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Novel mutations and a severe neurological phenotype in Sjögren-Larsson syndrome patients from Iran

Ariana Kariminejad[a,*], Mohammadreza Barzgar[b], Bita Bozorgmehr[a], Elham Keshavarz[c], Mohamad Hasan Kariminejad[a], Dana S'Aulis[d], William B. Rizzo[d]

[a] Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran
[b] Skin Research Center Shahid Beheshti University of Medical Sciences, Shohad e Tajrish Hospital, Tehran, Iran
[c] Radiology Department, Shahid Beheshti University of Medical Sciences, Mahdieh Hospital, Tehran, Iran
[d] Department of Pediatrics, UNMC Child Health Research Institute, University of Nebraska Medical Center, Omaha, NE, USA

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ABSTRACT

Sjögren-Larsson syndrome (SLS) is a rare autosomal recessive disorder characterized by ichthyosis, spasticity and intellectual disability. The disease is caused by mutations in the *ALDH3A2* gene that encodes fatty aldehyde dehydrogenase. We describe 7 Iranian SLS patients from 5 unrelated consanguineous families. Sequencing of *ALDH3A2* identified 4 novel mutations, including a 26-bp deletion (c.25_50del), small in-frame deletion (c.370_372del; p.G124del), a termination (p.Q35Ter) and a missense mutation (p.Lys211Glu). Bacterial expression of the p.Lys211Glu and p.G124del mutations showed little or no detectable enzyme activity. Three of the patients exhibited an unusual neuro-regressive clinical course associated with seizures, which may reflect the presence of unidentified genetic or environmental modifiers in this consanguineous population. This cohort represents the largest group of Iranian patients with molecularly confirmed SLS and expands the mutational and clinical spectrum of this disease.

1. Introduction

Sjögren-Larsson syndrome (SLS) (MIM #270200) is a rare autosomal recessive disorder with characteristic clinical features of intellectual disability, spastic diplegia or quadriplegia, and ichthyosis (Rizzo, 2007; Fuijkschot et al., 2012). The ichthyosis is usually present at birth, whereas spasticity becomes apparent in the first year of life with delay in achieving motor milestones. Most patients exhibit spastic diplegia, which impairs their ability to walk. Intellectual disability ranges from profound to mild. The disease is usually considered a static neurologic disorder with slowly progressive spasticity. Additional clinical features include a distinctive retinopathy with perifoveal crystalline deposits (glistening white dots), photophobia, speech delay and preterm birth. Myelin abnormalities are often seen on brain neuroimaging, and MR spectroscopy of the white matter reveals the presence of abnormal lipid peaks (Willemsen et al., 2004). Despite the significant neurologic involvement, neuro-regression is unusual and SLS is typically considered a static encephalopathy.

SLS is caused by mutations in *ALDH3A2*, which encodes fatty aldehyde dehydrogenase (FALDH; EC1.2.1.48) (De Laurenzi et al., 1996). FALDH deficiency results in accumulation of long-chain aldehydes and alcohols, which are thought to be responsible for the cutaneous and neurologic symptoms (Rizzo, 2014). The diagnosis of SLS is confirmed by finding decreased FALDH activity in cultured skin fibroblasts (Rizzo and Craft, 1991) or the presence of pathogenic bi-allelic *ALDH3A2* mutations. Owing to limited availability of enzyme testing, DNA-based diagnosis of SLS is becoming increasingly used and the spectrum of identified mutations is enlarging. To date, more than 80 mutations have been described in the literature (De Laurenzi et al., 1996; Sillén et al., 1998; Cho et al., 2017; Rizzo et al., 1999; Kraus et al., 2000; Rizzo and Carney, 2005; Auada et al., 2006; Sakai et al., 2006, 2010; Didona et al., 2007; Engelstad et al., 2011; Sarret et al., 2012; Yiş and Terrinoni, 2012; Davis et al., 2013; Incecık et al., 2013; Hosseini et al., 2012; Burgueno-Montanes et al., 2014; Jain et al., 2015; Gaboon et al., 2015; Tanteles et al., 2015; Rashid et al., 2016; Tavasoli et al., 2016; Nagappa et al., 2017). Most mutations are private, however several common mutations have been found in patients from the Mideast (Rizzo and Carney, 2005), Brazil (Auada et al., 2006) and Europe (Sillén et al., 1998; Cho et al., 2017; Rizzo et al., 1999; Kraus et al., 2000; Rizzo and Carney, 2005; Didona et al., 2007; Sarret et al., 2012).

Abbreviations: FALDH, fatty aldehyde dehydrogenase; SLS, Sjögren-Larsson syndrome

* Corresponding author. Kariminejad-Najmabadi Pathology & Genetics Center, #2, 4th St, Hasan Seyf St. Shahrak Gharb, Tehran, Iran.
E-mail address: arianakariminejad@yahoo.com (A. Kariminejad).

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Here we describe 7 Iranian SLS patients from 5 unrelated consanguineous kindreds. The probands carry 5 homozygous *ALDH3A2* mutations and three of the patients exhibited an unusual neuro-regressive clinical course.

2. Methods

2.1. SLS subjects and mutation analysis

Informed consent was obtained from the parents of all SLS subjects. Ethics approval was obtained by the Kariminejad-Najmabadi Pathology & Genetics Center ethical committee.

Genomic DNA was prepared from blood using standard methods. Primers and PCR conditions used to amplify and sequence exons in *ALDH3A2* were previously described (Rizzo et al., 1999).

2.2. Site-directed mutagenesis and FALDH expression

Two mutations (c.631A > G and c.370_372del) were introduced into the most abundant isoform of human *ALDH3A2* cDNA (containing an amino-terminal His tag) within a bacterial expression vector (pEXP1-DEST, Invitrogen Life Technologies) using the Quick-Change II XL Site Directed Mutagenesis Kit (Agilent Technologies) and confirmed by DNA sequencing. The mutant and wild-type vectors were transformed into *E. coli* and expression of FALDH was induced overnight with isopropyl-β-thiogalacopyranoside. The bacteria were collected by centrifugation and FALDH solubilized using P-PER Bacterial Protein Extraction Reagent (Thermo Scientific) at 4 °C. FALDH proteins were purified by affinity chromatography using the Capturem His-Tagged Purification Kit (Clontech). Enzyme activity was measured fluorometrically by measuring the octadecanal-dependent production of NADH at 37 °C (Rizzo and Craft, 1991). Enzyme activity of the mutant and wild-type FALDH was normalized for protein and the activity measured in the mutant proteins was expressed as a percentage of wild-type activity. The assay detection limit was 0.5% of mean wild-type activity.

3. Case reports

3.1. Family 1

Family 1 is highly consanguineous (inbreeding coefficient F = 1/64) and has 7 individuals affected with SLS (Fig. 1)

Patient 1 (Individual VI-1) is a 3-year-old male born to related parents. He was born at full term gestation by vaginal delivery. Ichthyosis was present at birth, but there was no collodion membrane. Hyperkeratosis was more severe on limbs and milder on face and trunk. During the first year, his major milestones were only mildly delayed: he held his head up at 6 months, sat with support at 8 months, clapped and imitated parents at 11 months, held toys and started saying words at 12 months. His first seizure (tonic clonic) was at the age of 4 days but seizures were not controlled despite treatment with different anticonvulsants. At 2 years of age he lost the ability to say any words, hold objects, clap, crawl, stand or walk. Spasticity increased over time. Examination at 3 years of age showed a small boy with height, weight and head circumference −1.881SD. He showed generalized ichthyosis involving the face, neck, palms, soles, trunk and back. He had hyperreflexia in the lower extremities with up going plantar reflexes and spastic paraplegia. He was unable to sit unsupported and had photophobia.

Patient 2 (Individual IV-15) is the second born male to related parents (inbreeding coefficient F = 1/16). He was born after an uneventful pregnancy and delivery. Generalized ichthyosis was noted at birth. Motor and cognitive milestones were delayed in the first year. He sat at 10 months, started saying words at 2–3 years and walked at 3 years. He never had seizures, but spasticity became increasingly prominent in the lower limbs from 3 years onwards, and by 6 years of age he could only crawl. Nevertheless, he was able to perform routine physical tasks including taking care of his personal hygiene, washing, dressing and eating by himself. Physical examination at 42 years showed severe spasticity of lower limbs, generalized ichthyosis with sparing of the face and mild to moderate intellectual disability. He could speak and easily communicate with complete sentences. Other than losing his ability to walk he has remained stable.

3.2. Family 2

Patient 3 (Individual IV-2) is a 3-year-old male child of first cousin parents (inbreeding coefficient 1/64) (Fig. 1). He was born by vaginal delivery at 32 weeks gestation after premature rupture of membranes. Ichthyosis with dark brown scales was noticed at birth. Motor and cognitive milestones were delayed; he held his head up at 4.5 months, sat at 15 months, crawled at 15 months and started saying words at 2 years. Physical examination at 3 years of age showed generalized ichthyosis, spastic diplegia with hyperreflexia and inability to walk. He had a vocabulary of 20 words. Spasticity has not increased with age and parents claim that there is a slow improvement of his general condition.

3.3. Family 3

Patient 4 (individual V-1) is the first female child of related parents (inbreeding coefficient (F = 1/16) (Fig. 1). She was born by vaginal delivery at 36 weeks gestation because of premature rupture of membrane. There was no collodion membrane, but generalized erythematous ichthyosis was present. She developed jaundice after 5 days, which resolved with phototherapy. She was able to hold her head and roll over at 4 months, but other milestones were severely delayed. She achieved the ability to say a few words by 1.5 years of age. After months of physiotherapy she was able to sit with support at 18 months, crawled at 3 years, spoke three word sentences at 3.5 years, sat without support at 4.5 years and could walk about 100 steps with assistance at 4.5 years. The parents had noted myoclonic jerks beginning at 4 months and the diagnosis of myoclonic seizures was established at 15 months of age with an abnormal electroencephalogram. She was placed on anticonvulsant (Phenobarbital) treatment, without complete control, and developed refractory tonic clonic seizures at the age of 4.5 years that failed to be controlled with carbazepine, prednisolone, nitrazpam, lomotrigine, and diazepam.

With onset of these seizures she showed cognitive and motor regression and lost the ability to sit unsupported, crawl, and walk with help. Her speech also regressed and ceased entirely at 6 years. Spasticity worsened after the first tonic clonic seizure and has increased more over the years. Physical examination at 9 years of age showed a low weight −1.1645 SD, height −1.881 SD and head circumference 1.645 SD. She could not sit without support, had no speech and did not interact. She showed hyperreflexia in the lower extremities, but at rest she exhibited mild generalized hypotonia in the upper body. She had ichthyosis involving the face, trunk and extremities, which was notably pruritic. Her facial appearance was coarse with short nose and proptosis. She had photophobia and perifoveal glistening white dots on the retina at ophthalmic exam (Fig. 2d). Brain MRI at 10 years of age showed mild periventricular white matter disease but no atrophy (Fig. 2a,b).

3.4. Family 4 has 2 individuals with SLS (Fig. 1)

Patient 5 (individual IV-3) is a 3-year-old boy, the first and only child of first cousin parents (inbreeding coefficient F = 1/32). He was born full term. At birth, he had generalized ichthyosis and developed mild neonatal jaundice, which responded to phototherapy. Motor milestones were delayed; he held his head at 8 months, sat at 2 years-3 months, crawled and stood with assistance at 2.5 years, but was unable to walk or speak words. Examination at 3 years of age revealed normal