Research Letter

Deficient stratum corneum intercellular lipid in a Japanese patient with lamellar ichthyosis by a homozygous deletion mutation in *SDR9C7*

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Supplementary file

Autosomal recessive congenital ichthyosis (ARCI) is an umbrella term for inherited non-syndromic ichthyosis, which includes harlequin ichthyosis, lamellar ichthyosis (LI), congenital ichthyosiform erythroderma and pleomorphic ichthyosis (also called self-healing/self-improving collodion baby).[1] The clinical diversity is matched by genetic heterogeneity, with 11 genes currently implicated in the pathobiology of ARCI,[2,3] including the most recent discovery of two missense mutations in *SDR9C7* in three consanguineous Lebanese families.[4] Here, we describe a case of ARCI (LI phenotype) that has a previously unreported homozygous deletion mutation in *SDR9C7*. We extend the spectrum of clinical features associated with *SDR9C7* mutations, and identify deficient intercellular lipid and malformation of intercellular lipid layers in the stratum corneum.

The patient is a 72-year-old Japanese woman, the youngest of 11 siblings born to non-related parents. At birth, she was noticed to have collodion membrane and presented with symptoms of ichthyosis since after birth. She was treated with systemic retinoid. However, it was ineffective and her scales got worse. Her other medical history included diabetes mellitus and hypertension. On examination, she had large, whitish to light brown scales without erythroderma on her trunk and extremities (Fig. 1a and b). There was palmoplantar keratoderma. She had no hair, nail, dental or mucosal abnormalities, and vision and hearing were normal. There was no family history of any

skin disorder.

Following ethical approval, informed written consent was obtained in compliance with the Declaration of Helsinki guidelines. A skin biopsy specimen obtained from the left lower leg showed compact hyperkeratosis with a normal-appearing granular layer (Fig. 1c). Neither parakeratosis nor dyskeratosis was observed and no inflammatory cell infiltration was present; these features are compatible with LI. Electron microscopy revealed markedly compact hyperkeratosis (Fig. 1d and e), and a minimal amount of lipid was observed between the cornified cells and only very thin or absent intercellular lipid layers were seen within the cornified cell layers. Of note, almost no lipid droplets were found in the cornified cells. Lamellar materials (lipid contents) were markedly reduced in the lamellar granules, although lamellar granules seemed to fuse normally with the cell membrane.

Genomic DNA from the patient was used for whole-exome sequencing (WES) analysis, using methodology described elsewhere.[5] No potentially pathogenic mutations were noted in known ARCI genes. In total, 472 previously unreported mutations were identified by WES, 44 homozygous and 428 heterozygous. Within these variants, there was a previously unreported homozygous deletion mutation in *SDR9C7*, c.897delT, which was then confirmed by Sanger sequencing (Fig. S1a). Further details of the mutation

detection and immunohistochemical analyses can be found in the Supplementary files.

SDR9C7 encodes short chain dehydrogenase/reductase family 9C, member 7. SDR9C7 is known to display weak conversion of all-trans-retinal to all-trans-retinol in the presence of NADH, but has not been shown to have retinoid or dehydrogenase activities.[6] How mutations in *SDR9C7* lead to the clinical phenotypes of ARCI, however, is uncertain. In cultured cells, expression of both the mutant SDR9C7 proteins (missense mutations) was markedly reduced compared with that of the wild-type protein, suggesting possibly instability of the protein.[4] The present mutation, c.897delT (p.Phe300Serfs*3), is different and causes a slightly truncated SDR9C7 protein that lacks 12 residues at the C-terminus (Fig. S1b), potentially underscoring the functional importance of the highly conserved C-terminus.

Our ultrastuctural findings also provide some insight into disease pathogenesis. Of note, compact hyperkeratosis with a normal granular layer was seen in the patient's skin, and we observed markedly reduced intercellular lipid and defective intercellular lipid layers within the stratum corneum; the lipid contents of the lamellar granules in the granular layer cells were also defective. From these findings, we infer that, rather than the vitamin A metabolic pathway abnormality proposed by Shigehara *et al.*,[4] based on SDR9C7 converting retinal into retinol,[6] the pathomechanism of LI caused by SDR9C7

deficiency probably involves abnormal metabolism and defective synthesis of lamellar granule lipid contents in keratinocytes, resulting in malformation of the intercellular lipid layers in the stratum corneum.

With regard to genotype/phenotype correlation, our patient showed light brown to shiny whitish scales which were somewhat different from the large erythematous scales reported in the Lebanese patients;[4] in addition, our case lacks palmoplantar keratoderma seen in those cases. These differences might be explained by the nature of the mutations (missense vs frameshift). Clinically, however, all ARCI patients with *SDR9C7* mutations have experienced recurrent fungal infections, and it is possible that the defective intercellular lipid layers in the stratum corneum might generate a dysfunctional epidermal barrier that facilitates this type of infection.

In summary, our findings expand the molecular basis of ARCI in identifying a patient with LI that has a homozygous frameshift mutation in *SDR9C7* with evidence for defective intercellular lipid layers in the stratum corneum contributing to the clinical phenotype.

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Figure legends

Figure 1. Clinical and morphological features of the proband

(a, b) Large light brown to whitish shiny scales on the trunk (a) and the right upper arm (b). (c) A skin biopsy sample shows compact hyperkeratosis with a slightly thin granular layers. No inflammatory cell infiltration was seen in the dermis or the epidermis. Hematoxylin-eosin stain. Scale bar = 50μm. (d) Ultrastructurally, remarkably compact, thick stratum corneum is seen and almost no lipid droplet is observed in the cornified cells. (e) Lamellar contents of the lamellar granules (arrows) are deficient in the granular layer cells. Only a small amount of lipid materials are secreted from the lamellar granules and the stratum corneum intercellular lipid layers is absent or extremely thin. Scale bars = 1μm.