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Sjögren–Larsson syndrome: Novel mutations in the *ALDH3A2* gene in a French cohort

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ABSTRACT

Sjogren–Larsson syndrome (SLS) is a rare autosomal recessive disorder characterized by ichthyosis, spastic di- or tetraplegia and mental retardation due a defect of the fatty aldehyde dehydrogenase (FALDH), related to mutations in the *ALDH3A2* gene. In this study, we screened a French cohort of patients with Sjögren–Larsson syndrome (SLS) for mutations in the *ALDH3A2* gene. The five unrelated patients with typical SLS all present mutations in this gene. Three novel mutations were identified whereas three other ones were previously described. We also realized functional analyses at the mRNA level for two splice site mutations to study their deleterious consequences. Two of the previously described mutations had already been identified in the same region of Europe, suggesting a putative founder effect. We suggest that, (1) when clinical and MR features are present, direct sequencing of the *ALDH3A2* gene in SLS is of particular interest without necessity of a skin biopsy for enzymatic assay in order to propose genetic counsel and (2) identification of mutations already described in the same population with putative founder effects may simplify genetic analysis in this context.

1. Introduction

Sjögren–Larsson syndrome (SLS: MIM#270200), an autosomal recessive disorder characterized by the triad: congenital ichthyosis, mental retardation and spastic diplegia, is due to a deficiency of the fatty aldehyde dehydrogenase (FALDH), a microsomal enzyme that oxidizes long chain aliphatic aldehydes derived from fatty alcohols, leading to the accumulation of alcohols such as hexadecanol, octadecanol and leukotrienes B4 [1]. Despite a high prevalence in Northern Sweden, SLS is found in all ethnic groups [2]. Pruritic ichthyosis is present during the first months with scales, erythema or hyperkeratosis, sparing the face [1,3]. Cutaneous biopsy reveals non specific hyperkeratosis, papillomatosis, acanthosis and a sligh inflammatory infiltrate of the upper dermis with lack of lamellar content in lamellar granules and deficiency of specific ceramides [4]. Spasticity appears during the first years and mental retardation is variable [5]. Cerebral MRI shows non specific dysmyelination and proton spectroscopy reveals two pathognomonic lipidic peaks at 1.3 and 0.9 ppm in white matter corresponding to methyl and methylene groups [1]. Macular "glistening white dots" due to accumulation of lipids in retina are frequent after four years of age. The FALDH activity measurement in fibroblasts is considered as a reference for diagnosis [5]. However, sequence analysis of the *fatty aldehyde dehydrogenase 3A2* (*ALDH3A2*) gene has identified mutations for most patients and more than 70 mutations are known in this gene [6].

2. Patients

Five unrelated patients, all born in France with parents native to France, Italy or Northern Africa, with typical clinical and radiological SLS were referred for genetic diagnosis after written informed consent. This study was conducted according to the Declaration of Helsinki Principles. Patients' data are summarized in Table 1. All patients presented with pruritic ichthyosis at birth or in the first three months. Patient 3 had a collodion membrane aspect at birth (Fig. 1A.a). Pruritic ichthyosis was persistent in all patients (Fig. 1A.b). All developed spastic paraplegia before the age of two years and had psychomotor delay. The three older patients (1 to 3) had walking impairment and glistening white dots in retina. The youngest patients (4 and 5) did not have yet eye fundus examination. All patients had characteristic dysmyelination on MRI and pathognomonic peaks on

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Table 1
Clinical and MR features, FALDH activity and molecular results of five patients with SLS.

Pt	Age[a]	Sex	Origin	Cons	Ichthyosis	Mental delay	SPG	Glistening white dots	WM change	Lipid peaks[b]	FALDH activity[c]	Intron / Exon	Nucleotide change[d]	Transcript change	Predicted protein	Hp[e]
1	7	F	France	No	+	+	+	+	+	+	<5%	Int 3	c.471+1delG homozygous	r.154-471del r.386-471del r.471delG	p.Ser52-Gln157del p.Asn130SerfsX5 p.Asn158IlefsX16	2/2
2	14	F	France	No	+	+	+	+	+	+	<10%	Int 3	**c.472-1G>T**	r.472-504del	p.Asp158-Glu168del	3
												Ex 5	c.733G>A		p.Asp245Asn	1
3	18	F	France Italy	No	+	+	+	+	+	+	<5%	Int 3	c.471+1delG		p.Ser52-Gln157del p.Asn130SerfsX5 p.Asn158IlefsX16	2
												Ex 4	c.619-620insG[f]		p.Glu207GlyfsX10	3
4	2	F	Algeria	Yes	+	+	+	NA	+	+	NA	Ex 2	**c.370G>A** homozygous		p.Gly124Arg	1/1
5	1	M	Tunisia	Yes	+	+	+	NA	+	+	NA	Ex 4	**c.648_649insAT** homozygous		p.Asp217LeufsX13	2/2

MR: magnetic resonance, Pt: patient, Cons: consanguinity, SPG: spastic paraplegia, WM: white matter, Int: intron, Ex: exon, Hp: haplotype, Ref: references, NA: not available.
[a] Age in years.
[b] In ppm.
[c] Fibroblast enzyme activities were provided by Dr RJA Wanders, University of Amsterdam and are expressed in% of the normal value.
[d] Mutation numbering is based on the cDNA NCBI RefSeq sequence isoform 2 NM_000382.1 with nucleotide +1 corresponding to the A of the ATG translation initiation codon. Novel mutations are shown in bold.
[e] Haplotypes in *ALDH3A2* were constructed with 4 intragenic SNPs as described by Rizzo et al. [7]: haplotype 1: [c.153+39T;c.471+31C;c.940+53C;c.1446T]; haplotype 2: [c.153+39C; c.471+31T;c.940+53G;c.1446T]; haplotype 3: [c.153+39C;c.471+31T;c.940+53C;c.1446A]; haplotype 4: [c.153+39C;c.471+31T;c.940+53C;c.1446T].
[f] This mutation has already been described but misnamed c.620-621insG by Rizzo et al. [7].

proton spectroscopy independently of their age at MRI achievement (Fig. 1A.c and d). Three patients had been previously confirmed with low FALDH activity but patients 4 and 5 were directly tested for genetic analysis.

3. Genetic analysis

gDNA was purified from blood samples using the Nucleon BACC kit (Amersham). *ALDH3A2* exons were amplified by PCR using primers derived from flanking intronic sequences of each exon and previously described by Rizzo et al. [7]. PCR was performed in 25 µl using 100 ng of gDNA (PCR conditions available on request). PCR products were purified using Exonuclease I and Calf Intestinale Alkaline Phosphatase (Fermentas) and directly sequenced with PCR primers using the BigDye Terminator V1.1 sequencing kit (Applied Biosystems) and analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). PCR results were controlled twice for validation. Total RNAs were isolated from cultured fibroblasts using Trizol reagent (Invitrogen). Reverse transcription reactions were performed using conditions previously described by Kraus et al. [8]. *ALDH3A2* cDNAs were amplified from exons 1 to 10 and 3 to 6 by PCR using the following primers: Ex1F 5′ GTGGGTTGACGGTGGAGAC3′, Ex10R 5′GGTTCATTAACCATTTAAAAGAGGA3′, Ex3F 5′GGCAAAGCTTCTCCCTCAG3′ and Ex6R 5′TCAGGAGACTCTTTTATATTTTCTCCA3′. PCR was performed in 25 µl using 2 µl of cDNA (PCR conditions available on request). PCR products were purified from agarose gel using the DNA gel extraction kit (Millipore) and sequenced with PCR primers as described above.

4. Results and discussion

Sequence analysis of *ALDH3A2* revealed six mutations, three novel and three previously described ones, described in Table 1. The c.471+1delG splice site mutation was found at the homozygous state in patient 1 and at the heterozygous state in patient 3, native to France. This mutation, associated with haplotype 2 (with regard to haplotype constructed with four intragenic SNPs as described by Rizzo et al. [7]) causes the loss of exons 2 and 3, predicting a protein with a loss of 106 amino acids (AA) [7,8]. Surprisingly, the analysis of patient 1's *ALDH3A2* mRNA revealed three different transcripts: the shorter one (1.4 kb) shows exon 2 and 3 skipping, the intermediate one (1.5 kb) only exon 3 skipping and the longest one (1.6 kb) corresponds to a deletion of the last 3′ guanidine of exon 3 due to its use to replace the deleted guanidine of the splice donor site (Fig. 1B). All these transcripts are expected to lead to truncated proteins but this analyze was not performed because of lack of known monospecific antibodies against the ALDH3A2 protein. Patient 1 and the previously described patients carrying this mutation [6] had a FALDH activity<5% that strongly suggests that none of the transcripts lead to functional proteins. Interestingly, despite the absence of consanguinity, patient 1's parents and patient 3's mother, who carried this mutation, come from the same valley in the French Alps. This mutation, already described associated with the same haplotype in European patients from countries that border the Alps (Germany, Italy and Switzerland) by Rizzo et al. [7] and Kraus et al. [8] seems to be caused by a founder effect in this region.

Patient 3 and his father (native from Italy), carried the c.619_620insG mutation, associated with haplotype 3. A founder effect could also be evoked for this mutation described associated with the same haplotype in subjects native from northern Italy in a previous study [7]. Founder effects have already been reported in SLS by Rizzo et al. [9] and De Laurenzi et al. [10] for two mutations identified in northern Europe (c.1297_1298delGA, c943C>T) but most of the mutations in SLS are private [6].

The c.733G>A missense mutation associated with haplotype 1, found in patient 2 native to France, had been described associated with different haplotypes in European and Turkish families [5,7,11]. It has been reported to occur at CpG dinucleotides and has been supposed to originate independently at a mutational hotspot rather than being a consequence of a recombination in an ancestral SLS allele [7]. This patient also carried a novel c.472-1G>T splice site mutation associated with haplotype 3. At the mRNA level, this latest mutation alters the splice acceptor site of exon 4 and leads to the use of the next available downstream AG as a cryptic acceptor site in exon 4 generating a 33 bp loss and predicting a protein with a loss of 11 AA (Fig. 1B). The same mRNA and protein consequences have been reported for the c.472-2A>G mutation affecting the same splice