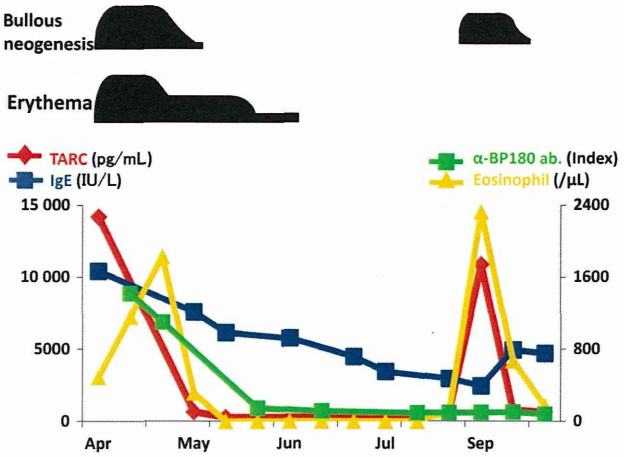

Figure 2 Chronological analysis of serum TARC/CCL17 levels and eosinophil numbers, IgE levels and anti-BP180 antibody levels during the disease course. Serum TARC/CCL17 levels decrease before any of the other three parameters decrease.

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.

R. Nin-Asai, Y. Muro,* A. Sekiya, K. Sugiura, M. Akiyama
Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan
*Correspondence: Y. Muro. E-mail: ymuro@med.nagoya-u.ac.jp
Funding sources: This study was supported in part by Grant-in-Aid for Scientific Research A 23249058 (to M.A.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1 Kakinuma T, Nakamura K, Wakugawa M *et al.* Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001; **107**: 535–541.
2 Kakinuma T, Wakugawa M, Nakamura K, Hino H, Matsushima K, Tamaki K. High level of thymus and activation-regulated chemokine in blister fluid and sera of patients with bullous pemphigoid. *Br J Dermatol* 2003; **148**: 203–210.
3 Saeki H, Tamaki K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J Dermatol Sci* 2006; **43**: 75–84.
4 Echigo T, Hasegawa M, Shimada Y, Inaoki M, Takehara K, Sato S. Both Th1 and Th2 chemokines are elevated in sera of patients with autoimmune blistering diseases. *Arch Dermatol Res* 2006; **298**: 38–45.
5 Dahlman-Ghozlan K, Heilborn JD, Stephansson E. Circulating levels of soluble E-selectin, ICAM-1 and VCAM-1 in bullous pemphigoid during low-dose methotrexate therapy. A prospective study. *Exp Dermatol* 2000; **9**: 336–340.

DOI: 10.1111/jdv.12719

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

Journal of Dermatological Science 80 (2015) 196–202

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

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

Akitaka Shibata[a], Kazumitsu Sugiura[a], Atsushi Suzuki[b], Takashi Ichiki[b], Masashi Akiyama[a,*]

[a] Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[b] Department of Pediatrics, Kainan Hospital, 396 Minami-Honda, Maegasu-cho, Yatomi 498-8502, Japan

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 20 September 2015
Accepted 1 October 2015

Keywords:
ABCA12
Exon deletion
Partial paternal uniparental disomy of a chromosome
Apparent homozygosity

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

* Corresponding author. Fax: +81 52 744 2318.
E-mail address: makiyama@med.nagoya-u.ac.jp (M. Akiyama).

http://dx.doi.org/10.1016/j.jdermsci.2015.10.003
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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

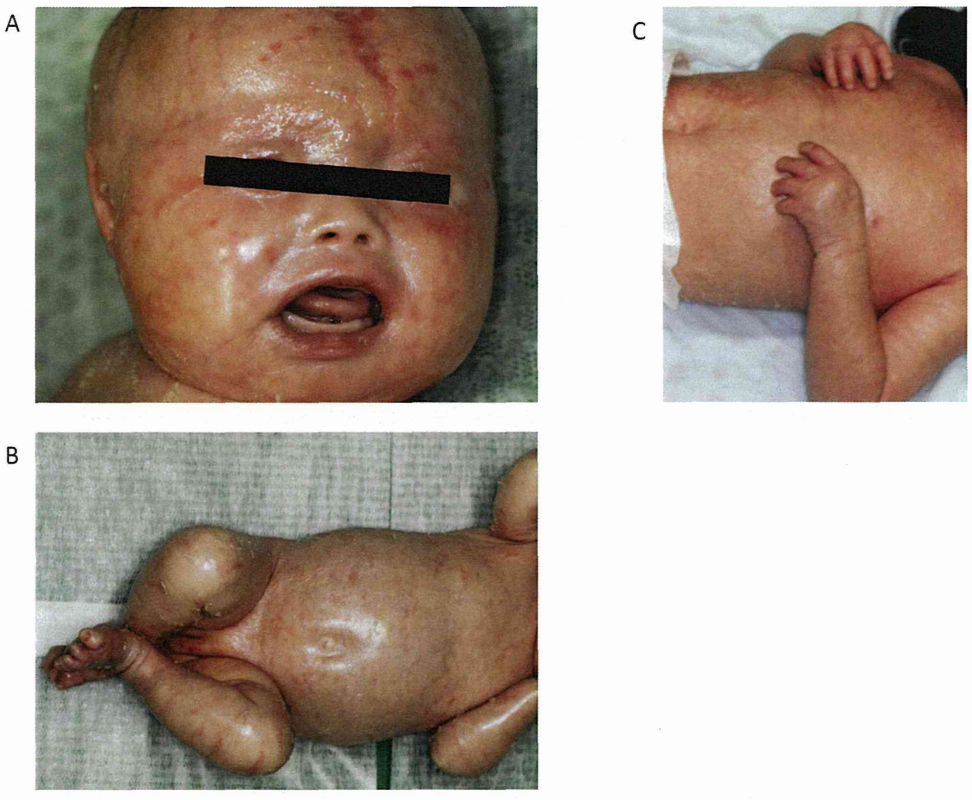

Fig. 1. The clinical features of the patient.
(A, B) At birth, she is seen to have thick plate-like scales with deep fissuring overlying erythrodermic skin. Severe eclabium and ectropion are seen.
(C) At a year and a half in age, she shows generalized erythroderma with desquamative scaling.

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′- TGGCCAGCAACTTGTCAATA).

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′-ATAGTACACATAGAAAAGGCAAGTGCT and 3′-ATAACTATTGTTAGCCATTCCCTTATAAAT).

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′-ACTCCTGCAGTCCACAATACG 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

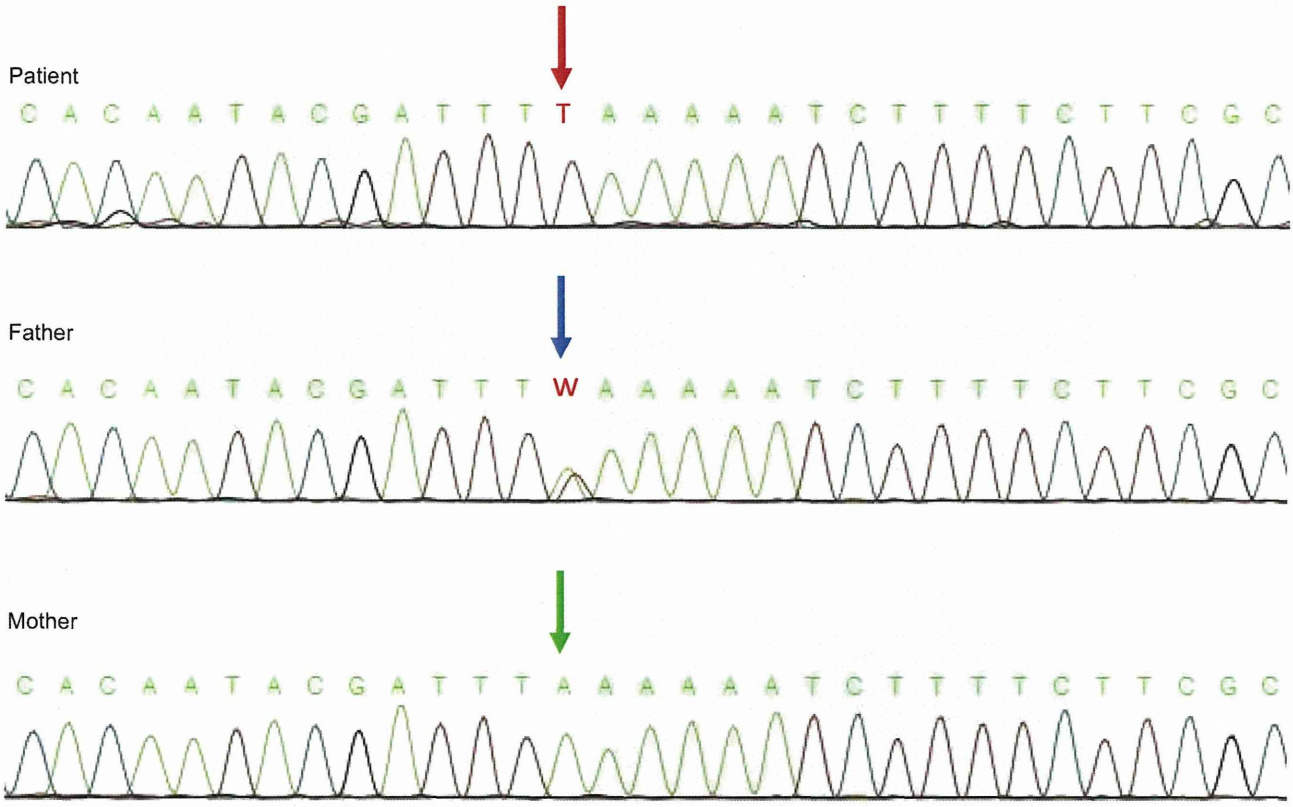

Fig. 2. Direct sequencing of the entire coding region, including exon-intron boundaries, of *ABCA12* from genomic DNA.
The previously unreported homozygous or monolithic nonsense mutation c.1216A>T in exon 11 of *ABCA12*, which results in an immediate stop codon (p.Lys406X), was present in the patient (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

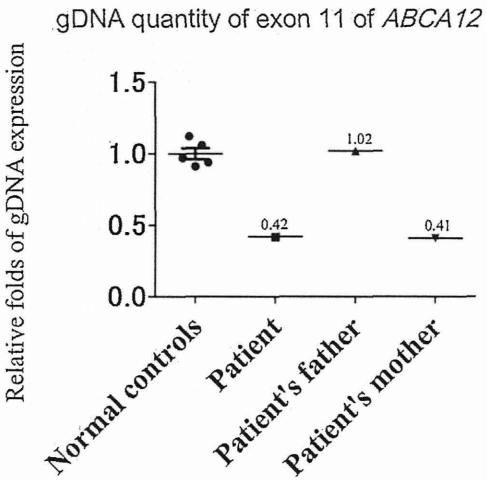

Fig. 4. qPCR analysis for exon 11 of *ABCA12* using gDNA in the patient and her mother.
The gDNA quantity in exon 11 of *ABCA12* is decreased in the patient and her mother.

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

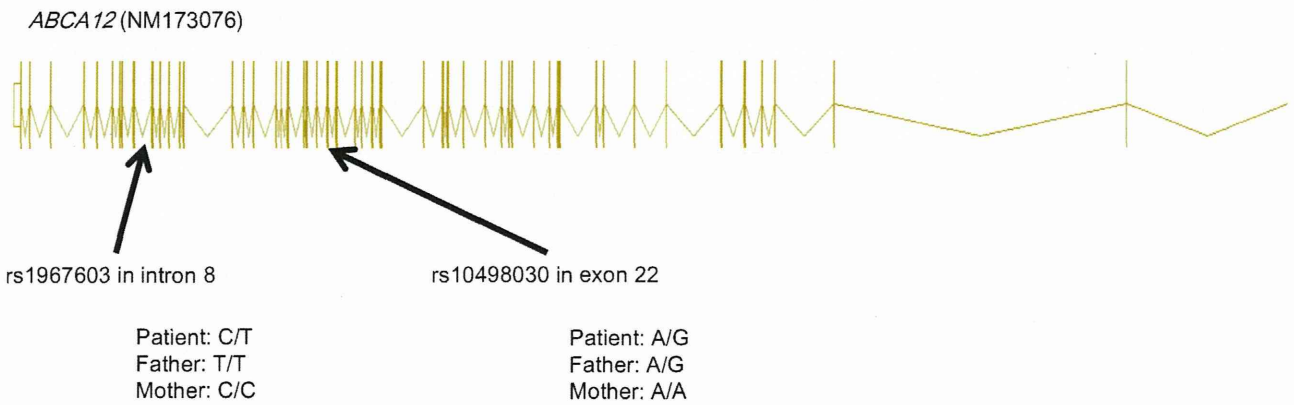

Fig. 3. Analysis of SNPs in *ABCA12* in the patient and her parents.
The patient is heterozygous for rs1967693 in intron 8 and rs10498030 in exon 22 of *ABCA12*.

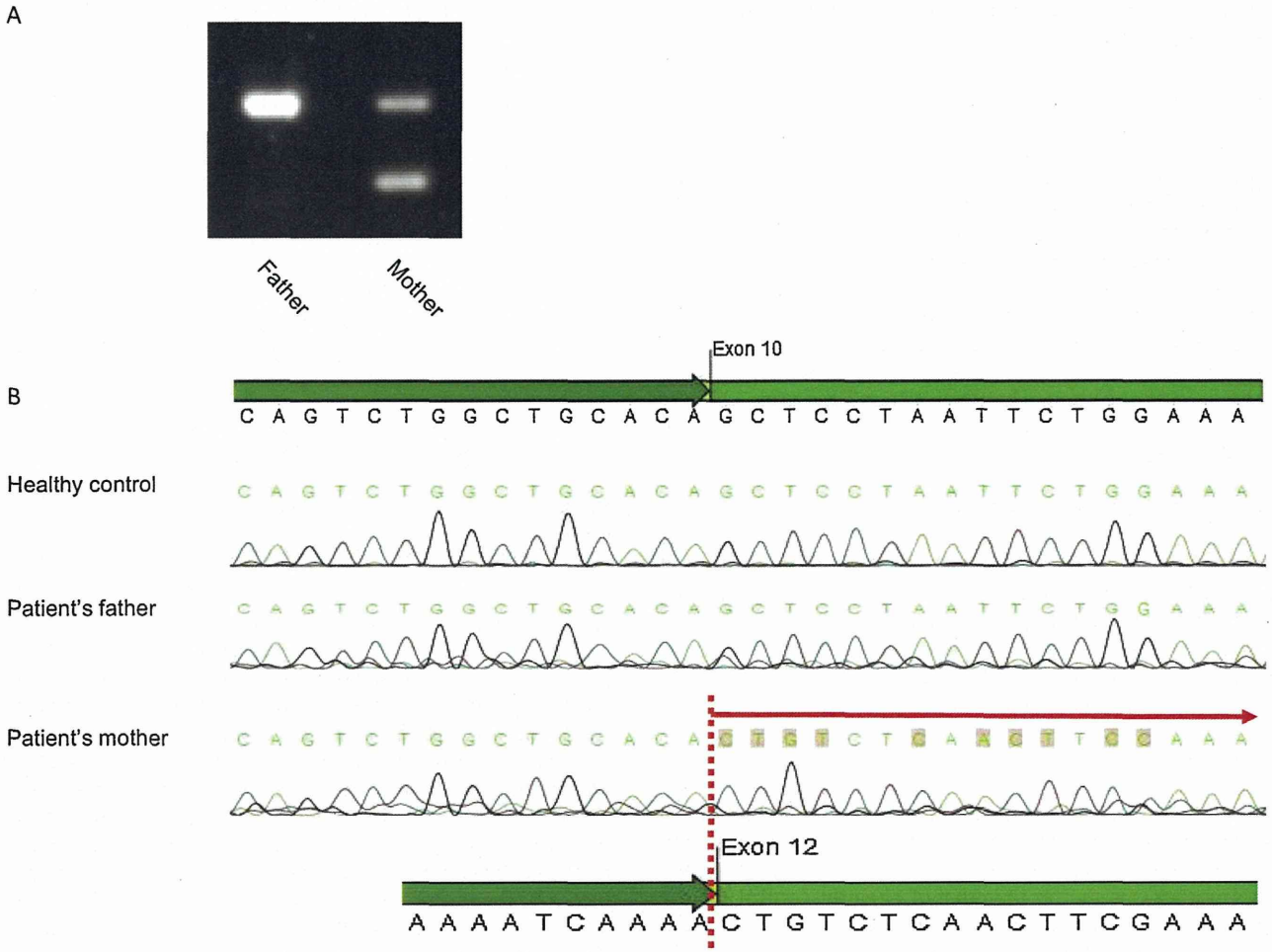

Fig. 5. Analysis of ABCA12 cDNA in the patient's mother.
(A) Short *ABCA12* cDNA was detected in the patient's mother, but not in the patient's father.
(B) Direct sequencing of the patient's mother's short cDNA revealed deletion of exons 10 and 11.

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

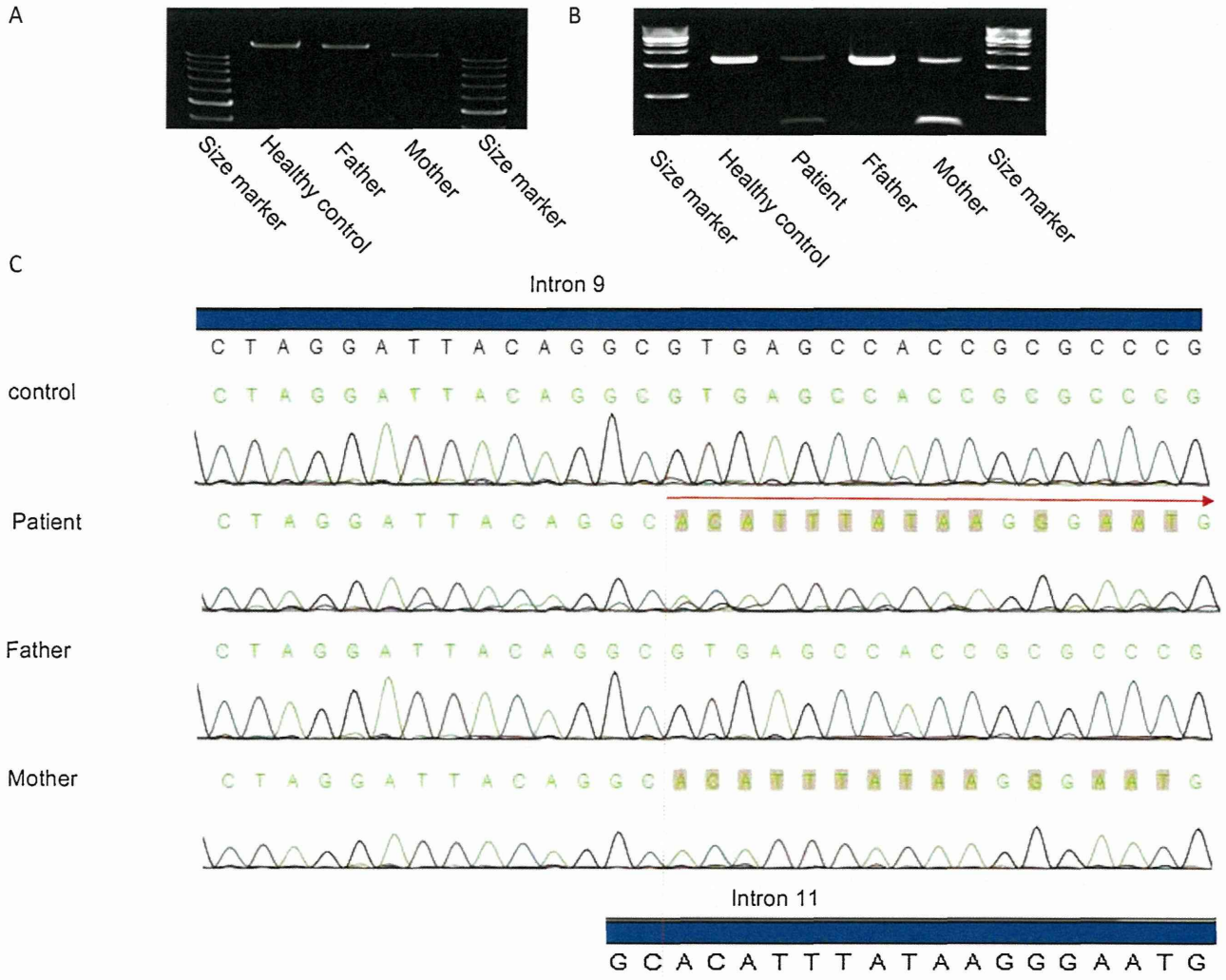

Fig. 6. The range of deletion in gDNA.
(A) The region between intron 8 and exon 13 was amplified from genomic DNA by PCR. PCR amplified 12,772 bp fragments in the patient's father and a normal control. In contrast, PCR amplified 11,000 bp fragments in the patient's mother.
(B) The region between intron 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).

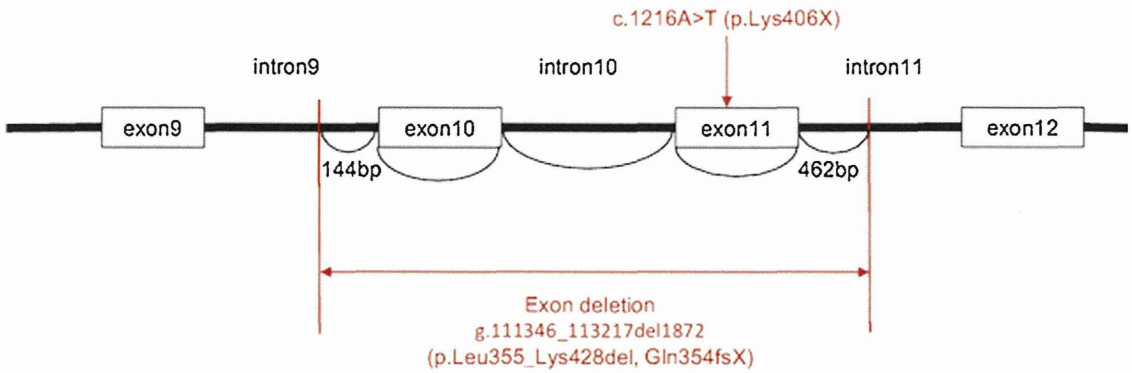

Fig. 7. The domain structure of *ABCA12* around the mutation site.
The patient was confirmed to be compound heterozygous for two overlapping *ABCA12* mutations: c.1216A>T (p.Lys406X) and g.111346_113217del1872 (p.Leu355_Lys428del, Gln354fs7*). Conventional Sanger sequence methods would easily lead one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

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research (B) to M.A. (15H04887) and a Grant-in-Aid for Challenging Exploratory Research to M.A. (15K15415), from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by a grant from the Ministry of Health, Labor and Welfare of Japan (Health and Labor Sciences Research Grants; Research on Intractable Diseases; H22-Nanchi-Ippan-177) to M. Akiyama.

References

[1] M. Akiyama, H. Shimizu, An update on molecular aspects of the non-syndromic ichthyoses, Exp. Dermatol. 17 (2008) 373–382.
[2] M. Akiyama, Y. Sugiyama-Nakagiri, K. Sakai, J.R. McMillan, M. Goto, K. Arita, et al., Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer, J. Clin. Invest. 115 (2005) 1777–1784.
[3] D.P. Kelsell, E.E. Norgett, H. Unsworth, M.T. Teh, T. Cullup, C.A. Mein, et al., Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis, Am. J. Hum. Genet. 76 (2005) 794–803.
[4] T. Annilo, S. Shulenin, Z.Q. Chen, I. Arnould, C. Prades, C. Lemoine, et al., Identification and characterization of a novel ABCA subfamily member, ABCA12, located in the lamellar ichthyosis region on 2q34, Cytogenet. Genome Res. 98 (2002) 169–176.
[5] P. Borst, R.O. Elferink, Mammalian ABC transporters in health and disease, Annu. Rev. Biochem. 71 (2002) 537–592.
[6] K. Sakai, M. Akiyama, Y. Sugiyama-Nakagiri, J.R. McMillan, D. Sawamura, H. Shimizu, Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes, Exp. Dermatol. 16 (2007) 920–926.
[7] F.M. Hantash, A. Rebuyon, M. Peng, J.B. Redman, W. Sun, C.M. Strom, Apparent homozygosity of a novel frame shift mutation in the CFTR gene because of a large deletion, J. Mol. Diagn. 11 (2009) 253–256.
[8] T. Takeichi, K. Sugiura, K. Matsuda, M. Kono, M. Akiyama, Novel ABCA12 splice site deletion mutation and ABCA12 mRNA analysis of pulled hair samples in harlequin ichthyosis, J. Dermatol. Sci. 69 (2013) 259–261.
[9] E. Engel, A new genetic concept: uniparental disomy and its potential effect, isodisomy, Am. J. Med. Genet. 6 (1980) 137–143.
[10] E. Engel, A fascination with chromosome rescue in uniparental disomy: Mendelian recessive outlaws and imprinting copyrights infringements, Eur. J. Hum. Genet. 14 (2006) 1158–1169.
[11] D. Castiglia, M. Castori, E. Pisaneschi, M. Sommi, C. Covaciu, G. Zambruno, et al., Trisomic rescue causing reduction to homozygosity for a novel ABCA12 mutation in harlequin ichthyosis, Clin. Genet. 76 (2009) 392–397.
[12] R. Riveiro-Alvarez, D. Valverde, I. Lorda-Sanchez, M.J. Trujillo-Tiebas, D. Cantalapiedra, E. Vallespin, et al., Partial paternal uniparental disomy (UPD) of chromosome 1 in a patient with Stargardt disease, Mol. Vis. 13 (2007) 96–101.
[13] K. Sasaki, H. Mishima, K. Miura, K. Yoshiura, Uniparental disomy analysis in trios using genome-wide SNP array and whole-genome sequencing data imply segmental uniparental isodisomy in general populations, Gene 512 (2013) 267–274.
[14] D. Wu, M. Wang, X. Wang, N. Yin, T. Song, H. Li, et al., Maternal transmission effect of a PDGF-C SNP on nonsyndromic cleft lip with or without palate from a Chinese population, PLoS One 7 (2012) e46477.
[15] C.A. Stratakis, S.E. Taymans, D. Schteingart, B.R. Haddad, Segmental uniparental isodisomy (UPD) for 2p16 without clinical symptoms: implications for UPD and other genetic studies of chromosome 2, J. Med. Genet. 38 (2001) 106–109.
[16] A. Diana, R. Tesse, A.M. Polizzi, T. Santostasi, A. Manca, G. Leonetti, et al., A large deletion causes apparent homozygosity for the D1152H mutation in the cystic fibrosis transmembrane regulator (CFTR) gene, Gene 497 (2012) 90–92.
[17] R. da Silva Martins, A.C. Fonseca, F.E. Acosta, T.W. Folescu, L.Y. Higa, I.R. Sad, et al., Severe phenotype in an apparent homozygosity caused by a large deletion in the CFTR gene: a case report, BMC Res. Notes 7 (2014) 583.
[18] V. Neocleous, P.K. Yiallouros, G.A. Tanteles, C. Costi, M. Moutafi, Apparent Homozygosity of p. Phe508del in CFTR due to a Large Gene Deletion of Exons 4–11, Case Rep. Genet. 2014 (2014) 613863.
[19] M.L. Huie, K. Anyane-Yeboa, E. Guzman, R. Hirschhorn, Homozygosity for multiple contiguous single-nucleotide polymorphisms as an indicator of large heterozygous deletions: identification of a novel heterozygous 8-kb intragenic deletion (IVS7-19 to IVS15-17) in a patient with glycogen storage disease type II, Am. J. Hum. Genet. 70 (2002) 1054–1057.
[20] G.R. Villani, M. Grosso, G. Pontarelli, A. Chierchia, R. Sessa, M. Sibilio, et al., Large deletion involving exon 5 of the arylsulfatase B gene caused apparent homozygosity in a mucopolysaccharidosis type VI patient, Genet. Test. Mol. Biomarkers 14 (2010) 113–120.
[21] K.J. Nowak, P. Walsh, R.L. Jacob, R.D. Johnsen, J. Peverall, E.M. McNally, et al., Severe gamma-sarcoglycanopathy caused by a novel missense mutation and a large deletion, Neuromuscul. Disord. 10 (2000) 100–107.
[22] M.A. Torralba, P. Alfonso, J.I. Perez-Calvo, A. Cenarro, G.M. Pastores, P. Giraldo, et al., High prevalence of the 55-bp deletion (c. 1263del55) in exon 9 of the glucocerebrosidase gene causing misdiagnosis (for homozygous N370S (c. 1226A > G) mutation) in Spanish Gaucher disease patients, Blood Cells Mol. Dis. 29 (2002) 35–40.

Acta Derm Venereol 2015; 95: 872–874

LETTER TO THE EDITOR

What are the "True" Pathogenic Anti-desmoglein Antibodies?

Yoshinao Muro, Kazumitsu Sugiura and Masashi Akiyama

Division of Connective Tissue Disease and Autoimmunity, Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. E-mail: ymuro@med.nagoya-u.ac.jp

Sir,

We read with interest the reports by Li et al. (1) and Demitsu et al. (2). The former showed that, in some populations of pemphigus vulgaris, there is a significant gap in anti-desmoglein (Dsg) 3 antibody enzyme-linked immunosorbent assay (ELISA) index as measured by conventional ELISA vs. that measured by ethylenediaminetetraacetic acid (EDTA)-treated ELISA. The latter report demonstrated that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor, but not the mature, forms of Dsg1 and Dsg3. Both studies suggest that anti-Dsg1 and Dsg3 antibodies are heterogeneous and can be categorized into pathogenic and non-pathogenic. However, even these recent methods for evaluating pathogenic anti-Dsg1/3 antibodies have not completely clarified the discrepancy between the disease activity and the ELISA index.

The usefulness of anti-Dsg1/3 ELISAs and indirect immunofluorescence (IIF) for the monitoring of pemphigus, based on a possible correlation between autoantibody levels and disease activity, remains controversial (3, 4). A recent study showed that ELISA index values can be a valuable tool for monitoring the disease, whereas IIF titres insufficiently reflect clinical activity (5). However, an interesting report was published recently in which a patient with pemphigus vulgaris in remission had a high anti-Dsg3 antibody ELISA index, but negative IIF results (6). The case presented a discrepancy between the disease activity, the ELISA index for Dsg3 and the IIF findings. The authors tried to resolve this discrepancy by devising a conformational ELISA index in which the EDTA-ELISA index is subtracted from the conventional ELISA index, which is a better indicator of disease activity than the conventional ELISA index. However, the patient's antibodies were found to be directed against $Ca2^{+}$-dependent epitopes even during remission, because the conformational ELISA index showed high levels even during remission. If the patient's antibodies were recognizing mainly precursor Dsg3, which is synthesized in the endoplasmic reticulum, and not recognizing mature Dsg3, then this could explain the negative findings in IIF. Using recombinant proteins, the patient's antibodies were shown to react preferentially with mature Dsg3. Thus, the mechanisms behind the inconsistency between the ELISA and IIF findings remain uncertain.

Some reports suggest that levels of the IgG_4 subclass and IgE class of anti-Dsg3 antibody are associated with active disease and that the IgG_1 levels are associated with remission (7, 8). Nagel et al. (8) demonstrated that, in addition to IgG_4, IgE autoantibodies to Dsg3 were present in a significant number of patients with acute-onset and active pemphigus vulgaris and that tissue-bound and serum IgE autoantibodies were detected in acute-onset pemphigus vulgaris by direct and indirect IF microscopy. This concept of class switch to IgG_1 during remission is also difficult to apply in the case described by Nakahara et al. (6), because there is almost no possibility that secondary antibodies against the human immunoglobulins used in IIF contain no anti-human IgG_1 antibodies.

Here, we propose a possible explanation of the discrepancy. We recently showed that the avidity of autoantibodies differs between a disease group and healthy individuals (HI) (9). Anti-dense fine speckles 70-kDa (DFS70) antibodies are anti-nuclear antibodies that are found more frequently in patients with atopic dermatitis (AD) than in HI, although the pathogenicity of these antibodies remains obscure. By comparing conventional anti-DFS70 ELISA values and antibody-stripping ELISA values by urea, we measured the avidity of anti-DFS70 antibodies in the AD and HI groups. The avidity of the antibodies was significantly higher in the patients with AD than in the HI, even though there was no difference in the avidity of anti-diphtheria toxoid between the 2 groups. A few reports have addressed the association between autoantibody avidity and disease activity. Regarding rheumatoid arthritis, Suwannalai et al. (10) reported that higher-avidity anti-citrullinated protein antibodies were observed only in symptomatic patients, whereas low-avidity anti-citrullinated protein antibodies were observed in both healthy subjects and patients with rheumatoid arthritis. Another study showed that, in some cases of vasculitis, myeloperoxidase anti-neutrophil cytoplasmic antibody had a high affinity that correlated with disease activity, irrespective of antibody titre (11). As far as we know, there are no reports investigating the avidity of anti-Dsg1/3 antibodies.

Autoantibody-defined epitopes are often conformation-dependent. For example, centromere protein-C (CENP-C), the main target of anti-centromere antibodies, has multiple epitopes formed from the C-terminal protein (12). Affinity-purified antibody against the dimer formation of the C-terminus in a liquid phase was found to be reactive only in IIF. We also demonstrated that a synthetic compound peptide composed of

 doi: 10.2340/00015555-2119
 ISSN 0001-5555

discontinuous sequences mimicked the conformation-dependent epitope found in patients with systemic lupus erythematosus (13). However, the polyclonal antibody against this peptide had weaker avidity than that of human autoantibody. The following are thought to cause affinity changes: a point mutation in an amino acid, post-transcriptional modifications and interactions with other molecules within epitopes.

Table I summarizes the reported heterogeneity of anti-Dsg1/3 antibodies. Further research into the avidities of anti-Dsg1/3 antibodies is required in order to elucidate the pathogenic mechanisms of pemphigus toward establishing a measurement for "true" pathogenic anti-Dsg1/3 antibodies.

The authors declare no conflicts of interest.

Table I. *Heterogeneity of anti-desmoglein antibodies*

Conventional enzyme-linked immunoassay (ELISA) vs. conformational ELISA
Autoantibodies to precursor desmogleins vs. mature desmogleins
Autoantibody subtypes: IgG1, IgG2, IgG4, IgA and IgE

Response to the Letter to the Editor by Muro et al.

Takashi Hashimoto[1], Norito Ishii[1] and Toshio Demitsu[2]

[1]Department of Dermatology, Kurume University School of Medicine, and Kurume University Institute of Cutaneous Cell Biology, 67 Asahimachi, Kurume, Fukuoka 830-0011, [2]Department of Dermatology, Jichi Medical University Saitama Medical Centre, Saitama, Japan. E-mail: hashimot@med.kurume-u.ac.jp

We read with much interest the Letter to the Editor on pathogenic (true) anti-desmoglein (Dsg) antibodies by Muro et al., which commented on our article (2) and another article by Li et al. (1). We reported that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor form, but not the mature form, of Dsg1 and Dsg3 (2).

It is occasionally found that the results of Dsg ELISAs and indirect immunofluorescence (IF) do not correlate with disease activity (5). In addition, individuals with no clinical features of pemphigus may show positive results in Dsg ELISA (2). In particular, we have encountered several patients with pemphigus vulgaris, who showed very high index values of Dsg3 ELISA even in the remission stage of the disease. To confirm the causes of the loss of pathogenicity of the serum autoantibodies, we considered 5 possible mechanisms, as described below, and summarized in Table I.

The first possibility is that pathogenic autoantibodies react with Ca^{++}-dependent conformational epitopes on Dsgs, because various autoantibodies have been shown to react with such conformation-dependent epitopes (13). This possibility can be confirmed by EDTA-treated Dsg ELISA or conformational ELISA index in which the conventional ELISA index is subtracted by EDTA-treated ELISA index (14).

The second possibility is that non-pathogenic autoantibodies react with precursor fragment on immature Dsg, which is present in the endoplasmic reticulum. This possibility can be confirmed by immunoprecipitation (IP)-immunoblotting (IB) or ELISA of recombinant proteins (RPs) of immature and mature Dsg (15).

The third possibility is that pathogenicity depends on extracellular domains of Dsg. Several studies have suggested that pathogenic autoantibodies react with N-terminal domains of mature Dsg (EC1 or EC2 domain), and autoantibodies to EC3-EC5 are non-pathogenic. The best method to confirm this possibility is IP-IB of Dsg3/Dsg2 or Dsg1/Dsg2 domain swapping molecule RPs (16, 17).

The fourth possibility is that pathogenicity depends on IgG subclasses (IgG1-IgG4) (7). Alternatively, IgA

Table I. *Possible mechanisms for pathogenicity of anti-desmoglein (Dsg) antibodies along with methods to confirm each possibility*

No.	Possible mechanisms	Methods for confirmation
1	Ca^{2+}-dependent conformation	EDTA-treated ELISA Conformational ELISA (subtraction of conventional ELISA by EDTA-ELISA)
2	Autoantibodies to precursor fragment in immature Dsgs	IB or ELISA of immature Dsg RP
3	Dependency to extracellular Dsg domains, EC1–EC5	IP-IB or ELISA of Dsg1/Dsg2 and Dsg3/Dsg2 swapping molecule RPs
4	Dependency to immunoglobulin subtypes (IgG1–IgG4, IgA and IgE)	IF, IB or ELISA using subtype-specific antibodies
5	Autoantibodies to p38 MAPK activating domain within Dsg	p38 MAPK phosphorylation study p38 MAPK inhibitor study
6	Avidity (affinity) of anti-Dsg antibodies	Autoantibody-stripping ELISA by urea treatment or inhibition by self-antigen in a liquid phase

IP: immunoprecipitation; IB: immunoblotting; RP: recombinant protein; ELISA: enzyme-linked immunoassay; EDTA: ethylenediaminetetraacetic acid; MAPK; mitogen-activated protein kinases.