A Nonsense *ALMS1* Mutation Underlies Alström Syndrome in an Extended Mennonite Kindred Settled in North Mexico

Marisa Cruz-Aguilar,[1] Carlos Galaviz-Hernández,[2] José Hiebert-Froese,[3] Martha Sosa-Macías,[2] and Juan Carlos Zenteno[1,4]

Aim: Alström syndrome (AS) is a rare autosomal recessive multisystem disease caused by biallelic mutations in *ALMS1*, a gene encoding a widely expressed centrosomal/basal body protein. Although more than 200 pathogenic mutations in *ALMS1* have been identified to date in AS patients from various ethnic populations, there are very few reports of *ALMS1* founder mutations in isolated populations. Our aim was to describe the molecular characterization of a cohort of AS patients from an extended inbred Mennonite kindred settled in Mexico.
Methods: Genetic study included polymerase chain reaction amplification and direct nucleotide sequencing of the entire *ALMS1* gene in DNA from seven related AS patients.
Results: A homozygous single-nucleotide c.10480C>T substitution in exon 16, predicting a p.Q3494* nonsense mutation, was identified in all affected subjects.
Conclusions: To our knowledge, this is the first demonstration of a high prevalence of AS in Mennonites, a population group maintaining high levels of consanguineous marriage in their communities. Our findings provide an example of genetic isolation and consanguinity causing a high prevalence of AS and offer the opportunity for early clinical interventions and for genetic counseling of at-risk couples in this community.

Keywords: Alström syndrome, *ALMS1* gene, retinal dystrophy, Mennonites, consanguinity

Introduction

Alström syndrome (AS; OMIM No. 203800) is an uncommon autosomal recessive multisystem disorder that is clinically defined by childhood-onset cone-rod retinal dystrophy, sensorineural hearing loss, obesity, and insulin resistance (Alström *et al.*, 1959). Additional anomalies such as cardiomyopathy, type 2 diabetes mellitus, short stature in adulthood, recurrent pulmonary infections, and renal and hepatic dysfunction are frequently observed in affected individuals (Marshall *et al.*, 2007b). The disease is likely to be underdiagnosed because of its rarity, and available prevalence figures range from 1:10,000 (Minton *et al.*, 2006) to less than 1:1,000,000 (Marshall *et al.*, 2012). AS patients develop progressive cone-rod retinal degeneration, leading to visual impairment, photophobia, and nystagmus, with more than 90% of patients becoming totally blind by the age of 16 years (Russell-Eggitt *et al.*, 1998; Marshall *et al.*, 2005). Other endocrine disorders can include childhood obesity, hyperinsulinemia, hypertriglyceridemia, and hypergonadotrophic hypogonadism (Paisey *et al.*, 2004; Girard and Petrovsky, 2011; Bettini *et al.*, 2012).

Biallelic mutations in the Alström syndrome 1 (*ALMS1*) gene are causative of AS (Collin *et al.*, 2002; Hearn *et al.*, 2002). *ALMS1* is located at chromosome 2p13.1 and encodes a ubiquitously expressed protein of 4169 amino acids (Hearn *et al.*, 2002). The majority of *ALMS1* mutations predicts truncated proteins by nonsense and frameshift variations clustered in exons 8, 10, and 16 (reviewed in Marshall *et al.*, 2007a and Marshall *et al.*, 2015). In the photoreceptors, ALMS1 mutations lead to defective function of the connecting cilium (Collin *et al.*, 2005). To date, more than 200 pathogenic mutations in *ALMS1*, in either the homozygous or compound heterozygous state, have been identified in AS patients from various ethnic populations (Marshall *et al.*, 2015). Remarkably, very few instances of AS arising from an *ALMS1* founder mutation in isolated populations have been described (Minton *et al.*, 2006; Taşkesen *et al.*, 2012). Here,

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[1]Genetics Department-Research Unit, Institute of Ophthalmology, "Conde de Valenciana," Mexico City, Mexico.
[2]Instituto Politécnico Nacional, CIIDIR-Durango, Mexico.
[3]Clinica Medica Sur del Carmen, Durango, Mexico.
[4]Department of Biochemistry, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico.

we report the clinical and molecular findings in seven AS patients from an extended inbred Mennonite kindred settled in Mexico. Our study illustrates an example of genetic isolation causing a high prevalence of AS.

Materials and Methods

Patients

AS probands from an extended Mennonite family from Durango State (North Mexico) were clinically evaluated. Seven affected individuals (Fig. 1) as well as seven unaffected first-degree relatives were examined. Affected individuals are clustered in the fifth generation and are familiarly related, and we were unable to identify a probable common ancestor before the first generation depicted. The family pertains to an Old Colony Mennonite group community that migrated from Canada during the early 1920s. The first wave of immigrants arriving from Canada to found this village was composed of ~1000 subjects and currently, the population is composed by about 8000 inhabitants. The patients lived in a rural community and for this reason, specialized studies were not performed in most of them. The diagnosis of AS was given according to the presence of major and minor clinical criteria, as previously defined by Marshall *et al.* (1993, updated in 2012).

Genetic analysis

The study protocol was approved by the Institutional Ethics Committee at the Institute of Ophthalmology ''Conde de Valenciana'' (Mexico City). Informed consent was obtained from all participants. Genomic DNA was isolated from peripheral blood of participating individuals, and polymerase chain reactions (PCRs) were designed to amplify the exons of the *ALMS1* gene by using primers shown in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/gtmb). PCR conditions are available on re-

quest. Purified amplicons were sequenced by using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Samples were run in a 3130 Genetic Analyzer (Applied Biosystems), and sequences were compared manually against Ensembl Transcript ID ENST613296.4.

Results

Clinical evaluation

Patient no. 1: (V-17). ElRF is a 14-year-old female patient. She was the product of at a term gestation, with a birth weight of 3200 g. Horizontal nystagmus was noted at 8 weeks of life followed by photophobia and decreased visual acuity at the ages of 1 and 3 years, respectively. An ophthalmological examination at 6 months of age revealed nystagmus, reduced visual fixation, and photophobia. Overweight was noticed during the first year of life, and significant increase of weight was developed by the age of 4 years. Currently, her weight is 89.2 kg, height is 1.62 m, and body mass index (BMI) is 34. Blood biochemical analysis showed high triglyceride (251 mg/dL; normal values <150 mg/dL) and very low density lipoprotein (VLDL) (50 mg/dL; normal values 2–30 mg/dL) levels. Other parameters of her lipid profile were within normal limits. Hearing acuity was preserved, although her school performance was reported as inadequate.

Patient no. 2: (V-18). ARF is a 10-year-old female patient. Her birth weight was 3700 g and at 18 months of life, obesity was noted. She developed horizontal nystagmus by 12 weeks of life and by the age of 7 years, her visual acuity was severely impaired. No hearing problems were reported. Suppurative otitis media due to multidrug-resistant *Pseudomonas aeruginosa* was diagnosed at the age of 8 years. At present, she is able to copy texts and to recognize a maximum of 10 words only. Physical examination showed a weight of 70.2 kg, a height of
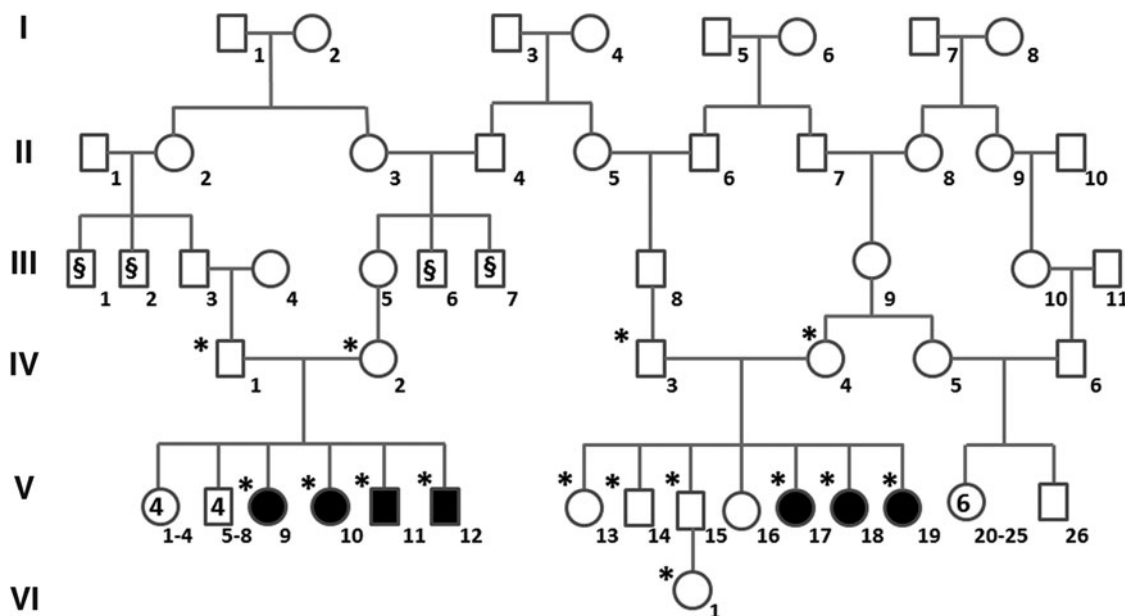

FIG. 1. Ancestral simplified Pedigree of an extended Mennonite family segregating Alström syndrome. *Squares* and *circles* indicate men and women, respectively. *Black symbols* represent affected members; *open symbols* represent the unaffected individuals; *indicates individuals who were molecularly analyzed in this study; and §signals four relatives who were reported with a history of blindness and obesity and, thus, probably suffering from the disease.
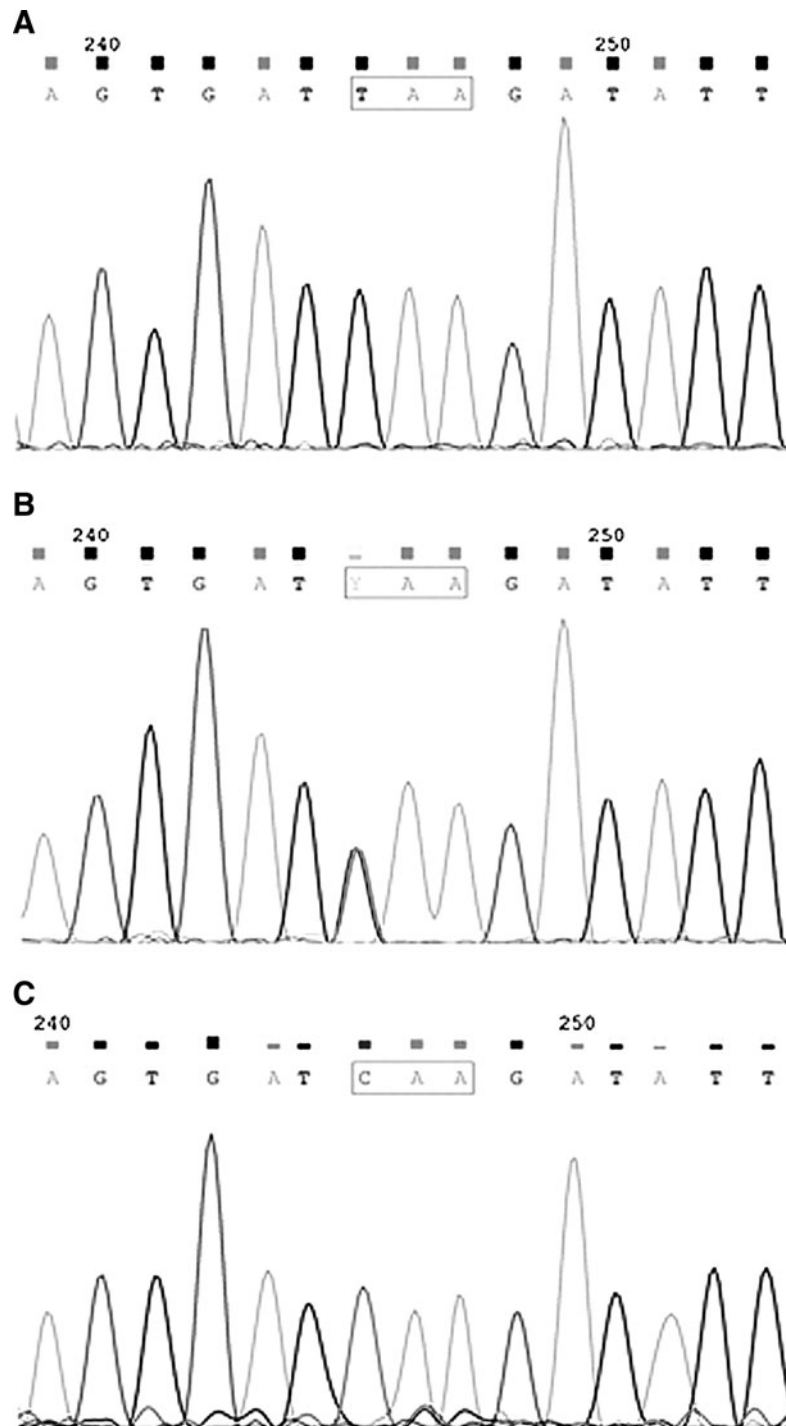
FIG. 2. *ALMS1* nucleotide sequence analysis in Alström syndrome family individuals. **(A)** Partial sequence analysis of exon 16 demonstrates a homozygous c.10480C>T transition in DNA from all seven affected subjects. This mutation predicts the introduction of a premature termination codon (p.Q3494*). **(B, C)** Show heterozygosity for the nonsense *ALMS1* mutation and wild-type sequence, respectively.

1.55 m, and a BMI of 29.2. Horizontal nystagmus was evident in both eyes, whereas acanthosis nigricans was present in the neck. No blood biochemical analyses were available.

Patient no. 3: (V-19). EVRF is an 8-year-old girl who had a birth weight of 3700 g. She developed horizontal nystagmus and photophobia at 20 weeks of life. Bilateral hearing deficit was noted at the age of 7 years, but a formal audiometric test has not yet been performed. Overweight was noted at the age of 3 years. Her actual weight is 52.3 kg, height is 1.34 m, and BMI is 29.1. Acanthosis nigricans was evident around the neck. Blood biochemical analyses have not been performed.

Four relatives (subjects V-9 to V-12 in pedigree) of patients nos. 1–3, aged 14, 11, 5, and 2 years, respectively, were reported to exhibit a similar phenotype. They were clinically assessed by one of us (C.G.-H.), and their phenotypic features were compatible with a diagnosis of AS. All of them developed pendular nystagmus within the first year of life and at examination exhibited telecanthus, short palpebral fissures, photophobia, impaired photomotor reflex, and decreased osteotendinous reflexes. Acanthosis nigricans was observed in subjects V-9 and V-10, whereas overweight (elevated BMI) was demonstrated in all but subject V-12. Hypoacusia was reported only in patient V-10. No formal audiometric

tests or blood chemistry analyses had been performed in any of them.

Genetic analysis

Sanger sequencing of the *ALMS1* gene performed in DNA from the seven AS-affected individuals from this Mennonite family allowed the identification of a homozygous transition at position 10480 (c.10480C>T) in exon 16, in all diseased subjects (Fig. 2). This variant is predicted to introduce a premature termination codon at residue 3494 (p.Q3494*) of ALMS1, resulting in a truncated protein. Unaffected parents (individuals IV-1, IV-2, IV-3, and IV-4) were heterozygous carriers of the mutant allele, whereas healthy sibs were either heterozygous for the mutation (V-13 and V-14) or homozygous (V-15 and VI-1) for the wild-type allele.

Discussion

AS is a rare and severe multisystem disorder in which impairment of sensory functions results in visual and audiological deficiency in association with a variety of metabolic disorders. Here, we report the identification of the molecular cause of AS in an extended Mennonite pedigree settled in Mexico. As anticipated, all seven affected subjects from this isolated population carried an identical homozygous mutation in the *ALMS1* gene. The identified p.Q3494* mutation is a truncating variant, as virtually all pathogenic mutation that have been associated so far with the disease (Marshall *et al.*, 2015). The p.Q3494* mutation has been previously described (designated as p.Q3493X) in two AS reports (Khoo *et al.*, 2009; Edwards *et al.*, 2015). Although no functional studies have been performed yet on this variant, it is located near the beginning of the highly conserved ALMS1 motif and probably disrupts ALMS1 critical functions in microtubule organization, particularly in the formation and maintenance of cilia.

The study of populations with geographic or cultural isolation in which endogamy and/or consanguineous marriages are still frequent has been extremely valuable for the identification of the molecular bases of a number of human genetic diseases (Zlotogora, 1994; Boycott *et al.*, 2008; Sherwin *et al.*, 2008). In such populations, genetic variation decreases with subsequent generations, thus increasing the chances of recessive disease (Alkuraya, 2013; Erzurumluoglu *et al.*, 2016). Mennonites are Anabaptists who descended from Swiss, Dutch, and German ancestors. Due to religious persecutions, Mennonite communities migrated to various parts of Europe, seeking regions that were tolerant of their traditions and customs. The first Mennonites (Swiss/German, also known as Old Order Mennonites) immigrated to North America in the late 18th century, settling primarily in Southern Ontario and Pennsylvania (Puffenberger, 2003). The Dutch/German Mennonites (or Old Colony Mennonites) eventually settled in Russia and Ukraine and then immigrated to Canada in two major waves, with the first one arriving in the early 1870s and the other one arriving between 1922 and 1930 (Orton *et al.*, 2008). Because each population descended from a small number of founders, many rare alleles were likely introduced by one or a few common ancestors, and their sustained isolation has resulted in a high frequency of particular monogenic diseases within each subgroup (Puffenberger, 2003; Boycott *et al.*, 2008; Orton *et al.*, 2008). Settlements maintain strict adherence to religious customs,

and because members rarely marry outside the church, consanguinity is still common (Strauss and Puffenberger, 2009; Schroeder *et al.*, 2016).

The AS Mennonite family described in this work settled in Mexico three generations ago, by 1924. Their ancestors migrated from the Manitoba region in Canada and belong to the Old Colony Mennonite group. To our knowledge, this is the first demonstration of a high prevalence of AS in a Mennonite community. Previously, founder mutation effects were identified in Mennonite communities, rendering a high prevalence of some recessive diseases, including, among others, primary immunodeficiency, Fanconi anemia, and torsion dystonia due to *ZAP70*, *FANCC*, and *ATM* gene mutations, respectively (de Vries *et al.*, 2012; Saunders-Pullman *et al.*, 2012; Schroeder *et al.*, 2016). The identification of founder mutation effects in isolated populations has important implications for the development of opportune detection and genetic counseling programs in such communities.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Juan Carlos Zenteno, MD, PhD
Genetics Department-Research Unit
Institute of Ophthalmology
''Conde de Valenciana''
Chimalpopoca 14, Col. Obrera
Mexico City CP 06800
Mexico

E-mail: jczenteno@institutodeoftalmologia.org