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Apparent homozygosity due to compound heterozygosity of one point mutation and an overlapping exon deletion mutation in *ABCA12*: A genetic diagnostic pitfall

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ABSTRACT

Background: Harlequin ichthyosis (HI), one of the most severe genetic skin disorders, is autosomal recessively inherited. Mutations in *ABCA12*, which encodes ATP-binding cassette transporter A12 (ABCA12), are known to be the cause of HI. It is very difficult to make precise genetic diagnosis when an exon deletion mutation overlaps the site of another causative point mutation. This combination of mutations may lead us to conclude incorrectly that the patient has the point mutation homozygously, a phenomenon called "apparent homozygosity".

Objective: To demonstrate that the present HI patient has apparent homozygosity in *ABCA12* mutations.

Methods: We performed direct sequencing of gDNA in the entire coding region, including exon–intron boundaries, of *ABCA12* in the HI patient and her parents. To further elucidate the mutations in the patient, parental mutation segregation study was done and SNP analysis was conducted on the region flanking *ABCA12* in the patients and her parents. Quantitative PCR of gDNA in exon 11 of *ABCA12* was also performed. Direct sequencing of cDNA from exon 9 to exon 13 and of gDNA between intron 9 and intron 11 of *ABCA12* was done in the HI patient and her parents.

Results: Direct sequencing of gDNA in the entire coding region, including exon–intron boundaries, of ABCA12 seemed to indicate that the patient had the novel homozygous nonsense mutation c.1216A>T (p.Lys406X) in exon 11. However, mutation segregation analysis, SNP analysis, qRTPCR of gDNA in exon 11 of ABCA12 and direct sequencing of cDNA from exon 9 to exon 12 of ABCA12 and of gDNA between intron 9 and intron 11 of ABCA12 in the HI patient and her parents demonstrated that the present patient was compound heterozygous for two *ABCA12* mutations: c.1216A>T (p.Lys406X) in exon 11 and g.111346_113217del1872 (p.Leu355_Lys428del, Gln354fs7*) which was overlapping exon deletion mutations involving exons 10 and 11.

Conclusion: When direct sequencing indicates that a patient from a non-consanguineous family has an apparently homozygous non-founder point mutation, the homozygosity may be "apparent homozygosity", and we should keep in mind the possibility of overlapping exon deletion mutation.

1. Introduction

Harlequin ichthyosis (HI; OMIM#242500) is an extremely severe congenital autosomal recessive ichthyosis. The clinical features at birth include severe ectropion, eclabium, flattened ears and stiffed skin surface with large thick plate-like scales over the entire body [1,2]. HI-specific harlequin complexion is characterized by ectropion and eclabium.

In 2005, we and another research group identified mutations in *ABCA12*, which encodes ATP-binding cassette transporter A12 (ABCA12), as the cause of HI [2,3]. ABCA12 is a member of the large superfamily of ATP-binding cassette transporters [4], which bind and hydrolyze ATP to transport various molecules across limiting membranes or into vesicles [5]. ABCA12 is apparently localized in the membrane of the trans-Golgi network and lamellar granules of keratinocytes in the upper epidermis, mainly in the uppermost

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spinous and granular layer cells. It is suggested that ABCA12 works in the transport of lipids into the trans-Golgi network and lamellar granules, to accumulate lipids that are essential to skin barrier formation [6].

In HI families, most mutations are truncation mutations, including nonsense mutations, frame-shift deletions/insertions and splice-site mutations, in *ABCA12*. Other mutations reported in HI families are missense mutations and in-frame deletions/insertions. However, there are some patients in whom only a single mutant allele is detected by direct sequencing of the entire coding region, including the exon–intron boundaries. These patients are assumed to have heterozygous exon-deletion mutations or mutations in non-coding regions—for example, in the upstream promoter regions or introns—which cannot be detected by conventional sequencing of the coding regions. Among patients who seem to have a homozygous mutation by direct sequencing, there are some cases in which only one parent has the mutation, as in the case in this report. In those cases, it is recommended to confirm the presence of partial uniparental disomy (UPD). We should keep another possibility in mind: that the patient might have an exon deletion mutation involving an exon in which another point mutation exists. Deletion mutations of large genomic regions involving several exons in the heterozygote state are not revealed by conventional molecular analysis such as Sanger sequencing. This combination of mutations may lead us to conclude incorrectly that the patient has the point mutation homozygously, a phenomenon called "apparent homozygosity" [7]. To our knowledge, there has been no reported case of apparent homozygosity in dermatology. Here, we report a HI case of apparent homozygosity who is compound heterozygous for a novel *ABCA12* nonsense mutation p.Lys406X in exon 11 and an exon deletion mutation involving exons 10 and 11.

2. Material and methods

2.1. Patient

A Japanese female was born at 37 weeks gestational age by caesarean section because of breech presentation. The birth weight was 1826 g. She had no family history of consanguinity or any skin disorder. At birth, she had thick plate-like scales with deep fissuring overlying the erythrodermic skin. Severe eclabium and ectropion were seen. These clinical features are typical of HI (Fig. 1A, B). The thick scales desquamated gradually during the first 5 months of life, resulting in generalized erythroderma and overlying fine scaling. She was treated with systemic retinoid (1.0 mg/kg/day) from postnatal day 6. At a year and a half in age, she showed generalized erythroderma with desquamative scaling, persistent mild eclabium and ectropion (Fig. 1C).

The study was approved by the Ethics Committee of Nagoya University and was carried out according to *The Declaration of Helsinki Principles*. The participants gave written informed consent. We obtained written informed consent from the next of kin on behalf of the patient.

2.2. Direct sequencing of genomic DNA in the entire coding region, including the exon–intron boundaries, of ABCA12

We extracted genomic DNA from saliva of the patient using the DNeasy Micro DNA extraction kit (Qiagen, Hilden, Germany). We extracted genomic DNA from peripheral blood samples from the patient's parents and normal control individuals using the DNeasy Mini DNA extraction kit (Qiagen, Hilden, Germany). Oligonucleotide primers and polymerase chain reaction (PCR) conditions used for amplification of all *ABCA12* exons and exon–intron borders

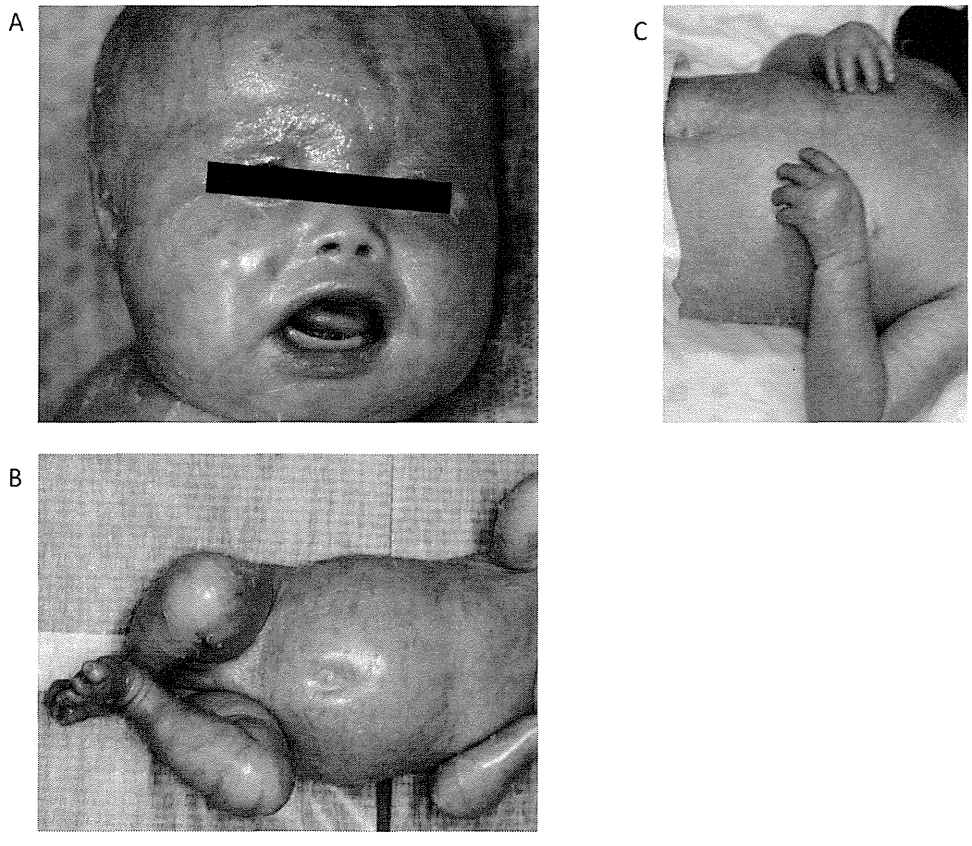

Fig. 1. The clinical features of the patient.
(A, B) At birth, she is seen to have thick plate-like scales with deep fissuring overlying erythrodermic skin. Severe eclabium and ectropion are seen.
(C) At a year and a half in age, she shows generalized erythroderma with desquamative scaling.

were described previously [8]. The *ABCA12* sequence was according to GenBank accession no. NG_007074.1.

2.3. Reverse transcription (RT)-PCR amplification and cDNA sequencing

We extracted total RNA from plucked hairs from the patient, the patient's parents and normal control individuals using the RNeasy RNA extraction kit (Qiagen, Hilden, Germany) [8]. Then, we obtained cDNA by RT-PCR using the PrimeScript RT reagent Kit (Perfect Real Time) (Takara, Otsu, Japan), according to the manufacturer's protocol. A 856-bp-long PCR product was amplified from cDNA exons 9–13 of wild-type *ABCA12* with a specific primer pair (5′-CCACGTTTTGCAACTAACGA and 3′- TGGCCAG-CAACTTGTCAATA).

2.4. SNP analysis

Haplotypes were studied using microsatellite markers flanking *ABCA12* (rs1967603 in intron 8 and rs10498030 in exon 22).

A specific primer pair (5′-AACTCCTGACCTCGTGATCC and 3′-CTGTACTTGGACCAGAGACC) was used for analyzing rs1967603.

The same primers used for direct sequencing of the exon 22 and exon 22–intron 22 borders were also used for analyzing rs10498030.

2.5. Direct sequencing of two regions of ABCA12 genomic DNA: intron 8–exon 13, and intron 9–intron 11

A 12,772-bp-long PCR product was amplified from gDNA of wild-type *ABCA12* between intron 8 and exon 13 with a specific primer pair (5′-TAAAATAATGTAGTGGCTTGATGCACAGAC and 3′-TGATATTTTCCAAGTAATTCATTTGCAGTG). A 2302-bp-long PCR product was amplified from gDNA of wild-type *ABCA12* between intron 9 and intron 11 with a specific primer pair (5′-ATAGTACA-CATAGAAAAGGCAAGTGCT and 3′-ATAACTATTGTTAGCCATTCCCT-TATAAAT).

2.6. Quantitative PCR (qPCR) of gDNA

We performed gDNA qPCR by LightCycler 480 real-time system using the LightCycler 480 SYBR green 1 Master Kit (Roche Applied Science, Basel, Swiss). A specific PCR primer pair (5′-ACTCCTG-CAGTCCACAATACG and 3′-AGGAACTGGAGGAAAGTAATCTTCA) was used for amplification of a 78 base pair fragment in exon 11 of *ABCA12*. As a control, we amplified GAPDH as a housekeeping gene. The qPCR procedures were according to the manufacturer's protocol.

3. Results

3.1. Direct sequencing of genomic DNA in the entire coding region, including the exon–intron boundaries, of ABCA12

We searched for mutations in *ABCA12* in the affected girl and her parents. The entire coding region, including the exon–intron boundaries, of *ABCA12* were amplified from genomic DNA by PCR, and direct sequencing revealed a previously unreported homozygous or monolithic nonsense mutation, c.1216A>T (p.Lys406X), in exon 11 of *ABCA12* in the patient. Parental segregation study

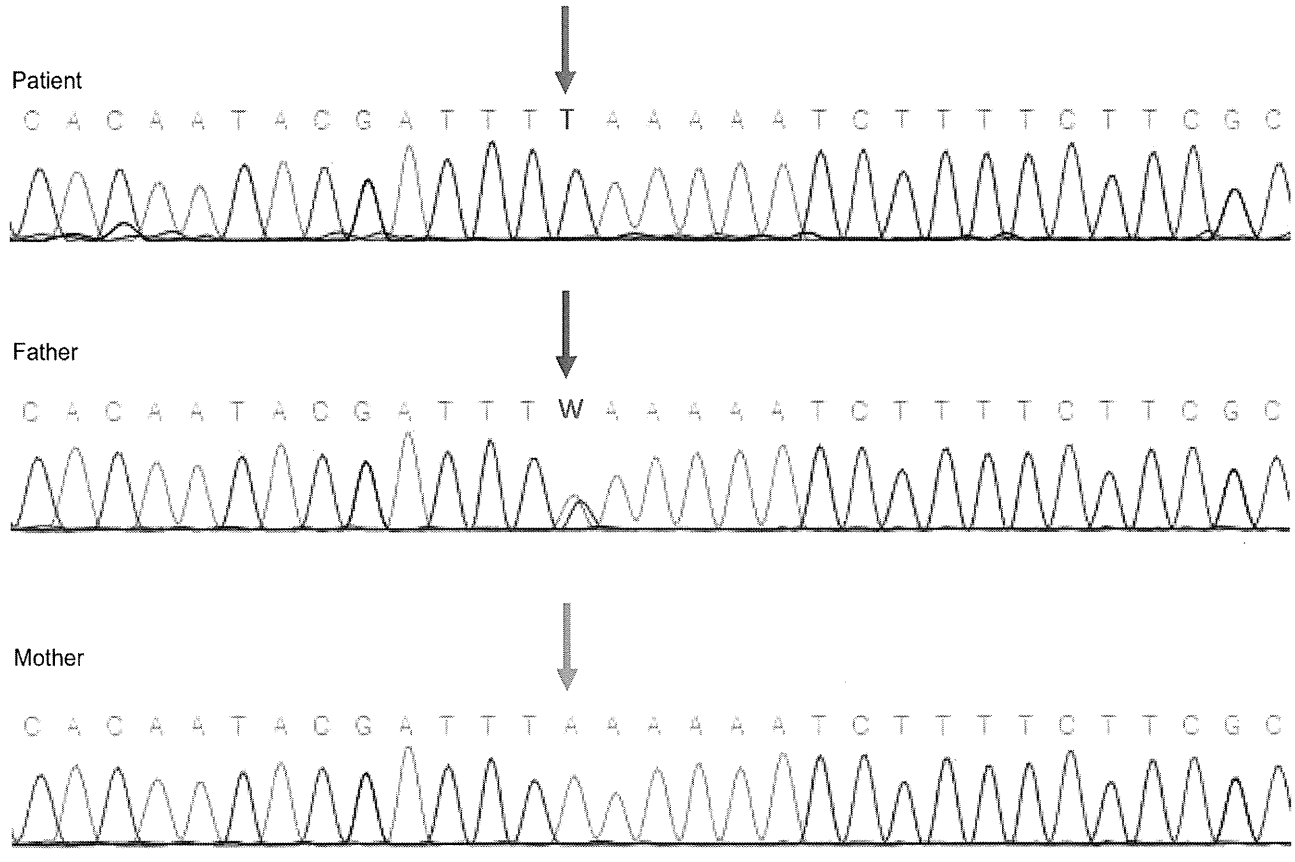

Fig. 2. Direct sequencing of the entire coding region, including exon-intron boundaries, of *ABCA12* from genomic DNA.
The previously unreported homozygous or monolithic nonsense mutation c.1216A>T in exon 11 of *ABCA12*, which results in an immediate stop codon (p.Lys406X), was present in the patient (a red arrow).
Parental segregation study showed the mutation to be present in the father heterozygously (blue arrow) but absent in the mother (green arrow).

showed the mutation to be present in the father heterozygously but absent in the mother (Fig. 2).

3.2. SNP analysis

In direct sequencing study, the patient seemed to have the homozygous nonsense mutation of *ABCA12*, although only the father, and not the mother, had that mutation. Thus, we thought that partial UPD should be considered in the present patient. Therefore, haplotype analysis was conducted on the region flanking *ABCA12* in the patient and her parents. rs1967603 in intron 8 and rs10498030 in exon 22 were found to be heterozygous in the patient (Fig. 3). This result did not completely rule out the possibility of partial paternal UPD between intron 8 and exon 22 in *ABCA12*. However, the interval between intron 8 and exon 22 is less than 35 kb (34,512 base pairs). 35 kb is thought to be too short to cause partial UPD.

3.3. Genomic DNA qPCR in exon 11 of ABCA12

Next, we suspected an exon deletion that included the exon of the p.Lys406X mutation. qPCR analysis for exon 11 of *ABCA12* using genomic DNA revealed the genomic DNA quantity of exon 11 of *ABCA12* to be decreased in the patient and her mother (Fig. 4). These results suggested the possibility of an *ABCA12* exon deletion involving exon 11 in the patient and her mother.

3.4. cDNA sequencing

We performed cDNA sequencing in the parents and a normal control individual to detect the maternal exon deletion mutation between exons 9 and 13. cDNA from exon 9 to exon 13 from the patient's mother was shorter than that of a healthy control and the patient's father (Fig. 5A). Direct sequencing of her cDNA revealed the deletion of exons 10 and 11 in cDNA (Fig. 5B), which resulted in frameshift and truncation shortly downstream from the deletion site (p.Leu355_Lys428del, Gln354fs7*). These results suggested that the *ABCA12* exon deletion involving exon 10 and exon 11, derived from mother, was inherited by the patient.

3.5. Direct sequencing of genomic DNA of ABCA12 between intron 9 and intron 11

Subsequently, we studied the deleted region in the genomic DNA of the patient and her mother. The genomic DNA region between intron 8 and exon 13 was amplified from genomic DNA by PCR. PCR amplified 12,772 bp fragments using genomic DNA

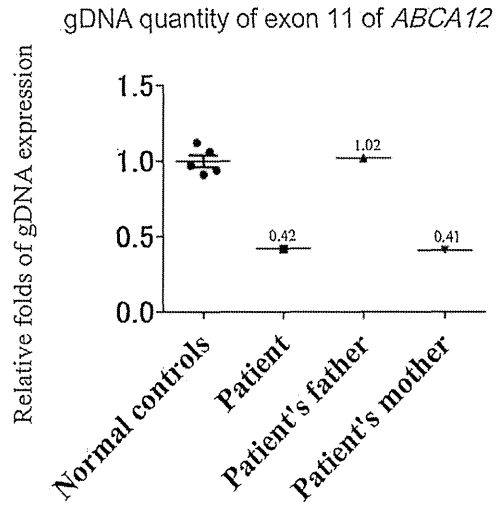

Fig. 4. qPCR analysis for exon 11 of *ABCA12* using gDNA in the patient and her mother.
The gDNA quantity in exon 11 of *ABCA12* is decreased in the patient and her mother.

samples from the patient's father and a normal control. PCR amplified about 11,000 bp fragments in the patient's mother (Fig. 6A). The deleted region was assumed to be about 1700 bp in length. The region between intron 9 and intron 11 was amplified from genomic DNA by PCR. PCR amplified 2302 bp fragments in the patient's father and a normal control. PCR amplified 2302 bp and about 600 bp fragments in the patient and the patient's mother (Fig. 6B). A direct sequence of about 600 bp fragments extracted from agarose gel revealed the deleted sequence to have a total deficiency of 1872 bp, consisting of 144 bp at the 3′ end of intron 9; the entire sequences of exon 10, intron 10 and exon 11; and 462 bp at the 5′ end of intron 11 (Fig. 6C). From the findings described above, the patient was confirmed to be compound heterozygous for two *ABCA12* mutations: c.1216A>T (p.Lys406X) and g.111346_113217del1872 (p.Leu355_Lys428del, Gln354fs7*) (Fig. 7). Conventional Sanger sequencing methods would easily have led us to misunderstand the patient as being homozygous for the point mutation.

4. Discussion

In the present case, the entire coding region, including the exon–intron boundaries, of *ABCA12* were amplified from genomic DNA by PCR, and direct sequencing revealed the previously unreported homozygous or monolithic mutation c.1216A>T in exon

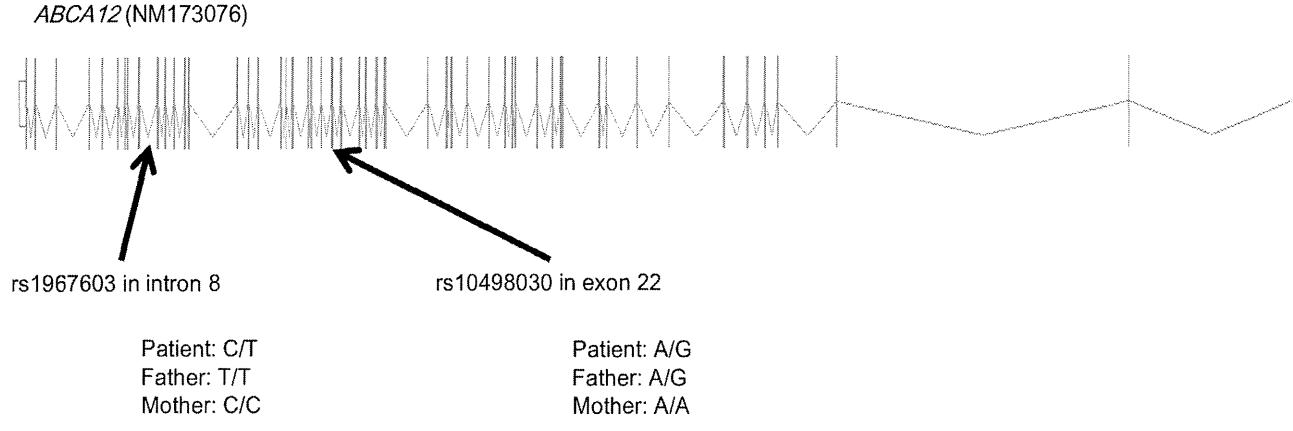

Fig. 3. Analysis of SNPs in *ABCA12* in the patient and her parents.
The patient is heterozygous for rs1967693 in intron 8 and rs10498030 in exon 22 of *ABCA12*.

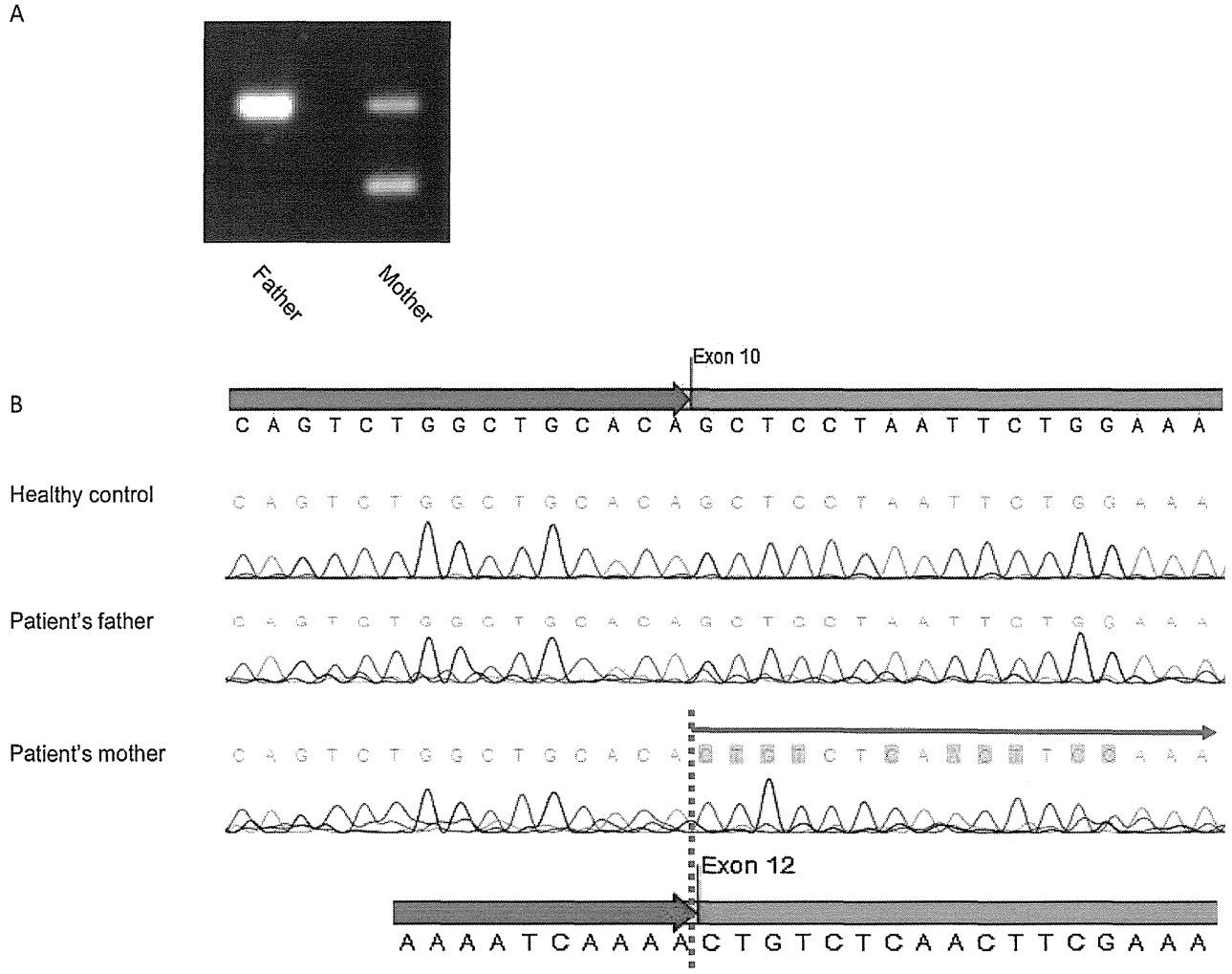

Fig. 5. Analysis of ABCA12 cDNA in the patient's mother.
(A) Short *ABCA12* cDNA was detected in the patient's mother, but not in the patient's father.
(B) Direct sequencing of the patient's mother's short cDNA revealed deletion of exons 10 and 11.

11 of *ABCA12*, resulting in an immediate stop codon (p.Lys406X). Parental segregation study showed the mutation to be present in the father heterozygously, but not in the mother.

Thus, presence of partial UPD was suspected and, in the present case, we performed SNP analyses in order to exclude the possibility of UPD. UPD is an exceptional event leading to the non-Mendelian inheritance of autosomal recessive disorders. The concept of UPD was first introduced in 1980 by Engel [9] to explain the paradoxical inheritance of two copies of a mutant allele from one parent only. Since then, a growing number of examples of UPD resulting in homozygosity of an autosomal recessive mutation have been described [10]. Chromosome 2 has been shown to be one of the hotspots for UPD, resulting in a number of recessive diseases [10], and *ABCA12* is in chromosome 2. Actually, one case of HI caused by complete UPD has been reported [11]. The results of haplotype analysis in our case could not completely rule out the possibility of partial paternal UPD between intron 8 and exon 22 in *ABCA12*; the interval is less than 35 kb. In ABC families, a 4400-Mb-long partial UPD case was reported in the *ABCA4* gene in a patient with Stargardt disease [12]. However, there have been no reports of HI caused by partial UPD. Sasaki et al. reported that the rate of partial UPD in general populations is estimated to be one per 3806 chromosome pairs (0.026%) [13]. As for a size of partial UPD, a case of 14 kb-sized small partial UPD was reported in chromosome 4 [14]. However, in chromosome 2 in which ABCA12exists, reported partial UPD cases seems to have at least 100 kb-sized partial UPD [15]. Thus, the 34-kb interval in the present case is too short to have caused partial UPD.

Deletion mutations of large genomic regions involving several exons in the heterozygote state are not revealed by conventional molecular analysis including Sanger sequencing. This combination of mutations may lead us to conclude incorrectly that the patient has a homozygous point mutation, a phenomenon called "apparent homozygosity [7]". Apparent homozygosity has been reported mainly in the cystic fibrosis transmembrane conductance regulator gene which causes cystic fibrosis [7,16–18]. To date, cases of apparent homozygosity have been reported in a small number of other molecules/diseases, lysosomal enzyme acid alpha-glucosidase which causes glycogen storage disease type 2 [19], arylsulfatase B which causes mucopolysaccharidosis [20], gamma-sarcoglycan which causes gamma-sarcooglycanopathy [21], and glucocerebrosidase which causes Gaucher disease [22]. Thus, in the present case, we suspected an exon deletion involving exon 11 of *ABCA12*, where the one detected mutation existed, although there have been no reported cases of apparent homozygosity in dermatology to our knowledge.

We performed qPCR for the genomic DNA sequence of exon 11 in *ABCA12* to detect an exon deletion involving exon 11. We performed cDNA analysis by RT-PCR and confirmed an exon deletion involving exon 10 and exon 11. Finally, we used sequence analysis of genomic DNA to specify the region of deletion as being a total deficiency of 1872 bp, consisting of 144 bp at the 3′ end of

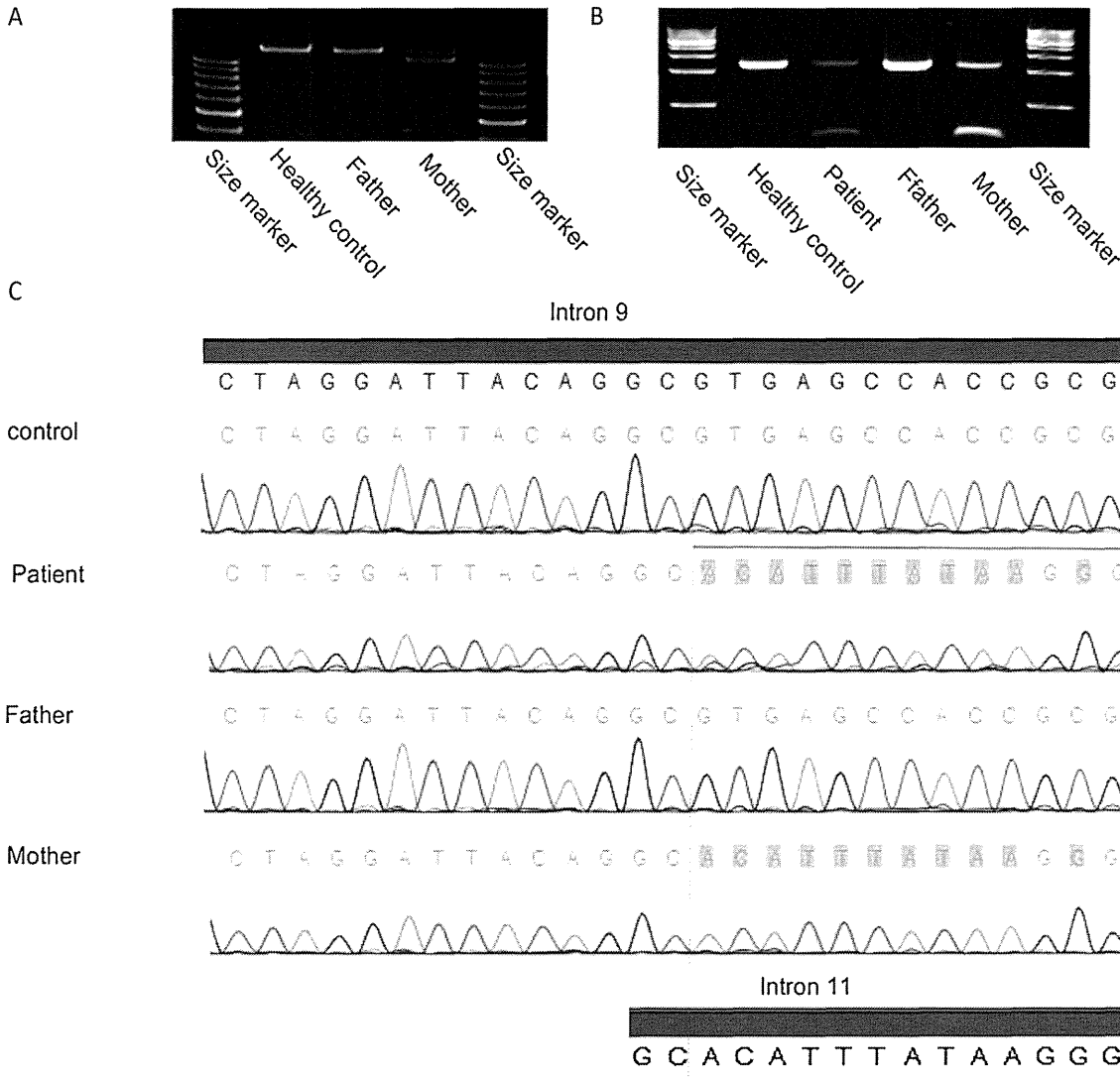

Fig. 6. The range of deletion in gDNA.
(A) The region between intron 8 and exon 13 was amplified from genomic DNA by PCR. PCR amplified 12,772 bp fragments in the patient's father and a normal control. In contrast, PCR amplified 11,000 bp fragments in the patient's mother.
(B) The region between intron 9 and intron 11 was amplified from genomic DNA by PCR. PCR amplified 2302 bp fragments in patient's father and a normal control. In contrast, PCR amplified about 2302 bp and about 600 bp fragments in the patient and patient's mother
(C) Direct sequence of about 600 bp fragments extracting from agarose gel revealed deficiency in 144 bp in the 3′ end of intron 9, exon10, intron10, exon11 and 462 bp in the 5′ end of intron 11 (total 1872 bp deficiency).

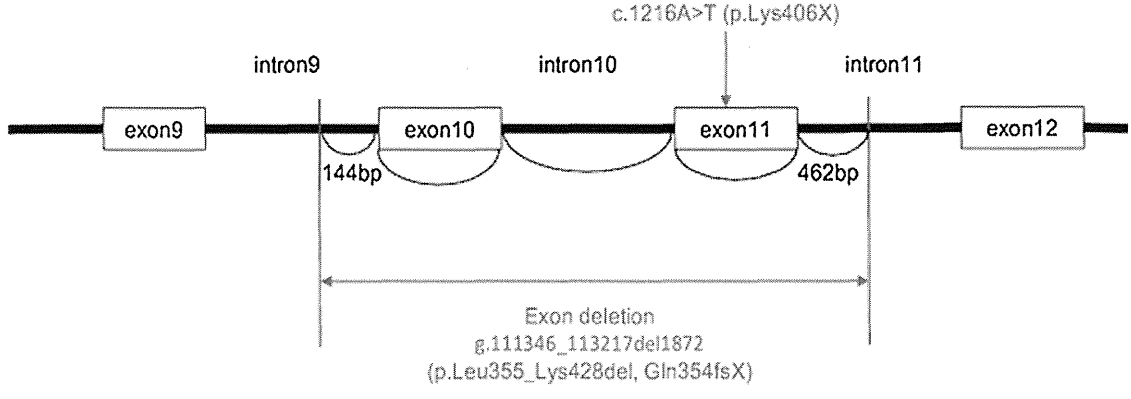

Fig. 7. The domain structure of *ABCA12* around the mutation site.
The patient was confirmed to be compound heterozygous for two overlapping *ABCA12* mutations: c.1216A>T (p.Lys406X) and g.111346_113217del1872 (p.Leu355_Lys428del, Gln354fs7*). Conventional Sanger sequence methods would easily lead one to mistake the patient as being homozygous for the point mutation, as phenomenon we call "false homozygosity".

intron 9, the entire sequences of exon 10, intron 10 and exon 11, and 462 bp at the 5′ end of intron 11.

In conclusion, it is very difficult to make a precise genetic diagnosis when an exon deletion mutation overlaps the site of another causative point mutation. In such cases, we are prone to mistake the patient as homozygous for the point mutation from the results of direct sequencing, a phenomenon called "apparent homozygosity." Our case is the first reported case of apparent homozygosity in the dermatology field to our knowledge.

In diseases for which no founder mutation has been reported, such as HI, patients in non-consanguineous families rarely have homozygous mutations. When direct sequencing indicates that a patient from a non-consanguineous family has an apparently homozygous non-founder point mutation, the homozygosity may be "apparent homozygosity", and we should keep in mind the possibility of not only UPD, but also of overlapping exon deletion mutation.

Conflict of interest

The authors declare no conflict of interest.

Funding source

None.

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Letter to the Editor

Striate palmoplantar keratoderma: Report of a novel *DSG1* mutation and atypical clinical manifestations

Keywords
Striate palmoplantar keratoderma
Desmoglein 1

Dear Editor

Striate palmoplantar keratoderma (SPPK; OMIM #148700) is a rare autosomal dominant genodermatosis that is clinically characterized by linear hyperkeratosis on the volar aspects of the fingers with focal thickening of the palmoplantar skin [1]. SPPK is caused by heterozygous mutations in the genes encoding desmoglein 1 (*DSG1*), desmoplakin (*DSP*) or keratin 1 (*KRT1*) [1]. Although these proteins are abundantly expressed by keratinocytes on the whole body, the lesions of SPPK are usually confined to the palms and soles [1]. Since only 24 genetically confirmed cases of SPPK have been reported [1–3], however, the clinical features remain poorly understood. Here, we report a case of SPPK carrying a novel *DSG1* mutation and showing atypical clinical manifestations.

A 27-year-old Japanese man presented with a more than 20-year history of hyperkeratotic skin lesions on the palmoplantar regions. The patient had no other significant past medical history. Physical examination revealed focal areas of marked hyperkeratosis with fissures on the palms and soles, especially on the weight-bearing areas of the soles (Fig. 1a). Striate and longitudinal hyperkeratotic bands were noted on the palmar aspects of all fingers (Fig. 1b). Moreover, the patient showed hyperkeratotic erythematous lesions on the dorsal aspects of the fingers and big toes (Fig. 1c). Palmar hyperlinearity was also noted (Fig. 1d), but no other skin, hair, nail, dental or cardiac abnormalities were found. He had a positive family history, as five other individuals in the family – his grandfather, father, uncle, elder sister and younger brother – reportedly had similar skin lesions, suggesting that the disease was inherited in an autosomal dominant manner. Histology of the hyperkeratotic lesion sampled from his right palm showed marked orthohyperkeratosis, papillomatosis, acanthosis and widened intercellular spaces between keratinocytes in the stratum spinosum and granulosum (Fig. 1e). From these findings, a diagnosis of SPPK was highly suspected. However, involvement of the dorsal aspects of the fingers and big toes was unusual and atypical for SPPK. To confirm the diagnosis, mutation analysis was performed. The patient gave written informed consent for mutation analysis in compliance with the *Declaration of Helsinki Principles*. The study was approved by the local Committee of Medical Ethics. Genomic DNA was obtained from peripheral blood using the QIAamp DNA Blood Maxi Kit (Qiagen, Maryland, USA). All exons and exon-intron boundaries of *DSG1*,*DSP* and *KRT1* were sequenced (primer sequences are available upon request), which led to the identification of a novel heterozygous 1-bp deletion mutation *c.587delT* in exon 6 of *DSG1* (Fig. 2). This mutation introduces a premature termination codon (PTC) at 3 codons downstream (*p.Phe196Serfs*3*). Sanger sequencing confirmed that the mutation was absent in 50 control individuals. No pathogenic mutations were identified in either *DSP* or *KRT1*. Thus, the diagnosis of SPPK was confirmed.

In this study, we identified a novel frameshift mutation in *DSG1*. To our knowledge, this is the first mutation identified in SPPK in an Asian population except for Pakistani. To date, 19, 4 and 1 mutations in *DSG1*, *DSP* and *KRT1*, respectively, have been identified as causes of SPPK [1–3]. The previously reported mutations in *DSG1* include 8 nonsense, 6 frameshift and 5 splice-site mutations [1–3]. Of these, all of the nonsense and frameshift mutations are predicted to be loss-of-function, since they cause a PTC, while mRNA variants resulting from most of the splice-site mutations remain undetermined. The *DSG1* mutation identified here yields a PTC, presumably causing degradation of the mutant mRNA by nonsense-mediated mRNA decay and haploinsufficiency of DSG1. DSG1 binds to Erbin and decreases signaling through the RAS/MAPK pathway to promote stratification and differentiation of keratinocytes [4]. Notably, palmoplantar keratoderma is frequently found in RASopathies, which are a group of genetic diseases involving the RAS/MAPK pathway [5]. Therefore, haploinsufficiency of DSG1 is highly likely to cause SPPK via elevated Ras activity [5].

To date, only two cases with SPPK showing involvement of non-palmoplantar skin have been reported in the English literature [1,6]. Milingou et al. reported a 12-year-old Libyan patient who showed hyperkeratotic plaques on the knees, ankles and the dorsa of the digits [6]. Zamiri et al. reported a 40-year-old Scottish

Abbreviations: DSG1, desmoglein 1; DSP, desmoplakin; FLG, filaggrin; KRT1, keratin 1; PTC, premature termination codon; SAM syndrome, severe dermatitis, multiple allergies and metabolic wasting syndrome; SPPK, striate palmoplantar keratodermaa.

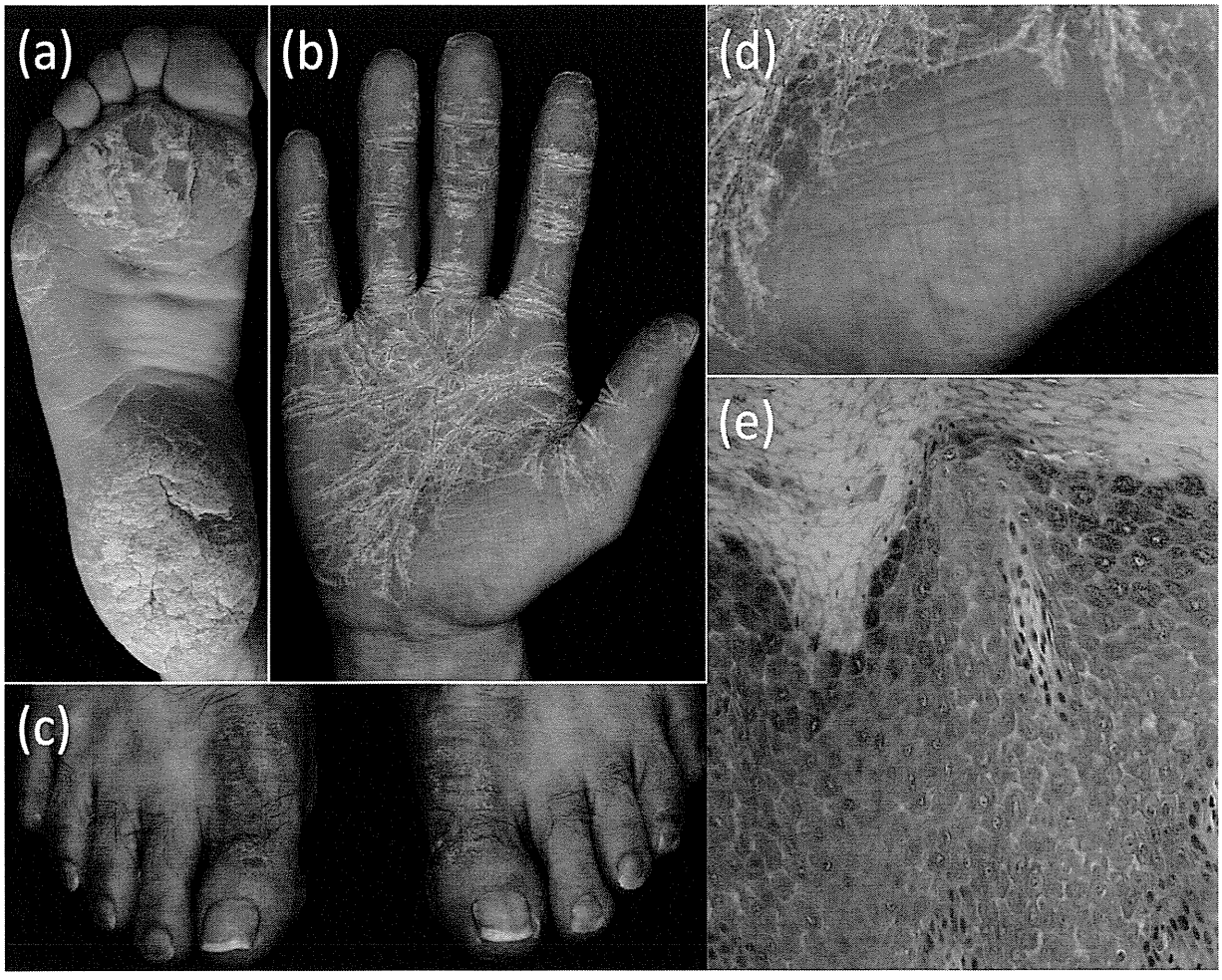

Fig. 1. (a) Thick cracked calluses on the soles, especially on the weight-bearing areas. (b) Marked hyperkeratosis on the palms. Linear hyperkeratosis along the flexor aspect of the fingers, with particular prominence on the creases of the palms, is also noted. (c) Erythema and hyperkeratosis on the dorsal aspect of the big toes extending to the feet. (d) Increased palmar markings on the thenar eminence. (e) The stratum granulosum and spinosum show widened intercellular spaces (hematoxylin and eosin, original magnification ×200).

patient who exhibited mild hyperkeratosis on the knees [1]. Both patients carry a heterozygous loss-of-function mutation in *DSG1*; the former carries *c.121insT* (*p.Trp41Leufs*10*) and the latter *c.430A>T* (*p.Arg144Ter*) [1,6]. Notably, our patient, who is heterozygous for *c.587delT* in *DSG1*, also exhibited non-pruritic hyperkeratotic lesions on the dorsal skin of the fingers and big toes. Given that the lesions have existed since childhood without remission and that DSG1 is expressed in the epidermis of the

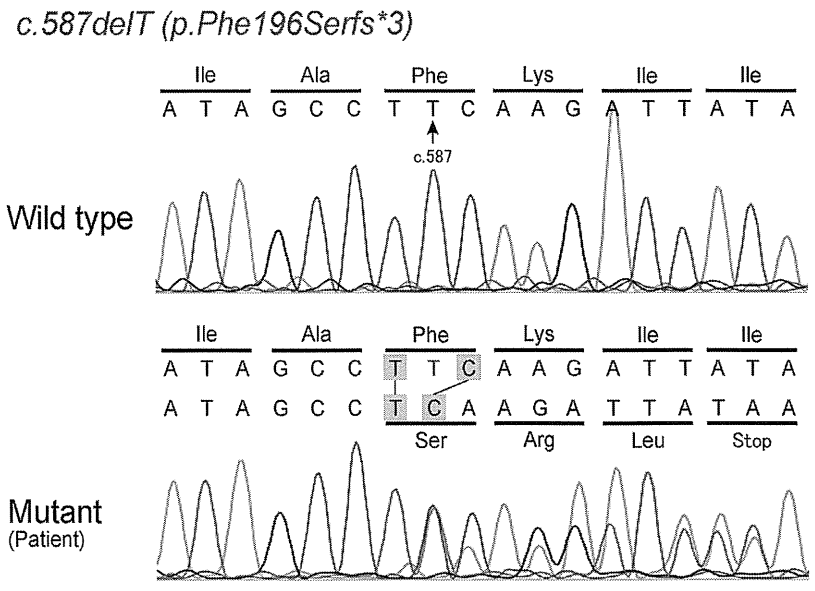

Fig. 2. The patient is heterozygous for a frameshift mutation c.587delT in exon 6 of DSG1, which results in a premature termination codon at 3 amino acids downstream of the mutation (*p.Phe196Serfs*3*).

whole body, we presume that the lesions might be part of the clinical features of SPPK. This speculation should be verified histologically in future study, since no skin biopsy was performed from the non-palmoplantar lesions in our case, nor in the above two cases. However, the widespread involvement of non-palmoplantar skin in severe dermatitis, multiple allergies and metabolic wasting syndrome (SAM syndrome), which is caused by homozygous null mutations in *DSG1* [7], and pemphigus foliaceus, which is caused by autoantibody against DSG1 [8], may support our presumption that SPPK can affect non-palmoplantar skin.

Palmar hyperlinearity was also observed in the present case. Hyperlinearity of the palms is known to be indicative of mutations in the gene encoding filaggrin (*FLG*) that cause ichthyosis vulgaris and predispose to atopic dermatitis [9]. However, the patient has never suffered from either disease. Moreover, we sequenced all coding regions of *FLG* as described previously [10], but the patient does not harbor any *FLG* null mutations. These facts collectively suggest there might be the possibility that palmar hyperlinearity is a feature that is also associated with SPPK, although such an association has not been reported. Further studies are warranted to validate this finding.

In conclusion, we report the identification of a novel frameshift mutation in *DSG1* and atypical clinical features in a patient with SPPK, which would expand the mutational and phenotypic spectrum of the disease. Our results indicate that hyperkeratotic lesions on non-palmoplantar skin and palmar hyperlinearity might be observed in a patient with SPPK due to a *DSG1* mutation.

Conflicts of interest

None declared.

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Serum thymus and activation-regulated chemokine (TARC/CCL17) levels reflect the disease activity in a patient with bullous pemphigoid

Editor

Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by eosinophilia and high serum IgE levels. It is thought to be a Th2-dominant inflammatory skin disease. Thymus and activation-regulated chemokine (TARC)/CCL17 is a Th2 chemokine. We report a BP patient who showed high levels of serum TARC/CCL17 that correlated with the disease activity.

A 66-year-old man presented with bullous eruptions on his palms. A month later, new blisters developed on nearly the whole body. He was initially given 0.25 mg/kg/day oral prednisone, increased to 0.5 mg/kg/day, in a dermatology clinic. However, the bullous lesions spread over the entire body, and he was referred to our hospital.

On admission, he presented bullae and erythema on almost the whole body surface (Fig. 1a) without mucosal involvement. Skin biopsy showed subepidermal blisters with dermal inflammatory cell infiltration (Fig. 1b). Direct immunofluorescent examination showed the linear deposition of IgG and C3 along the basement membrane zone. Laboratory findings revealed a white blood cell count of 16 300/μL (normal range: 3800–8500), eosinophils of 3.0% (1.0–6.0), CRP of 3.52 mg/dL (<0.30), IgE of 10 419 IU/mL (<202) and LDH of 184 IU/L (119–229). The serum anti-BP180 antibody level was 1420 index (<9). According to these findings, he was diagnosed with BP. We sequentially measured serum levels of TARC/CCL17 by enzyme-linked immunoassay (Allerport TARC, Shionogi, Tokyo, Japan). TARC/CCL17 was elevated to 14 200 pg/mL. Computed tomography and gastrointestinal endoscopy showed no internal malignancy.

Although the oral prednisone dosage was increased to 1.0 mg/kg/day, new blisters continued to form. Cyclosporin at 200 mg (3.3 mg/kg/day) was added, and the disease activity was controlled successfully. He was discharged with 0.33 mg/kg/day prednisone and cyclosporin at 200 mg/day. The serum TARC/CCL17 was decreased to 210 pg/mL. When the dosage of prednisone was tapered to 0.17 mg/kg/day, a few bullae appeared on the extremities within 2 months after discharge. At this time, the serum TARC/CCL17 was elevated to 10 870 pg/mL. The anti-BP180 antibody level did not change (96 index). We increased the prednisone dosage to 0.25 mg/kg/day, and blister formation ceased. Then, the TARC/CCL17 level gradually decreased. We chronologically analysed the disease activity, serum TARC/CCL17 levels, eosinophil counts, IgE and anti-BP180 antibody levels during the patient's disease course (Fig. 2). Serum TARC/CCL17 level fluctuated in parallel with disease activity as well as with eosinophil counts.

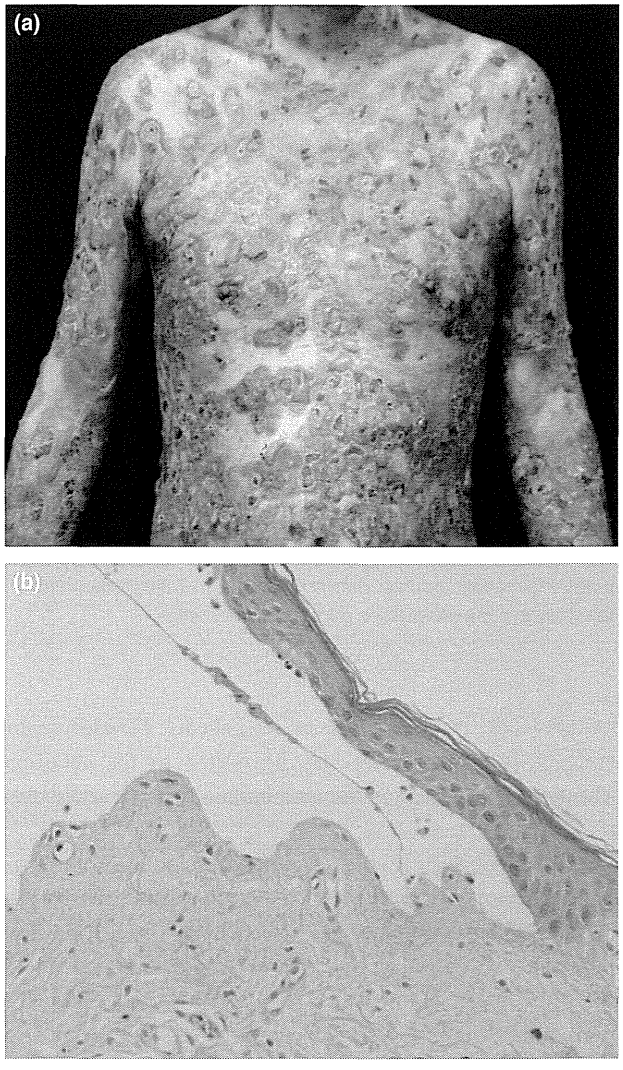

Figure 1 (a) Clinical features upon hospital admission. Blistering, eczematous exanthema is seen on the chest and upper extremities. (b) Histological image of a skin biopsy specimen from the edge of a blister (haematoxylin & eosin ×200). A subepidermal bulla is found with upper dermal inflammatory infiltrates.

TARC/CCL17 is a member of the CC chemokine group. We revealed the rapid decrease of serum TARC/CCL17 levels after remission of BP in our patient. This phenomenon is very similar to that observed in patients with atopic dermatitis.[1] The pathological relation between TARC/CCL17 and BP has been suggested in a few reports.[2–4] Kakinuma *et al.*[2] reported that serum TARC/CCL17 levels in patients with BP are significantly high and that they decrease after disease remission. Serum TARC/CCL17 levels in BP patients were correlated with eosinophil counts in the peripheral blood. Their immunohistochemical studies showed infiltrating cells expressing CCR4, a receptor for

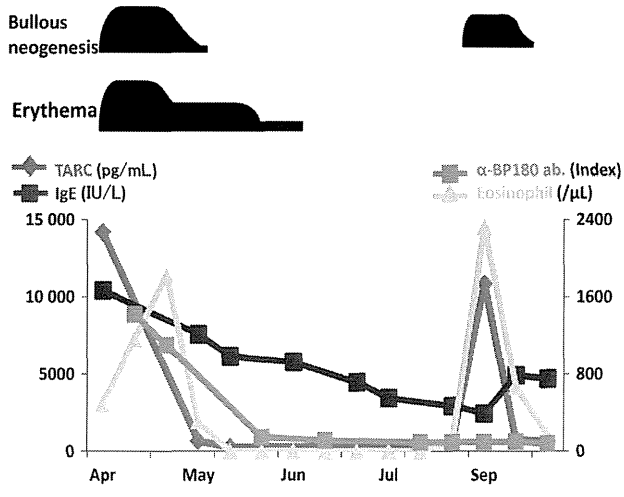

Figure 2 Chronological analysis of serum TARC/CCL17 levels and eosinophil numbers, IgE levels and anti-BP180 antibody levels during the disease course. Serum TARC/CCL17 levels decrease before any of the other three parameters decrease.

TARC/CCL17, beneath bullae of BP patients. Elevated serum levels of Th2 chemokines may be related to the abnormal activation of Th2 cells and humoral immunity in the active stage of BP.

Dahlman-Ghozlan K *et al.*[5] reported that soluble isoforms of cellular adhesion molecules such as sE-selectin and soluble vascular cell adhesion molecule-1 were elevated in BP, and that the elevated sE-selectin levels correlated with the number of blisters. Interestingly, in our patient, the serum TARC/CCL17 level decreased before decreases in any of the three other parameters: eosinophil count, serum IgE level and anti-BP180 antibody level. Further studies using more longitudinal BP sera are needed to determine whether serum TARC/CCL17 can be used as a sensitive indicator of disease activity.

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Acquired perforating dermatosis associated with sorafenib therapy

Editor

Sorafenib, approved in metastatic renal cell and hepatocellular carcinoma, inhibits Raf serine/threonine kinases, receptor tyrosine kinases and suppresses metalloproteinase-9 expression.[1,2] Skin manifestations of toxicity are heterogeneous and may occur in 91% of patients.[1]

We describe three patients with different forms of acquired perforating dermatosis (APD) which developed during *sorafenib* treatment.

Patient 1: A 49-year-old patient had a metastatic renal cell carcinoma diagnosed in 2008. He brought a bag every day, which he carried on his right shoulder. In 2011, he started *sorafenib* treatment. Two months later, he developed pruriginous umbilicated papules, some of them containing a keratotic plug, in areas of friction of the right lumbar and inguinal region (Fig. 1a). *Sorafenib* was stopped and replaced with *axitinib*. One month later, his eruption had resolved spontaneously (Fig. 1b).

Patient 2: A 75-year-old man with hepatocellular carcinoma had been treated with *sorafenib* since 2009. He had a progressively worsening cholestatic hepatitis (gamma-glutamyl transferase 1114 UI/l, total bilirubin 19 μmol/L, alkaline phosphatase 1823 UI/l) and diffuse cutaneous pruritus since the beginning of 2012. Four months later, he experienced a diffuse skin eruption, which had developed gradually over the previous 2 months (Fig. 1c). *Sorafenib* was stopped. A 50% improvement in skin lesions was observed within 1 month.

Patient 3: A 51-year-old man was diagnosed with hepatocellular carcinoma in 2011. *Sorafenib* was initiated in 2012. Four months later, he experienced a pruriginous eruption on the buttocks and thigh. The eruption had started 2 months previously and consisted of painful, hyperkeratotic, umbilicated papules, some of them containing a keratotic plug (Fig. 1d). Because of the positive therapeutic response, *sorafenib* treatment was continued. Protective measures against microtrauma including

tologist, 56% maxillofacial surgeon, 43% plastic surgeon, 34% GP, 18% specialist nurse, 15% multidisciplinary team, 5% gynaecologist, 23% another specialist, 3% no current specialists. Patient-desired changes included improved support from healthcare professionals (32%), additional treatment options (27%), improved access to healthcare services (20%) and improved support for patient groups (13%).

This study represents the largest national survey of GS patients to date. Our findings corroborate previous studies that suggest problems relating to BCCs and OKTs impact most upon quality of life and that GS is associated with emotions including depression (up to 50% patients), frustration, fear and anxiety.[4,5]

In summary, patients perceive that primary care physicians and the general public have a poor knowledge of GS. GS and associated treatments impacts markedly upon psychosocial functioning, which should also be considered by the diverse array of specialists responsible for the holistic management of this group of patients.

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Anti-transcription intermediary factor 1-γ antibody-positive clinically amyopathic dermatomyositis complicated by interstitial lung disease and breast cancer

Editor

A 63-year-old Japanese woman noted facial erythema, and, around the same time, a mass in her left breast (Fig. 1), which was diagnosed as breast cancer. Subsequently, she underwent partial excision of the left breast and sentinel lymph node biopsy, which confirmed early-stage breast cancer. A therapeutic regimen of two courses of adriamycin and cyclophosphamide chemotherapy, in addition to trastuzumab therapy was conducted. Thereafter, radiation therapy at 50 Gy was administered to the left axilla. The patient remained disease-free at the 3-year follow-up. One year after the onset of facial erythema, the patient began to experience general fatigue that continued for 1.5 years. She was then referred to our clinic. At the first visit, she showed Gottron's sign and facial erythema (Fig. 2). No muscle weakness was detected by manual muscle testing. She had no significant medical history or a family history of rheumatic disease. Laboratory parameters were as follows: white blood cells, 3300/μL; platelets, 157 000/mm; C-reactive protein (CRP), 0.1 mg/dL; creatine kinase (CK), 47 IU/L; aldolase, 3.9 IU/L; serum myoglobin, 32 ng/mL; KL-6, 304 U/mL. Serum antinuclear antibody test results were positive (×40; speckled pattern). Using an enzyme-linked immunosorbent assay (ELISA) that we developed, serum anti-TIF1-γ antibodies, but not anti-melanoma differentiation-associated gene 5 (MDA5) antibodies, were detected.[1] No serum anti-aminoacyl transfer RNA synthetase (ARS) antibodies were detected using a commercial ELISA kit (MBL, Nagoya, Japan). Based on these findings, the patient was diagnosed with clinically amyopathic dermatomyositis (CADM). She was prescribed topical tacrolimus for the skin lesions. At a follow-up visit 3 months after her first visit, her serum KL-6 level had increased to 2326 U/mL (Fig. 2). High-resolution computed tomography (CT) of the chest showed a slight ground glass pattern bilaterally at the lung bases, which was suspected to be an early sign of interstitial lung disease (ILD). However, she had no respiratory symptoms and the CT findings were not pathognomonic. Thus, follow-up periodic blood tests and chest CT scans were conducted for 1 year without administering any treatment. The serum KL-6 levels gradually decreased, although they were still above the normal limit (>1000 U/mL). Serum surfactant protein-D levels were persistently high. Serum CK, aldolase and CRP levels were within the normal ranges. Restricted areas showing ground glass patterns were detected at

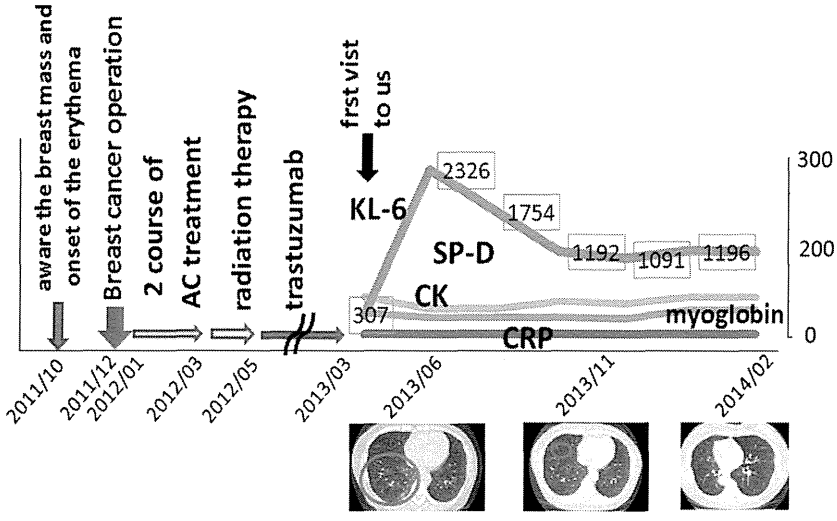

Figure 1 Clinical course of the patient and associated computed tomography (CT) imaging. Three months after her first visit to our hospital, the KL-6 level had increased to 2326 U/mL and a chest CT image showed ILD, although she had no respiratory symptoms. The serum KL-6 level was persistently high, but no remarkable change was observed on CT. Areas showing ground glass patterns are indicated by red circles. Creatine kinase, aldolase and C-reactive protein levels remained low.

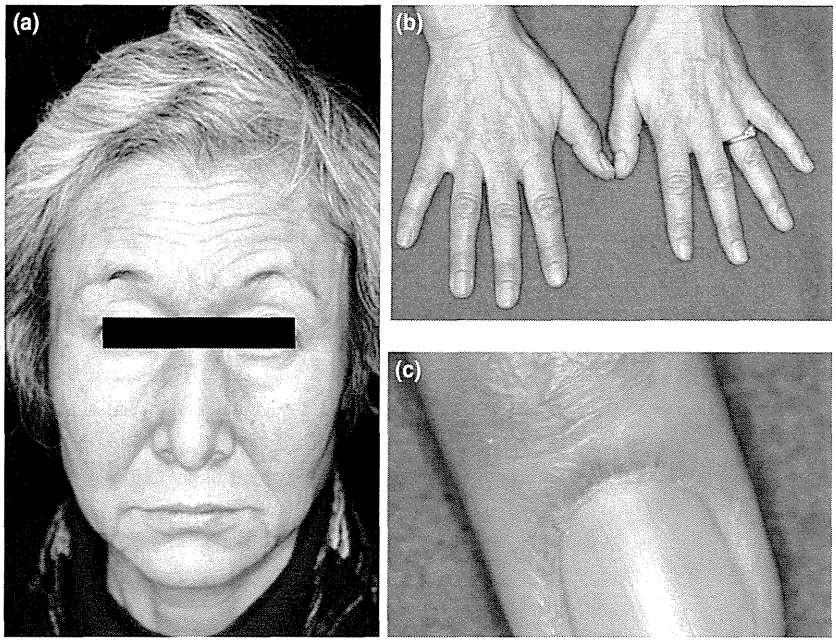

Figure 2 The patient's clinical features. The patient showed facial erythema (a), Gottron's sign (b) and periungual capillary dilation (c).

various sites on all follow-up chest CT images (Fig. 1). Overall, the ILD remained stable. The patient's cutaneous symptoms were exacerbated by sunlight exposure. Nearly half of all dermatomyositis (DM) cases are complicated by ILD, which is one of the most important prognostic factors despite appropriate treatment, making it difficult to predict the prognosis of each patient. Accumulation of data on the correlations between patient prognosis and myositis-specific autoantibody positivity, such as anti-ARS and anti-MDA5 antibodies, may to some extent allow us to predict the course of ILD in DM patients.[2] The presence of anti-ARS and anti-MDA5 antibodies is often associated with recurrent ILD and acute refractory ILD respectively. Anti-TIF1-γ antibodies are associated with cancer or juvenile DM. However, a few reports on the clinical features of anti-TIF1-γ-positive DM including ILD have been published.[3] The prognosis of ILD in anti-TIF1-γ-positive DM patients should be clarified in the near future. Drug-induced ILD can be induced by trastuzumab or other anti-neoplastic agents.[4] Therefore, we cannot deny the possibility of drug-induced ILD in the patient. To our knowledge, this is the first detailed report of the clinical course of a patient with anti-TIF1-γ autoantibody-positive CADM, complicated by ILD and breast cancer. The findings of this case suggest that ILD in a patient with anti-TIF1-γ autoantibody-positive CADM can be managed without aggressive immunosuppressive treatment.

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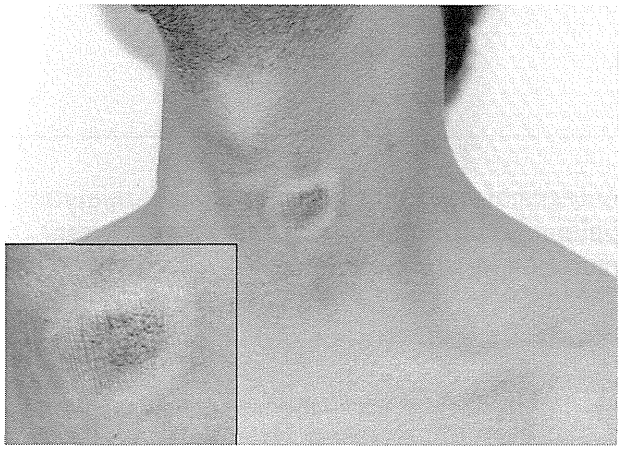

Figure 1 Pigmented macule of the anterior aspect of the neck surrounded by a halo-phenomenon.

Lichen sclerosus mimicking halo-naevus comedonicus

Editor

Lichen sclerosus (LS) is a chronic inflammatory skin disease, which more frequently involves females in the genital area. Extragenital LS is less common than the genital variant. We reported a particular extragenital LS mimicking a halo-naevus comedonicus.

A 50-year-old man presented with a pigmented lesion of the anterior aspect of the neck of 1 year duration. At examination, the lesion was a well-defined plaque, 4 × 3 cm in diameter, with multiple comedones developed on a light brown pigmented macule. The pigmented area was delineated by a surrounding white ring without comedones (Fig. 1). The lesion was moderately indurated on palpation. The patient was free of any other skin lesion, no vitiligo or other halo-phenomenon was noticed. No topical treatment was applied. The patient was healthy and did not report any other disease or medication intake. The histological examination from a biopsy taken in the comedones bearing area showed a dilated pore (open comedone) associated with a dense compact infiltrate of corneocytes with hyperkeratosis of the walls of the follicular channel (Fig. 2a). A compact orthohyperkeratosis of the stratum corneum was found, thicker than the atrophic underlying epidermis (Fig. 2a–b). The papillary dermis was moderately oedematous in some areas associated with a moderate inflammatory infiltrate (Fig. 2c), and homogenized and sclerotic in others areas (Fig. 2a). A lichenoid infiltrate was found in localized areas (Fig. 2c). Thus, the diagnosis of lichen sclerosus mimicking a halo-naevus comedonicus in an adult patient was made. The patient denied any further investigation and was lost of follow-up.

LS is most common on the neck, the shoulders and the upper part of the trunk.[1] It is generally asymptomatic but pruritus may be present. Our patient was asymptomatic. Extragenital LS generally appears as white or ivory multiple macules associated with skin atrophy. The lesion of the present patient was particular as the peripheral ivory ring was delineating a central slight brown

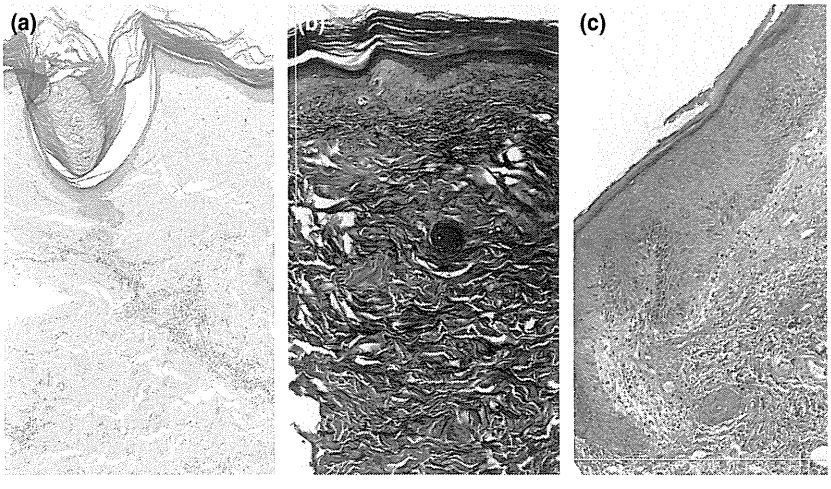

Figure 2 (a) Histology of a comedone-like structure, with homogenized papillary dermis and atrophic epidermis with orthohyperkeratosis (haematin and eosin staining, original magnification ×400). (b) Densification of the papillary dermis, with horizontalization of the elastic fibres (orcein staining, original magnification ×400). (c) Oedema and moderate inflammatory infiltrate of the papillary dermis associated with dermal-epidermal lichenoid pattern (orcein staining, original magnification ×400).

Research letter

Autosomal dominant diffuse nonepidermolytic palmoplantar keratoderma due to a recurrent mutation in aquaporin-5

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Dear Editor, Autosomal dominant diffuse nonepidermolytic palmoplantar keratoderma (NEPPK; MIM 600231) is a clinically and genetically heterogeneous disorder, one form of which is associated with a whitish spongy appearance upon immersion in water. Using linkage data in combination with whole-exome sequencing in families with NEPPK, heterozygosity for five different missense mutations in *AQP5* (encoding aquaporin-5) was identified recently in affected members of seven Swedish families, three British families and a Scottish family.[1,2] All the mutations segregated with disease in the respective families and were not found in the dbSNP or 1000 Genomes Project databases. A further gain-of-function mutation in *AQP5* was subsequently reported in a large NEPPK pedigree of Chinese Han descent.[3]

Aquaporins are cell membrane channels that conduct water or sugar alcohol molecules (aquaglyceroporins).[4] The protein family member aquaporin-5 is predominantly expressed in epithelial cells, such as in the lung and cornea, and helps

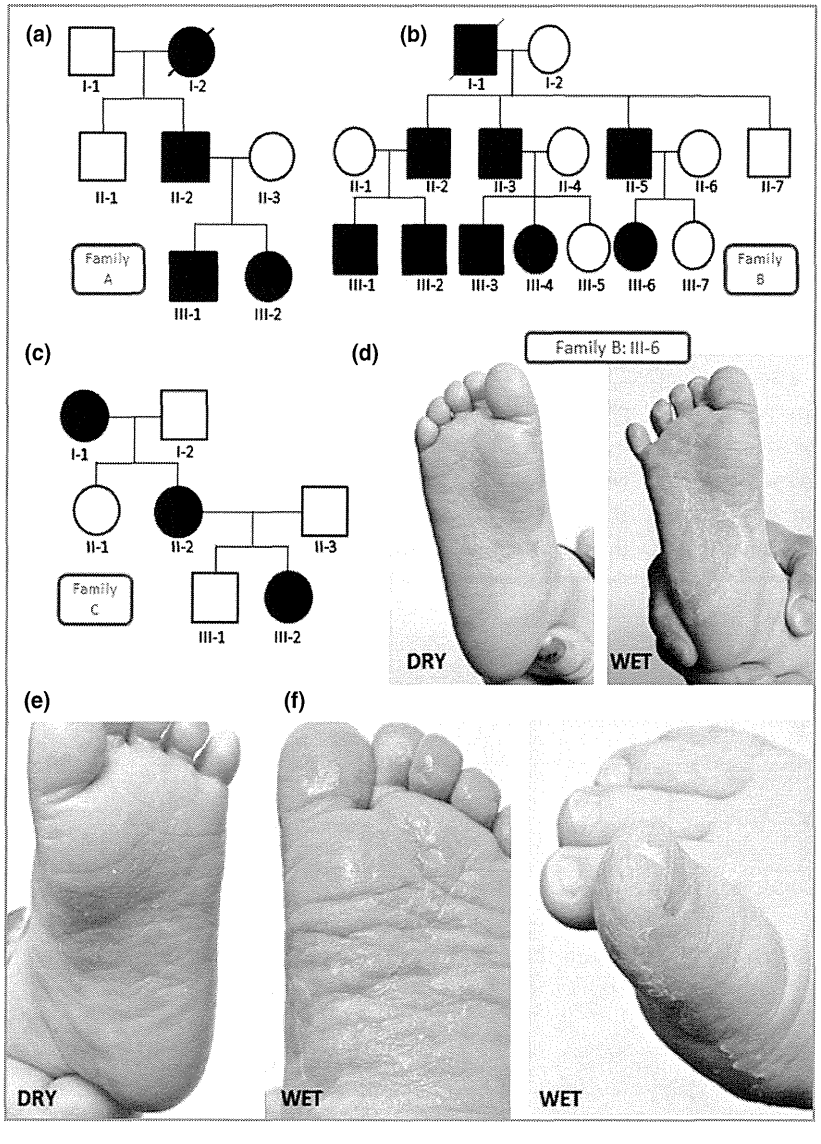
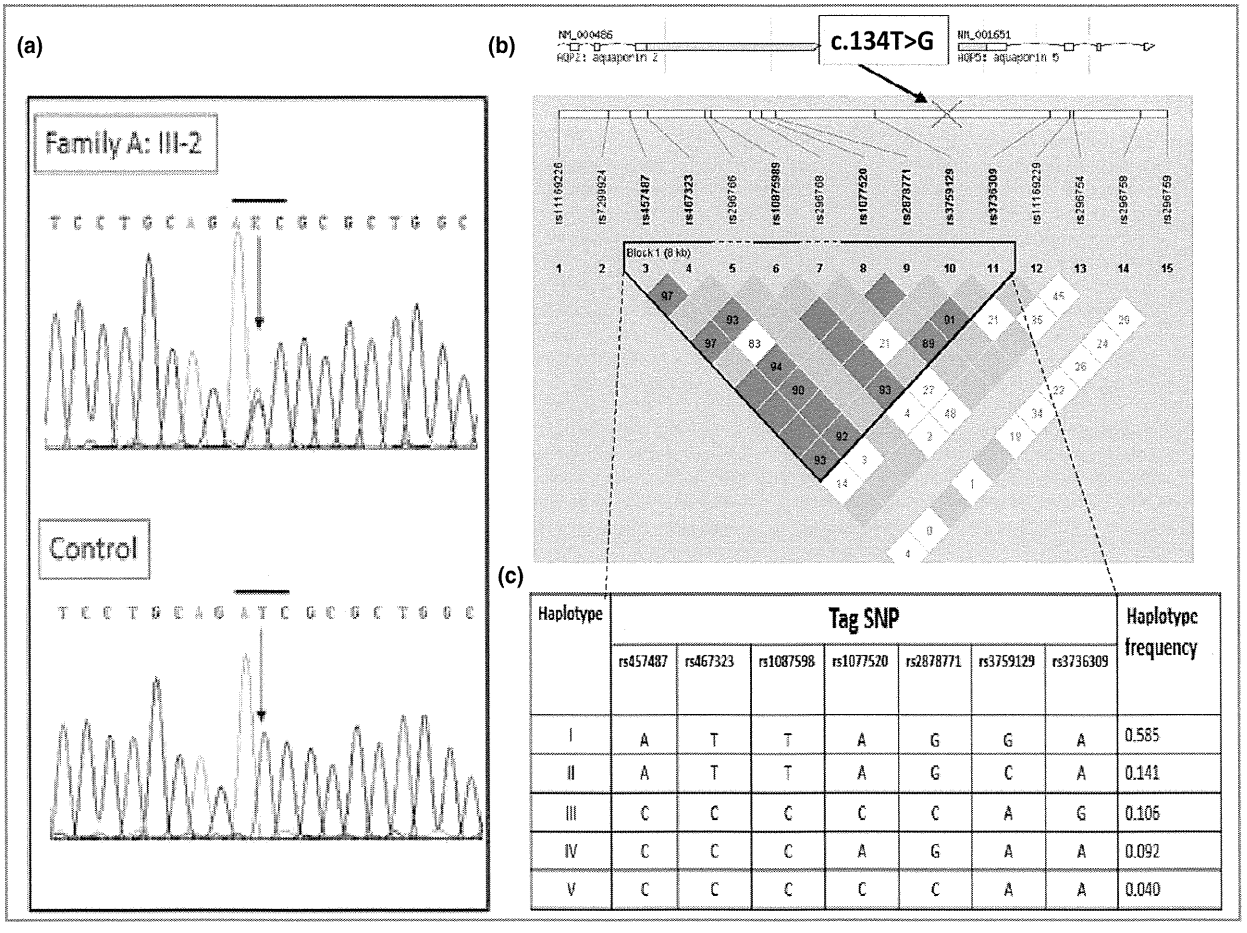


Fig 1. Pedigrees and clinical features. (a) Pedigree of Family A; (b) pedigree of Family B; (c) pedigree of Family C; (d) clinical illustration of the sole of the right foot in individual III-6 from Family B before and after immersion in water for 10 min; (e) further clinical illustration from this individual's right foot illustrating the diffuse keratoderma and (f) the subsequent maceration and white spongy appearances after water exposure.

regulate water excretion from exocrine glands including salivary, lacrimal and sweat glands.[5] The *AQP5* mutation studies also indicated a significant role in palmoplantar skin with increased water uptake through the plasma membrane of keratinocytes in the granular layer. Skin immunohistochemistry revealed that mutant aquaporin-5 retained the ability to traffic to the plasma membrane (gain-of-function).[1] The mutations in *AQP5* also led to an increase in acetylated alpha-tubulin in patient palmar skin compared with control, suggesting increased levels of microtubule stabilization in this type of NEPPK.[1]

We now report three further NEPPK pedigrees of white British descent with NEPPK. Three seemingly unrelated autosomal dominant pedigrees are depicted in Figure 1a–c. Typically, affected individuals developed mild palmoplantar keratoderma and acral desquamation during the first year of life, sometimes as young as 3 months (Fig. 1d). Each subject experienced increased sweating at acral sites and developed prominent white spongy plaques following ~ 5 min immersion in water (Fig. 1d and e). The keratoderma was of varying severity (mild to severe) but diffuse and yellowish with a well-demarcated inflammatory and sometimes papular erythematous margin. Extension of the hyperkeratosis onto the dorsae of the fingers and toes was noted in some cases. Most affected individuals reported frequent secondary dermatophyte infections, which responded to oral terbinafine.

Following informed consent, genomic DNA from affected individuals in all three pedigrees was used for polymerase chain reaction and Sanger sequencing of the coding exons and flanking introns of *AQP5*. Surprisingly, initial screening of an affected individual from each pedigree identified the same heterozygous mutation, c.134T>G (p.Ile45Ser) in exon 1 (Fig. 2a). We then confirmed segregation of the mutation with phenotype in the respective families. The mutation p.Ile45Ser has been reported previously in two other British families with NEPPK, suggesting that this mutation is recurrent within the British population.[1] These two families originated from London/Essex (personal communication, Professor Edel O'Toole, March 2015) and the three pedigrees in our report all emanate from the south of England, collectively suggesting the possibility of a shared ancestral mutant allele. We therefore constructed linkage disequilibrium (LD) blocks containing *AQP5* using genotype data from the HapMap database (Fig. 2b).[6] The haplotype structure with its tag-single nucleotide polymorphisms (SNPs) was determined using Haploview (Fig. 2c).[7] We genotyped seven tag-SNPs using Sanger sequencing. In all subjects from our three pedigrees with NEPPK, the locus containing the *AQP5* mutation c.134T>G had



Haplotype	Tag SNP							Haplotype frequency
	rs457487	rs467323	rs1087598	rs1077520	rs2878771	rs3759129	rs3736309	
I	A	T	T	A	G	G	A	0.585
II	A	T	T	A	G	C	A	0.141
III	C	C	C	C	C	A	G	0.106
IV	C	C	C	A	G	A	A	0.092
V	C	C	C	C	C	A	A	0.040

Fig 2. Sanger sequencing and haplotype analysis. (a) Sanger sequencing identifies a recurrent heterozygous missense mutation in *AQP5*, c.134T>G (p.Ile45Ser). (b) *AQP5* structure and the linkage disequilibrium block around *AQP5* were evaluated using genotype data from the HapMap database. (c) The haplotype structure with seven tag-single-nucleotide polymorphisms (SNPs) was determined using Haploview.

an identical haplotype I (ATTAGGA), thus implying the likelihood of a common founder mutation that probably also extends to the already reported individuals with this mutation.[1] Collectively, our findings add further support to mutations in *AQP5* being responsible for this particular subtype of NEPPK and also have implications for the optimal initial genetic screening of other British individuals with this clinical diagnosis.

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Letter to the Editor

Bi-allelic nonsense mutations in *ABHD5* underlie a mild phenotype of Dorfman-Chanarin syndrome

Keywords
Dorfman-Chanarin syndrome
ABHD5

Dear Editor,

Dorfman-Chanarin syndrome (DCS) (neutral lipid storage disease with ichthyosis) is an autosomal recessive disorder [1,2]. Nearly all cases present with skin manifestations of moderate to severe congenital ichthyosiform erythroderma (CIE). Other organs might be affected in the form of fatty liver, myopathy, hearing loss, sub-capsular cataract and mental retardation. Since 2001, ~30 mutations in *ABHD5* (also termed *CGI-58*), which encodes αβ hydrolase domain-containing 5 (ABHD5), have been found in DCS [3]. Here, we report compound heterozygous nonsense mutations in *ABHD5* (p.Arg234* and p.Arg280*) associated with particularly mild DCS that has implications for genotype–phenotype correlation.

The patient is a 9-year-old Chinese girl. She was born by Caesarean section. From the age of 2–3 months, she developed dry skin. She has no family history of consanguinity or skin disorders. On examination, she has generalized scaling with erythroderma on her face, trunk and extremities (Fig. 1a–c). She had no pruritus and no hair or nail abnormalities were observed. There was neither apparent cognitive impairment nor evidence of growth retardation. The patient had no subjective symptoms of muscle weakness, ear anomalies, hearing involvement, or cataract. We were not able to perform further investigations including peripheral blood film, lipid profile, cataract screening, electrocardiogram and echocardiogram. Routine serum biochemistry showed the following abnormal parameters: AST, 75 IU/L (normal range, 0–41); ALT, 88 IU/L (normal range, 0–45); LDH, 287 IU/L (normal range, 100–250). We checked titers of the anti-viral antibodies to rule out viral hepatitis caused by hepatitis type A, B, C, herpes simplex, cytomegalovirus and Epstein–Barr virus. All the antibodies were negative. Four months later, these mildly elevated enzymes spontaneously reverted to normal. Light microscopy of a lesional skin revealed marked hyperkeratosis with only a small number of parakeratotic cells. Intra-cytoplasmic lipid droplets within epidermal keratinocytes were not observed.

Following informed consent, and in accordance with the Declaration of Helsinki principles, genomic DNA from the patient was used for whole-exome sequencing analysis, using methodology described elsewhere [4]. In the patient, compound heterozygous *ABHD5* mutations were identified: a previously reported nonsense mutation c.700C > T (p.Arg234*) in exon 5 (Fig. 1d) and a novel nonsense mutation c.838C > T (p.Arg280*) in exon 6 (Fig. 1e) (GenBank accession no. NM_016006). Neither mutation was detected in DNA from 674 normal control individuals.

Most of the molecular pathology in DCS involves truncation mutations in *ABHD5*, although 8 missense mutations also have been reported (www.hgmd.cf.ac.uk). Of note, compound heterozygosity for two mutations c.700C > T (p.Arg234*) and c.245A > G (p.His82Arg) was previously reported in 42-year-old man with CIE, muscle weakness (including elevated creatine phosphokinase), progressive hearing loss and bilateral sub-capsular cataracts, although liver enzymes were normal [5]. With regard to the more severe phenotype in that case compared to our patient, we hypothesized that the different consequences of the second mutant allele (p.Arg280* vs p.His82Arg) might relate to the differences in clinical features. However, we cannot exclude the possibility that some features related to other organ systems onset in the later stage of the present case. Thus, in DCS cases, close monitoring for systemic involvements is recommended even if their phenotypes are mild in the childhood.

At present, genotype–phenotype correlation in DCS is uncertain. Previous reports have shown that certain homozygous missense mutations in *ABHD5* (p.Gln130Pro and p.Glu260Lys) underlie severe phenotypes of DCS [3]. It is known that Gln^{130} and Glu^{260} are important residues in the interaction with adipocyte triglyceride lipase, and these interactions are important for normal physiology of the cell [6]. In addition, amino acids 69–87 in ABHD5 form a highly hydrophobic region corresponding to the lipid-binding domain of protein [7]. The present nonsense mutations p.Arg234* and p.Arg280* cause premature termination of translation and result in truncated amino acid peptides instead of the normal protein. Thus, the reported active sites within the encoded transcript may be spared if some truncated mutant protein is synthesized. Nonsense mutations may also result in RNA decay unless sited in the last exon or distal third of the penultimate exon. Given that p.Arg280* is located within exon 6 of this 7 exon gene, we hypothesize that there may be some residual ABHD5 enzyme activity in this case, which contributed to her mild DCS phenotype.

The issue of transiently elevated liver enzymes is also difficult to explain based on the mutation findings. We previously reported a

Abbreviations: DCS, Dorfman-Chanarin syndrome; CIE, congenital ichthyosiform erythroderma; ABHD5, αβ hydrolase domain-containing 5.