

After the first passage, keratinocytes started to proliferate with a constant doubling time of 2 d. The same procedure was used to establish long-term keratinocyte cultures from mouse embryos with a different genetic background (Caldelari *et al*, unpublished data). It should be mentioned in addition that no special culture and handling conditions were required, contrary to the findings of Hager *et al* (1999). Namely, glass pipettes were routinely used, no differences were observed when stopping trypsinization with medium containing a low or a high calcium concentration, and cells were cultured under normal CO₂ content (5%). We observed, however, that keratinocyte proliferation was improved when cultured at 34°C instead of 37°C.

To assess the differentiation potential of our mouse keratinocytes, we introduced a change in the calcium concentration in the medium from 0.07 mM to 1.2 mM. Elevation of the calcium level in culture has been shown to induce differentiation of mouse and human keratinocytes (Hennings *et al*, 1980; Jensen *et al*, 1990). Onset of differentiation is characterized by the establishment of cell-cell contacts, due to assembly of adherens junctions followed by formation of desmosomes (for review see Garrod *et al*, 1996; Kowalczyk *et al*, 1999). Terminally differentiated cells finally express involucrin, a major protein of the cornified envelope, which was defined as marker for keratinocyte terminal differentiation (Gandarillas and Watt, 1997; Zhu and Watt, 1999). In our mouse keratinocytes, calcium elevation in the medium induced similar differentiations changes in passage 22 up to passage 61, as seen by the formation of a highly ordered keratin network, the stabilization of adhesion components at the plasma membrane within 6 h (Fig 1A), and the expression of the terminal differentiation marker involucrin after 3 d (Fig 1B). The amount of involucrin expressed was independent of the presence or absence of growth factors in the culture medium prior to elevation of the calcium concentration. Involucrin expression was also similar when supplemented Williams' Medium E containing 1.8 mM calcium (Wilkinson *et al*, 1987) was used during differentiation. Conversely, cells cultivated in low calcium medium showed prominent intercellular spaces as seen in phase contrast microscopy (data not shown) and exhibited low expression of keratin and adhesion molecules (Fig 1A). Involucrin was not

expressed at detectable levels at this stage. (Fig 1B).

In summary, we report the establishment of long-term mouse keratinocyte cultures which were grown up to passage 61 without losing their capacity to respond to the elevation of the calcium concentration in the medium by development of a well-organized keratin network, establishment of intercellular adhesion, and finally expression of the terminal differentiation marker involucrin.

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A Novel Point Mutation of the *FALDH* Gene in a Japanese Family with Sjögren–Larsson Syndrome

To the Editor:

Sjögren–Larsson syndrome (SLS; MIM# 270200) is an autosomal recessive disorder characterized by mental retardation, di- or tetraplegia and congenital ichthyosis (Rizzo, 1993). The ichthyosis in SLS is usually present at birth, but there have been reports of a much later onset of ichthyosis, at an age of < 1 y of age. Mental retardation is variable, but is severe (IQ < 50) in about 70% of cases. Additional clinical features include glistening white dots on the retina, seizures, short stature, and speech defects. Patients with SLS have deficient activity of fatty aldehyde dehydrogenase (*FALDH*), an enzyme that is necessary for the oxidation of fatty alcohol to fatty acid through its involvements as a component of the fatty alcohol:NAD⁺ oxidoreductase enzyme complex (Rizzo and Craft, 1991).

The *FALDH* gene has been mapped to the SLS locus on 17p11.2 (Rogers *et al*, 1995). It is approximately 31 kb and consists of 10 exons, resulting in a cDNA of 1455 bp, which is translated into a protein with 485 amino acids (Rogers *et al*, 1997). Here we report a novel point mutation in the *FALDH* gene (A→G transversion at nucleotide 1157) resulting in the change of asparagine to serine at amino acid 386, i.e., N386S, and show the successful DNA diagnosis in a family.

A 1 y old girl visited our university clinic on July 17 1999. She presented with congenital ichthyosis, and showed spastic tetraplegia and mental retardation. Histologic examination revealed orthohyperkeratosis, acanthosis, and papillomatosis, and the granular cell layer of the epidermis was slightly thickened. Alcohol dehydrogenase staining was performed on the skin of this patient and a normal control after freezing the samples in an OCT compound according to the method of Judge *et al* (1990), and revealed a reduction in alcohol dehydrogenase activity only in the patient's skin. Her parents were not relatives and both of them were healthy.

After informed consent, blood samples were collected and genomic DNA were extracted from the buffy coats (QIAGEN, Hilden, Germany). Genomic DNA from normal healthy Japanese

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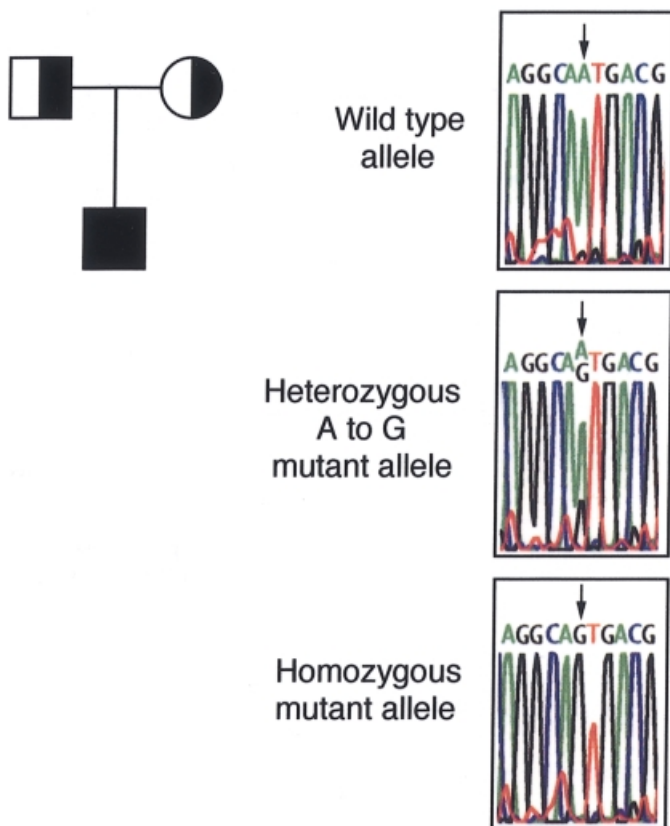


Figure 1. Sequence analysis of the *FALDH* gene. DNA sequence of exon 8 of the *FALDH* gene is shown from a healthy individual, a heterozygous parent, and an affected child. The arrow denotes nucleotide position 1157 in the *FALDH* cDNA.

people were used as controls. A 213-bp polymerase chain reaction fragment containing exon 8 of *FALDH* gene was amplified using specific primers (SLS-E8F, 5'-ACTTCACTGACCTGGACACC-T-3' and SLS-E8R, 5'-GCAGCCCATAACAATCCACTCA-3'). Amplification conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 45 s, 65°C for 30 s, and 72°C for 1 min, and then final extension at 72°C for 7 min. The patient's amplified fragment as well as that from the father and the mother were directly sequenced by using the ABI-PRISM dye terminator and the 373 sequencer (Applied Biosystems, Foster City, CA). The sequence from the patient revealed a homozygous point mutation (1157A→G) in exon 8 of the *FALDH* gene, which changes codon 386 from asparagine to serine. The heterozygosity was demonstrated in both of her parents (Fig 1). This mutation was excluded to be present in 50 normal unrelated individuals.

Since the first report about the mutations in *FALDH* gene of the SLS patients, 20 different pathogenic mutations to date have been identified, seven with small deletions, one with gross deletion, three with insertions, one with deletion/insertion, and eight with missense mutations (De Laurenzi *et al*, 1996; Sillen *et al*, 1997). Interestingly, nonsense mutation was not detected in this gene. Missense mutations have been reported in 38 patients, of which 30 have C→T transversion at nucleotide 943 resulting in the change of proline to serine at amino acid 315, and it is indicated that this mutation is the major cause of SLS in the inbred Swedish families (De Laurenzi *et al*, 1997). So far, nothing is described about the mutation (N386S) found in this case. A BLAST database search shows that the asparagine residue at position 386 is highly conserved in a variety of species and different types of aldehyde dehydrogenases, e.g., succinate semialdehyde dehydrogenase in man, the rat, and *Bacillus subtilis*, and aldehyde dehydrogenase in *Cladosporium herbarum*. In addition, the analysis of the secondary structure of the rat class 3 aldehyde dehydrogenase revealed that Asn 388, which corresponds to Asn 386 in human *FALDH*, appears to stabilize adjacent elements of secondary structure (Liu *et al*, 1997). These findings therefore suggest that the substitution of this strictly conserved amino acid residue could account for the enzyme deficiency of *FALDH* in SLS patients.

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