

Nonsense Variant in *CYP4F22* Causes Malformation of Corneocyte Lipid Envelopes in a Lamellar Ichthyosis Patient

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Cytochrome P450 4F22 (*CYP4F22*), encoded by *CYP4F22*, is a protein proposed to be integral to the healthy formation of the corneocyte lipid envelope (CLE) (1). *CYP4F22* deficiency clinically results in lamellar ichthyosis (LI), a type of autosomal recessive congenital ichthyosis (ARCI) (1). A number of variants in *CYP4F22* have been identified as being pathogenic for LI, with the majority of reports originating from families with Mediterranean or Middle-Eastern backgrounds (2).

Here, we report a patient with LI carrying a novel *CYP4F22* variant. In-depth analysis successfully demonstrated the loss of CLE in the stratum corneum, which has not been reported previously.

CASE PRESENTATION

The patient, a 14-year-old girl originally from Afghanistan, presented with erythematous, dry, scaly skin since birth. On examination, there was scaling over almost the entire body, with mild pain and itching (Fig. 1A, B). She was born to consanguineous parents (cousins). Two of her 9 uncles were reported to have similar symptoms. Neither she nor her affected uncles had received any treatment previously.

She was started on treatment with emollients and topical steroids. The treatment improved her erythema and scaling to

some extent, although she never achieved full remission. She has remained on regular follow-up at our hospital since.

MATERIALS, METHODS AND RESULTS

This study was reviewed and approved by the institutional ethics committee. Informed consent was obtained from the patient and her parents, and all research was performed in accordance with the Declaration of Helsinki.

Genetic analysis was performed using DNA extracted from peripheral blood samples of the patient and her parents. Whole-exome sequencing (WES) was performed to identify pathogenic variants to explain the clinical symptoms, using previously described methods and equipment (2). Variants were filtered according to the characteristics of an autosomal recessive inheritance pattern, and variants with an allele frequency of over 1% in all populations were excluded. Once the candidate variant was identified through WES, Sanger sequencing was undertaken. The region of interest was amplified by polymerase-chain reaction (PCR) using forward (5'-AGCCAACTGCCTGAAATCAT-3') and reverse (5'-TCAAATGACCCTTCCTCTGG-3') primers for *CYP4F22* exon 4. AmpliTaq Gold 360 DNA Polymerase (Thermo Fischer Scientific, Waltham, MA, USA) was used in the PCR reaction. The resulting PCR products were purified using illustra ExoProStar (Cytiva, Marlborough, MA, USA). Sequencing analysis was performed using the computer software CLC Main Workbench (QIAGEN, Hilden, Germany).

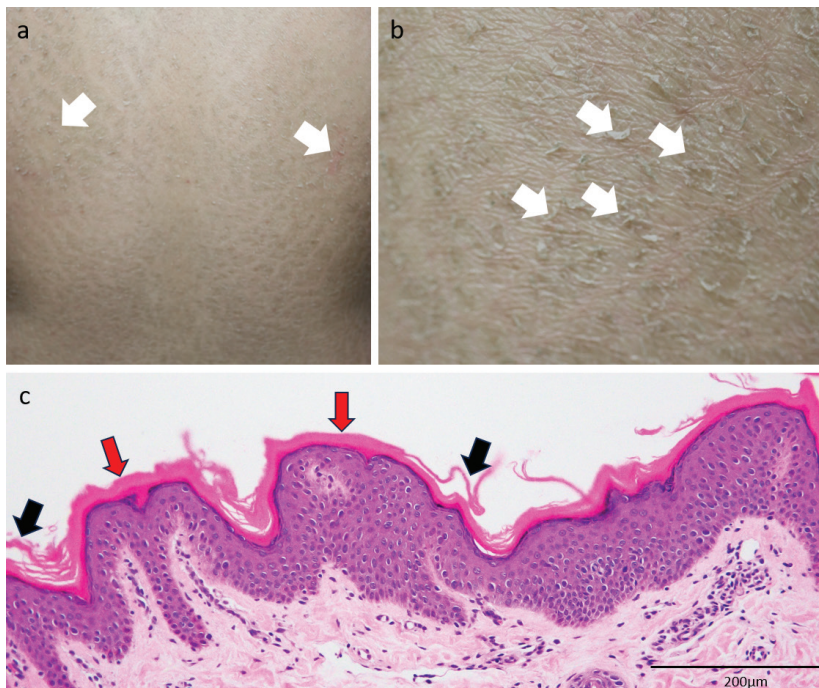


Fig. 1. Clinical and histopathological features of the patient. (A) Photograph of the back of the patient. Some erythema and significant amounts of scaling are seen (white arrows). Similar symptoms were seen over almost the entire body. (B) Photograph of the lower back of the patient. Significant amounts of scaling are seen. Each scale is approximately 1–2 cm in diameter, and the sizes are consistent throughout the back (white arrows). (C) Histopathological photograph of a skin biopsy sample from the right lower back (haematoxylin-eosin staining, magnification x 200). Compact hyperkeratosis can be seen in the majority of the stratum corneum (red arrows), with areas of detachment of the piled stratum corneum cells (black arrows). The epidermis is not thickened, and the stratum granulosum has an unremarkable appearance.

Transmission electron microscopy (TEM) was performed using skin biopsy samples from the patient. The samples were fixed in 2.5% (w/v) glutaraldehyde solution, post-fixed in 0.5% (w/v) ruthenium tetroxide (RuO₄) (3), then fixed in an osmium tetroxide solution for 3 h. Subsequent dehydration was performed using ethanol (samples immersed once for 10 min each at concentrations of 50%, 70%, 80%, 90%, 95%, 99%, and 3 times at 100%), before being further immersed in propylene oxide for resin embedding. Once embedding was complete, sections were cut to a thickness of 80 nm using an ultramicrotome UC7k (Leica Microsystems, Wetzlar, Germany) and placed on copper grids. The sections on the copper grids were observed and photographed using an electron microscope JEM-1400PLUS (JEOL, Akishima, Tokyo, Japan).

Immunohistochemical analysis was performed on skin biopsy samples from the patient. The antibodies used were anti-IL-17C, anti-IL-36 γ , and anti-TNF α using techniques described previously (2). The resulting images were observed and qualitatively assessed. Quantitative analysis was performed using CellProfiler (Broad Institute, Cambridge, MA, USA), software designed to assist image analyses of biological samples. The images were first resized, and redundant areas were crudely cropped and mounted onto the software. A freely available pipeline validated for IHC analysis was used for the software commands (4). The outcomes of interest were the area of visible 3,3'-diaminobenzidine (DAB) staining, the total area of the image, and the stained area as a percentage of the total area (i.e., the area of visible 3,3'-diaminobenzidine staining/total area of the image). This procedure was repeated 3 times, and the average percentage of stained area for each antibody was calculated.

Histopathological analysis of lesional skin biopsied from the right lower back demonstrated an unremarkable appearance of the epidermis. The stratum corneum showed compact hyperkeratosis with less basket-weave appearance (Fig. 1C).

WES identified a homozygous nonsense variant, c.296G>A (p.Trp99Ter), in *CYP4F22* in the patient, and the monoallelic identical variant in both of her parents. Given the clinical presentation and the identified variant, the patient was diagnosed with LI.

TEM demonstrated an absence of the CLE around the corneocytes (Fig. S1A, B). Notably, at lower magnification, areas of loosening and scaling could be observed between the layers of corneocytes, suggesting easy skin shedding. Inclusion bodies were also seen in the corneocyte cytosol. In comparison, TEM images of the stratum corneum of a healthy control demonstrated the presence of CLE around the corneocytes (Fig. S1D). All layers of the epidermis except the stratum corneum were found to be intact, and no areas of loosening, scaling, or inclusion bodies were seen (Fig. S1C).

Immunohistochemical analysis with anti-IL-17C, anti-IL-36 γ , and anti-TNF α antibodies demonstrated an inflammatory response in the patient's skin compared with controls (Fig. S2). Quantitative analysis using CellProfiler found the area of DAB-stained regions (in pixels) for each antibody to be greater in the patient's skin (IL-17C 294406px, IL-36 γ 83109px, TNF α 122562px) than in the controls (IL-17C 69358px, IL-36 γ 1158px, TNF α 4135px). Furthermore, the average percentage across the 3 measurements for the DAB-stained area was higher for each antibody in the patient's skin (IL-17C 57.9%, IL-36 γ 18.3%, TNF α 28.7%) than in the controls (IL-17C 14.7%, IL-36 γ 0.2%, TNF α 0.8%). A paired *t*-test showed a statistically significant difference in the percentage of DAB-stained areas for all antibodies (*p*=0.01).

DISCUSSION

Patients with LI due to *CYP4F22* variants have been reported to possess low levels of acylceramide, required for normal CLE formation (1, 5). In our case, the identi-

fied variant in *CYP4F22* was thought to lead to absent or reduced CYP4F22 activity, and we hypothesize that less acylceramide would be synthesized, which would explain the absence of CLE. This may be because a premature stop codon appears relatively early in the sequence, which would most likely result in mRNA decay. Even if nonsense-mediated decay did not occur and a truncated protein terminating at the amino acid position 99 were produced, this would still lack a significant proportion of the functional domains of CYP4F22, including the substrate-binding regions (amino acid positions 149–160 and 469–480) (6), and hence would likely be rendered non-functional.

Our preparation of the samples for TEM observation did not use pyridine extraction. Pyridine extraction is used in TEM preparation to enhance the appearance of the CLE and to visualize covalently bound CLE (7). Unbound CLE can be observed without pyridine treatment, which may be the case here. The unbound CLE consists of ultralong-chain acylceramides, cholesterol, and free fatty acids. Of these, the ultralong-chain acylceramides do not have a specific ultrastructure (1, 7). Therefore, even if unbound CLE (mainly free ultralong-chain acylceramides) remained, we think that it is still possible to visualize CLE.

In milder forms of ARCI, such as self-healing collodion baby (SHCB), some production of acylceramide remains, which may contribute to the spontaneous improvement of the ichthyotic phenotype of SHCB with age (2). In contrast, we postulate that a complete lack of CLE, as demonstrated in our case, leads to a chronic phenotype like LI. Indeed, after 2 years of follow-up since the initial presentation, the ichthyotic symptoms persist.

The p.Trp99Ter variant was a nonsense variant that was submitted to ClinVar by 3billion, a South Korean for-profit organization that provides genetic testing services. This particular submission does not mention the clinical details in which it was found (8). It cites the evidence of pathogenicity based on the American College of Medical Genetics guidelines 2015 (9). Therefore, we believe that our description of the present patient and her family is the first published clinical report of this variant, providing further evidence attesting to its pathogenicity and the correlation between mutation severity and phenotype.

The IL-17 family, IL-36 family, and TNF α cytokines have been found to be upregulated in various ARCIs, including Netherton syndrome (10), *NIPAL4*-associated ichthyosis (11), and SHCB (2). Paller et al. (12) reported significant upregulation of IL-17C and IL-36 γ in LI patients, although TNF α did not show a significant difference. In our case, TNF α was also significantly increased in lesional skin. The synergistic effect between TNF α and IL-17C has been implicated in various inflammatory conditions (13, 14). The TNF α signalling pathways (e.g., NF- κ B, MAPK) are associated with significant tissue inflammation (14, 15), and we feel this may have contri-

buted to the ichthyosis phenotype and may also explain why TNF α was upregulated, unlike previous studies.

In summary, we reported a case of LI arising due to a nonsense variant in *CYP4F22*. This is the first report in which, through TEM evidence, absent or reduced activity of CYP4F22 has been confirmed to be associated with an absence of CLEs in the stratum corneum. Furthermore, we have confirmed through immunohistochemistry the significant upregulation of pro-inflammatory cytokines associated with ichthyoses as a result of the *CYP4F22* variant. We feel that our case provides further clinical evidence of the pathogenesis of LI due to *CYP4F22* variants. Analysis of CLEs and other ultrastructural features by TEM might be useful in ARCI diagnosis and in the prediction of long-term prognosis.

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