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Sjögren–Larsson syndrome in Brazil is caused by a common c.1108-1G → C splice-site mutation in the *ALDH3A2* gene

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Sjögren–Larsson syndrome (SLS; OMIM 270200) is an autosomal recessive disorder characterized by the presence of pruritic ichthyosis, mental retardation, spastic diplegia or tetraplegia, retinal perimacular ‘glistening white dots’ and photophobia.¹ SLS is caused by mutations in the *ALDH3A2* gene, which codes for fatty aldehyde dehydrogenase (FALDH), a microsomal enzyme that catalyses the oxidation of medium- and long-chain aliphatic aldehydes.² More than 70 mutations have been identified in this disease.³ Most mutations are unique to each SLS family, but several common mutations have been reported among patients from Europe and the Middle East. In the first molecular genetic analysis of a cohort of patients with SLS from Brazil, we now report a common disease-causing *ALDH3A2* mutation, delineate its associated phenotypic spectrum and describe a diagnostic screening test using restriction enzyme digestion.

Case and methods

Nine patients with SLS from three apparently unrelated Brazilian kindreds native to the south and southeastern part of the country were studied. Patients 1, 2 and 3 were previously reported⁴ and were born to consanguineous parents; patients 4, 5 and 6 are sisters and patients 7 and 8 are their cousins. Patient 9 was also born to first cousins. Blood specimens,

skin biopsies and clinical data were collected after obtaining informed consent and with approval by the Institutional Review Board.

Total genomic DNA was extracted from blood using standard phenol-chloroform methods. *ALDH3A2* exons and their flanking sequences were amplified by polymerase chain reaction (PCR) and sequenced.² To detect the c.1108-1G → C mutation more conveniently, exon 8 was digested with *DdeI* according to the manufacturer's instructions and restriction products separated by agarose gel electrophoresis. *ALDH3A2* haplotypes were determined using four intragenic single nucleotide polymorphisms as described.² FALDH enzyme activity was measured in cultured skin fibroblasts.⁵

Results and discussion

Biochemical and molecular characterization

All patients had <10% of normal FALDH enzyme activity in cultured skin fibroblasts (Table 1). Sequencing of the *ALDH3A2* gene revealed a homozygous c.1108-1G → C mutation in intron 7 of all of the affected individuals. Their parents were heterozygous for the mutation, except for the parents of patient 9 who were not tested. The c.1108-1G → C mutation abolishes a *DdeI* restriction enzyme cut site in the wild-type gene. *DdeI* digestion of a 275-bp PCR product of exon 8 and its flanking intron 7 sequence yielded a 181-bp fragment from normal control subjects, but a 263-bp fragment from the patients with SLS (Fig. 1b). This constitutes a convenient screening test for the mutation. Haplotype analysis of the *ALDH3A2* gene showed that patients from each family were homozygous for haplo-type 1 (see Rizzo *et al.*²).

The c.1108-1G → C mutation alters the splice acceptor site at the junction of intron 7 and exon 8. Like many exons, exon 8 does not end between codons, but rather ends with part of a codon, which is completed when exon 8 is spliced to exon 9. Consequently, skipping of exon 8 results in a frameshift of all subsequent codons in exon 9 and 10. The results of this mutation, as previously reported,² are two abnormal mRNA transcripts. One transcript is missing exon 8 and is predicted to cause a deletion of amino acids 370–403 with frameshift and premature termination of translation, whereas the other transcript is missing exons 8 and 9, which leads to deletion of amino acids 370–481.² Both abnormal proteins should be devoid of catalytic activity, because they are missing amino acid residues that are critical for forming the catalytic domain and binding of the protein to the microsomal membrane. The small amount of residual FALDH activity in our patients with SLS is probably due to the presence of another related aldehyde dehydrogenase enzyme.⁶

In addition to our Brazilian patients, a 28-year-old SLS proband from Spain has been reported with the c.1108-1G → C mutation, also associated with haplotype 1.⁷ As the Brazilian patients have the same intragenic *ALDH3A2* haplotype, it is tempting to speculate that a common ancestor carrying the c.1108-1G → C allele migrated to Brazil from the Iberian Peninsula of Europe in the 16th century and that subsequent inbreeding, as seen in each of our three families, resulted in the appearance of homozygous patients with SLS.

Clinical and histological features

The relationship between the *ALDH3A2* genotype and the clinical phenotype of SLS has been difficult to establish, because most mutations are unique for each family and typically affect only one or two siblings. Except for the Swedish patients with SLS who are homozygous for c.943C → T, no studies have examined a large group of patients with the same genotype.⁸ Our Brazilian patients therefore provide a unique opportunity to define the phenotypic variation associated with c.1108-1G → C.

The clinical and histological data on the nine patients with SLS are summarized in Table 1. In general, the patients exhibited a moderate-to-severe form of SLS with respect to mental retardation, spastic diplegia and ichthyosis. Within this phenotypic context, patients varied, even within a family, in the presence of pruritus, thickness of the granular layer, retinal glistening white dots and photophobia. All of our patients had moderate-to-severe ichthyosis with marked yellow-brown and lichenified hyperkeratosis (Fig. 1a). Pruritus was present only in six patients. Histologically, the cutaneous pathology revealed variation in the nature of the hyperkeratosis (focal, compact or loose) and the thickness of the granular layer. Macular glistening white dots, which are a pathognomonic feature of SLS,¹ were seen in only two of our patients. Four of six patients who had photophobia did not have glistening dots, suggesting that more subtle macular changes are responsible for the photophobia in SLS.

In summary, our Brazilian patients with SLS possess a common mutation that can be easily detected by *DdeI* digestion. The patients exhibit a strikingly similar moderate-to-severe neurocutaneous phenotype in which variation in cutaneous and ocular symptoms may be ascribed to unknown genetic and/or environmental factors.

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Fig. 1. Mutation detection and appearance of the ichthyosis in the Brazilian patients with Sjögren–Larsson syndrome. (a) The antecubital region of the arm (patient 5) shows thickened skin markings, and a posterior view of the neck (patient 4) shows a marked yellow-brown lichenified hyperkeratosis. (b) The c.1108-1G → C mutation abolishes a *DdeI* restriction enzyme cut site in the wild-type gene. Digestion of a 275-bp DNA fragment of intron 7/exon 8 with *DdeI* restriction enzyme demonstrates the c.1108-1G → C mutation. The control DNA was cut into three fragments (181 + 82 + 12 bp), whereas the mutation eliminates a *DdeI* cut site, resulting in formation of 263-bp and 12-bp fragments. The smaller DNA fragments have run off the gel and are not visualized. Lane M, molecular weight markers; lane PCR, undigested DNA; lanes C, 4, 5, 6 and 9, control DNAs digested with *DdeI*; lanes 1, 2, 3, 7 and 8, digested DNAs from patients 1, 2, 3, 7 and 8, respectively. Numbers to the right of the gel indicate the size of the DNA fragments in bp.

Table 1
Clinical, histological and enzymatic findings in the patients with Sjögren–Larsson syndrome

Patient	1	2	3	4	5	6	7	8	9
Sex/age (years)	F/8	F/6	M/4	F/25	F/10	F/14	F/30	M/28	M/21
Dermatological									
Ichthyosis	Severe	Severe	Moderate	Moderate	Moderate	Moderate	Severe	Moderate	Moderate
Pruritus	Severe	Severe	Moderate	–	–	Focal	Focal	–	+
Neurological									
Mental retardation	Severe	Severe	Moderate	Severe	Moderate	Severe	Moderate	Severe	Moderate
Tendon reflexes	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent
Muscular tone	Increased	Increased	Increased	Increased	Increased	Increased	Increased	Increased	Increased
Spastic palsy	Diplegia, confined to wheelchair	Diplegia, confined to wheelchair	Paraparesis, ambulatory with support	Diplegia, confined to wheelchair	Diplegia, confined to wheelchair	Diplegia, confined to wheelchair	Paraparesis, ambulatory with support	Diplegia, confined to wheelchair	Diplegia, confined to wheelchair
Ophthalmological									
Visual acuity	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Reduced	Reduced	Normal
Glistening dots	–	–	–	+	–	–	–	–	+
Photophobia	–	–	–	+	+	+	+	+	+
Histological									
Biopsy site	Abdomen	Abdomen	Abdomen	Lower back	Lower back	Lower back	Arm	Arm	Arm
Hyperkeratosis	+, loose	+	+	+, focal	+, compact	+, loose	+	+	+
Acanthosis	+	focal	+	+, marked	+	+, focal	+, focal	+	+
Papillomatosis	+	+	+, focal	+, marked	+	+	+	+	+
Cell layers in stratum granulosum	1-5	1-5	1-5	3-0	1-5	1-3	4-0	4-0	Not tested
Other clinical features	Hypersalivation	Hypersalivation	Hypersalivation				Hypothyroidism	Hypothyroidism	
FALDH activity ^a	Not tested	2%	1%	3%	8%	3%	<1%	<1%	9%

+, present; –, absent.

^aFatty acid dehydrogenase (FALDH) activity measured in cultured fibroblasts is expressed as a percentage of mean normal activity (8850 pmol min^{–1} mg^{–1} protein).