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Identification of a novel causative mutation in *KRT1* in diffuse palmoplantar keratoderma, facilitated by whole-exome sequencing

(Original title: "Whole-exome sequencing facilitates causative mutation detection in diffuse palmoplantar keratoderma")

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We enrolled a large four-generation Japanese diffuse palmoplantar keratoderma (DPPK) pedigree of 14 individuals (Fig. 1a). After informed consent was obtained, genomic DNA from the participants was used for Sanger sequencing (SS) of *KRT1* and/or whole-exome sequencing (WES). The proband (III-3) underwent WES and SS; eight other family members (II-2, II-3, III-1, III-4, III-5, III-6, IV-1 and IV-2) underwent only SS. There were DNA samples from 9 family members: 6 with DPPK, and 3 unaffected individuals (Figure 1a). SS of *KRT1* and WES were performed as previously reported [1].

The proband (Ⅲ-3) of the pedigree was a 31-year-old Japanese woman who presented with diffuse hyperkeratosis on the palms and soles shortly after birth, although she did not develop skin fragility or blistering. Her wrists and the dorsal aspects of the extremities were unaffected. All the individuals with DPPK in the pedigree displayed mild to severe hyperkeratosis confined to the palms and soles, which was similar to the index patient. The proband's two sons (IV-1 and IV-2) had blistering on the legs from the first week of life. The blisters later disappeared, but diffuse hyperkeratosis remained on the palms, soles and axillae (*figure 1b, c*).

Based on the clinical features and the autosomal-dominant inheritance of the present family, the most likely candidate gene was *KRT1*. However, we could not completely exclude the possibility of *KRT9* or *DSG1*. WES identified the previously unreported heterozygous mutation c.1318_1319delinsAT (p.Ala440Ile) in exon 7 of *KRT1* (NM_006121) in the proband (III-3). The mutation was verified by SS (*figure 1d*). We did not identify any other potentially pathogenic variants in the 32 genes other than *KRT1* that are implicated in inherited PPK, including *KRT9* or *DSG1* [2] in the proband (III-3). SS of *KRT1* in 9 individuals from this pedigree showed the co-segregation of the p.Ala440Ile substitution with the disease phenotype. The pathogenicity of this novel heterozygous mutation was supported by the Protein Variation Effect Analyzer (PROVEAN; score: -4.844, deleterious), the Sorting Intolerant From Tolerant algorithm (SIFT; score: 0.0, deleterious), the Protein Analysis Through Evolutionary Relationships (PANTHER; score: -6.81644, deleterious) and Polymorphism Phenotyping v2 (PolyPhen2; score: 1.0, probably damaging). The outcomes predicted for the present mutation indicated the genetic defect to be disease-causing. The mutation was not found

in the gnomAD database.

WES may contribute to the identification of genetic mutations missed by SS and to correct clinical diagnoses [3]. Indeed, there are many cases showing unexpected results different from the initial clinical diagnosis in the daily work of a molecular diagnostic laboratory. WES has a serious impact on diagnostics in genetic skin disease clinics. [4] WES also has the advantage of enabling us to analyze all the genes implicated in inherited palmoplantar keratoderma (PPK), and mutations/variants in other genes that possibly modify the phenotype can occasionally be detected in addition to main causative mutations. In fact, two PPK patients were reported to have potentially phenotype-modifying additional mutations in *SERPINB7* as well as the main disease-causative mutations in *DSG1* [5] and *KRT9* [6].

The majority of *KRT1* pathogenic variants have been found in either the 1A helix initiation motif or the 2B helix termination motif of keratin 1 (K1), and most of these variants result in keratinopathic ichthyosis [7]. However, some pathogenic variants in *KRT1* result in DPPK [7]. The present heterozygous mutation, p.Ala440Ile, is also located in the 2B helix termination motif of K1, which is thought to be critical for keratin intermediate filament assembly [8]. This motif interacts with the distal helix of keratin 10 (K10) to form a parallel coiled-coil heterodimer with a predominantly hydrophobic intermolecular interface. In the literature, the mutation p.Leu437Pro in K1, which is near our mutation, leads to the loss of hydrophobic interactions with the amino acid residues Tyr400, Gln403 and Leu404 in K10, and to the loss of hydrogen bonds with the amino acid residue Gln403 (adjacent to Cys401) in K10 [8]. The present mutation, p.Ala440Ile, in K1 may also affect hydrogen bonds and act as a disease-causing mutation.

In conclusion, for genetic skin disorders such as PPK, using WES for an initial genetic analysis can save the time, cost and labor involved in detecting causative mutations without any additional invasive examinations.

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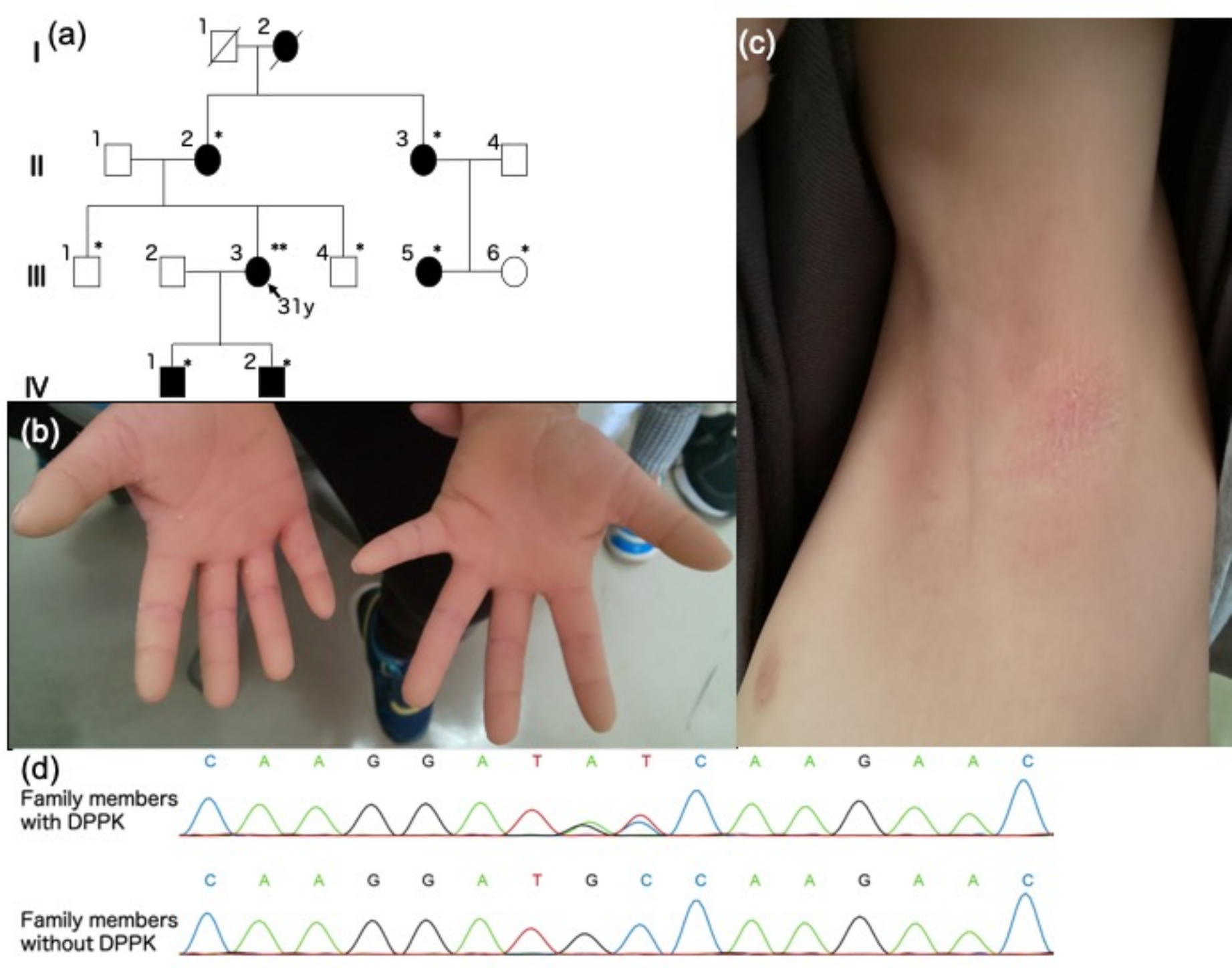
Figure Legends

Figure 1. The pedigree, clinical features and the KRT1 mutation in the present family. (a) The pedigree of the present family of 14 members, 7 of whom were diagnosed with DPPK. An arrow indicates the index patient (proband, III-3). **WES and Sanger sequencing were performed; *only Sanger sequencing was performed. (b, c) Mild to moderate diffuse hyperkeratosis with an erythematous border on the palms (b) and the axilla (c) of a patient (IV-1). (d) Sanger sequencing reveals the previously unreported heterozygous mutation c.1318_1319delinsAT in *KRT1* (NM_006121) in the proband (III-3).


(a)
I
II
III
IV
31y
(b)
(c)
(d)
Family members with DPPK
C A A G G A T A T C A A G A A C
Family members without DPPK
C A A G G A T G C C A A G A A C