Novel ALDH3A2 Heterozygous Mutations Are Associated with Defective Lamellar Granule Formation in a Japanese Family of Sjögren–Larsson Syndrome

To the Editor:

Sjögren–Larsson syndrome (SLS: MIM# 270200) is an autosomal recessive disorder characterized by congenital ichthyosis, mental retardation, and spastic paresis (Rizzo, 1993). Rizzo *et al* (1988) demonstrated that long-chain fatty alcohol was deposited in cultured fibroblasts, white blood cells, and serum in SLS patients. Later, De Laurenzi *et al* (1996) reported that mutations in the fatty aldehyde dehydrogenase (FALDH) gene (ALDH3A2) were responsible for the development of SLS. However, the exact pathomechanisms of this ichthyosis in SLS is not fully understood. In this study, we report novel heterozygous mutations in ALDH3A2 in a Japanese family with SLS. In this family, a combination of heterozygous mutations is associated with defective lamellar granules and abnormal intercellular lipid in the stratum corneum.

Case 1: A 6-year-old girl with congenital ichthyosis, mental retardation, and spastic paresis in both her lower extremities, visited our clinic (Fig 1*A*). Physical examination revealed xerosis and fine scales over her whole body, and lamellar-shaped scales on her dorsal hands and feet. Her lower extremities were hypertonic. Brain magnetic resonance imaging (MRI) demonstrated a high-intensity area in the postangular area of the left parietal lobe. Ophthalmologic examination was unremarkable.

Case 2: A 1-year-old boy, the younger brother of case 1, was brought to our clinic suffering from congenital ichthyosis, mental retardation, and spastic paresis on both his lower extremities. Physical examination revealed brown-colored pigmentation with mild to moderate hyperkeratosis on his neck, with fine, dark scales on the dorsal feet (Fig 1*B*). No other abnormalities, including ophthalmologic problems, were observed.

To elucidate the genetic abnormality of the patients, blood samples were collected from both patients and their parents. All the experiments, skin biopsies and blood sampling were performed with the parents' written informed consent and with the institutional approval of Hokkaido University Graduate School of Medicine for experiments handling human matter in accordance with Helsinki Principles. The ALDH3A2 gene was amplified by the methods previously reported by Rizzo *et al* (1999). DNA sequencing of all the PCR products was carried out using a Genetic Analyzer 310A automatic sequencer (Perkin-Elmer Life Sciences-ABI, Foster City, California). In both children, we detected a combination of heterozygous mutations in exon 4 and 7 (Fig 1*C*). The mutation in exon 4 (481delA) was only present in their mother, and the mutation in exon 7

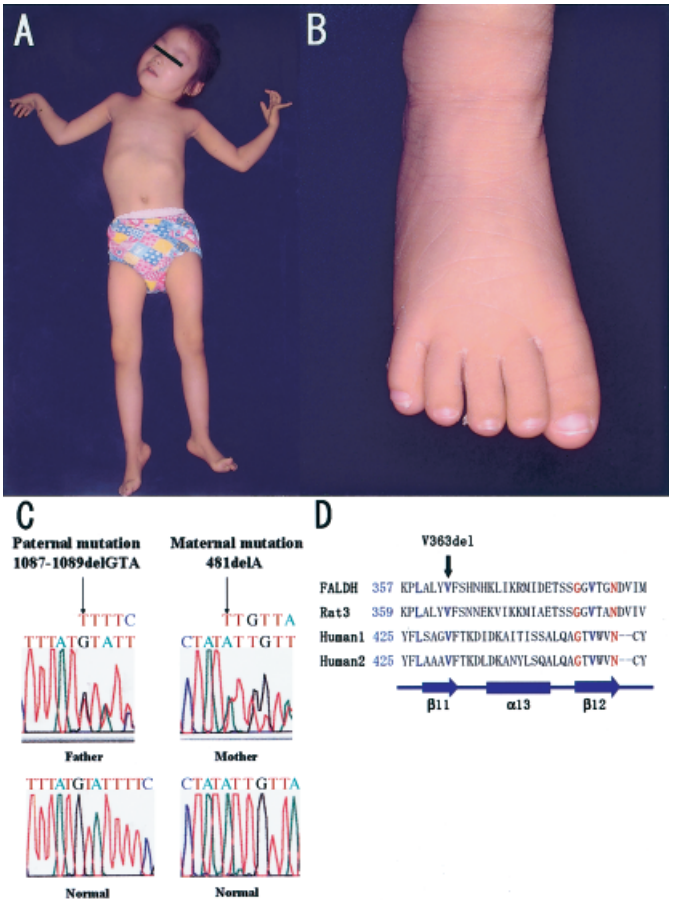

Figure 1
Clinical features and fatty aldehyde dehydrogenase (FALDH) gene (ALDH3A2) mutations. (*A*) Low magnification clinical view of case 1. Xerotic skin and fine scales over the entire body and spastic paresis on both lower extremities. (*B*) High magnification clinical view of case 2. Fine gray scales present on the dorsal feet. (*C*) Sequence analysis of the ALDH3A2. A combination of heterozygous mutations derived from their mother (481delA in exon 4) and father (1087–1089delGTA in exon 7) were detected. (*D*) A sequence alignment between the FALDH, rat class 3 and human class 1 and class 2 ALDH showing the relative locations of key residues in these enzymes. Strictly conserved residues are highlighted in red, while highly conserved residues are shown in blue. Secondary structure components found in the class 3 rat ALDH structure are presented in blue, bars represent α-helices and arrows represent β-strands. (Modified from the paper by Liu *et al*, 1997.)

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

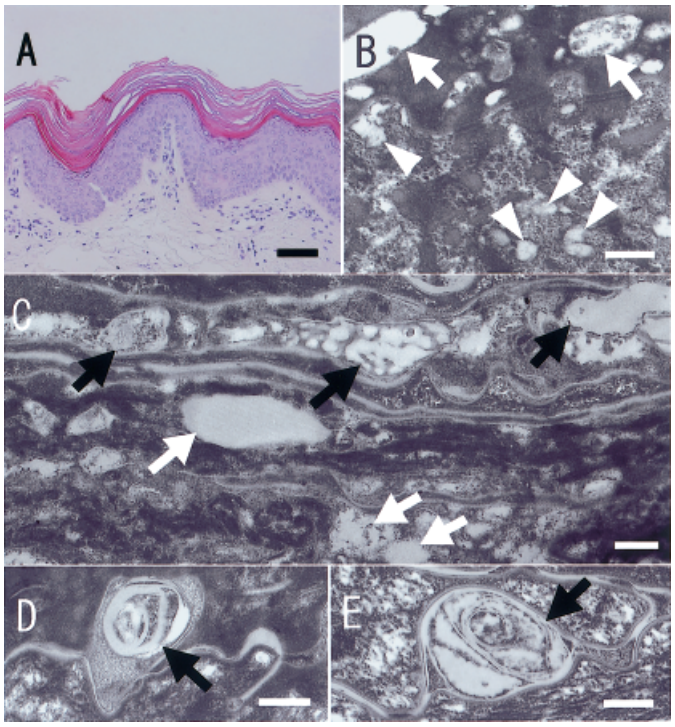

Figure 2
Morphological features of the patient's epidermis. (*A*) Histopathology of ichthyotic skin lesion of case 1. Orthohyperkeratosis with mild hypergranulosis was observed. (*B*) Ultrastructurally, at the stratum granulosum/stratum corneum interface, abnormal apparently empty lamellar granules (*white arrowheads*) were seen in the granular cells and lipid vacuoles (*white arrows*) were observed in the cornified cells. (*C–E*) Vacuoles, presumably lipid droplets (*white arrows*) and irregularly shaped abnormal intercellular materials (*black arrows*) were apparent in the stratum corneum layers. *Scale bars* = 50 (*A*) and 0.3 μm (*B–E*).

(1087–1089delGTA) was only demonstrated in their father. The presence of both these mutations was excluded in 100 alleles of 50 normal unrelated Japanese individuals.

Histopathological examination of the skin biopsy specimens obtained from both cases revealed orthohyperkeratosis with mild hypergranulosis (Fig 2*A*). Electron microscopic examination of osmium tetroxide-fixed samples from the lesional skin of both patients, demonstrated the abnormal lamellar granules lacking the normal lamellar contents (Fig 2*B*). Some of the defective lamellar granules secreted their components into the intercellular space in the stratum corneum. Irregularly shaped granular and electron-lucent materials were also deposited in the dilated intercellular space between corneocytes (Fig 2*C–E*). Variously sized empty vacuoles, presumably containing electron lucent lipid, were also seen in the stratum corneum (Fig 2*B*, *C*).

Immunofluorescent staining for keratin 1, 10, loricrin, and involucrin, performed as described previously (Akiyama *et al*, 1998), revealed that all of these molecules were normally distributed in the epidermis (data not shown). The distribution pattern of a *trans*-Golgi network marker, TGN-46 and a lamellar granule component, cathepsin D (Ishida-Yamamoto *et al*, 2004) were normal, although immunofluorescent staining for glucosylceramides revealed an abnormal, irregular or granular distribution of glucosylceramides in the patients' stratum corneum (data not shown).

Normal epidermal transglutaminase activity was confirmed in both patients by the *in situ* transglutaminase activity assays previously described elsewhere (Hohl *et al*, 1998; Raghunath *et al*, 1998; Akiyama *et al*, 2001) (data not shown).

FALDH is a microsomal NAD-dependent enzyme, which is necessary for the oxidation of long-chain aliphatic aldehydes to fatty acids (Kelson *et al*, 1997). Until now, several mutations in ALDH3A2 have been shown to be responsible for SLS around the world (Rizzo *et al*, 1999).

In our cases, a heterozygous combination of two novel mutations has been identified. The maternal mutation 481-delA in exon 4 resulted in a frame-shift leading to a stop codon at codon 522. This premature translation termination eliminates the downstream 64% of ALDH3A2 coding sequences. The paternal mutation 1087–1089delGTA in exon 7 resulted in a deletion of valine at position 363 of FALDH protein. According to a comparison of 145 full-length ALDH-related sequences by Perozich *et al* (1999), this valine is highly conserved among many of the ALDH family members, and participates in one of the ten most conserved sequence motifs in ALDH. In addition, analysis of the crystallized 3-D structure of the related class 3 rat cytosolic ALDH revealed that this valine is located at one of the six parallels of β-strands, β11, comprising the catalytic domain of the molecule (Fig 1*D*) (Liu *et al*, 1997). These findings strongly suggest that valine at position 363 is important for structural folding of the catalytic domain and are therefore essential for the normal function of the FALDH protein.

Previously, abnormal lamellar or membranous inclusions in the cornified cells were observed in the lesional skin of a SLS patient, 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 addition to these previous observations, we revealed that the malformed lamellar granule components were secreted into the intercellular space, and irregular-shaped granular and electron-lucent materials were deposited in the irregularly dilated intercellular space in stratum corneum. Immunofluorescence studies revealed an abnormal distribution of glycosylceramide, a lamellar granule component, in the patients' stratum corneum. These observations together suggest defective lamellar granule formation in these patients.

Similarly, a large number of abnormal lamellar granules associated with disturbed intercellular lamellar structures were observed in ichthyotic skin in a patient with Dorfman–Chanarin syndrome (Akiyama *et al*, 2003). Furthermore, in harlequin ichthyosis, lamellar granules are completely absent or, if present, are not correctly secreted into the intercellular space (Milner *et al*, 1992). Thus, the present ultrastructural and immunofluorescence findings suggest that formation of defective lamellar granule contents and defective intercellular lipids, presumably related to the mutations in ALDH3A2, may lead to the ichthyotic skin developed in SLS patients.

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