CYP4F22 p.V215D is a novel variant causative for lamellar ichthyosis

Running title: A new CYP4F22 variant of lamellar ichthyosis

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Yoshihiro Ishida[1], Kazuko Saeki[2], Marina Ueda[1], Hyeon-Cheol Lee-Okada[2], Hiromi Doi[1], Naotomo Kambe[1], Saeko Nakajima[1], Takehiko Yokomizo[2], and Kenji Kabashima[1]

[1]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

[2]Department of Biochemistry, Graduate School of Medicine, Juntendo University, Tokyo, Japan

Correspondence should be addressed to:

Yoshihiro Ishida MD, PhD

Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

Phone: +81-75-751-3310, **Fax:** +81-75-751-4949

E-mail: yishida@kuhp.kyoto-u.ac.jp

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Ethics: The study was approved by the institutional review board at Kyoto University Hospital. The patients in this manuscript have given written informed consent to the publication of their case details.

Dear Editor:

Ichthyoses encompass many skin diseases that result from abnormalities in cornification. Lamellar ichthyosis (LI) represents a rare subset of the ichthyoses. Nine genes, including *CYP4F22*, that engage in lipid metabolism are known to cause LI[1]; however, our understanding of the genetic basis of LI is likely incomplete because of its rarity. Here, we report *CYP4F22* p.V215D as a novel allele causative for LI by combining exome sequencing and liquid chromatography-electrospray ionisation tandem mass spectrometry (LC-MS/MS).

A Japanese male with LI agreed to receive molecular testing at the age of 62. He reported that he was born from non-consanguineous parents and had no family history of congenital skin diseases. The patient's recollections of his birth indicated prematurity coupled with erythroderma, although the records at birth were lost to time. Physical examination showed brown, plate-like scales on the forehead and legs, fine scales on the back, ectropion of the eyelids and palmoplantar hyperkeratosis (Fig. 1a-c). There was neither erythema nor abnormalities of hair or nail plates.

Exome sequencing of the genomic DNA extracted from the patient's peripheral blood nominated 23,338 germline variants. Among them was one variant with the pathogenic annotation in the ClinVar database (heterozygous for *LIPH*: NM_139248: p.H248N), but it was likely coincidental, judging by the irrelevance to the patient’s phenotype. Subsequently, our focus pivoted towards genes implicated in LI, revealing that the patient was homozygous for *CYP4F22* p.V215D (chromosome

19:g.15648777T>A[hg19])[2]. The variant was unreported in dbSNP or the human genetic variation database, a compilation of 3,248 Japanese exomes. SIFT and Polyphen2 predicted the variant deleterious to the protein's function. To rule out the possibility of sequencing artefacts, we validated the variant by Sanger sequencing with the custom primers (forward: TGCATGTGAGTCCTAAGGCT, reverse: TCCCATAGGCCAGAGTTGTC).

CYP4F22 encodes a member of the cytochrome P450 superfamily of enzymes, which catalyses the hydroxylation of the ω-carbon of ultra-long-chain fatty acids (FAs)[3]. To ascertain the pathogenicity of the variant, we analysed the lipid profile of the patient's skin by performing an LC-MS/MS analysis of tape-stripped skin as described previously[4]. Two healthy individuals were also analysed as controls. As expected, the patient showed a build-up of non-hydroxy ceramides and a marked reduction of ω-hydroxy ceramides and esterified ω-hydroxy ceramides compared to the healthy control (Fig. 2). Given that CYP4F22 is an essential enzyme in ω-carboxylation of ultra-long-chain of FAs, the lipid profile demonstrates that the enzymatic activity of CYP4F22 is virtually absent in this patient. Combined with the results from the genetic analysis, our data point to the pathogenicity of the newly identified allele, *CYP4F22* p.V215D. In line with the previous report, this missense mutation did not result in severe manifestation of autosomal recessive congenital ichthyosis, such as collodion membranes[2]. Given the novelty of the variant, we suspect that the patient's parents are descendants of a single Japanese founder. However, we could not confirm the

hypothesis because the patient declined to have his pedigree examined. It is worth noting that known pathogenic variants of *CYP4F22* lack a conspicuous pattern; more than half of the reported mutations are missense mutations located in various exons of *CYP4F22*[2,5,6]. Thus, interpreting novel variants of *CYP4F22* will likely remain challenging. In addition to reporting a new allele, our study exemplifies that combining lipidomic and genomic approaches is useful in studying very rare variants that result in altered lipid synthesis in the skin.

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Figure legends

Fig 1 Clinical pictures of the patient with lamellar ichthyosis

Clinical pictures showing the leg (a), palm and (b) right eye (c) of the patient with lamellar ichthyosis

Fig 2 Lipid profile of the patient's skin

The diagram on the left shows schematics of ceramide synthesis. The heatmap on the right shows the LC-MS/MS data of the skin tape strips. Each column represents a sample, and each row represents a ceramide species. Each color of the left bars corresponds to the ceramide classes and subclasses. Right bars indicate saturation status and carbon chain length of fatty acids colored in blue in the structural formulas. HC: healthy control, AH: α-hydroxy-6-hydroxysphingosine ceramide, AP: α-hydroxy- phytosphingosine ceramide, AS: α-hydroxy-sphingosine ceramide, ADS: α-hydroxy-dihydrosphingosine ceramide, NH: non-hydroxy-6-hydroxysphingosine ceramide, NP:

97 non-hydroxy-phytosphingosine ceramide, NS: non-hydroxy-sphingosine ceramide, NDS:

98 non-hydroxy-dihydrosphingosine ceramide, OH: ω-hydroxy-6-hydroxysphingosine ceramide, OP:

99 ω-hydroxy-phytosphingosine ceramide, OS: ω-hydroxy-sphingosine ceramide, ODS:

100 ω-hydroxy-dihydrosphingosine ceramide, EOH: esterified ω-hydroxy-6-hydroxysphingosine

101 ceramide, EOP: esterified ω-hydroxy-phytosphingosine ceramide, EOS: esterified

102 ω-hydroxy-sphingosine ceramide, EODS: esterified ω-hydroxy-dihydrosphingosine ceramide,

103 MUFA: monounsaturated fatty acid, SFA: saturated fatty acid, FA2H: fatty acid 2-hydroxylase,

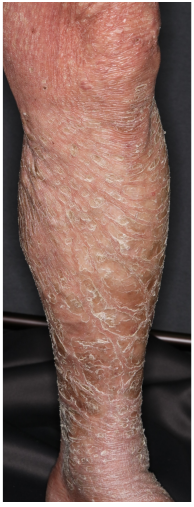
104 CYP4F22: cytochrome P450 family 4 subfamily F member 22, ACS: acyl-CoA synthetase, CERS:

105 ceramide synthase, PNPLA1: patatin-like phospholipase domain-containing protein 1, ABHD5:

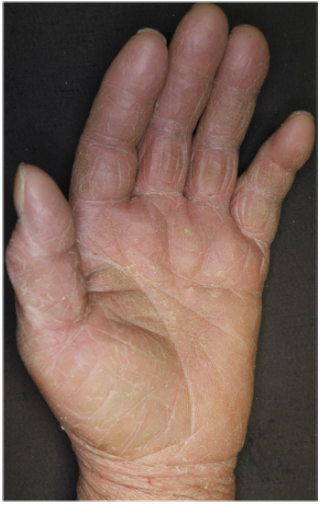
106 α/β-hydrolase domain-containing protein 5.

Figure 1

a

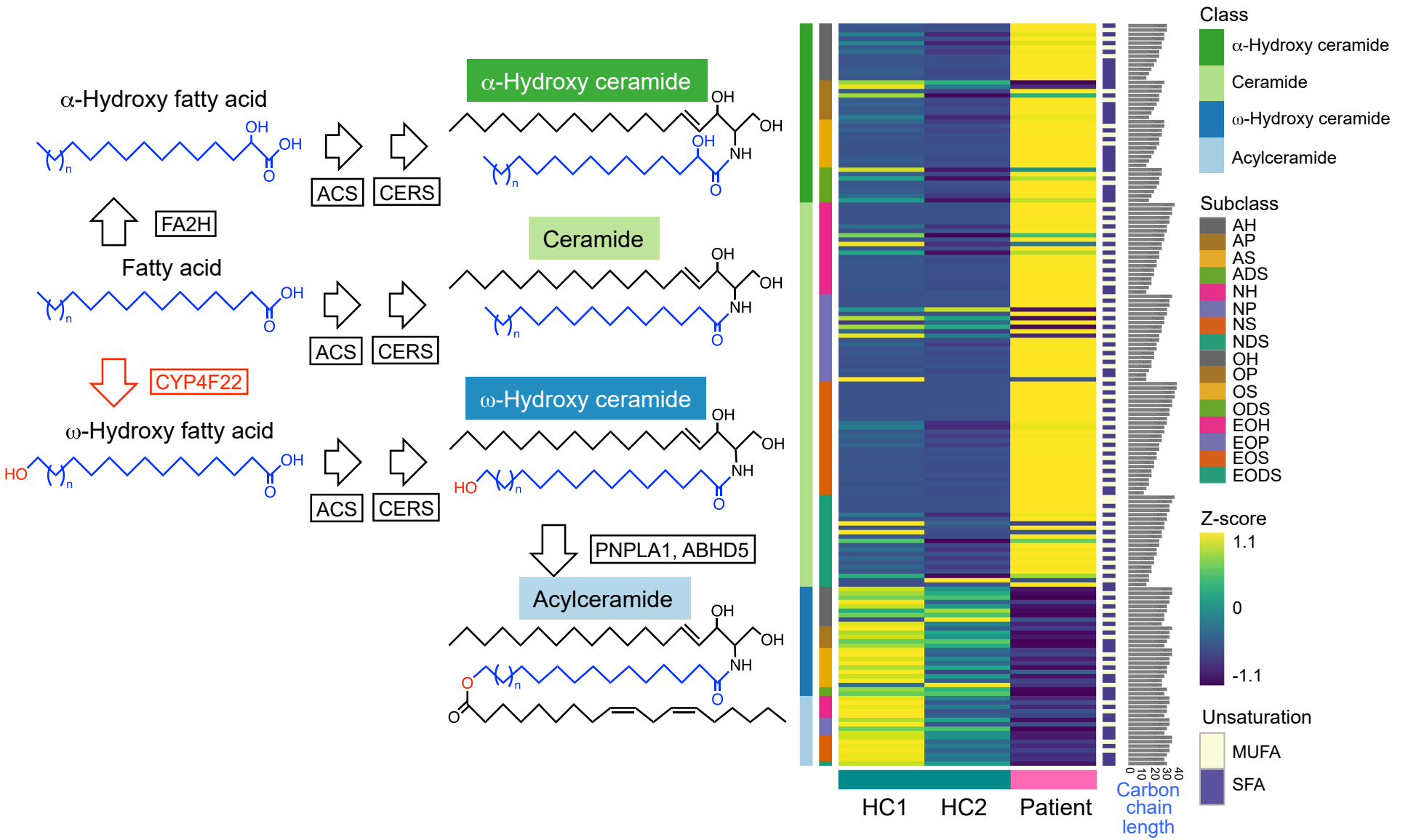


b



c

Figure 2


α-Hydroxy fatty acid
FA2H
Fatty acid
CYP4F22
ω-Hydroxy fatty acid
ACS
CERS
α-Hydroxy ceramide
Ceramide
ω-Hydroxy ceramide
PNPLA1, ABHD5
Acylceramide
Class
α-Hydroxy ceramide
Ceramide
ω-Hydroxy ceramide
Acylceramide
Subclass
AH
AP
AS
ADS
NH
NP
NS
NDS
OH
OP
OS
ODS
EOH
EOP
EOS
EODS
Z-score
1.1
0
-1.1
Unsaturation
MUFA
SFA
HC1
HC2
Patient
Carbon chain length