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Large contiguous gene deletions in Sjögren–Larsson syndrome

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ABSTRACT

Sjögren–Larsson syndrome (SLS) is an autosomal recessive disorder characterized by ichthyosis, mental retardation, spasticity and mutations in the *ALDH3A2* gene for fatty aldehyde dehydrogenase, an enzyme that catalyzes the oxidation of fatty aldehyde to fatty acid. More than 70 mutations have been identified in SLS patients, including small deletions or insertions, missense mutations, splicing defects and complex nucleotide changes. We now describe 2 SLS patients whose disease is caused by large contiguous gene deletions of the *ALDH3A2* locus on 17p11.2. The deletions were defined using long distance inverse PCR and microarray-based comparative genomic hybridization. A 24-year-old SLS female was homozygous for a 352-kb deletion involving *ALDH3A2* and 4 contiguous genes including *ALDH3A1*, which codes for the major soluble protein in cornea. Although lacking corneal disease, she showed severe symptoms of SLS with uncommon deterioration in oral motor function and loss of ambulation. The other 19-month-old female patient was a compound heterozygote for a 1.44-Mb contiguous gene deletion and a missense mutation (c.407C>T, P136L) in *ALDH3A2*. These studies suggest that large gene deletions may account for up to 5% of the mutant alleles in SLS. Geneticists should consider the possibility of compound heterozygosity for large deletions in patients with SLS and other inborn errors of metabolism, which has implications for carrier testing and prenatal diagnosis.

1. Introduction

Sjögren–Larsson syndrome (SLS; OMIM 270200) is an autosomal recessive inborn error of metabolism caused by mutations in the *ALDH3A2* gene for fatty aldehyde dehydrogenase (FALDH) [1,2]. Clinical features of SLS include ichthyosis, spastic diplegia or tetraplegia, mental retardation, seizures and a distinctive retinal crystalline maculopathy characterized by perifoveal glistening white dots. The ichthyosis is usually congenital in onset and is often pruritic in nature. Neurological symptoms of mental retardation and spasticity typically develop by the 2nd year of life and present with delay in achieving motor and cognitive milestones. The symptoms of SLS vary from mild to profound, and are generally non-progressive.

FALDH catalyzes the oxidation of fatty aldehyde to fatty acid, and is a necessary component of the fatty alcohol:NAD^+ oxidoreductase enzyme complex that catalyzes the sequential oxidation of fatty alcohol to fatty acid [3–5]. SLS patients consequently have elevated fatty alcohols in plasma, urine and cultured cells [6–9]. The symptoms of SLS are thought to arise directly or indirectly from accumulation of fatty aldehyde, fatty alcohol or related lipid products in the skin and brain [10].

The *ALDH3A2* gene is located on chromosome 17p11.2. More than 70 mutations have been discovered in SLS patients, including small deletions or insertions, missense mutations, splicing defects and complex mutations composed of deletion/insertions and nucleotide substitutions [11]. Intragenic deletions of one or more exons have also been rarely described [12–14]. Most mutations in SLS are private, and many patients have been found to be homozygous due to consanguinity or founder effects.

We now present 2 unique SLS patients in whom the disease was caused by unusually large deletions involving *ALDH3A2* and surrounding genes on chromosome 17p11.2.

2. Materials and methods

The Institutional Review Board at the University of Nebraska Medical Center approved this research, and all subjects consented to the study.

Abbreviations: CGH, comparative genomic hybridization; FALDH, fatty aldehyde dehydrogenase; FISH, fluorescence *in situ* hybridization; LDI-PCR, long distance inverse-PCR; OMIM, Online Mendalian Inheritance in Man; SLS, Sjögren–Larsson syndrome; SMS, Smith–Magenis syndrome; SNP, single nucleotide polymorphism.

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2.1. Patient descriptions

2.1.1. Patient 1

This 24-year-old female was born at 32 weeks gestation to consanguineous first-cousin Pakistani parents. She was noted to have erythematous dry scaly skin at birth, but had no collodion membrane. She developed several seizures at 6 weeks of age, which were associated with hypocalcemia. Delays in achieving motor milestones and speech were noted in infancy. After 2 febrile illnesses at 6–7 months of age, she lost the ability to roll over and her limbs became very stiff. A brain CT at 9 months of age showed moderate cerebral atrophy. However, she began to sit unsupported and crawl at about 18 months. Physical examination at 3 years of age showed developmental delay, spastic diplegia and generalized ichthyosis. Speech consisted of occasionally saying "mama" and "baba." Laboratory studies were normal, including karyotype, EEG, electroretinogram, thyroid function tests, plasma phytanic acid, urine amino acids and metabolic screen. Her ichthyosis responded well to etretinate therapy, which was subsequently switched to isotretinoin and then discontinued several years later because of concerns about retinoid toxicity. By 10 years of age, she was no longer speaking and began having difficulty swallowing. Physical exam showed a pruritic, generalized ichthyosis along with spastic diplegia, leg contractures and ankle clonus (Fig. 1A). She had photophobia and avoided bright lights. She was able to ambulate only with a walker using a crouched gait. Brain MRI revealed bilateral, symmetrical abnormal T2-weighted signal involving the fronto-parietal and superior periventricular regions. Several months later she developed increased drooling with tongue protrusion and dysphagia. Oral motor incoordination and loss of swallowing slowly progressed and necessitated placement of a gastrostomy tube at 12 years of age. Bilateral hamstring lengthening was also performed at this age. At 14 years, she was noted to have spastic tetraplegia and be non-ambulatory. At 17 years of age, ophthalmologic examination under sedation showed chronic blepharitis, but no cataracts, retinal or corneal abnormalities. The diagnosis of SLS was confirmed by demonstrating FALDH deficiency (8% of mean normal activity) in cultured fibroblasts.

2.1.2. Patient 2

This 19-month-old female infant was born at 36 weeks gestation to a 27-year-old mother and non-consanguineous father. Mother was of Irish and Cherokee Indian descent; father was English and American Indian. At birth, the infant was noted to have a collodion membrane with shiny, "tight" skin that transformed into dry, scaling skin after 2–3 weeks. Over time, she began scratching continually, often to the point of bleeding. At 10 months of age, a skin biopsy revealed hyperkeratosis, papillomatosis and acanthosis.

At 12 months of age, physical examination revealed height, weight, and head circumference within normal age limits. Significant findings included ichthyotic skin, ears adherent to the scalp bilaterally, and shortened fifth fingers bilaterally. She was noted to have spastic diplegia. Truncal hypotonia prevented her from sitting up for more than short periods of time. A Denver II Developmental Assessment found her to have social, language, fine motor, and gross motor abilities consistent with an age of 9, 7, 6, and 4 months old, respectively. Ophthalmologic examination revealed no ocular or visual abnormalities, including no evidence of macular glistening white dots. Her skin was dry with hyperkeratosis or scaling on the extremities, trunk, neck, palms, and soles (Fig. 2D). The hyperkeratosis was most severe on the patient's legs, forearms, neck and trunk, where it was reminiscent of lamellar ichthyosis. Her hair and fingernails were normal. At 19 months of age, the patient suffered a tonic–clonic seizure, which subsequently recurred on several occasions.

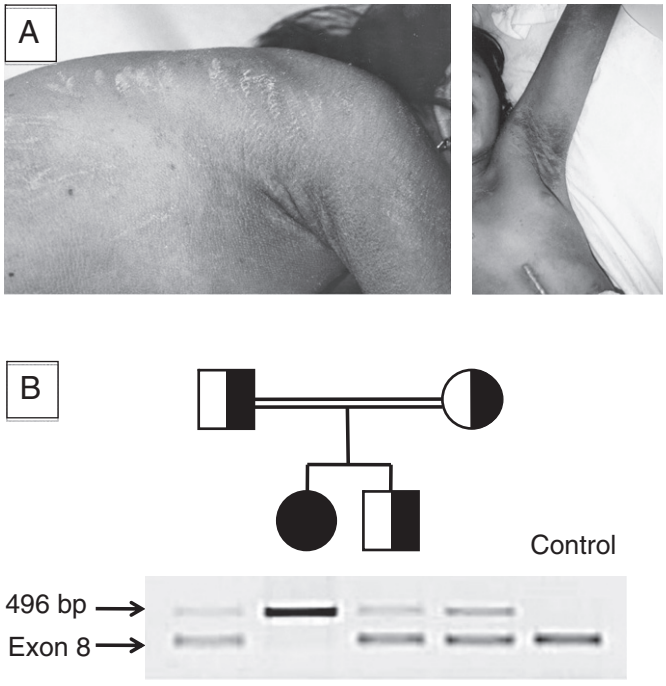

Fig. 1. Phenotypic appearance and mutation detection in Patient 1. A. Left photo: At 14 years of age, note the generalized pruritic ichthyosis with excoriations on the trunk. Right photo: Dark scales in the axillary region and an abdominal scar from gastrostomy tube placement are evident. B. Multiplex PCR amplification of the deletion mutation in genomic DNA generated a 496 PCR product from Patient 1, her mother and father, and heterozygous brother. A control PCR product (exon 8) was produced using DNA from the parents and heterozygous brother, but not from Patient 1.

2.2. Fibroblast culture

Cultured skin fibroblasts were grown from skin biopsies in Dulbecco's minimal essential medium containing 10% fetal bovine serum, penicillin, and streptomycin at 37 °C in an atmosphere of 5% CO_2. The cells were collected by trypsinization and washed twice with phosphate-buffered saline. Cell pellets were stored at −70 °C for DNA isolation.

2.3. FALDH assay

Fibroblasts were homogenized and assayed for FALDH activity using octadecanal as substrate as described [5], except that all reactions were run at 37 °C in a 96-well fluorescent plate reader (Molecular Dynamics) in a total volume of 0.38 ml. Cell protein was determined according to Lowry et al [15]. Enzyme activity was determined as pmol/min/mg cell protein, and expressed as percentage of normal mean enzyme activity.

2.4. DNA isolation

Genomic DNA was purified from cultured fibroblasts and blood using the Wizard Genomic DNA Purification kit (Promega). Buccal DNA was collected from controls and family members for PCR as described [16].

2.5. Deletion characterization by long distance inverse-PCR (LDI-PCR)

LDI-PCR was performed essentially as described by Willis et al [17]. A PstI restriction enzyme cut site was identified in the DNA sequence flanking one end of the deletion of Patient 1. One microgram of genomic DNA was therefore digested with 0.5 Unit of PstI in a total volume of 25 µl according to the manufacturer's instructions (New England Biolabs). The DNA digest was purified using a QIAquick PCR