Novel ALDH3A2 Heterozygous Mutations in a Japanese Family with Sjögren–Larsson Syndrome

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TO THE EDITOR

Sjögren–Larsson syndrome (SLS: OMIM No. 270200) is an autosomal-recessive hereditary disorder characterized by congenital ichthyosis, mental retardation, and spastic diplegia or tetraplegia (Rizzo, 1993). Rizzo *et al.* (1988) revealed that long-chain fatty alcohol was abnormally accumulated in cultured fibroblasts, white blood cells and serum in SLS patients. In 1996, De Laurenzi *et al.* (1996) reported that mutations in the fatty aldehyde dehydrogenase (FALDH) gene (*ALDH3A2*) underlie SLS. In the present study, we report novel compound heterozygous mutations in *ALDH3A2* in a Japanese family with SLS.

A 2-year-old Japanese girl was suffering from congenital ichthyosis, mental retardation, spastic tetraplegia, and recurrent epileptic seizures. The patient was the first child of non-consanguineous, healthy Japanese parents. Ichthyosis was not seen in any other family members. She was born as a pre-term baby at 34 weeks 3 days of gestation (body weight, 2392 g), but not a collodion baby. Physical examination revealed fine scales and slight erythema over her whole body (Figure 1). Hyperkeratosis was also seen on the palms and soles. Her hair, nails, and teeth were normal. The skin manifestations were consistent with non-bullous congenital ichthyosiform erythroderma. Her extremities were hypertonic. T2-weighted magnetic resonance imaging demonstrated a high-intensity area in her brain periventricular white matter. Ophthalmologic examination revealed retinal crystals, generally referred to as ''glistening white dots''.

To elucidate the precise genetic abnormality in the family, mutational analysis was performed in the affected girl and her parents. Briefly, genomic DNA isolated from peripheral blood cells was subjected to PCR amplification, followed by direct automated sequencing using an ABI PRISM 3100 genetic analyzer (ABI Advanced Biotechnologies, Columbia, MD). Oligonucleotide primers and PCR conditions used for amplification of all exons and exon–intron borders of *ALDH3A2* were originally derived from the report by Rizzo *et al.* (1999) and were partially modified for our study (Shibaki *et al.*, 2004). The entire coding region including the intron/exon boundaries for both forward and reverse DNA strands from the patient, her parents and 100 healthy Japanese controls were sequenced. In the patient, a combination of two novel heterozygous mutations, 332G>A in exon 2 and 636T>G in exon 4, were identified (Figure 2a). The mutation 332G>A was present in her father, and the mutation 636T>G was demonstrated in her mother. The presence of both mutations was excluded in 200 alleles of 100 normal unrelated Japanese individuals. The medical ethical committee of the Hokkaido University approved all described studies. These studies were conducted according to the Declaration of Helsinki Principles. The patient's parents gave their written informed consent.

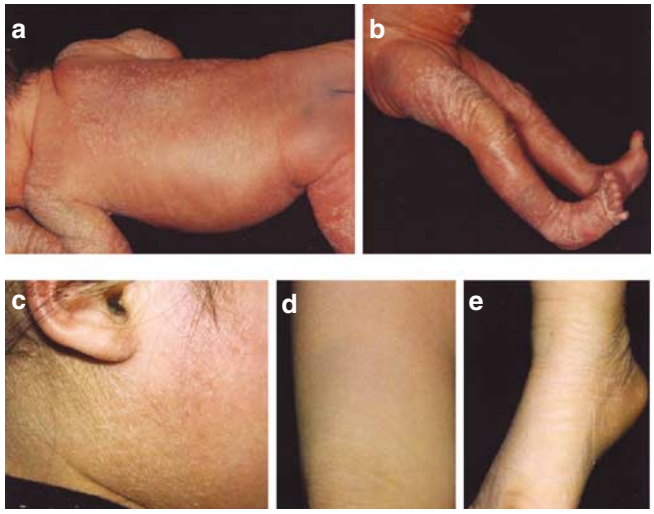

Figure 1. Clinical features of the SLS patient. (**a, b**) In the perinatal period, xerotic skin, and fine scales were apparent over the (**a**) proband's trunk and (**b**) hyperkeratosis was severe on her legs. (**c, d, e**) At 2 years of age, fine, whitish scales were seen on her (**c**) cheek, (**d**) thigh, and the (**e**) dorsal foot.

The paternal mutation 332G>A in exon 2 leads to an alteration of the tryptophan residue at codon 111 into a stop codon (nonsense mutation W111X) and this premature translation termination eliminates approximately 80% of the length of FALDH polypeptide (loss of 77.3% length of the FALDH major splice variant). Thus, this nonsense mutation is expected to seriously abolish FALDH function.

The maternal mutation 636T>G in exon 4 resulted in an alteration of a serine at codon 212 to arginine (S212R). FALDH amino-acid sequence alignment shows that this serine residue at codon 212 is conserved among several diverse species (reference sequences: CR457422, XP 511337, NP 113919, CAI25890, NP 001016537, AAK49120) (Figure 2b).

In addition, according to a comparison of 145 full-length aldehyde dehydrogenase-related sequences by Perozich *et al.* (1999), this serine is

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

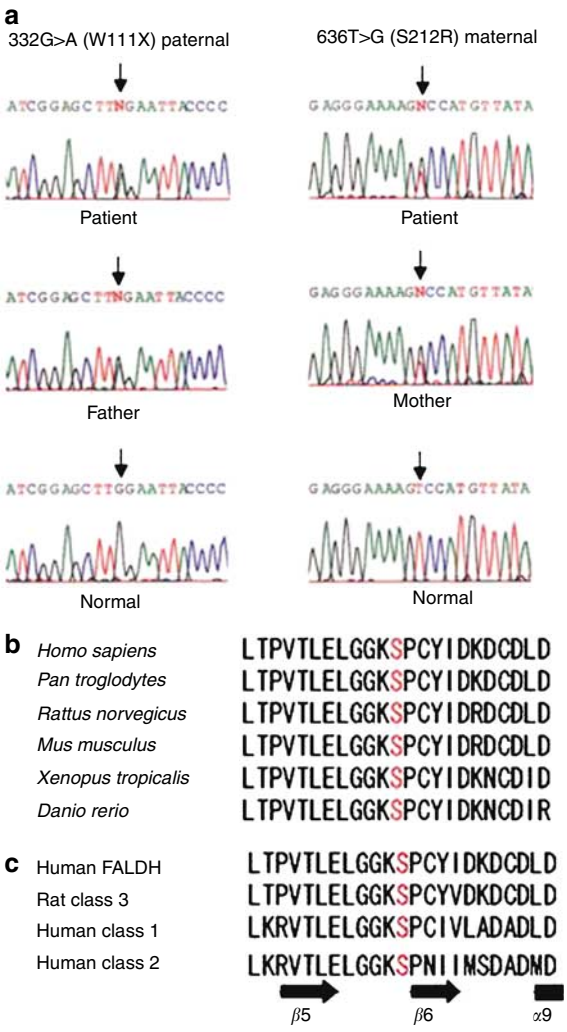

Figure 2. *ALDH3A2* mutations in the present SLS patient and sequence alignments around the missense mutation. (**a**) Sequence analysis of the *ALDH3A2*. A combination of heterozygous mutations derived from their mother (332G>A (W111X) in exon 2) and father (636T>G (S212R) were detected. (**b**) FALDH amino-acid sequence alignment shows the level of conservation in diverse species of the amino-acid S212 (red characters), which was altered by the missense mutation in the present family. (**c**) A sequence alignment between the FALDH, rat class 3 and human class 1 and class 2 ALDHs showing the relative locations of key residues in these enzymes. Serine residue at codon 212 of FALDH is strictly conserved. Secondary structure components found in the class 3 rat ALDH structure are presented with a bar and arrows. The bar represents an α-helix and arrows represent β-strands. (Modified from the paper by Liu *et al.*, (1997).)

highly conserved among many of the ALDH family members, and participates in one of the 10 most conserved sequence motifs in ALDHs (Figure 2c). In addition, analysis of the crystallized three-dimensional structure of the related class 3 rat cytosolic ALDH revealed that this serine is located adjacent to the first β-strand, β6, of the six parallels of β-strands, comprising a significant portion of the catalytic domain of the molecule (Liu *et al.*, 1997) (Figure 2c). These findings strongly suggest that the serine at codon 212 is important for connecting the α/β structure of a dinucleotide-binding Rossmann fold (Freshney *et al.*, 1994) to the catalytic domain and/or for structural folding of the catalytic domain, and is therefore essential for the normal function of the FALDH protein. The fact that the present patient harboring the missense mutation S212R showed typical SLS phenotypic features strongly suggests that this serine residue is essential for FALDH enzymatic function.

FALDH is a microsomal NAD-dependent enzyme, which is necessary for the oxidation of long-chain aliphatic aldehydes into fatty acids (Kelson *et al.*, 1997). The FALDH gene (*ALDH3A2*) located on chromosome 17p11.2 (De Laurenzi *et al.*, 1996) comprises 11 exons, and is widely expressed in a variety of tissues (Chang and Yoshida, 1997; Rogers *et al.*, 1997). Until now, a number of mutations in *ALDH3A2* have been shown to be responsible for SLS over the world (Rizzo *et al.*, 1999). Founder effects were observed in certain areas and races (Rizzo *et al.*, 1999; Kraus *et al.*, 2000; Rizzo and Carney, 2005). In Japanese patients with SLS, one homozygous mutation 1157A>G (N386S) in *ALDH3A2* was reported in a patient of one family (Aoki *et al.*, 2000) and two other mutations, 481delA and 1087_1089delGTA, were reported in another family (Shibaki *et al.*, 2004). We have detected two additional mutations, 332G>A (W111X) and 636T>G (S212R), in the present family. All the mutations detected in Japanese families were distinct one another and we therefore speculate there is no founder effect in Japanese cases with *ALDH3A2* mutations causing SLS.

The pathogenesis of the ichthyosis in SLS includes abnormal lamellar or membranous inclusions in cornified cells, which were reported in a patient with SLS, although causative genetic abnormalities were not known in that particular case (Ito *et al.*, 1991). The inclusions were speculated to be lamellar granule-in-origin. Later, a deficiency in acyl-ceramides in the lipid layer in the stratum corneum was also reported in SLS patients (Paige *et al.*, 1994). In a previous report (Shibaki *et al.*, 2004), we observed ultrastructurally abnormal lamellar granules lacking normal lamellar contents in the upper spinous and granular layers. In addition, we showed some malformed lamellar granule components were secreted into the intercellular space in the stratum corneum (Shibaki *et al.*, 2004). These observations suggest defective lamellar granule formation in SLS. SLS is thought to be a form of ichthyoses caused by defective

lipid barrier in the stratum corneum (Akiyama, 2006).

As illustrated here, mutation analysis of the *ALDH3A2* gene is a highly sensitive method to confirm a diagnosis of SLS, which does not require a skin biopsy and can complement or replace FALDH enzymatic assays or analysis in SLS.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Kaori Sakai[1], Masashi Akiyama[1], Tomoyuki Watanabe[2], Kazunori Sanayama[2], Katsuo Sugita[3], Mari Takahashi[4], Keisuke Suehiro[4], Kazuhiko Yorifuji[4], Akihiko Shibaki[1] and Hiroshi Shimizu[1]

[1]Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [2]Department of Pediatrics, Japanese Red Cross Narita Hospital, Narita, Japan; [3]Division of Child Health, Faculty of Education, Chiba University, Chiba, Japan and [4]Department of Dermatology, Japanese Red Cross Narita Hospital, Narita, Japan.
E-mail: akiyama@med.hokudai.ac.jp

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Novel Hairless *RET*-Transgenic Mouse Line with Melanocytic Nevi and Anagen Hair Follicles

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TO THE EDITOR

The c-*RET* proto-oncogene encodes a receptor-tyrosine kinase and glial cell line-derived neurotrophic factor ligands, including glial cell line-derived neurotrophic factor, neurturin, artemin, and persephin, have been reported to be ligands of RET (Takahashi, 2001). *RFP/RET* is a hybrid oncogene between c-*RET* and *RFP* that was isolated by NIH3T3 transfection assays (Takahashi *et al.*, 1985). Previously, we established a metallothionein-I/*RFP-RET* (*RET*)-transgenic mouse line (242) that spontaneously develops systemic skin melanosis without macroscopic tumors (Iwamoto *et al.*, 1991; Kato *et al.*, 1999). Generally, most hair follicles in adult wild-type mice are in telogen (Kato *et al.*, 2001). It is basically impossible to induce continuous anagen hair follicles in adult wild-type mice, although temporal anagen hair follicles are inducible by shaving hairs (Kato *et al.*, 2001). Interestingly, adult *RET*-transgenic mice have continuous anagen hair follicles with hyper melanin production (Kato *et al.*, 2001, 2004). Moreover, hair growth of adult transgenic mice was promoted compared with that of control C57BL/6 mice (Kato *et al.*, 2001). These results suggest that a continuous anagen phase of hair follicles plays an important role in hair growth.

We also established another *RET*-transgenic mouse line (304/B6) (Kato