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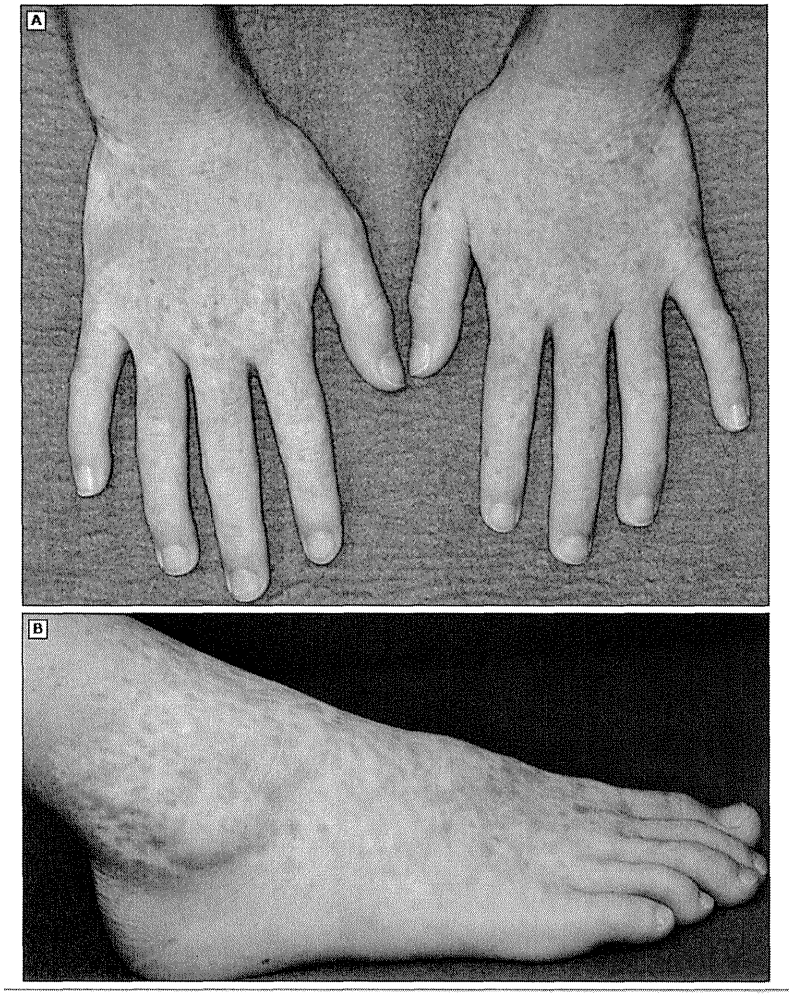
Topic 15525 Version 4.0

GRAPHICS

Dyschromatosis symmetrica hereditaria



A mixture of hypopigmented and hyperpigmented macules approximately 5 mm in diameter on the dorsum of the hands and feet in a patient with dyschromatosis symmetrica hereditaria.

Courtesy of Michihiro Kono, MD, PhD.

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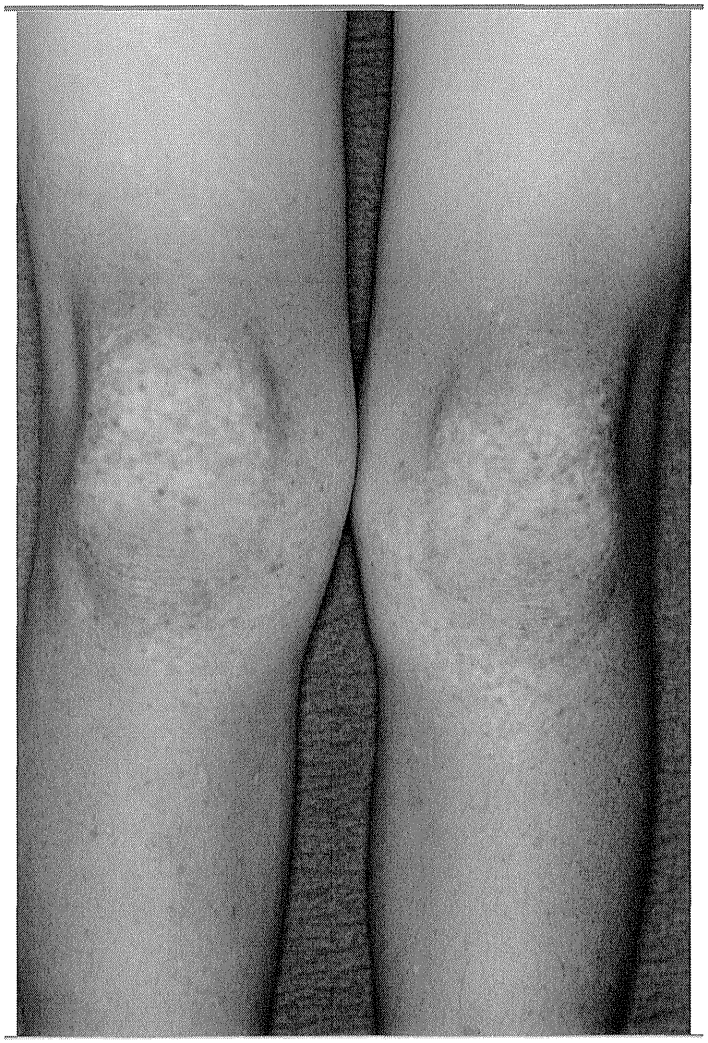
Dyschromatosis symmetrica hereditaria

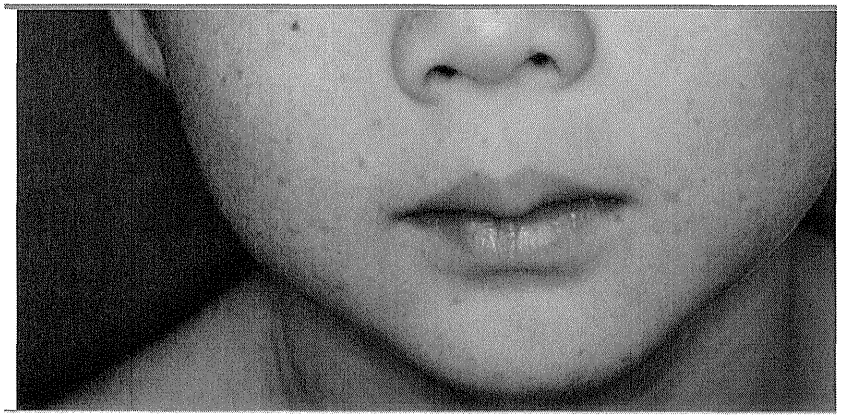


A mixture of hypopigmented and hyperpigmented macules approximately 5 mm in diameter on the knees of a child with dyschromatosis symmetrica hereditaria.

Courtesy of Michihiro Kono, MD, PhD.

Graphic 105810 Version 1.0

Dyschromatosis symmetrica hereditaria

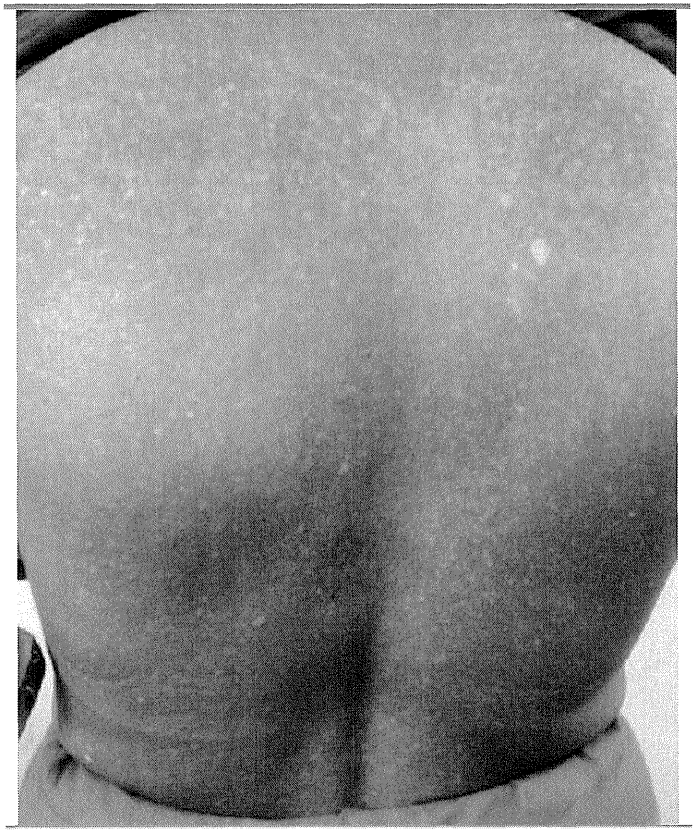


Freckle-like lesions on the face of a child with dyschromatosis symmetrica hereditaria.

Courtesy of Michihiro Kono, MD, PhD.

Graphic 105811 Version 1.0

Dyschromatosis universalis hereditaria (DUH)

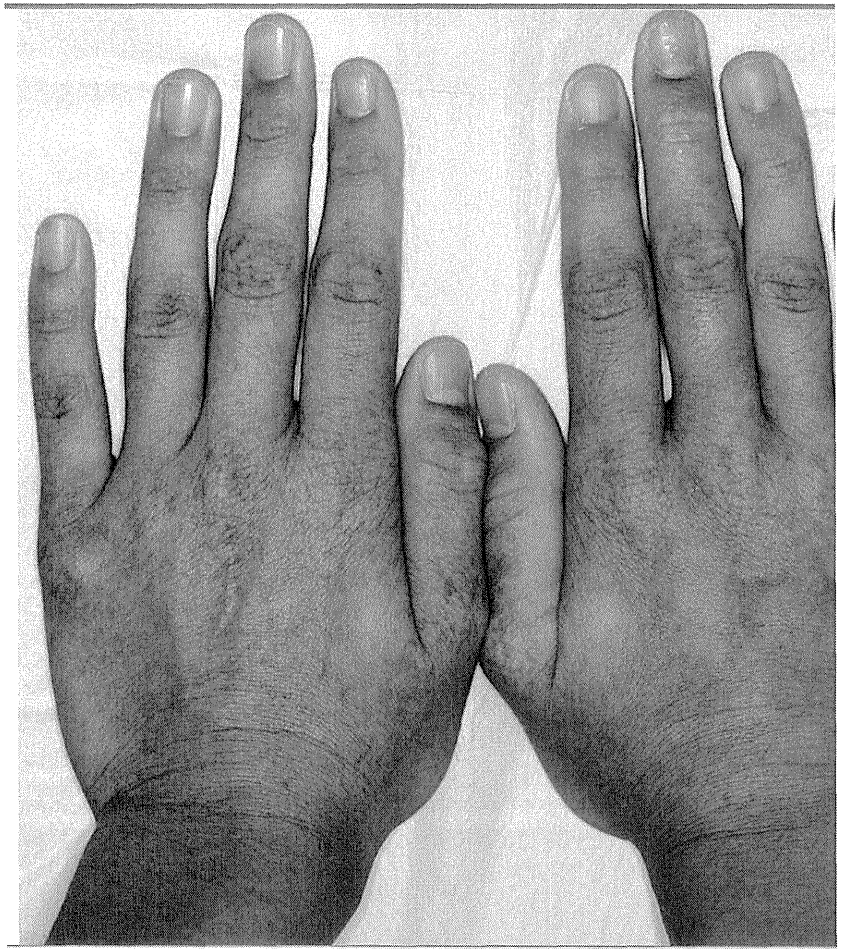


Alternating hypo- and hyperpigmented macules of varying size on the back of a patient with dyschromatosis universalis hereditaria.

From: Sardana K, Goel K, Chugh S. Reticulate pigmentary disorders. Indian J Dermatol Venereol Leprol 2013; 79:17. DOI: 10.4103/0378-6323.104665. Reproduced with permission from Wolters Kluwer - Medknow. Copyright © 2013 Indian Association of Dermatologists, Venereologists and Leprologists. Unauthorized reproduction of this material is prohibited.

Graphic 93763 Version 1.0

Reticulate acropigmentation of Kitamura

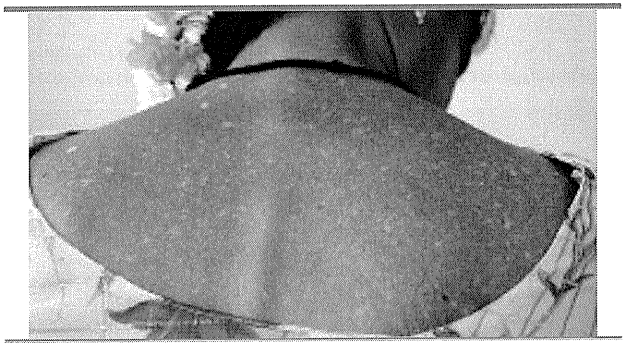


Pigmented macules on the dorsum of the hands of a patient with reticulate acropigmentation of Ki

Courtesy of Michihiro Kono, MD, PhD.

Graphic 105812 Version 1.0

Amyloidosis cutis dyschromica

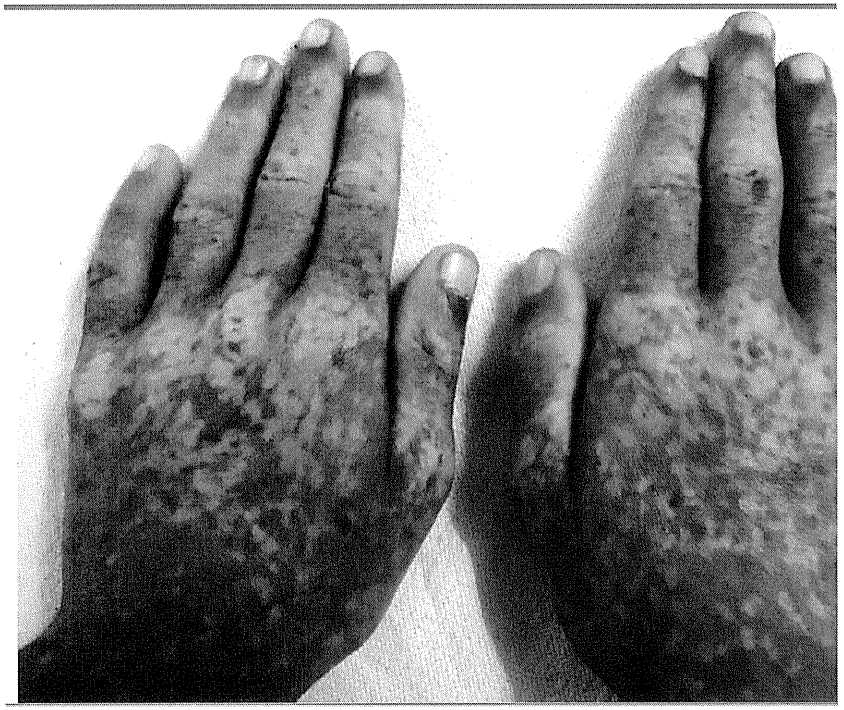


Multiple hypopigmented and depigmented macules are visible on the upper back of a 26-year-old woman with amyloidosis cutis dyschromica.

From: Kurian SS, Rai R, Madhukar ST. Amyloidosis cutis dyschromica. Indian Dermatol Online J 2013; 4:344. DOI: 10.4103/2229-5178.120678.

Graphic 94996 Version 1.0

Dyschromatosis symmetrica hereditaria (Reticulate acropigmentation

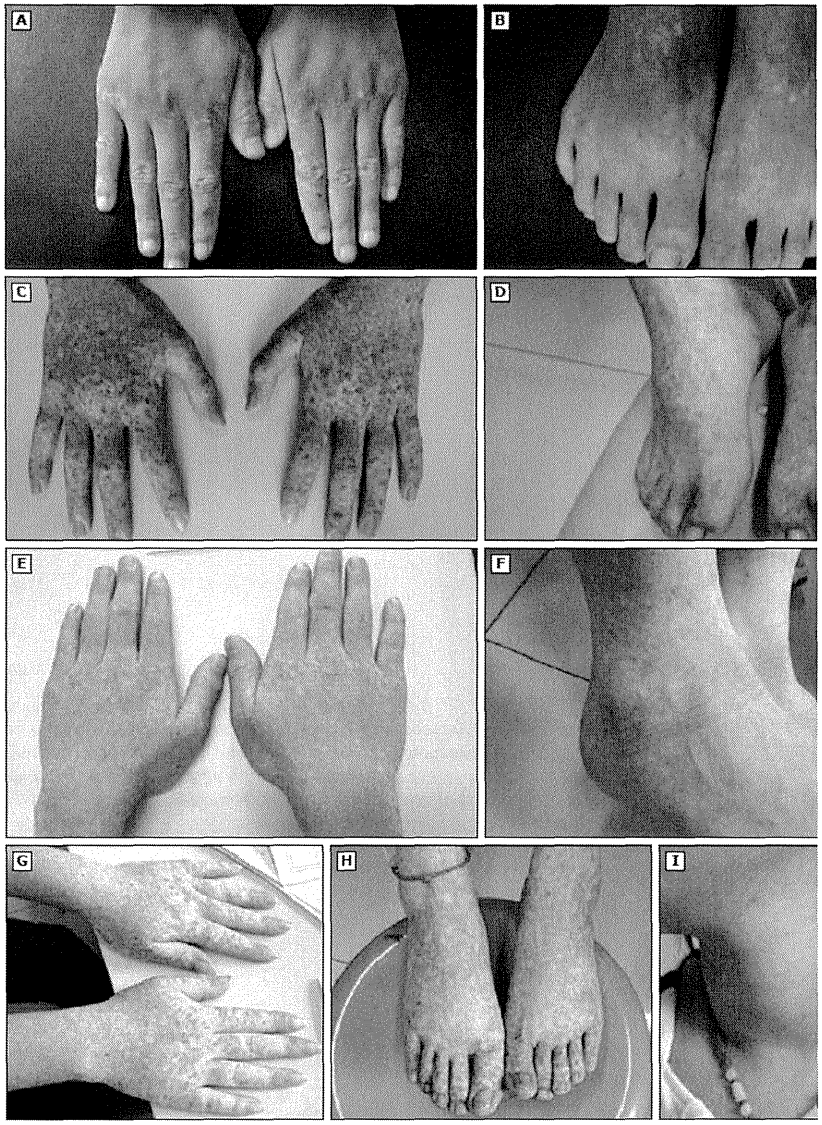


Hyperpigmented and hypopigmented macules over the dorsum of the hands in a 10-year-old boy v dyschromatosis symmetrica hereditaria.

From: Mohana D, Verma U, Amar AJ, Choudhary RK. Reticulate acropigmentation of dohi: A case report genodermatoses with mottled pigmentation. Indian J Dermatol 2012; 57:42. DOI: 10.4103/0019-5154.9

Graphic 94173 Version 1.0

Dyschromatosis symmetrica hereditaria



Hyperpigmented and hypopigmented macules on the back of hands and feet of patients with dysch
symmetrica hereditaria.

*From: Yuan C, Liu H, Fu X, et al. Two novel mutations of the ADAR1 gene in Chinese patients with dysch
symmetrica hereditaria. Indian J Dermatol Venereol Leprol 2012; 78:746. DOI: 10.4103/0378-6323.102*

Graphic 94994 Version 1.0

Contributor Disclosures

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Conflict of interest policy

SHORT REPORT

Loss-of-function mutations in the gene encoding filaggrin underlie a Japanese family with food-dependent exercise-induced anaphylaxis

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Abstract

Background Food-dependent exercise-induced anaphylaxis (FDEIA) is a serious food allergy in which anaphylaxis develops when exercise is performed within several hours after food intake. The precise mechanism underlying allergic sensitization in FDEIA has been an important issue but remains poorly understood.

Objectives We aimed to elucidate the pathomechanism including the route of allergen sensitization involved in FDEIA.

Methods A Japanese family with wheat-dependent exercise-induced anaphylaxis (WDEIA), a specific form of FDEIA, were clinically examined. Mutation analysis of the gene encoding filaggrin (*FLG*) was also performed.

Results Two of the family members were confirmed as WDEIA on the basis of their medical history and positive provocation test results. Notably, the two affected individuals in the family had concomitant ichthyosis vulgaris. Mutation analysis of *FLG* revealed that they carry one or more loss-of-function mutations that have not been described in the Japanese population.

Conclusion These results indicate that *FLG* mutations might be involved in the pathogenesis of WDEIA in the present case.

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Conflicts of interest

None declared.

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Introduction

Food-dependent exercise-induced anaphylaxis (FDEIA) is an IgE-mediated hypersensitivity characterized by development of serious systemic allergic reactions including anaphylaxis when exercise is performed within several hours after ingesting certain foods. The precise mechanism underlying allergic sensitization in FDEIA has been an important issue, but it remains poorly understood. However, a growing body of evidence has indicated that epidermal barrier defect plays a crucial role in percutaneous allergic sensitization in systemic allergies, including food allergies.[1,2] Indeed, loss-of-function mutations in the gene encoding filaggrin (*FLG*), a key epidermal protein in skin barrier function, are known to be a significant predisposing factor to the development of atopic diseases (eczema, asthma and rhinitis) and even peanut allergy.[1,3–6] This indicates a role for skin barrier dysfunction in the pathogenesis of systemic allergic diseases as well as skin diseases, which raises the notion that a skin barrier defect may play an important role in FDEIA. To date, however, no case–control studies have been reported, and not even a single case report suggesting a causal link between *FLG* mutation and FDEIA has been available. Therefore, the association of *FLG* null mutations with FDEIA remains unclear. Here, we report a Japanese family in which two individuals carrying *FLG* null mutations developed wheat-dependent exercise-induced anaphylaxis (WDEIA), a specific form of FDEIA.

[†]These authors contributed equally to this work.

Report of a case

A 29-year-old Japanese woman presented with a 4-year history of repeated episodes of urticaria and anaphylactic shock. She developed itching, generalized urticaria, dyspnoea and/or short-term loss of consciousness within a few hours after ingesting wheat-containing products, such as noodles and bread, and then performing mild exercise, whereas exercise alone was tolerated. Based on the anaphylactic shock episodes after the combination of wheat intake and exercise, we suspected WDEIA. Laboratory measures revealed elevated total IgE of 665.5 IU/mL (normal; 0–295) and specific IgE to wheat of 24.50LC (normal; 0.00–1.39) and to gluten of 0.92UA/mL (normal; 0.00–0.34) in her serum, although specific IgE to ω-5 gliadin was within normal limits. Prick tests were then performed using commercial wheat extract (Torii Pharmaceutical Co., Tokyo, Japan). Histamine dihydrochloride of 10 mg/mL was used as a positive control. Reactions were read at 15 min, and then compared with positive histamine controls and scored (1+: 25% of the area of the histamine-induced wheal; 2+: 50%; 3+: 100%; 4+: 200%). She had a positive prick test to wheat (3+). She showed no symptoms when challenged with wheat alone, aspirin alone, exercise alone or any combination of the two of them, performed on separate days. To enhance the challenge test reaction, aspirin was also orally administered. Provocation tests with intake of 100 g of wheat and 500 mg of aspirin in combination with exercise induced widespread wheals and pruritus. Taken together, the diagnosis of WDEIA was confirmed. Notably, she had a positive family history of WDEIA, as her mother also reported experiencing similar episodes of urticaria, dyspnea and sudden loss of consciousness. We also diagnosed the mother as having WDEIA, based on a positive prick test to wheat (3+), elevated specific IgE to ω-5 gliadin and a positive challenge test. The mother and daughter had no history of gastrointestinal hypersensitivity. Remarkably, physical examination also revealed both of them to have marked dry skin and palmar hyperlinearity, which are suggestive of ichthyosis vulgaris (IV; OMIM 146700) (Fig. 1a,b). The daughter also suffered from atopic eczema (AE) and asthma. Mutation analysis of *FLG* was subsequently performed. Briefly, genomic DNA of the two patients were obtained from peripheral blood using the QIAamp DNA Blood Maxi Kit (Qiagen, Maryland, USA) and all exons and exon–intron boundaries of *FLG* were amplified and sequenced as described previously.[4]

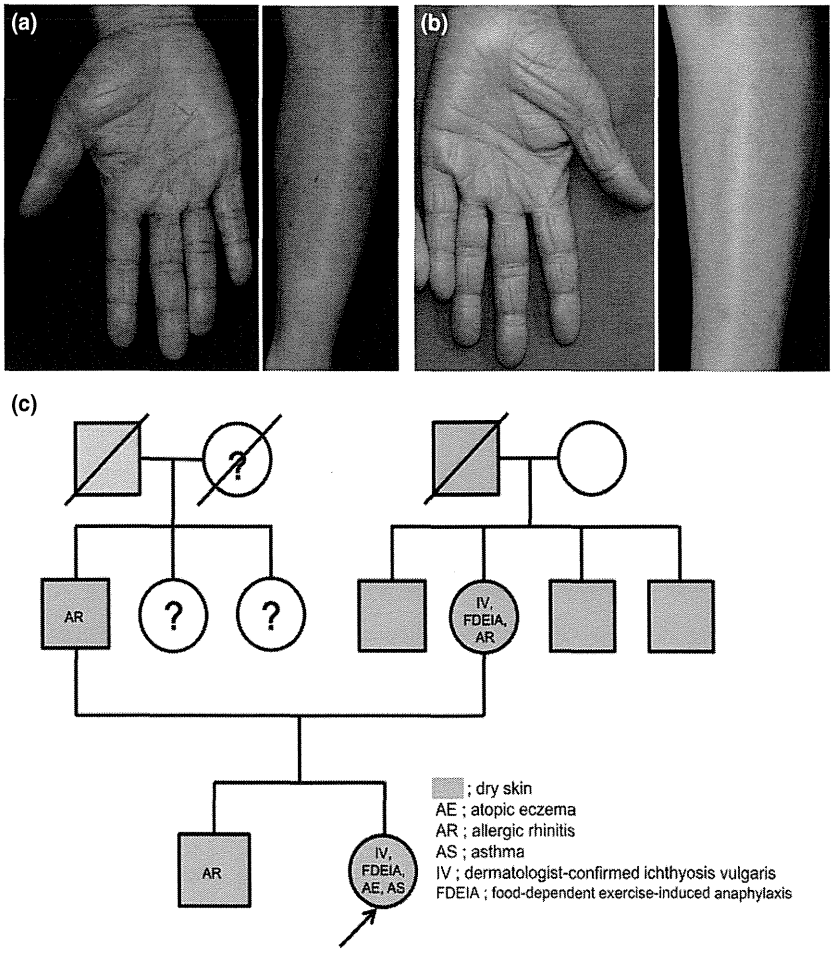

Figure 1 (a, b) Both the proband (a) and her mother (b) showed palmar hyperlinearity and marked dry skin that were suggestive of concomitant ichthyosis vulgaris. (c) A family history of allergies and dry skin.

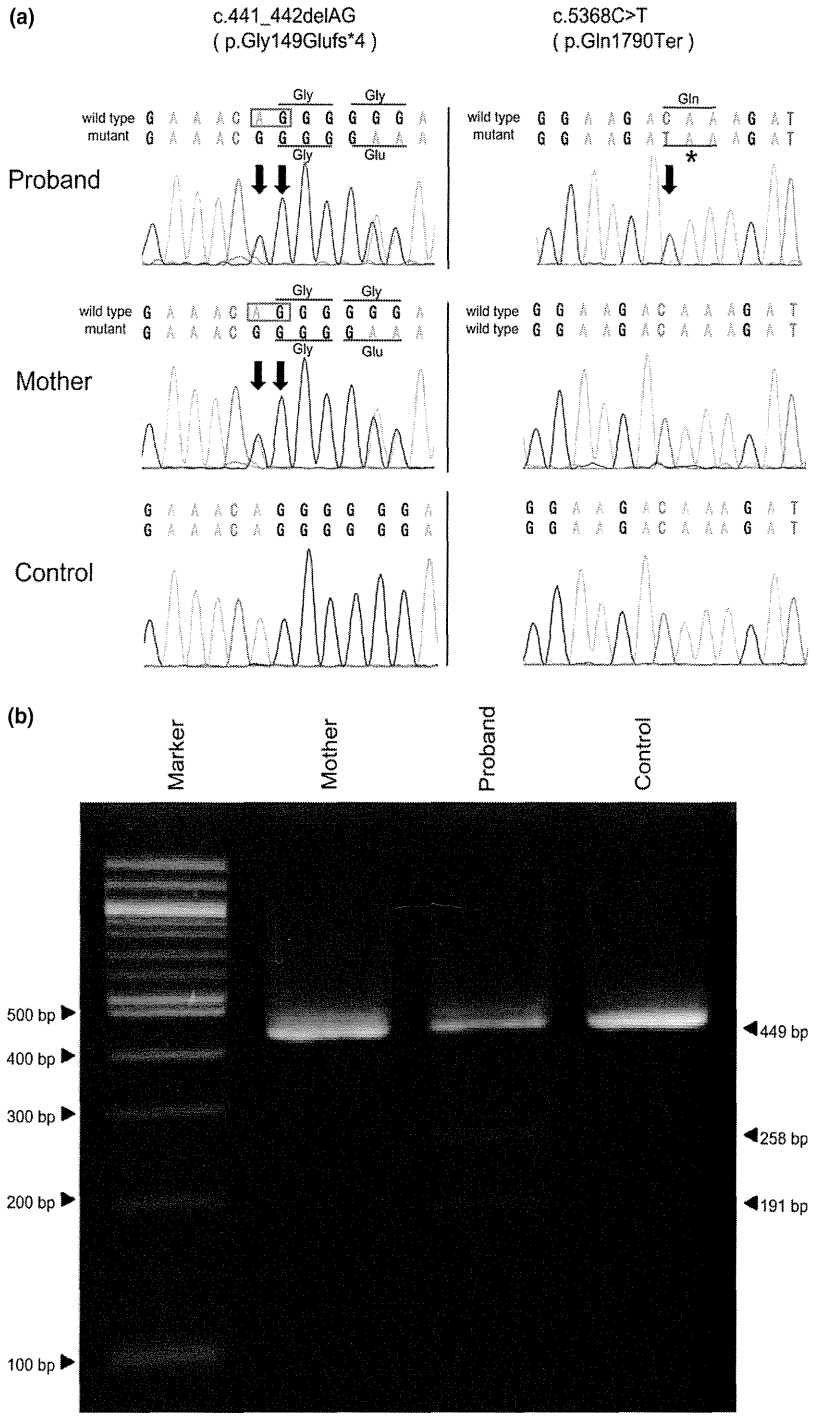

Figure 2 (a) A heterozygous 2-bp deletion mutation in *FLG* was identified in the proband and her mother, resulting in c.441_442delAG (p.Gly149Glufs*4). Normal control sequence from filaggrin repeat 0 in exon 3, corresponding to codons 436-448 (left panels). A heterozygous transition mutation c.5368C>T in *FLG* was also identified in the proband, resulting in p.Gln1790Ter, while her mother was wild type for this mutation. Normal control sequence from filaggrin repeat 5 in exon 3, corresponding to codons 5362-5374 (right panels). (b) Mutation p.Gln1790Ter was further confirmed by *Bsa*BI restriction digest. The mutant allele was resolved as 258-bp and 191-bp fragments, whereas the wild-type allele gave a 449-bp fragment. Mutant-specific bands (258-bp and 191-bp) were identified only in the proband.

The mother and daughter gave written informed consent for mutation analysis in compliance with the Declaration of Helsinki Principles. The study was approved by the Medical Ethics Committee of the Hokkaido University Graduate School of Medicine. Mutation analysis of *FLG* revealed the daughter to be compound heterozygous for c.441_442delAG

(p.Gly149Glufs*4) and c.5368C>T (p.Gln1790Ter) and the mother to be heterozygous for c.441_442delAG (Fig. 2a). The mutation p.Gln1790Ter creates a *Bsa*BI restriction site. A 449-bp PCR fragment was amplified with forward primer 5′ GTAGTCGGAGACAGTGGAA 3′ and reverse primer 5′ ACATCAGACCTTTCCTGGGAC 3′. After purification using the QIAquick PCR purification kit (Qiagen), the PCR product was digested with 50U of *Bsa*BI (New England Biolabs, Ipswich, USA) at 60°C for 6 h. Digests were resolved on 4% (w/v) agarose gels. The mutant allele was resolved as 258-bp and 191-bp fragments, whereas the wild-type allele gave a 449-bp fragment (Fig. 2b). Thus, mutation p.Gln1790Ter was confirmed by restriction enzyme digestion. Both of the mutations were absent in 50 ethnically matched control individuals. Genomic DNA from the remaining members of the family was not available for the mutation analysis. The family history of allergies and dry skin is summarized in Fig. 1c.

Discussion

WDEIA is a distinct form of severe food allergy whose onset requires both physical exercise and ingestion of wheat-containing products within the preceding several hours. It causes anaphylaxis and is potentially life threatening, but the pathogenesis has yet to be elucidated. The route of allergen sensitization to wheat or wheat-derived antigens in patients with WDEIA is speculated to be via either the gastrointestinal tract or the skin, but it remains undetermined. Interestingly, recent reports have indicated that WDEIA can be induced by the use of soap containing hydrolyzed wheat protein.[7,8] These observations strongly suggest the importance of percutaneous and/or rhinoconjunctival sensitization in the pathogenesis of WDEIA.

In the present family, the affected individuals had not used such soap before the onset of WDEIA, but it is of note that both of the patients have concomitant IV and carry one or more *FLG* mutations. Since filaggrin plays a crucial role in epidermal barrier formation, loss-of-function mutations in *FLG* are believed to disrupt the outside-inside skin barrier.[2] Indeed, *FLG* mutations have been extensively shown to be a significant predisposing factor to AE, atopic asthma, allergic rhinitis and elevated IgE by a number of case–control studies.[1,4–6] Interestingly, children with AE, up to 45% of whom carry one or more *FLG* mutations,[4] have a higher prevalence of food allergy.[9] *FLG* mutations were also shown to have a strong and significant association with IgE-mediated peanut allergy, with an odds ratio of 5.3 ($P = 3.0 \times 10^{-6}$; 95%CI, 2.8–10.2), in a large case–control study.[3] Furthermore, the recent analysis of a filaggrin-deficient murine model supports the idea of a prominent role of epithelial deficiency as a facilitating early event in allergic priming.[2] Taken together, we speculate that impaired epidermal barrier resulting from *FLG* mutations might allow the penetration of wheat-derived antigens into the skin, possibly resulting in increased percutaneous sensitization to wheat and the development of WDEIA. This speculation warrants further investigation.

Two *FLG* mutations, c.441_442delAG and p.Gln1790Ter, were identified in the family. Both of the mutations have recently been identified in Chinese IV patients[10] but have never been described in the Japanese population. To date, eight *FLG* null variants have been reported in the Japanese population.[11,12] This study brings the total number of *FLG* null mutations identified in the Japanese population to ten. Notably, most of the *FLG* mutations identified in the Japanese population have also been identified in the Chinese population, and vice versa.

To our knowledge, this is the first detailed report of a familial case of WDEIA. *FLG* mutations might be involved in the pathogenesis of WDEIA in the present case. Further investigation would shed light on the exact mechanism involved in this life-threatening disorder.

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BJD
British Journal of Dermatology

Progressive hyperpigmentation in a Taiwanese child due to an inborn error of vitamin B12 metabolism (cblJ)

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None declared.

T.T. and C.K.H. are joint first authors.

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Summary

The physiology of human skin pigmentation is varied and complex, with an extensive melanogenic paracrine network involving mesenchymal and epithelial cells, contributing to the regulation of melanocyte survival and proliferation and melanogenesis. Mutations in several genes, involving predominantly the KIT ligand/c-Kit and Ras/mitogen-activated protein kinase signalling pathways, have been implicated in a spectrum of diseases in which there is hyperpigmentation, hypopigmentation or both. Here, we report on a 12-year-old girl from Taiwan with a 6-year history of diffuse progressive skin hyperpigmentation resulting from a different aetiology: an inborn metabolic disorder of vitamin B12 (cobalamin), designated cblJ. Using whole-exome sequencing we identified a homozygous mutation in *ABCD4* (c.423C>G; p.Asn141Lys), which encodes an ATP-binding cassette transporter with a role in the intracellular processing of cobalamin. The patient had biochemical and haematological evidence of cobalamin deficiency but no other clinical abnormalities apart from a slight lightening of her previously black hair. Of note, she had no neurological symptoms or signs. Treatment with oral cobalamin (3 mg daily) led to metabolic correction and some reduction in the skin hyperpigmentation at the 3-month follow-up. This case demonstrates that defects or deficiencies of cobalamin should be remembered in the differential diagnosis of diffuse hyperpigmentary skin disorders.

What's already known about this topic?

- Inherited defects affecting vitamin B12 (cobalamin, cbl) metabolism can result in various haematological and neurological abnormalities, as well as occasional changes in the skin.
- Nine different metabolic defects in the intracellular processing of cbl have been reported, which led to isolated methylmalonic aciduria and/or isolated homocystinuria.
- The most recently described disease subtype is cblJ, resulting from mutations in *ABCD4*; three individuals with *ABCD4* mutations have been described.

What does this study add?

- We identified a homozygous missense mutation in *ABCD4* in a 12-year-old girl with progressive hyperpigmentation.
- *ABCD4* mutations can lead to skin hyperpigmentation in the absence of any neurological abnormalities, thus *ABCD4* is a further candidate gene for familial progressive hyperpigmentation.
- Treatment with oral cbl can reduce the hyperpigmentation that results from *ABCD4* mutations over several months.

Progressive skin hyperpigmentation is a genetically heterogeneous disorder with two key signalling cascades, the KIT ligand/c-Kit and Ras/mitogen-activated protein kinase pathways, implicated in the pathophysiology of several clinical syndromes.[1] Hyperpigmentation can also result from vitamin B12 (cobalamin, cbl) deficiency, although the associated hyperpigmentation is not usually diffuse. Typically, it is more evident on the palms, soles and mucosae and sites of pressure, and there may be additional clinical abnormalities such as glossitis.[2–6] In this report, we describe a patient with diffuse progressive skin hyperpigmentation resulting from an autosomal recessive disorder of cbl metabolism, further expanding the differential diagnosis in such cases.

Case report

A 12-year-old girl, the only child of nonconsanguineous Han Chinese parents, presented with a 6-year history of asymptomatic progressive generalized skin hyperpigmentation. Examination revealed diffuse mottled hyperpigmentation affecting all of her skin, including sun-covered sites; her hair was dark brown rather than black (Fig. 1a,b). No mucosal or nail pigmentation was noted and her tongue was normal. Biopsy of hyperpigmented skin showed increased melanin within basal keratinocytes and in numerous melanophages in the papillary dermis (Fig. 1c,d). Transmission electron microscopy revealed melanophages around capillaries (Fig. 1e), and heavily melanized melanosomes packed in phagocytic vacuoles (Fig. 1f). She was otherwise in good health with no neurological or cardiovascular symptoms. Her parents and other family members reported no similar skin changes.

To identify the genetic basis of the hyperpigmentation, and following informed consent, whole-exome capture was performed (peripheral blood genomic DNA) by in-solution hybridization using the SureSelect All Exon 50 Mb Version 4.0 (Agilent, Santa Clara, CA, U.S.A.) followed by massively parallel sequencing (HiSeq2000; Illumina, San Diego, CA, U.S.A.) with 100-bp paired-end reads. Over 8·4 gigabases of mappable sequence data were generated, such that > 93% of the coding bases of the exome defined by the GENCODE Project (http://www.gencodegenes.org/) were represented by at least 20 reads.

In total 25 454 single-nucleotide substitutions were identified in the patient: 10 925 homozygous and 14 529 heterozygous. Within these variants, a nonsynonymous homozygous mutation was identified in *ABCD4* (c.423C>G; p.Asn141Lys). This mutation has been reported previously, in a 14-year-old Taiwanese boy with skin hyperpigmentation and neurological abnormalities.[7] *In silico* analysis with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) predicts the mutation to be 'probably damaging', and the SIFT program (http://sift.jcvi.org/) predicts it to be 'damaging', thus functionally relevant. We further confirmed the mutation by Sanger sequencing and restriction endonuclease digestion (MwoI) (Fig. 2); both parents were shown to be heterozygous carriers. Next, we screened for this missense change in unrelated Taiwanese DNA samples (MwoI digest), but the mutant allele was not detected in any of 498 unrelated control chromosomes.

Biochemically, the patient was found to have elevated plasma homocysteine (52·0 μmol L^{-1}, reference 3·7–17·2 μmol L^{-1}) and low serum cbl (187·7 pg mL^{-1}, reference 250–900 pg mL^{-1}); a macrocytic anaemia (mean corpuscular volume of 102·6 fL) and haemoglobin of 11·5 g dL^{-1} were also noted. Tandem mass spectrometry analysis revealed a slightly decreased methionine level (17·5 μmol L^{-1}, reference 18–42 μmol L^{-1}), and urine organic acid analysis revealed the presence of extremely high levels of methylmalonic acid (Fig S1; see Supporting Information). The plasma homocysteine and serum cbl in parental blood samples were normal. The patient was treated with 3 mg of vitamin B12 orally per day, and biochemical correction of homocysteine and methylmalonic acid levels was noted: after 12 weeks of therapy her serum cbl was 344·6 mg mL^{-1}, with a haemoglobin of 14·0 g dL^{-1} and mean corpuscular volume of 93·3 fL. Over 3 months of follow-up there was a slight reduction in skin pigment levels. Of note, in the other reported Taiwanese case with this mutation in *ABCD4*, the skin hyperpigmentation resolved after 12 months of vitamin B12 therapy.

Discussion

ABCD4 [ATP-binding cassette, subfamily D (ALD), member 4] encodes a member of the superfamily of ATP-binding cassette (ABC) transporters that transport various molecules across extra- and intracellular membranes. It also has a putative role in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle, and in peroxisome biogenesis. Its relevance to

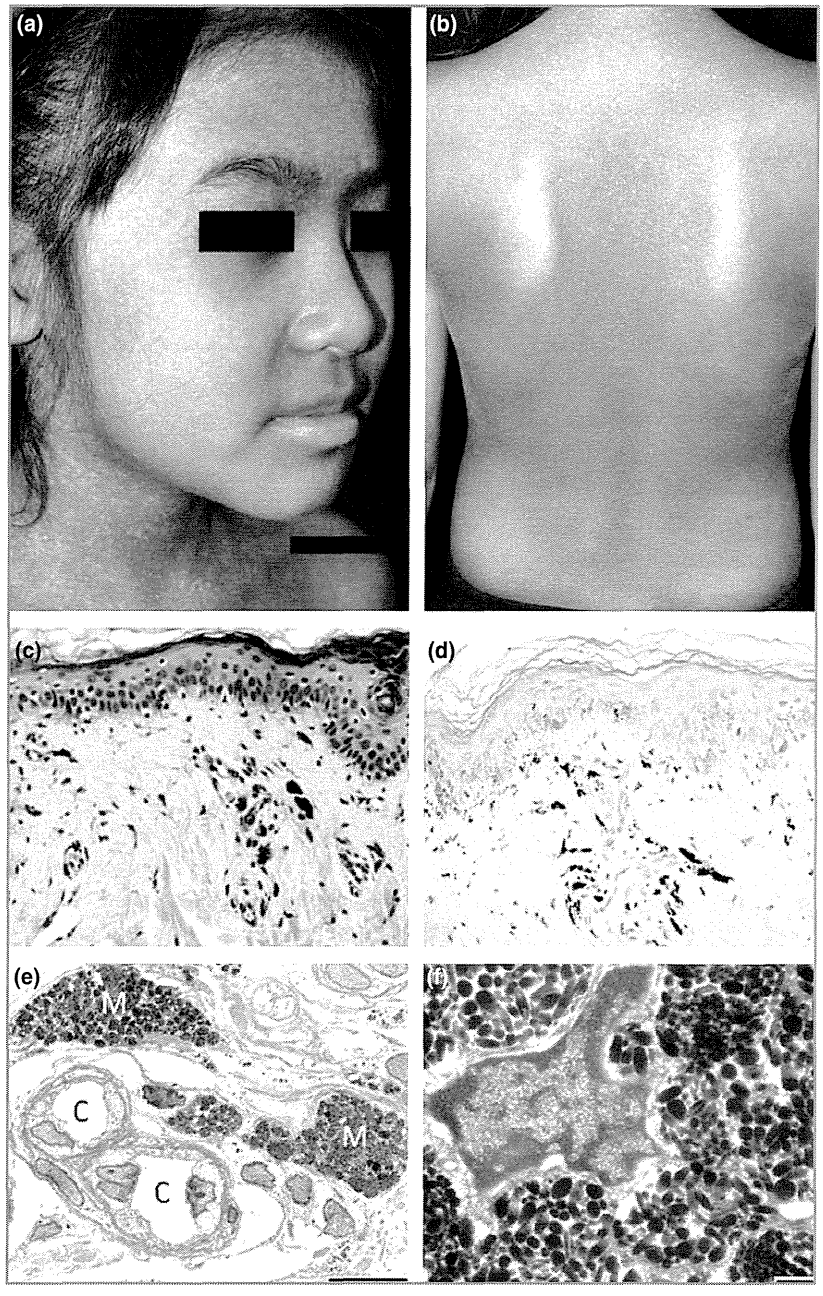

Fig 1. Clinicopathological studies of this 12-year-old Taiwanese girl. (a, b) Diffuse mottled hyperpigmentation on the face, neck and trunk. (c) Light microscopy reveals increased melanization in the lower epidermis and numerous melanophages in the papillary dermis. (d) Fontana–Masson staining highlights the presence of increased melanin. (e) Ultrastructural images showing melanophages near capillary vessels. C, capillary; M, melanophages. Magnification ×2500. (f) High magnification revealing heavily melanized melanosomes packed in phagocyte vacuoles (×30 000).

human health/disease was realized only in 2012 with the discovery of human mutations in *ABCD4* through whole-exome sequencing.[8] ABCD4 has been shown to colocalize with lysosomal proteins, contributing ATP-ase activity for the intracellular processing of cbl, and thus permitting its release from lysosomes into the cytoplasm.[1] Cbl is then converted into two active cofactors: methylcobalamin (needed for methylation of homocysteine to methionine) and adenosylcobalamin (which helps process methylmalonyl-CoA to succinyl-CoA). Biochemically, inborn errors of cbl therefore lead to elevated blood and urine levels of homocysteine and/or methylmalonic acid.[8,9] Mutations in *ABCD4* underlie the disease subtype cblJ (MIM 614857).[7]

The first two reported cases with mutations in *ABCD4* presented with feeding difficulties, hypotonia, developmental delay, bone marrow suppression and cardiovascular defects in early life.[8] Skin hyperpigmentation was not evident in these children. The main phenotypic abnormalities in the one further case reported (from Taiwan) were neurological (dizziness, headache and transient ischaemic attacks), although some skin hyperpigmentation and greying of the hair were noted as minor additional clinical features.[7] In contrast, skin hyperpigmentation was the predominant sign in our case.

With regard to genotype–phenotype correlation, the recurrent missense mutation in the Taiwanese cases may be less disruptive to the ABCD4 protein than the splice site and

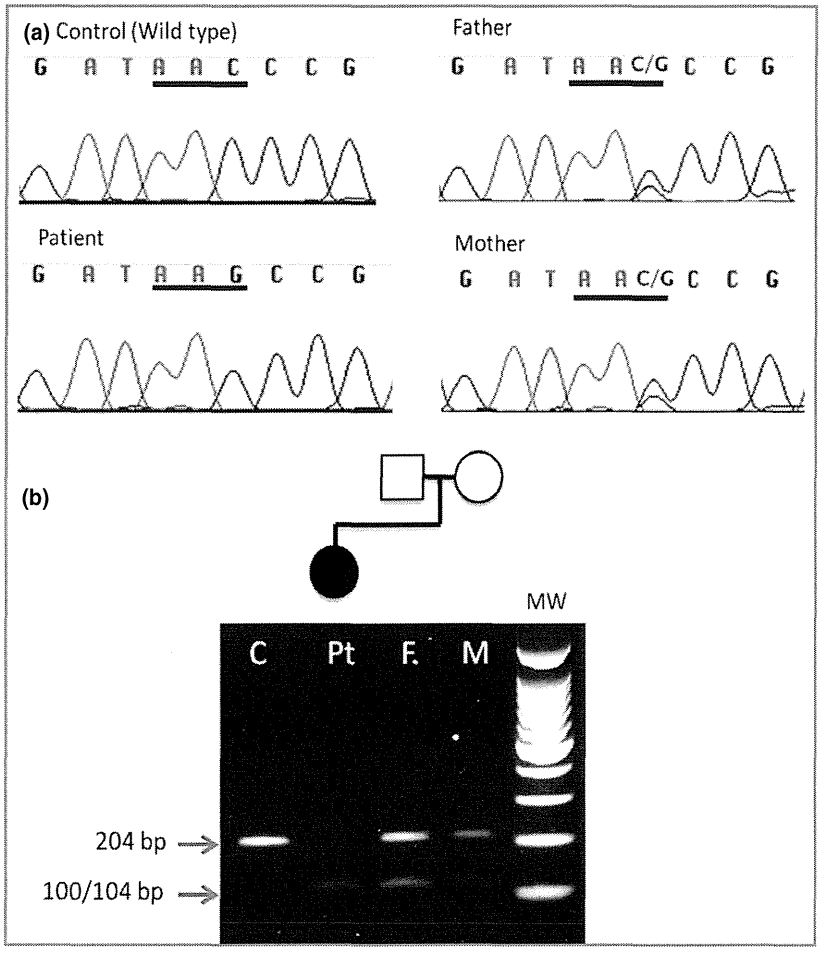

Fig 2. Molecular pathology of this case. (a) The proband has a homozygous single-nucleotide substitution, c.423C>G, in *ABCD4*, which leads to the amino acid change p.Asn141Lys; both parents are heterozygous carriers of this missense mutation. (b) Verification of the mutation by MwoI restriction enzyme digestion. The mutation c.423C>G creates a new (solitary) cut size such that the 204-bp band is cleaved into 104-bp and 100-bp fragments (which cannot be separated on this gel). For the patient (Pt) only the lower cleaved band is present, whereas in the father (F) and mother (M) two discrete bands are seen, consistent with heterozygosity for this mutation. For the control DNA (C) only the single undigested upper band is visible. MW, molecular weight ladder.

frameshift mutant alleles in more severely affected individuals, although clearly there is phenotypic disparity (so far unexplained) between the two Taiwanese subjects both homozygous for p.Asn141Lys. However, it is possible that early diagnosis and treatment in our case has pre-empted and prevented the other potential disease manifestations and complications. This amino acid substitution appears to be exclusive to Taiwanese subjects, as it has not been observed or reported elsewhere (or detected in > 1500 in-house exome datasets), although we failed to detect its presence in 498 control Taiwanese chromosomes. None of the parents of the Taiwanese cases was known to be related to one another, and all came from different parts of Taiwan; therefore c.423C>G in *ABCD4* may represent a rare mutant allele in this population.

Skin pigmentation is dependent on the type and amount of melanin present; this is regulated by tyrosinase and tyrosinase-related enzyme activity and by other proteins that influence the size, number and distribution of melanosomes within keratinocytes.[1] For our case, the main differential diagnosis was familial progressive hyperpigmentation (MIM 614233; 145250), characterized by progressive hyperpigmentation, but without hypopigmentation. The latter is a feature of other related disorders such as familial progressive hyperpigmentation and hypopigmentation, and dyschromatosis universalis hereditaria type 2 (MIM 612715). Familial progressive hyperpigmentation appears to be genetically heterogeneous, although a mutation has previously been reported in *KITLG*.[10] In our case, the mechanism underlying how mutations in *ABCD4* led to skin hyperpigmentation is unknown. Possible aetiologies include cbl deficiency leading to lower levels of reduced-type glutathione (which has a tyrosinase-inhibiting effect), or disruption of lysosomes in melanophages, although both hypotheses are highly speculative.

In summary, our case highlights the protean nature of progressive hyperpigmentation, and emphasizes that disorders of vitamin B12 metabolism should also be remembered in the differential diagnosis. Early recognition of this clinicopathological disorder is important, as oral treatment with vitamin B12 may not only improve the appearance of the skin, but also prevent the major neurological and cardiovas-

cular complications associated with inborn errors of cbl metabolism.

Acknowledgments

The authors thank Professor Chi-Chan Shieh and Dr Michihiro Kono for academic discussions and Ms Hui-Ping Pan for technical support.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Raw laboratory data showing extremely high levels of methylmalonic acid. This is not given any precise quantification, but the peak extends to the top of the screen and is reported as 'extremely high'.

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SHORT COMMUNICATION

TRPS1 Haploinsufficiency Results in Increased *STAT3* and *SOX9* mRNA Expression in Hair Follicles in Trichorhinophalangeal Syndrome

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Trichorhinophalangeal syndrome types I and III (TRPS1, OMIM 190350; TRPS3, OMIM 190351) are rare hereditary diseases with autosomal dominant inheritance (1, 2). The first case was reported in 1966 (3). In 2000 the *TRPS1* gene was identified as one of its causative genes and mapped to chromosomal region 8q24.1 (1).

These syndromes have characteristic sparse and slow-growing hair, craniofacial abnormalities, such as bulbous pear-shaped nose, and skeletal abnormalities (3–5). We report here the effects of TRPS1 protein deficiency in a case of TRPS1.

CASE REPORT

A 26-year-old Japanese woman was referred to us for sparse scalp hair from birth. Physical examination revealed characteristic symptoms of TRPS1 (Fig. 1a–c). Her serum creatinine level was 0.71 mg/dl (normal range <0.70 mg/dl). Radiologically, cone-shaped epiphyses were found at the phalanges (Fig. S1[1]). She had never had growth hormone treatment. She was 158 cm tall, roughly the mean height of Japanese females, and had no growth retardation. None of her family members showed hypotrichosis or skeletal abnormalities. The patient was suspected of having TRPS, and a *TRPS1* mutation search was performed. The ethics committee of Nagoya University approved the studies described below, which were conducted according to the principles of the Declaration of Helsinki. The participants gave written informed consent. Direct sequencing of the entire coding regions and exon-intron boundaries of *TRPS1* revealed the patient to be heterozygous for the previously unreported nonsense mutation c.2191G>T in *TRPS1*, resulting in an immediate stop codon (p.Glu732X) (Fig. 1d). This mutation was not found in 100 healthy Japanese control individuals. No other mutation was found in the *TRPS1* gene of the patient. TRPS III has missense mutations specifically in the GATA DNA-binding zinc finger of the TRPS1 protein, located in the region, the amino acid positions 896–920. TRPS III shows similar clinical features to TRPS I, except that TRPS III presents more severe brachydactyly and growth retardation (2). From the clinical features and causative gene mutation, we diagnosed the patient as TRPSI. In our patient, we analysed mRNA levels of TRPS1 and TRPS1-related molecules expressed

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1948

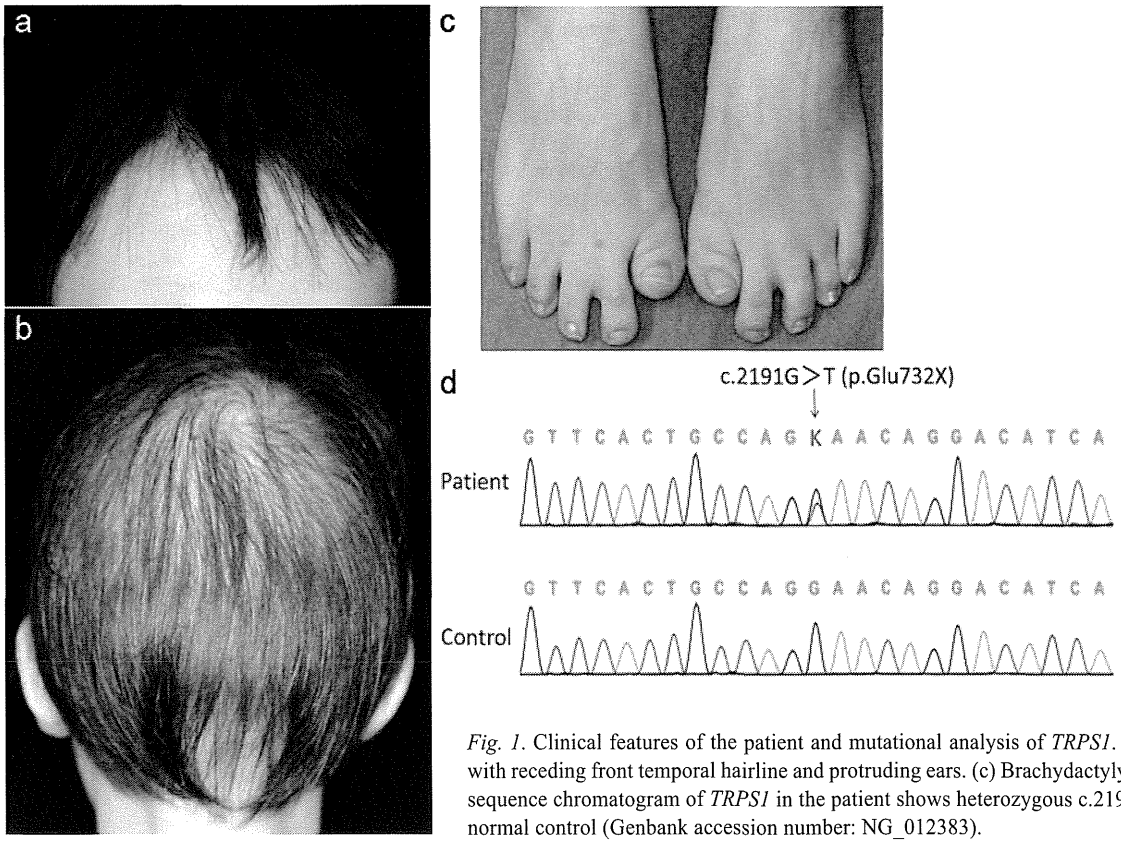

Fig. 1. Clinical features of the patient and mutational analysis of *TRPS1*. (a, b) Sparse scalp hair with receding front temporal hairline and protruding ears. (c) Brachydactyly of the big toes. (d) The sequence chromatogram of *TRPS1* in the patient shows heterozygous c.2191G>T compared with a normal control (Genbank accession number: NG_012383).

by cutaneous epithelial cells using total RNA samples extracted from plucked hairs of the patient and 7 normal control individuals. *TRPS1* was down-regulated and *STAT3*, *SOX9* and *CTNNB1* were up-regulated in plucked hairs from the patient compared with those in normal controls (Fig. S2 a, b[1]). *FGF5*, *TGFB1*, *STAT6* and *STAT1* were not up-regulated (Fig. S2c[1]).

DISCUSSION

The *TRPS1* gene encodes a zinc-finger transcription factor TRPS1 protein composed of 1,281 amino acids with 9 putative zinc-finger motifs (1). The *IKAROS*-like sequence consists of the last 2 zinc-finger motifs (motifs 8 and 9) and mediates the transcription repressive function. The 7th zinc-finger motif binds to the GATA consensus *cis* element and also mediates repressive activity (6). For example, Trps1 is assumed to be a regulator of chondrocyte proliferation and survival via the control of Stat3 expression (7). Indeed, up-regulated expression of STAT3 was observed in the outer root sheath of hair follicles in a TRPS1 patient with a TRPS1 mutation by immunohistochemistry (8). The Sox9 gene is known to regulate the proliferation and survival of hair follicle stem cells. Trps1 also regulates epithelial proliferation in the developing hair follicle via its control of Sox9 expression by the binding GATA sequence (9). β-catenin drives a hair shaft formation signal and is a key component of canonical Wnt signalling. Recent evidence suggests that the Wnt/β-catenin pathway cross-talks with STAT3 signalling to regulate the survival of retinal pigment epithelial cells (10). In mice model, Trps1is also reported to interact with 2 histone deacetylases, Hdac1 and Hdac4, thereby increasing their activity. Loss of Trps1 results in histone H3 lysine 9 (H3K9) hyperacetylation, which is maintained during mitosis (11).

We confirmed that mRNA decay occurred in *TRPS1* in plucked hairs. The GATA-type zinc-finger is resident at position 896–920. Thus, it is thought that transcription repressive function would be lost and haploinsufficiency would occur. We assumed that TRPS1 would also directly repress STAT3 and SOX9 activity by binding the GATA sequence in human hair follicles as in the mice model. The mutant TRPS1 in the present case was unable to repress *STAT3* and *SOX9* mRNA expression. The present results show that the elevation of CTNNB1 in mRNA level indicated that crosstalk of STAT3 and Wnt/β-catenin would also occur in hair follicles. Thus, TRPS1 would also repress Wnt/β-catenin signalling by repressing STAT3. In light of this, we think that the present TRPS1 loss-of-function mutation leads to the up-regulation of Sox9 and STAT3 and that the increased STAT3 signal results in the activation of hair shaft formation signalling by Wnt/β-catenin and the depletion of progenitor cells for the hair follicle epithelium. This depletion might be associated with the hypotrichosis phenotype in TRPS1. We speculate that FGF-5 and TGF-β1, which are degradation period shift signals in hair cycle, STAT6 and STAT1 were not directly influenced by TRPS1. Furthermore, loss of TRPS1 function may result in H3K9 hyperacetylation, which is maintained during mitosis (11) and supposedly lead to hypotrichosis.

In conclusion, we clearly demonstrate for the first time that haploinsufficiency of TRPS1 leads to the up-regulation of STAT3 and SOX9, and that activated STAT3 and β-catenin signalling might be associated with the hypotrichosis phenotype in TRPS1 (Fig. S2d[1]). Our data give us further insight into the function of TRPS1 in hair signalling and into the pathomechanisms of TRPS.

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Pustular psoriasis-like lesions associated with hereditary lactate dehydrogenase M subunit deficiency without interleukin-36 receptor antagonist mutation: long-term follow-up of two cases

DOI: 10.1111/bjd.13590

Dear Editor, Lactate dehydrogenase (LDH) is a key enzyme involved in the catalysis of the interconversion of pyruvate and lactate in the final step of anaerobic glycolysis, and is present in almost all cells. It exists as five isozymes composed of tetramers with two different subunits: H (heart) and M (muscle).[1] The isozymes composed mainly of the M subunit (LDH4 and LDH5) are predominant in tissues that undergo anaerobic metabolism, such as skin, liver and muscle. LDH M subunit deficiency, first reported in two Japanese families,[2] is characterized by fatigability and myalgia with myoglobinuria and high creatine kinase after strenuous exercise. The diagnosis of LDH M subunit deficiency is usually based on the electrophoretic pattern of LDH, which shows a band for LDH1 only. Erythematosquamous skin lesions were documented first,[3] and several types of eruptions have since been reported, for example desquamating erythematosquamous lesions and annular erythematous plaques with desquamating borders.[4,5] Here, we report two patients with LDH M subunit deficiency with generalized pustular psoriasis (GPP)-like lesions, and include the immunological aspects of their long-term follow-up. Patient 2 is the same patient reported previously by Yoshikuni *et al.* in 1986.[3]

Patient 1, a 64-year-old man, was initially referred to us in October 2006 for evaluation of annular erythematous plaques (Fig. 1a), with pustules on the peripheries (Fig. 1b). He had suffered from asymptomatic scaly papules and erythematous patches on the elbows and knees since childhood, which were exacerbated in the summer. Laboratory tests revealed moderately elevated levels of aspartate transaminase (70 U L^{-1}; normal range 10–35 U L^{-1}) and alanine aminotransferase (79 U L^{-1}; normal range 5–40 U L^{-1}). The electrophoretic pattern of LDH showed 100% LDH1 and 0% LDH2–LDH5. A skin biopsy taken from abdominal skin showed a subcorneal infiltrate of neutrophils in the psoriasiform epidermis with spongiform pustules of a Kogoj-like pattern (Fig. 1c). The patient had been treated intermittently with oral ciclosporin 3·5 mg kg^{-1} daily, and with topical corticosteroid and calcipotriol for 3 years. In order to avoid the adverse effects of ciclosporin, the cessation period of ciclosporin treatment was taken into consideration during treatment. However, his pustular lesions worsened. Therefore, intravenous infliximab 5 mg kg^{-1} was started, and marked therapeutic effectiveness was seen.

Patient 2, at the age of 50 years, had suffered from small follicular erythematous papules with scales and large desquamating erythematous plaques on the elbows and legs since early childhood, with myalgia after exercise (Fig. 1d).[3] At the age of 56 years, pustules emerged on large desquamating ery-

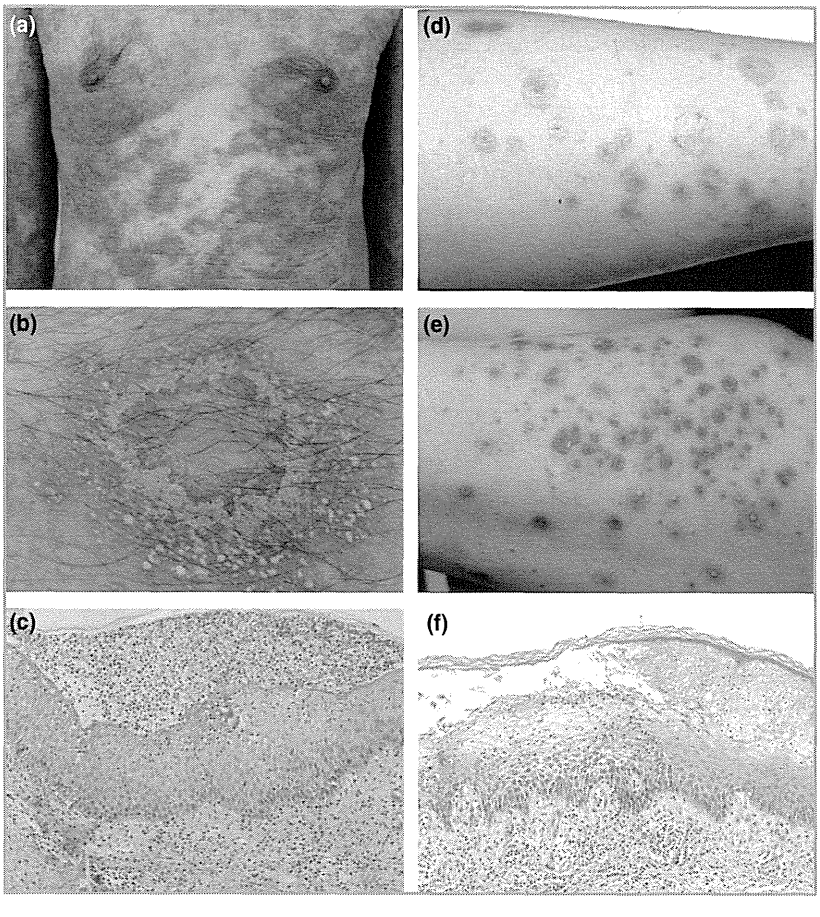

Fig 1. (a, b) Erythematous plaques with pustules on the trunk and extremities of patient 1. (c) Biopsy specimen from patient 1 showing subcorneal infiltration of neutrophils (spongiform pustules of Kogoj) in a psoriasiform epidermis with perivascular infiltration of lymphocytes and a few neutrophils in the dermis. (d) Initial skin lesions in patient 2, showing small follicular erythematous papules with scales and large desquamating erythematous plaques on her elbows and legs. (e) Skin lesions in patient 2 at the age of 56 years, which spread to the lower extremities with pustules. (f) Biopsy specimen from patient 2, showing subcorneal infiltration of neutrophils (spongiform pustules of Kogoj) and lymphocyte infiltration in the upper dermis.

thematous plaques (Fig. 1e). A skin biopsy revealed spongiform pustules of Kogoj and a lymphocytic infiltrate in the upper dermis (Fig. 1f).

After obtaining written consent, genomic DNA was prepared from peripheral blood of both patients. Seven fragments containing seven exons and exon–intron junctions were amplified and subjected to direct DNA sequencing for *LDHA*. Exon 6 of *LDHA* showed a 20-base pair deletion in both cases, which is the most common mutation in LDH M subunit deficiency (Fig. 2a), resulting in frame shift and premature termination. Six of eight Japanese patients who suffered from LDH M subunit deficiency had the same mutation (Table S1; see Supporting Information).

Intracytoplasmic cytokine expression was analysed for patient 1. Peripheral blood mononuclear cells, collected upon the appearance of pustules, were stained with mouse monoclonal antibodies to human interleukin (IL)-17A, IL-22 and interferon (IFN)-γ (BD Bioscience, Franklin Lakes, NJ, U.S.A.), as reported previously.[6] As CD4 expression on T cells is downregulated in the presence of stimulants, T helper cell (Th)17 cells were expressed as IL-17A$^+$CD3$^+$ and IL-17A$^+$CD8$^-$ cells. The percentages of IL-17A$^+$CD8$^-$ T cells and IL-22$^+$CD8$^-$ T cells were 23·2% (Fig. 2b; normal 0·4%) and 15·4% (Fig. 2c; normal < 1%), respectively. Even compared with drug eruptions,[6] which show high numbers of Th17 cells, the percentage of Th17 cells in this case was extremely high.

IL-8 is a well-known chemokine in neutrophil biology, and chemerin attracts plasmacytoid dendritic cells in relation to the pathogenesis of psoriasis.[7] In patient 2, the serum levels of IL-8 ($P = 0·02$; Fig. 2d) and chemerin ($P = 0·01$; Fig. 2e) were significantly higher than those in healthy participants ($n = 3$), as assessed by enzyme-linked immunosorbent assay.

A recent study revealed that the majority of GPP is caused by a deficiency in the IL-36 receptor antagonist due to mutations of *IL36RN*.[8] Neither of our patients had mutations of *IL-36RN*.

LDH catalyses the interconversion of pyruvate and lactate in the final step of anaerobic glycolysis.[9] Therefore, the lack of LDH activity might affect keratinocyte metabolism via impaired adenosine triphosphate (ATP) production in the anaerobic stage. Recent studies have revealed that physical and chemical damage induces the extracellular release of ATP,

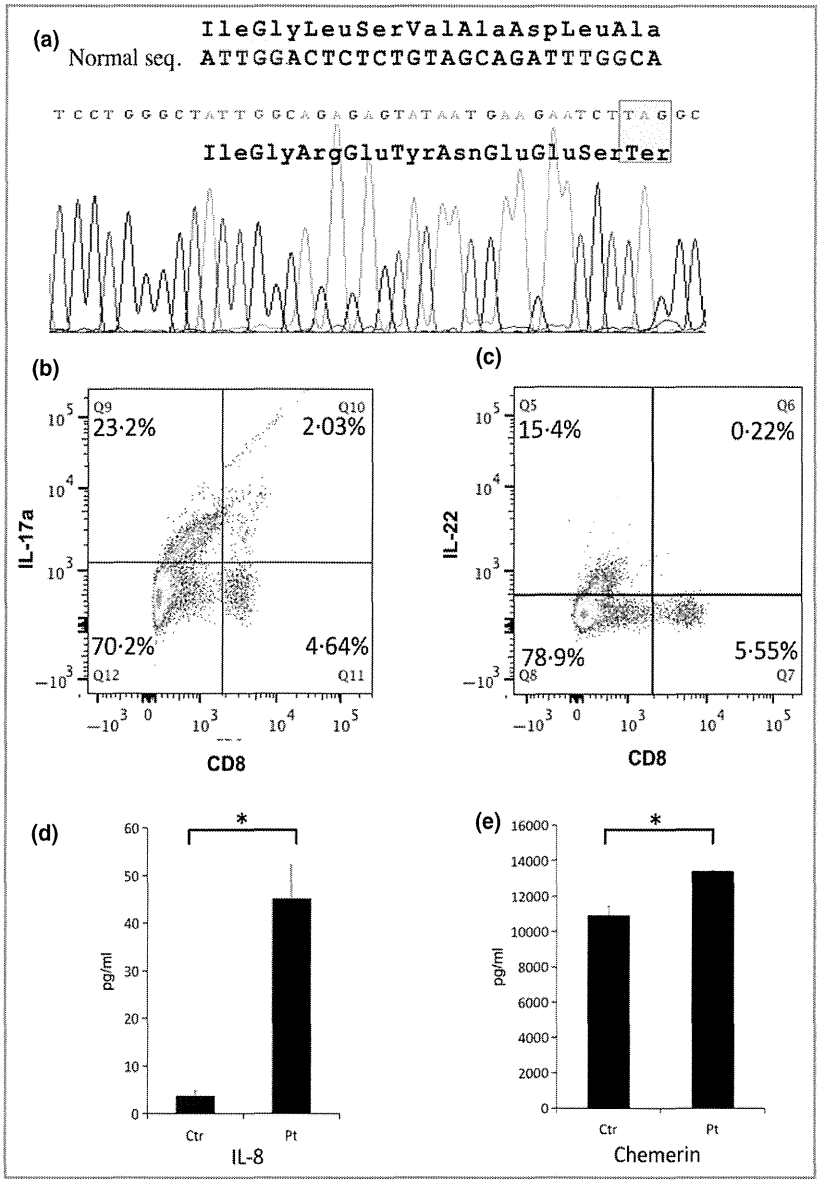

Fig 2. (a) Direct DNA sequencing of exon 6 of *LDHA* showing a 20-base pair deletion in exon 6. (b, c) Intracytoplasmic cytokine analysis showing that the frequencies of interleukin (IL)-17A$^+$CD8$^-$ cells (representing IL-17A$^+$CD4$^+$ T cells) and IL-22$^+$CD8$^-$ cells (representing IL-22$^+$CD4$^+$ T cells) were 23·3% and 15·4%, respectively, in patient 1. (d, e) Enzyme-linked immunosorbent assay data of the serum levels of chemerin and IL-8 in patient 2, which are significantly higher than those in a healthy participant (* $P = 0·02$ and $P = 0·01$, respectively). Ctr, control; Pt, patient.

followed by the production of cytokines and chemokines.[10] In association with this change, keratinocytes might release various psoriatic pathogenic factors, such as IL-8, cathelicidin LL-37 and vascular endothelial growth factor. Moreover, inflammatory and/or plasmacytoid dendritic cells can be stimulated to produce IL-23 or tumour necrosis factor-α and IFN-α, respectively.[7] It is possible that these alterations induce pustular lesions in patients with LDH M subunit deficiency. Our study suggests that abnormal LDH activity is involved in the pathogenesis of GPP.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Reported cases with hereditary lactate dehydrogenase M subunit deficiency.

Combining biologics with methotrexate in psoriasis: a systematic review

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Dear Editor, Biological drugs ('biologics') have revolutionized the treatment of patients with extensive and therapy-resistant psoriasis.[1] Unfortunately, biologics [the antitumour necrosis factor (TNF)-α agents adalimumab, infliximab and etanercept, and the anti-p40 (interleukin12/23) ustekinumab] appear to lose efficacy over time and many patients are eventually switched to another biologic.[2]

For the monoclonal antibody-based anti-TNF-α drugs, this loss of efficacy has been partly attributed to immunogenicity. Neutralizing antidrug antibody (ADA) formation against the biologic drug leads to inhibition of function and formation of drug–antibody complexes, resulting in accelerated clearance from the circulation.[3]

To overcome both these efficacy problems, the feasibility of off-label therapies that combine biologics with traditional systemic agents are being explored. However, there is insufficient evidence to suggest that these agents can significantly prevent immunogenicity in psoriasis.[4] In contrast, combined treatment with methotrexate (MTX) may improve short-term clinical efficacy and drug survival in particular. The latter effect may be due to MTX's ability to reduce neutralizing ADA formation and thus maintain adequate biologic drug levels. However, European S3 guidelines on the systemic treatment of psoriasis do not recommend this combination therapy.[5] Herein, we review the combined therapy of biologics and MTX in psoriasis.

Pivotal electronic databases were searched up to 27 October 2014 to identify studies on the combination therapy of MTX and biologics for the treatment of psoriasis (Fig. 1). The searches were limited to English-language articles. Two independent investigators performed a preliminary selection of eligible trials based on the title and abstract, followed by a second selection based on the full text (see Fig. 1). Any discrepancies were resolved by discussion or by referral to a third investigator.

Eight studies were selected and reviewed (see Table 1). These studies generally showed that combination therapy of a biologic and MTX had higher efficacy than biologic monotherapy. Combination therapy was well tolerated and not associated with higher rates of clinically relevant adverse events.

However, the following limitations of the studies reviewed should be taken into account. Firstly, most studies were performed with relatively small numbers of patients (range 11–32). The only exception was the randomized controlled trial (RCT) by Gottlieb *et al.*,[6] which prospectively investigated the efficacy and safety of etanercept monotherapy vs. MTX combination treatment in a relatively large number of patients (n = 239). Secondly, most treatment durations were short (24 weeks) and had a retrospective design, with the inherent