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Letter to the Editor

Deficient stratum corneum intercellular lipid in a non-Lebanese lamellar ichthyosis patient with a *SDR9C7* homozygous deletion mutation

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Short title: *SDR9C7* deletion mutation causes LI

Abbreviations: ARCI, Autosomal recessive congenital ichthyosis; LI, lamellar ichthyosis; SDR9C7, short chain dehydrogenase/reductase family 9C, member 7; WES, whole-exome sequencing

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To the Editor:

Autosomal recessive congenital ichthyosis (ARCI) is an umbrella term for inherited non-syndromic ichthyosis with autosomal recessive inheritance manner, which includes harlequin ichthyosis (HI), lamellar ichthyosis (LI), congenital ichthyosiform erythroderma and pleomorphic ichthyosis (also called self-healing/self-improving collodion baby) (Vahlquist, 2010). LI is a milder phenotype than HI, although the severity of the skin symptoms, including hyperkeratosis and scales, varies from patient to patient. Generally, LI does not show erythroderma, although several cases with very mild erythema have been reported (Takeichi and Akiyama, 2016). Very recently, Shigehara *et al.* reported that two missense mutations in *SDR9C7* causes ARCI in three consanguineous Lebanese families (Shigehara *et al.*, 2016).

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 activitie (Kowalik *et al.*, 2009). Shigehara *et al.* (2016) reported two missense mutations in *SDR9C7* in 3 families with ARCI. How mutations in *SDR9C7* lead to the clinical phenotypes of ARCI, however, is uncertain. Here, we describe a case of LI having a previously unreported homozygous deletion mutation in *SDR9C7* with her detailed

phenotype and extend the spectrum of clinical features of LI due to *SDR9C7* mutations. In addition, deficient intercellular lipid and malformation of intercellular lipid layers in the stratum corneum were observed in the patient skin by electron microscopy.

The patient is a 72-year-old Japanese, 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 history included diabetes mellitus and hypertension.

On examination, she showed large, whitish to light brown scales without erythroderma on her trunk and extremities (Fig. 1a and b). She did not show palmoplantar keratosis or alopecia. She had neither nail nor dental abnormalities. No mucosal change was seen. She did not have any visual or hearing difficulties. Recurrent fungal infections were seen in her eruptions. There was no family history of any skin disorder. A skin biopsy specimen obtained from the left lower leg showed compact hyperkeratosis with normal-appearing granular layers (Fig. 1c). Neither parakeratosis nor dyskeratosis was observed. Inflammatory cell infiltration was not seen. These features were compatible with LI.

Electron microscopy shows markedly compact hyperkeratosis (Fig. 1d and e).

Minimal amount of lipid was observed between the cornified cells and only very thin or absent intercellular lipid layers were seen in the cornified cell layers. Almost no lipid droplets was found in the cornified cells. Lamellar materials (lipid contents) were remarkably reduced in the lamellar granules, although lamellar granules seemed to fuse normally to the cell membrane.

Following ethical approval, informed written consent was obtained in compliance with the Declaration of Helsinki guidelines. Genomic DNA from the patient was used for whole-exome sequencing (WES) analysis, using methodology described elsewhere (Takeichi *et al.*, 2015). In total, 472 novel 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. 2a). For disease inheritance, we initially searched for potentially damaging compound heterozygous or heterozygous variants, not homozygote. However, the size of the homozygous block of homozygosity surrounding the present *SDR9C7* mutation was 4.37Mb (chr12:53647373_58019472). The sizes of the putative regions of homozygosity were estimated by the distance between the delimiting heterozygous variants identified by WES. This homozygous block suggests the possibilities of related parents of the proband or loss of heterozygosity of the

patient's genomic DNA. The WES data excluded pathogenic mutations in other ARCI-causative genes. This deletion mutation leads to a frame-shift, causing a premature stop codon 3 codons downstream from the substitution site (p.Phe300Serfs*3) (Fig. 2b) and resulting in a truncation of 12 residues from the C-terminus. SDR9C7 amino-acid sequence alignment shows that the 14 residues at the C-terminus are conserved among diverse species (Fig. 2c). From these findings, we finally diagnosed the present case as LI due to the previously unreported homozygous deletion mutation of *SDR9C7*.

Next, following informed consent, we conducted immunohistochemical analyses with two anti-SDR9C7 antibodies, anti-SDR-O antibody (ab90371; Abcam, Cambridge, U.K.) and anti-SDR9C7 antibody (LS-C172392; LifeSpan BioSciences, Inc.), in a lesional skin sample from the patient. The ab90371 is a rabbit polyclonal antibody, corresponding to C terminal amino acids 264-313 of human SDR9C7 and the LS-C172392 is a mouse monoclonal antibody. The immunohistochemical stainings showed reduced or almost absent expression of SDR9C7 in the patient's skin (Figure 2d and e) compared with the normal control skin (Fig. 2f and g).

So far, only two missense mutations, p.Arg72Trp and p.Ile200Thr, in *SDR9C7* have been reported as the pathogenic substitutions for ARCI. In cultured cells,

expression of both the mutant SDR9C7 proteins was markedly reduced compared with that of the wild-type protein, suggesting that the mutations severely affected a stability of the protein (Shigehara *et al.*, 2016). The present mutation c.897delT (p.Phe300Serfs*3) is the first deletion/truncation mutation, which causes a slightly truncated SDR9C7 protein which lacks the 12 residues at the C-terminus (Fig. 2b). Our findings suggest that the functional importance of C-terminus in SDR9C7.

Interestingly, systemic retinoids were not effective for the present patient. It has previously been reported that SDR9C7 is an enzyme to convert retinal into retinol (Kowalik *et al.*, 2009). Therefore, Shigehara *et al.* suggested that a potential role of vitamin A metabolism in terminal differentiation of the epidermis in humans. However, our clinical observation did not support their hypothesis. Histopathologcially, compact hyperkeratosis with normal granular layers was seen in the patient's skin. Notably, the electron microscopic observations revealed remarkably reduced intercellular lipid and defective intercellular lipid layers within the stratum corneum. Lipid contents of the lamellar granules in the granular layer cells were also defective. From these findings, we assume that the pathomechanisms of LI caused by SDR9C7 deficiency are abnormal metabolism and defective synthesis of lamellar granule lipid contents in the keratinocytes, resulting in malformation of the intercellular lipid layers in the stratum

corneum.

With regard to genotype/phenotype correlation, the present patient showed light brown to shiny whitish scales different from large erythematous scales reported in the Lebanese patients (Shigehara *et al.*, 2016). In addition, our case lacks palmoplantar keratoderma which were seen in the case previously published (Shigehara *et al.*, 2016). The difference between the causative mutations, the substitution versus the truncation, might be associated with difference in the phenotypes. The present case had recurrent episodes of cutaneous fungal infection, as was seen in previously reported ARCI patients with SDR9C7 mutations (Shigehara *et al.*, 2016). Considering the defective intercellular lipid layers in the stratum corneum in the present case, there might be a significant risk of fungal infection associated with epidermal barrier dysfunction in LI patients with *SDR9C7* mutations.

We report for the first time a patient with LI with a *SDR9C7* mutation in non-Lebanese population. In addition, the present mutation is the first documented frameshift mutation of *SDR9C7*. Although its truncation site is near the C-terminus of SDR9C7, extreme reduction of the protein expression of SDR9C7 was confirmed in the present patient's skin. Thus, the present case clearly suggests SDR9C7 functional deficiency leads to defective intercellular lipid layers in the stratum corneum, resulting

in the LI phenotype.

The authors have no conflicts of interest to declare.

Acknowledgments

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Figure legends

Figure 1. Clinical and morphological features of the proband

(a, b) The patient showed 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 = 50 μm. (d) Ultrastructurally, remarkably compact, thick stratum corneum is seen in the patient's skin. Almost no lipid droplet is observed in the cornified cells. (e) Lamellar contents of the lamellar granules (arrows) are deficient or malformed in the granular layer cells. Only a small amount of lipid materials are secreted from the lamellar granules and the intercellular lipid layers in the thick stratum corneum is absent or extremely thin. Scale bars = 1 μm.

Figure 2. Sequence data of *SDR9C7* and expression of SDR9C7 in the lesion of lamellar ichthyosis.

(a) Sequence data of *SDR9C7* in the patient and a control; the arrow indicates the homozygous mutation of c.897delT (p.Phe300Serfs*3). (b) Secondary structures of wild-type SDR9C7 and the mutant SDR9C7 produced from the mutant allele. The blue area (amino acids 31-38) indicates the reported the glycine rich motif TGxxxGxG, which

is necessary for cofactor binding. The acidic amino acid (p.59Glu) in key position near to cofactor binding region is marked by red arrow. The reported active center site YxxxK is marked by yellow area (amino acids 172-176). Sites of the previously reported mutations in ARCI are indicated with green arrows. (c) The truncated C-terminus are conserved among diverse species. (d-g) Immunohistochemistry of the affected lesion with the anti-SDR-O antibody (ab90371) (d) and the anti-SDR9C7 antibody (LS-C172392) (e). SDR9C7 staining is almost negative with both antibodies in the patient's skin (d, e) compared with the control skin (f, g). Scale bars = 100 □m.

Figure 1

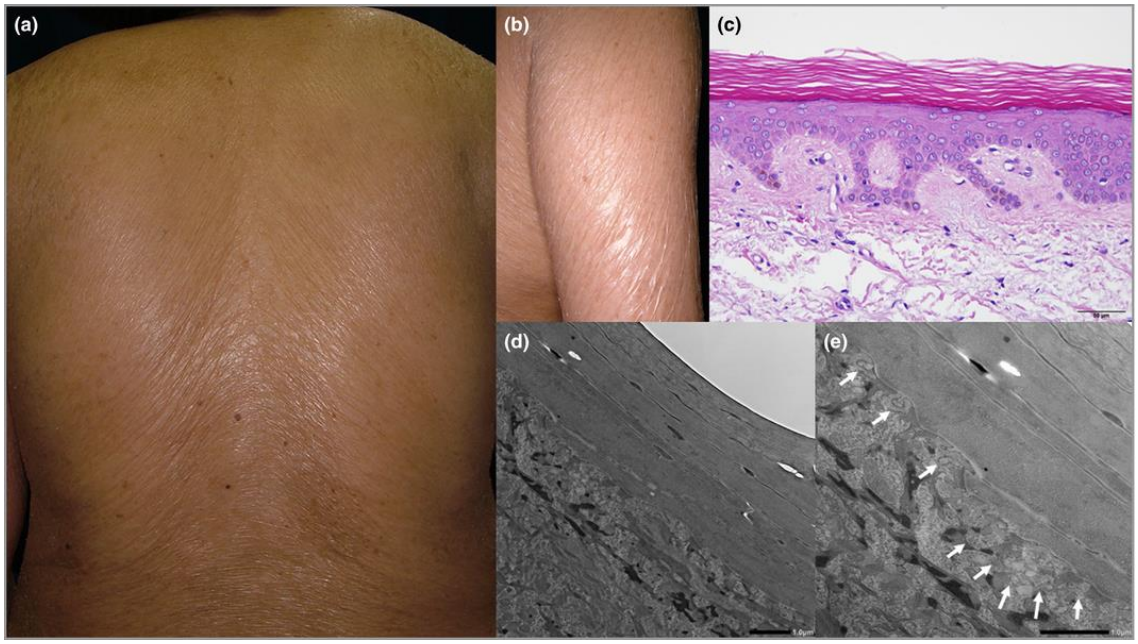

Figure 2