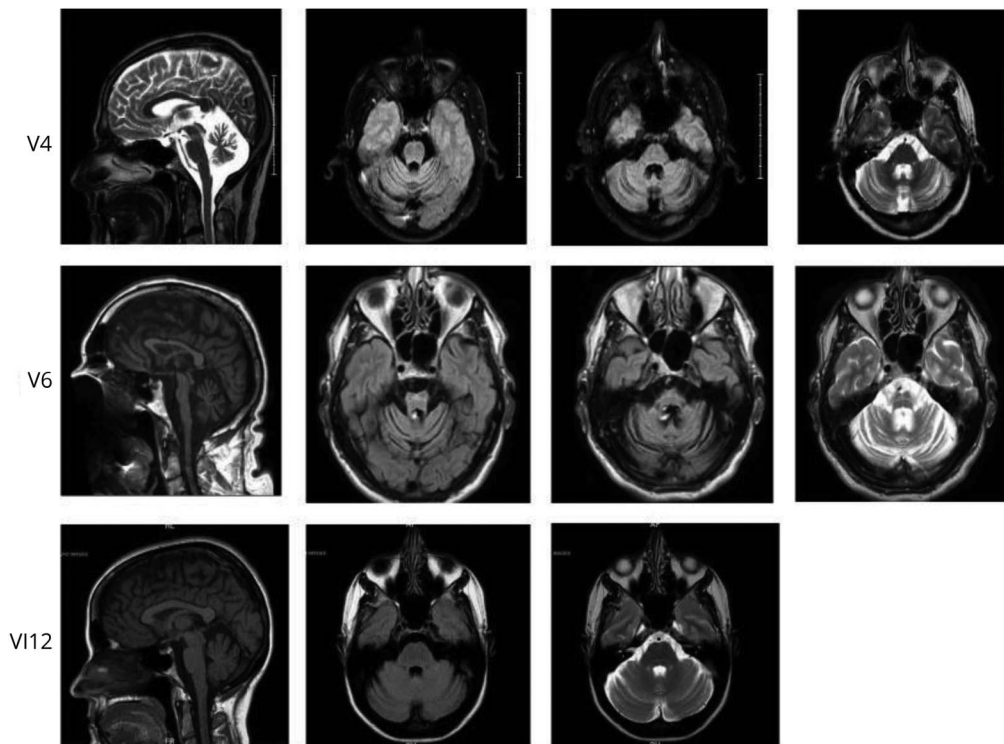
Figure 3 Location of Novel *ELOVL4* Mutation on Topographic Prediction of Protein



criteria and is nearby a previously reported mutation (P168F) in a large French Canadian family with SCA34. In addition, affected persons have relatively high incidence of pyramidal tract signs and oculomotor signs more consistent with those reported in the 2 Japanese families. Unlike the

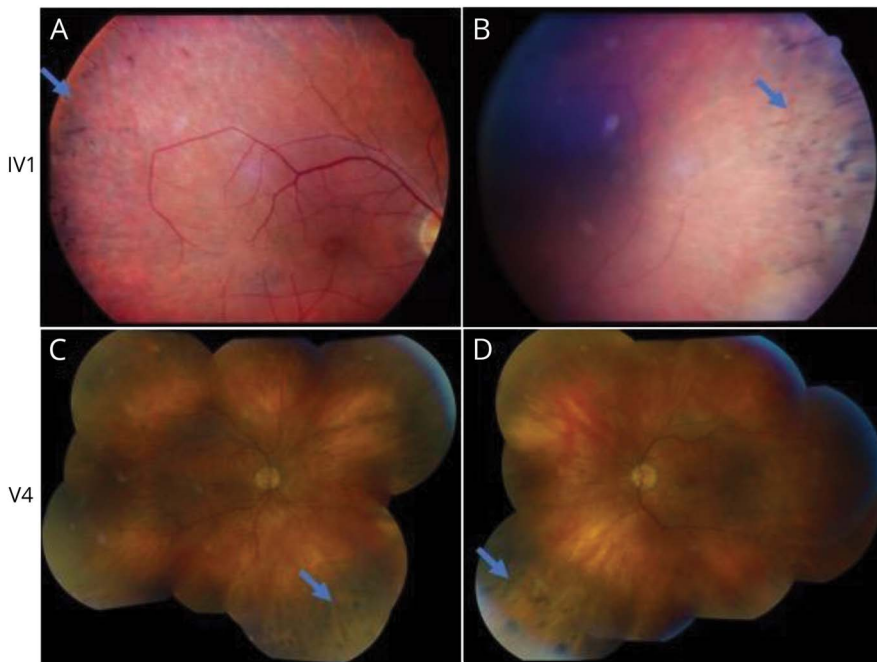
Japanese families, we did not see the loss of transverse pontine fibers on neuroimaging for members of our cohort. Several members of our family reported subjectively anosmia around the onset of gait ataxia. The clinical significance of this is unclear.

Figure 4 Brain MRIs of an American Family with Novel *ELOVL4* Mutation



MRI demonstrates cerebellar and pontine atrophy in 2 of 3 participants who were imaged. The third younger individual had mild changes.

Figure 5 Fundus Photographs of an American Family with Novel *ELOVL4* Mutation



Color fundus photographs demonstrating ophthalmic examination findings. Four patients were found to have ophthalmic findings consistent with retinitis pigmentosa. Color fundus photographs of patient IV1 (A, B) demonstrate bone spicule-like pigmentary changes. Montage color photographs of patient V4 demonstrate mild optic disc pallor, arteriolar attenuation, and peripheral retinal pigment epithelial changes (C = right eye, D = left).

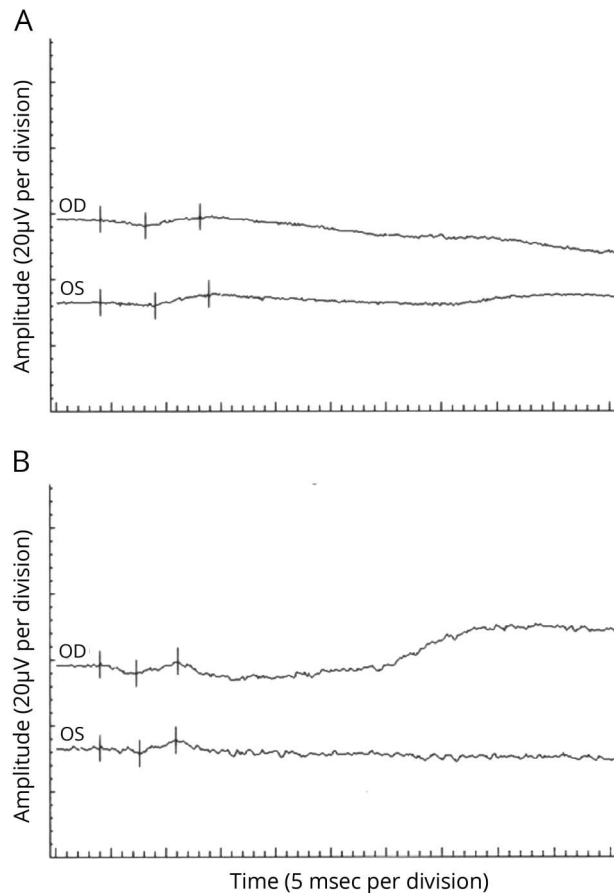
The relevant new finding in our family is the presence of retinitis pigmentosa. Although RP is not a typical presentation for Stargardt type 3, previous work on the more common autosomal recessive form of Stargardt disease, caused by mutations in the *ABCA4* gene, has shown RP and macular dystrophy to be part of the same phenotypic spectrum due to buildup of a toxic product in the retinal pigmented epithelium layer.⁶ The presence of inclusions in the retina of an *ELOVL4* transgenic mouse model could also point to a toxic gain of function mechanism.⁷

While *ELOVL4* mutations have been associated with autosomal dominant Stargardt-like macular dystrophy (OMIM 600110) in the past, ophthalmologic pathology in the previously reported SCA34 families has not been identified. The mechanism by which *ELOVL4* mutations lead to both Stargardt disease or SCA34 is currently unknown. *ELOVL4* encodes a protein that contributes to elongation of fatty acids longer than 26 carbons and is expressed in the retina, brain, testis, skin, and thymus.⁸ We did not measure very long chain fatty acids (VLCFA) due to technical constraints as standard assays cannot resolve the necessary carbon length. Further study is necessary to determine whether the pathogenesis of *ELOVL4* related disorders can be directly linked to VLCFA production. Based on the lack of overlapping ophthalmologic and neurologic phenotypes in affected patients with *ELOVL4* mutations, the mechanism for the 2 phenotypes is thought to be preferential disruption of

polyunsaturated (PUFA) vs saturated (SFA) very long chain fatty acid biosynthesis, important in retina and brain/skin respectively.⁹ This model predicts patients carrying the Thr171Ile mutation would have decreased PUFA and SFA synthesis while persons carrying previous mutations in SCA families would only have decreased SFA synthesis. Since our mutation is in close proximity to prior reported mutations in the 4th transmembrane domain of the *ELOVL4* protein, which were not associated with retinal phenotypes, it is unclear why it would produce a different functional effect. Recent work found *ELOVL4* synthesis of VLCFAs play an important role in tight junction stability in the blood-retinal barrier in mice.¹⁰ This could suggest blood-brain and blood-retinal barrier instability as a potential mechanism of disease in late-onset SCA. Speculation on potential mechanisms of cerebellar and retinal pathogenesis is limited by the lack of a robust biomarker for the synthesis of either VLC-PUFA or VLC-SFA in human subjects as well as limited understanding of the biological function of *ELOVL4* and its very long chain FA products on health and disease.

ELOVL4 mutations have previously been associated with inherited retinopathy and SCA independently of each other. We report an American family with concurrent spinocerebellar ataxia and retinal dystrophy due to an *ELOVL4* mutation. In addition, to supporting prior reports that *ELOVL4* mutations can cause an autosomal dominant spinocerebellar ataxia, our findings further broaden the spectrum of clinical presentations associated with SCA34.

Figure 6 Electroretinogram Data for Individual IV1



(A) Scotopic electroretinogram: After 30 minutes of dark adaptation, a bright flash stimulus resulted in b-waves of 37.8 μV OD (implicit time 45.5 msec) and 50 μV OS (implicit time 49.5 msec). Normal for age is greater than 229.2 μV with an implicit time of less than 53.4 msec. (B) Photopic electroretinogram: Under light-adapted conditions, a bright flash stimulus resulted in b-waves of 19.8 μV OD (implicit time 35.5 msec) and 21 μV OS (implicit time 34.5 msec). Normal for age is greater than 27.2 μV with an implicit time of less than 32.4 msec. The rod-selective responses were nonrecordable. The scotopic bright flash responses were markedly reduced in amplitude (A). The photopic responses were reduced in amplitude and delayed (B).

Study funding

No targeted funding reported.

Disclosure

Disclosures available: Neurology.org/NG.

Publication history

Received by *Neurology: Genetics* December 14, 2018. Accepted in final form July 8, 2019.

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Elaine M. Binkley, MD	University of Iowa, Iowa City	Author	Acquisition and interpretation of the data, drafted the figures, and revised the manuscript for intellectual content

Appendix (continued)

Name	Location	Role	Contribution
Jessica Rexach, MD, PhD	University of California Los Angeles, Los Angeles	Author	Acquisition of the data, drafted the figures, and revised the manuscript for intellectual content
Amy Knight-Johnson, GSC	University of Chicago, Chicago	Author	Major role in the acquisition of data
Pravin Khemani, MD	Swedish Medical Group, Seattle	Author	Major role in the acquisition of data
Brent L. Fogel, MD, PhD	University of California Los Angeles, Los Angeles	Author	Acquisition and interpretation of data; revised the manuscript for intellectual content
Soma Das, PhD	University of Chicago, Chicago	Author	Acquisition and interpretation of molecular data and revised the manuscript for intellectual content

Appendix (continued)

Name	Location	Role	Contribution
Edwin M. Stone, MD, PhD	University of Iowa, Iowa City	Author	Acquisition and interpretation of molecular data and revised the manuscript for intellectual content
Christopher M. Gomez, MD, PhD	University of Chicago, Chicago	Author	Designed and conceptualized study and revised the manuscript for intellectual content

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