RHEUMATOLOGY

Rheumatology 2012;51:800–804
doi:10.1093/rheumatology/ker408
Advance Access publication 30 December 2011

Concise report

Disappearance of anti-MDA-5 autoantibodies in clinically amyopathic DM/interstitial lung disease during disease remission

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Abstract

Objective. Autoantibodies against melanoma differentiation-associated gene 5 (MDA-5) are one of the serological markers for DM. Anti-MDA-5 antibodies are especially associated with rapidly progressive interstitial lung disease (ILD) in amyopathic DM (ADM). It is known that the antibody status of anti-ENAs does not generally change significantly with disease course. For anti-MDA-5 antibodies, however, few longitudinal studies have investigated such changes. This study aimed to establish a quantitative assay for anti-MDA-5 antibodies towards assessing the long-term outcome of ADM patients who had anti-MDA-5 antibodies.

Methods. We established ELISA for measuring anti-MDA-5 antibody levels using *in vitro* transcription and translation recombinant protein. The antibody levels were measured at different time points in 11 clinically ADM patients who tested positive for the anti-MDA-5 antibody on their first visit (range of follow-up 3 months to 16 years).

Results. At the stage of clinical remission, six patients received no medication and the four others received low-dose CS. ELISA showed that anti-MDA-5 antibodies disappeared in nine of the patients and fell to just above the cut-off in one patient; in the patient who died, the antibodies remained.

Conclusion. Our results suggest that anti-MDA-5 antibodies may be useful as a marker for monitoring disease activity in ILD complicated with ADM. Serial monitoring at short intervals is required to evaluate whether anti-MDA-5 antibody levels correlate with ADM disease activity.

Key words: amyopathic dermatomyositis, anti-MDA-5 antibody, interstitial lung disease, prognosis.

SCIENCE

Introduction

Myositis-specific autoantibodies are useful for diagnosing PM/DM. DM-specific autoantibodies against melanoma differentiation-associated gene 5 (MDA-5) and transcriptional intermediary factor 1-γ are particularly important, because they are closely associated with life-threatening complications such as rapidly progressive interstitial lung disease (ILD) and internal malignancies, respectively [1–4]. A subgroup of DM patients is known to have typical skin manifestations of DM but with little evidence of myositis, a condition known as clinically amyopathic DM (C-ADM). Initially, anti-MDA-5 antibodies were reported to be serological markers of clinically ADM with rapidly progressive ILD, especially in East Asia [5]; more recently they were found in Caucasian patients with ADM complicated with ILD [6]. Although it has been suggested that patients with anti-MDA-5 antibodies have a poor prognosis, few reports have tracked the long-term outcome of these patients [4, 7].

SLE is also an autoimmune rheumatic disease that is characterized by a fluctuating disease course and a variety of autoantibodies. Many autoantibody specificities (SSA/Ro, SSB/La, Sm, U1-RNP) in lupus patients remain constant over time, whereas reactivity to dsDNA may fluctuate with disease activity, although the pattern of change differs with autoantibody specificity [8, 9]. We have little information on an association between DM-specific

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Submitted 14 July 2011; revised version accepted 20 October 2011.

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autoantibodies and the long-term outcome of DM patients [10]. We established a quantitative assay of antibody levels and monitored anti-MDA-5 autoantibodies during long-term follow-up periods in order to assess the long-term outcome of ADM patients with anti-MDA-5 antibodies.

Materials and methods

Patients

The patients were seen or consulted in the Department of Dermatology, Nagoya University Graduate School of Medicine from 1994 to 2011. From our department serum bank, we used sera from 51 patients with DM, including 30 with C-ADM and 1 with C-ADM overlapping with scleroderma. These patients were diagnosed as having DM or C-ADM based on the criteria of Bohan *et al.* [11] and of Sontheimer [12], respectively. In general, C-ADM presents as typical skin lesions and amyopathy or hypomyopathy for >6 months. The ADM group included patients who developed fatal ILD within 6 months after disease onset. Of these 51 patients, 41 were characterized in our previous study [3]: 21 were anti-MDA-5 positive and 20 were negative. This study also included additional serum samples from 10 other DM patients with anti-MDA-5 antibodies, who were seen after our previous study [3] and defined by our immunoprecipitation assays with recombinant MDA-5. The anti-MDA-5-positive serum samples totalled 31 (male : female = 5 : 26). The mean age was 48.9 (range 11–80) years. One patient with JDM was included. Twenty sera were collected from healthy blood donors and used as normal controls.

In the 31 patients with anti-MDA-5 antibodies, sera from 10 patients with ADM were taken both at their first visit and at inactive disease periods after therapy. Serum from one other patient with ADM (female, aged 46 years) was taken at her first visit and just before death from ILD 3 months later. All the patients except one were female, and their ages ranged from 23 to 60 years. They were non-smokers and had no evidence of cancer. Ten of the patients developed ILD within 6 months after disease onset, whereas one patient had no lung involvement during the course. The first sera samples from all the patients were characterized as having had anti-MDA-5 antibodies previously [3]. The range of follow-up was 5–16 years, except for the patient who died. All the patients and healthy individuals in the present study gave fully informed consent for participation, including provision of sera samples. This study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine and conducted in accordance with the Declaration of Helsinki.

ELISA

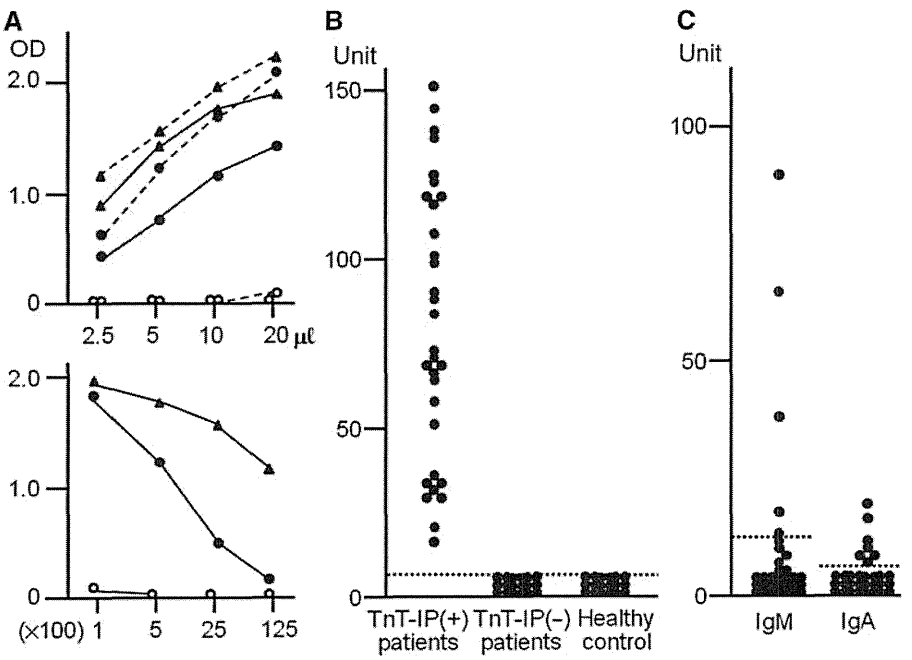
Specific binding of serum autoantibodies to recombinant MDA-5 was analysed using direct solid-phase ELISA. Biotinylated recombinant MDA-5 was produced from full-length MDA-5 cDNA using the TnT T7 Quick Coupled Transcription/Translation System (Promega, Madison, WI, USA) according to our protocol [3]. Nunc Immobilizer Streptavidin plates (Thermo Scientific Nunc, Roskilde, Denmark) to which streptavidin was covalently coupled via a spacer were pre-washed three times with PBS containing 0.05% Tween-20 (T-PBS) and were coated with biotinylated recombinant MDA-5 diluted with T-PBS (50 μl/well) and incubated for 1 h at room temperature. After three washes with T-PBS, the wells were blocked with 200 μl of a blocking buffer of 0.5% BSA (Wako, Osaka, Japan) in T-PBS for 1 h. Uncoated wells were used to measure the background levels for each sample. Diluted sample sera with blocking buffer (75 μl/well) were incubated for 1 h at room temperature, followed by incubation with anti-human IgG, IgM or IgA antibody conjugated with HRP (Dako, Glostrup, Denmark) as a secondary antibody (75 μl/well) at 1 : 30 000 dilution after five washes. After incubation for 1 h at room temperature, the plates were washed five times and incubated with Ultra TMB (Pierce, Rockford, IL, USA) (75 μl/well) as the substrate, according to the manufacturer's protocol. Then, optical density (OD) at 450 nm was determined using Multiskan FC (Thermo Scientific, Waltham, MA, USA). Each serum sample was tested in duplicate, and the mean OD subtracted background was used for data analysis. An in-house ELISA was used for measuring anti-diphtheria toxoid (DT). In brief, plates (Medisorp, Thermo Scientific Nunc) were coated with 50 μl/well DT (1 μg/ml in PBS) (List Biological Laboratories, Campbell, CA, USA) and blocked with 3% BSA in T-PBS. The sera samples were diluted 1 : 100 in 3% BSA in T-PBS. Anti-human IgG antibody conjugated with HRP and a substrate was used in the manner described above.

Results

ELISA with biotinylated recombinant MDA-5

To measure anti-MDA-5 antibodies in sera quantitatively, we tried to establish an ELISA that uses biotinylated recombinant MDA-5. Based on the results of two different anti-MDA-5-positive sera (Fig. 1A), we decided to use the 10 μl/well of TnT mixture and the diluted patient serum samples at 1 : 500 for measuring all samples. The unit of each sample was calculated as that sample's OD divided by the OD of the standard positive serum #1251 and then multiplied by 100. With the cut-off value determined as the mean value of 20 control sera + 3 S.D., 31 serum samples that had been identified as positive for anti-MDA-5 antibodies by immunoprecipitation also tested positive in these ELISA, and 20 serum samples from patients that were identified as being without anti-MDA-5 antibodies by immunoprecipitation also tested negative (Fig. 1B). We also measured IgM- and IgA-class antibodies using these assays as a positive control for the IgG anti-MDA-5 antibody level of #1251 (Fig. 1C). Both immunoglobulin classes of anti-MDA-5 antibodies were present, but in minor populations.

FIG. 1 ELISA using biotinylated recombinant MDA-5 protein. (**A**) Serial dilution of biotinylated protein (upper panel) or sera (lower panel) for ELISA. Closed circle and triangle: anti-MDA-5-positive sera in immunoprecipitation analysis. Open circle: healthy individual serum. In the upper panel, broken and solid lines denote sera diluted to 1 : 100 and 1 : 500, respectively. Recombinant protein was diluted with T-PBS to 50 μl of the final volume per well. In the lower panel, recombinant protein was applied at 10 μl diluted with 40 μl of T-PBS per well. Serum dilution was 1 : 100–1 : 12 500. (**B**) Measurement of anti-MDA-5 antibodies in 71 serum samples. All samples were classified as positive or negative for anti-MDA-5 antibodies, as determined by immunoprecipitation assay with biotinylated proteins. Broken line indicates the cut-off value (6.5 U), calculated from the mean OD values of 20 healthy controls + 3 S.D. (**C**) Isotype analysis of anti-MDA-5 antibodies. Thirty-one IgG anti-MDA-5-positive serum samples were also analysed for IgM and IgA class antibodies. Broken lines indicate the cut-off value (9.4 U for IgM and 6.0 U for IgA) calculated from the mean values of 20 healthy controls + 3 S.D.



Decline in anti-MDA-5 antibodies during remission

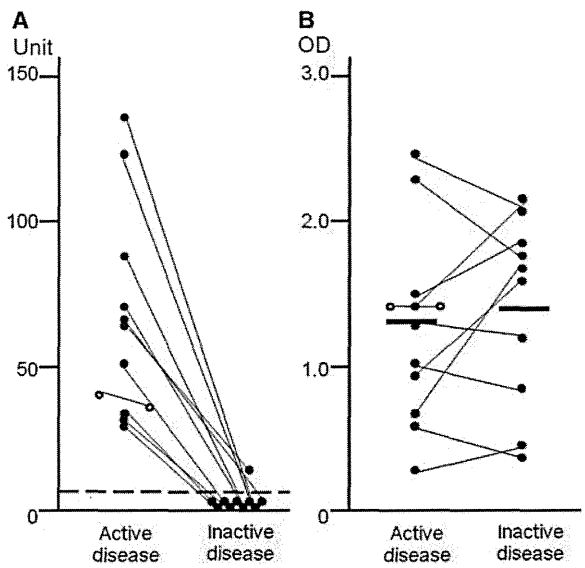
From 31 patients whose initial serum samples had anti-MDA-5 antibodies, sera were retaken during remission periods from the 10 patients with C-ADM. As a treatment for ILD in nine of these patients, methylprednisolone pulse therapy and immunosuppressive drugs were administered to eight patients and seven patients, respectively. The following immunosuppressive drugs were administered: ciclosporin to two patients, the combination of ciclosporin and i.v. CYC to two patients; ciclosporin, AZA and i.v. CYC to two patients and AZA and i.v. CYC to one patient. After initial therapy, 6 of the 10 patients were in clinical remission, which was defined as no evidence of active skin rash, myositis and lung involvement for >6 months without drug therapy. The remaining four patients also entered clinical remission, but with therapy of low-dose CS (prednisolone <7.5 mg/day). None of the 10 patients showed aggravated interstitial findings in their chest radiograph examinations for >5 years. The sampling of sera during remission ranged from 5 to 15 years after the first sampling. IgG-class anti-MDA-5 antibody levels were compared between serum samples at active and inactive disease states (Fig. 2A). Except for one patient who still had anti-MDA-5 antibodies but whose titre was dramatically reduced at 5 years from disease onset, in all the sera the anti-MDA-5 antibodies were absent during remission. These were also confirmed to be negative in the same ELISA plate with 20 sera samples from healthy individuals, and also by immunoprecipitation using this biotinylated protein (data not shown).

We also measured anti-DT antibodies in the same serum samples because we wondered whether the disappearance of anti-MDA-5 antibodies related to general immunosuppression. ELISA results showed that antibodies against DT remained at similar levels (Fig. 2B).

Discussion

In a Japanese multicentre study, 5-year survival in patients with anti-MDA-5 antibodies was 56% [4]. However, the long-term outcome of ADM patients has been seldom reported in terms of longitudinal serological findings. Since we recently examined >10 ADM patients

FIG. 2 Decrease of anti-MDA-5 antibody levels in remission. (**A**) Anti-MDA-5 antibody levels in 10 patients with ADM who were positive for serum anti-MDA-5 antibodies at their first visit decreased during inactive disease periods under the cut-off level (6.5 U), which is shown by the broken line. The open circle indicates the patient who died 3 months after disease onset. (**B**) Titres of anti-DT antibodies in ELISA. The same serum samples as used in (**A**) were measured. Horizontal bars show the mean values of OD for the sera group in the active stage (except for the patient indicated by the open circle) and for the sera group in the inactive stage.



with anti-MDA-5 antibodies who experienced clinical remission for >5 years, we investigated anti-MDA-5 antibodies in these surviving patients. Our results showed that all but one patient lost anti-MDA-5 antibodies in sera and went into remission.

Kuwana *et al.* [13] examined serial changes in anti-topo I antibody levels in patients with SSc and found that, in some patients with a favourable outcome, loss of anti-topo I antibodies occurred within 10 years after the first visit. Kinetic studies of *in vitro* T-cell proliferation indicated that the disappearance of anti-topo I antibodies was due to loss of activation of topo I-reactive T cells. Expressions of cryptic epitopes by protein cleavage are probably important for the autoantibody response. MDA-5, which plays important roles in the innate immune system during RNA viral infections, is degraded in cells infected with different picornaviruses [14]. Whether such cleavage might lead to autoimmune responses against MDA-5 needs further investigation.

In summary, we have identified the disappearance of anti-MDA-5 antibodies in ADM remission. The precise factors or mechanisms that define positive/negative immune response to MDA-5 among ADM patients remain unknown. Future studies should address whether anti-MDA-5 antibody levels are useful as indicators for response to therapy. To confirm anti-MDA-5 antibodies as a marker for increased disease activity, future studies would need to determine whether anti-MDA-5 antibodies reappear during disease activity.

Rheumatology key messages

- Anti-MDA-5 antibodies could be an important serological marker for ILD in ADM patients.
- The tracking of anti-MDA-5 antibodies could be useful for monitoring disease activity in ILD complicated with ADM.

Acknowledgements

Funding: This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan, and from the 24th General Assembly of the Japanese Association of Medical Sciences.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Sato S, Hoshino K, Satoh T *et al.* RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. Arthritis Rheum 2009;60:2193–200.

2 Kaji K, Fujimoto M, Hasegawa M *et al.* Identification of a novel autoantibody reactive with 155 and 140kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. Rheumatology 2007;46:25–28.

3 Hoshino K, Muro Y, Sugiura K *et al.* Anti-MDA5 and anti-TIF1-γ antibodies have clinical significance for patients with dermatomyositis. Rheumatology 2010;49:1726–33.

4 Hamaguchi Y, Kuwana M, Hoshino K *et al.* Clinical correlations with dermatomyositis-specific autoantibodies in adult Japanese patients with dermatomyositis: a multicenter cross-sectional study. Arch Dermatol 2011;147:391–8.

5 Sato S, Kuwana M. Clinically amyopathic dermatomyositis. Curr Opin Rheumatol 2010;22:639–43.

6 Fiorentino D, Chung L, Zwerner J *et al.* The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. J Am Acad Dermatol 2011;65:25–34.

7 Gono T, Kawaguchi Y, Satoh T *et al.* Clinical manifestation and prognostic factor in anti-melanoma differentiation-associated gene 5 antibody-associated interstitial lung disease as a complication of dermatomyositis. Rheumatology 2010;49:1713–9.

8 Tench CM, Isenberg DA. The variation in anti-ENA characteristics between different ethnic populations with systemic lupus erythematosus over a 10-year period. Lupus 2000;9:374–6.

9 Faria AC, Barcellos KSA, Andrade LEC. Longitudinal fluctuation of antibodies to extractable nuclear antigens in systemic lupus erythematosus. J Rheumatol 2005;32: 1267–72.

10 Marie I, Lahaxa L, Benveniste O *et al*. Long-term outcome of patients with polymyositis/dermatomyositis and anti-PM-Scl antibody. Br J Dermatol 2010;162:337–44.

11 Bohan A, Peter JB, Bowman RL *et al*. A computer-assisted analysis of 153 patients with polymyositis and dermatomyositis. Medicine 1977;56:255–86.

12 Sontheimer RD. Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis siné myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? J Am Acad Dermatol 2002;46:626–36.

13 Kuwana M, Kaburaki J, Mimori T *et al*. Longitudinal analysis of autoantibody response to topoisomerase I in systemic sclerosis. Arthritis Rheum 2000;43:1074–84.

14 Barral PM, Morrison JM, Drahos J *et al*. MDA-5 is cleaved in poliovirus-infected cells. J Virol 2007;81:3677–84.

LEMD3 mutation to tropoelastin expression. *Eur J Dermatol* 2010; **20**:693–7.
8 Mumm S, Wenkert D, Zhang X *et al.* Deactivating germline mutations in LEMD3 cause osteopoikilosis and Buschke–Ollendorff syndrome, but not sporadic melorheostosis. *J Bone Miner Res* 2007; **22**:243–50.
9 Couto AR, Bruges-Armas J, Peach CA *et al.* A novel LEMD3 mutation common to patients with osteopoikilosis with and without melorheostosis. *Calcif Tissue Int* 2007; **81**:81–4.
10 Zhang Y, Castori M, Ferranti G *et al.* Novel and recurrent germline LEMD3 mutations causing Buschke–Ollendorff syndrome and osteopoikilosis but not isolated melorheostosis. *Clin Genet* 2009; **75**:556–61.
11 Baasanjav S, Jamsheer A, Kolanczyk M *et al.* Osteopoikilosis and multiple exostoses caused by novel mutations in LEMD3 and EXT1 genes respectively – coincidence within one family. *BMC Med Genet* 2010; **11**:110.
12 Yuste-Chaves M, Cañueto J, Santos-Briz A *et al.* Buschke–Ollendorff syndrome with striking phenotypic variation resulting from a novel c.2203C>T nonsense mutation in LEMD3. *Pediatr Dermatol* 2011; **28**:447–50.
13 Hellemans J, Debeer P, Wright M *et al.* Germline LEMD3 mutations are rare in sporadic patients with isolated melorheostosis. *Hum Mutat* 2006; **27**:290–6.
14 Lin F, Morrison JM, Wu W, Worman HJ. MAN1, an integral protein of the inner nuclear membrane, binds Smad2 and Smad3 and antagonizes transforming growth factor-beta signaling. *Hum Mol Genet* 2005; **14**:437–45.
15 Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol* 2007; **127**:526–37.
16 Schiller M, Javelaud D, Mauviel A. TGF-beta-induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. *J Dermatol Sci* 2004; **35**:83–92.
17 Brown JJ, Bayat A. Genetic susceptibility to raised dermal scarring. *Br J Dermatol* 2009; **161**:8–18.

Funding sources: none.

Conflicts of interest: none declared.

Possible modifier effects of keratin 17 gene mutation on keratitis–ichthyosis–deafness syndrome

DOI: 10.1111/j.1365-2133.2011.10696.x

MADAM, Keratitis–ichthyosis–deafness (KID) syndrome (OMIM 148210, 242150) is a rare type of ectodermal dysplasia caused by mutations in the gap junction protein beta-2 gene (GJB2)[1] or beta-6 gene (GJB6).[2] On the other hand, mutations in genes encoding keratin 6a, 6b, 16 and 17 (*KRT6A*, *KRT6B*, *KRT16* and *KRT17*) are known to cause pachyonychia congenita (PC; OMIM 16720, 17210). PC and KID syndrome share similar symptoms, such as palmoplantar hyperkeratosis and onychodystrophy. This study reports a Japanese patient with atypical KID syndrome with the combined heterozygous mutations of a recurrent mutation in *GJB2* and a novel mutation in the V1 region of *KRT17*.

The proband was a 40-year-old Japanese woman. She was the child of healthy, nonconsanguineous parents. From childhood, she had shown diffuse mutilating palmoplantar hyperkeratosis (Fig. 1a), nail dystrophy (Fig. 1b), hypotrichosis, sensorineural hearing loss, and vascularized keratitis. Periorificial hyperkeratosis was not seen. From these findings, the diagnosis of KID syndrome was made. She had had recurrent bacterial and fungal skin infections. In her twenties, painful tumours appeared on her lower limbs. In her thirties, tumours on both buttocks developed to take on a papilloma-like appearance (Fig. 1c). Etretinate with topical or systemic antibiotics and antifungal agents did not alleviate her symptoms. Skin abrasion was repeatedly conducted on the tumours. Histopathology of the lesions revealed epidermal pseudocarcinomatous hyperplasia with dilation of vessels in papillary and reticular dermis accompanied by mixed immune cell infiltrates, excluding the involvement of squamous cell carcinoma (Fig. 1d). Vacuolated keratinocytes, suggesting human papillomavirus infection, were not detected.

Genomic DNA extracted from peripheral blood was used as a template for polymerase chain reaction (PCR) amplification. Direct sequencing of *GJB2*, *GJB6*, *KRT6A*, *KRT6B*, *KRT16* and *KRT17* was performed as described elsewhere.[3–5] The medical ethical committee of Hokkaido University approved all the described studies. The study was conducted according to the Declaration of Helsinki Principles. The proband gave her written informed consent.

Mutation analysis of the proband's genomic DNA revealed a c.148G>A transition (p.Asp50Asn) in *GJB2* (Fig. 2a), which is the most prevalent mutation in patients with KID syndrome.[1] Furthermore, the proband was found to be heterozygous for a c.177C>A transversion (p.Ser59Arg) in *KRT17* (Fig. 2b). Restriction enzyme digestion of the PCR products by *PvuII* was carried out to confirm the c.177C>A in *KRT17* (Fig. 2c). The c.177C>A in *KRT17* was novel and was not detected in 200 alleles from 100 normal Japanese individuals. Mutation screening on the proband's parents could not be performed because the father was not alive and the mother did not consent. Keratin 17 (K17) immunohistochemistry on skin samples from several different sites revealed K17 expression in whole epidermis although its expression level did not vary between nonlesional and lesional skin specimens (data not shown).

As the clinical manifestations of the proband were atypical and more severe than those of other patients with KID syndrome – as evidenced, for example, by diffuse mutilating palmoplantar hyperkeratosis and recurrent granulation tissue formation on the buttock – we hypothesized that mutations in other genes might have affected the proband's phenotype through modifier effects. Modifier genes are defined as genes that affect the phenotypic expression of another gene, and several studies have demonstrated that modifier genes are involved in manifestations of inherited disorders.[6] *KRT6A*, *KRT6B*, *KRT16* and *KRT17*, the causative genes of PC, which affects the nails and the palmoplantar area, were selected as candidates for modifier gene investigation in our case, although we cannot exclude the possibility that there are some other genes which modify KID syndrome phenotype.

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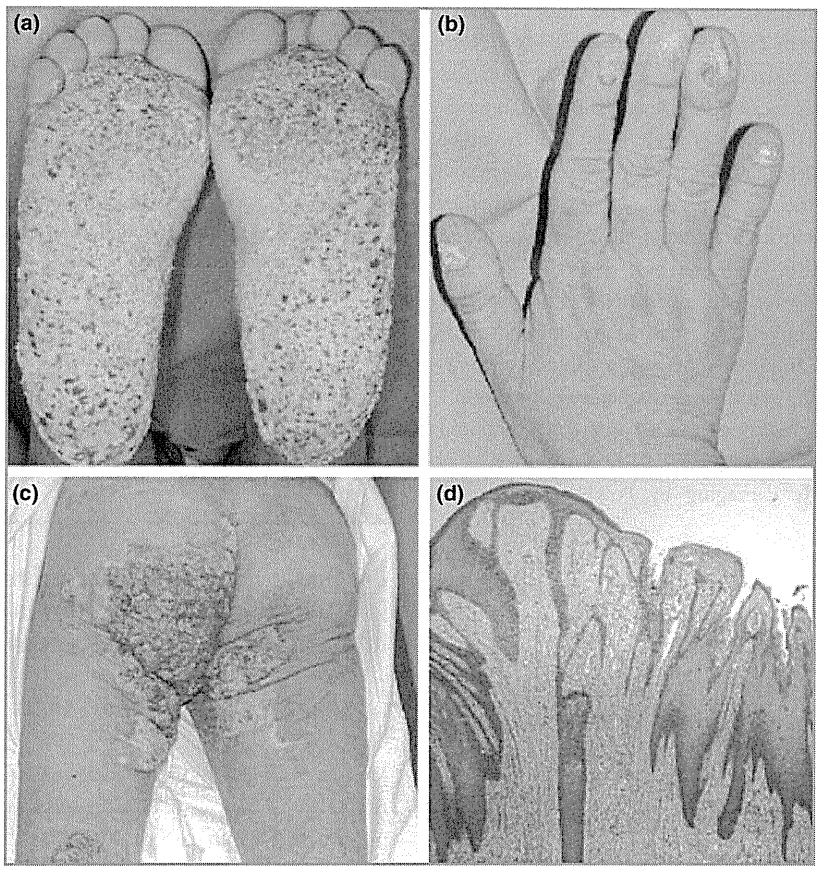

Fig 1. Clinical features of the proband. (a) Numerous erosive papules are coalesced into a hyperkeratotic plaque on the proband's soles. (b) Nail dystrophy is seen in the fingers. (c) A tumour is observed on the left buttock. Scars after skin abrasion are seen on the dorsal aspects of the thigh and on the right buttock. (d) Specimens from the tumour show pseudocarcinomatous hyperplasia of the epidermis. Dilated vessels with monocytic infiltrates are seen in the dermis (haematoxylin and eosin; original magnification × 100).

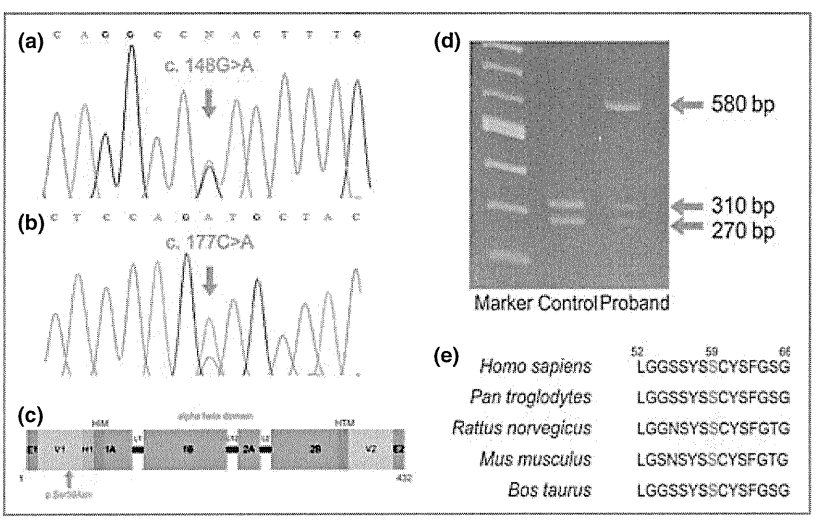

Fig 2. Mutation analysis. (a) The proband was heterozygous for a c.148G>A transition (p.Asp50Asn) mutation in *GJB2* (arrow). (b) c.177C>A (p.Ser59Arg) in *KRT17* was detected in the proband's genomic DNA (arrow). (c) PvuII restriction enzyme digestion of the polymerase chain reaction (PCR) products from genomic DNA of the proband and a normal control. c.177C>A resulted in the loss of a site for PvuII. PvuII restriction enzyme digestion of the PCR products from a normal controls reveals 270- and 310-bp bands. In contrast, 270-, 310- and 580-bp bands were detected in the proband, suggesting that she was heterozygous for c.177C>A. (d) A schematic of the structure of keratin 17. Note that Ser^{59} is located at the V1 region of the keratin molecule (arrow). HIM, helix initiation motif; HTM, helix termination motif. (e) Keratin 17 amino acid sequence alignment shows the level of conservation in diverse species of the amino acid Ser59 (red characters).

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Most of the keratin mutations are within the helix boundary motifs, which are crucial for keratin monomers to form dimers and subsequent keratin networks.[7] The KRT17 mutation found in the proband was located not within the helix boundary motifs but in the V1 region of K17 (Fig. 2d). In other keratin genes, such as KRT5 and KRT16, some mutations have been reported within the V1 region, and the phenotypes resulting from these mutations are milder than those resulting from the mutations within the helix boundary motifs.[7] The V1 regions of keratin intermediate filament have glycine loops[8] and it has been suggested that these structures modulate flexibility and other unknown physical attributes of keratin filaments by interacting with similar structures in loricrin.[9] Ser^{59} is located within a highly conserved segment composed of the glycine loop in K17 (Fig. 2e). p.Ser59Arg in K17 is predicted to be probably damaging by PolyPhen-2, with a score of 0·893.[10]

Based on these findings, it is conceivable that the p.Ser59-Arg variant in K17 has a modifying effect on the pathogenic GJB2 mutation p.Asp50Asn and may contribute the proband's phenotype. Nevertheless, the limited scope of this study (single case report) does not allow us to determine the clinical significance of p.Ser59Arg in K17, and the influence of other genetic and epigenetic factors cannot be excluded.

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References

1 Mazereeuw-Hautier J, Bitoun E, Chevrant-Breton J *et al.* Keratitis–ichthyosis–deafness syndrome: disease expression and spectrum of connexin 26 (GJB2) mutations in 14 patients. *Br J Dermatol* 2007; **156**:1015–19.
2 Jan AY, Amin S, Ratajczak P *et al.* Genetic heterogeneity of KID syndrome: identification of a Cx30 gene (GJB6) mutation in a patient with KID syndrome and congenital atrichia. *J Invest Dermatol* 2004; **122**:1108–13.
3 Richard G, White TW, Smith LE *et al.* Functional defects of Cx26 resulting from a heterozygous missense mutation in a family with dominant deaf-mutism and palmoplantar keratoderma. *Hum Genet* 1998; **103**:393–9.
4 del Castillo I, Villamar M, Moreno-Pelayo MA *et al.* A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002; **346**:243–9.
5 Kanda M, Natsuga K, Nishie W *et al.* Morphological and genetic analysis of steatocystoma multiplex in an Asian family with pachyonychia congenita type 2 harbouring a KRT17 missense mutation. *Br J Dermatol* 2009; **160**:465–8.
6 Nadeau JH. Modifier genes in mice and humans. *Nat Rev Genet* 2001; **2**:165–74.
7 Szeverenyi I, Cassidy AJ, Chung CW *et al.* The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases. *Hum Mutat* 2008; **29**:351–60.
8 Steinert PM, Mack JW, Korge BP *et al.* Glycine loops in proteins: their occurrence in certain intermediate filament chains, loricrins and single-stranded RNA binding proteins. *Int J Biol Macromol* 1991; **13**:130–9.
9 Terrinoni A, Puddu P, Didona B *et al.* A mutation in the V1 domain of K16 is responsible for unilateral palmoplantar verrucous nevus. *J Invest Dermatol* 2000; **114**:1136–40.
10 Adzhubei IA, Schmidt S, Peshkin L *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**:248–9.

Funding sources: none.

Conflicts of interest: none declared.

Clinically manifest X-linked recessive ichthyosis in a female due to a homozygous interstitial 1·6-Mb deletion of Xp22.31

DOI: 10.1111/j.1365-2133.2011.10685.x

Madam, X-linked recessive ichthyosis (XLI; OMIM 308100) is caused by mutations in or deletion of the entire STS gene coding for steroid sulfatase on Xp22.31.[1,2] Most patients (> 90%) have deletions, the most common one being approximately 1·6 Mb in size. The prevalence is about 1 : 1500 in males. The skin phenotype is fairly typical, consisting of mild erythroderma and generalized peeling or exfoliation of large, translucent scales within the first weeks after birth. Later, during infancy, large polygonal dark-brown scales develop on the extremities, trunk and neck. STS activity is reduced in female carriers, who can have dry skin in winter but otherwise have no recognizable phenotype. Three sisters with overt disease have previously been reported,[3] although without genetic analysis. We now describe a woman with clinically manifest XLI and elucidate the genetic basis.

The patient, of Dutch descent, presented to our department at the age of 18 years. She was born at term after prolonged delivery and, when she was a few weeks old, developed mild erythroderma with translucent scales that later on became larger and dark brown. Flexural areas were normal but she did have nuchal scaling. Her palms, soles and face were spared. There were no nail abnormalities, anosmia, or palmar hyperlinearity; psychomotor development was normal and she had no dysmorphic traits. Serum STS activity was zero. We diagnosed the patient as having XLI. The father, two of his brothers and several paternal first cousins also had been diagnosed with XLI. The same diagnosis had been established in several maternal uncles. Her mother and sister had dry scaling skin in the winter. We therefore surmised that XLI was segregating in both families.

In order to confirm the clinical diagnosis of XLI, fluorescent in situ hybridization (FISH) was carried out on metaphase

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fungal and radiographic studies were not thought to be necessary. A matrix biopsy was not performed because the patient and her family refused consent.

Comment. Samman[6] describes proximal nail loss without scarring as *periodic shedding* and categorized *autosomal dominant periodic nail shedding* as a subgroup of this category. Since 1897, there have been 4 case reports to our knowledge[2-5] of healthy adolescents or adults presenting with periodic shedding of their nails and a family history among several siblings or parents of the same patients. The first reported case of nonfamilial nail shedding was described in 2009 by Venugopal and Murrell.[7] Cases of idiopathic familial and sporadic onychomadesis are summarized in the **Table**.

The mechanism of nail matrix arrest in the setting of infection, fever, systemic disease, or drug exposure is unknown. Inhibition of cellular proliferation may occur and would be a logical explanation during treatment with antimitotic therapy. Another hypothesis is that the matrix activity and growth rate of the nails remains intact, but the quality of the manufactured nail plate differs, becoming thinner and dystrophic.

Idiopathic cases of onychomadesis can be classified as familial (autosomal dominant inheritance) or sporadic.

We describe herein a young, healthy female patient with no family history of onychomadesis and no seasonal variation in nail shedding. We suggest calling such unusual cases *idiopathic sporadic onychomadesis*.

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Financial Disclosure: None reported

1. Beau J. Note sur certains caracteres de semeiologie retrospective presentes par les ongles. *Arch Gen Med.* 1846;11(4):447-458.
2. Montgomery D. A case of hereditary and continuous shedding of the finger-nails. *J Cutan Dis.* 1897;12(3):252-256.
3. Oliver WJ. Recurrent onychoptosis occurring as a familial disorder. *Br J Dermatol Syph.* 1927;39:297-299.
4. Martin S, Rudolph AH. Familial dystrophic periodic shedding of the nails. *Cutis.* 1980;25(6):622-623.
5. Mehra A, Murphy RJ, Wilson BB. Idiopathic familial onychomadesis. *J Am Acad Dermatol.* 2000;43(2, pt 2):349-350.
6. Samman PD. *The Nails in Disease.* London, England: Heinemann Medical Books; 1965:183-184.
7. Venugopal SS, Murrell DF. Seasonal onychomadesis in an elderly gentleman. *Dermatol Rep.* 2009;1(3):10-11.

Perinatal Cytomegalovirus-Associated Bullae in an Immunocompetent Infant

Although perinatal cytomegalovirus (CMV) infection is common, symptomatic CMV infection is a rare complication in immunocompetent infants. We describe herein CMV-associated bullae accompanied by CMV hepatitis in an immunocompetent newborn infant is reported.

Report of a Case. The patient was a 1-month-old female infant of 39 weeks' gestation and vaginal delivery with no apparent complications. She presented with a history of crops of vesicles and bullae on the face, precordial area, and buttocks that began appearing at age 3 weeks and continued to appear. The lesions manifested as multiple 10-mm-diameter bullae with several crusts on the sites (**Figure 1**A and B).

Fluid content was purulent, and Tzanck test findings were negative. The patient had no fever, lymphadenopathy, or organomegaly, and her general condition and neonatal reflexes were normal. The findings of her neonatal screening test for metabolic diseases were negative. Birth weight was 2.99 kg, and she was normally breastfed.

A skin biopsy specimen from a newly formed blister in the skin overlying the lower jaw revealed severe spongiosis and reticular degeneration in the epidermis. Perivascular mixed inflammatory cell infiltration was seen, and the blood vessel wall was thickened in the superficial dermis (**Figure 2**). Cytomegalovirus was not detected by immunohistochemical staining of the skin specimen.

Laboratory tests demonstrated normal complete blood cell counts and normal biochemical findings except for levels of aspartate aminotransferase (AST) (406 U/L) and alanine aminotransferase (ALT) (325 U/L), suggesting active hepatitis (to convert AST and ALT to microkatal per liter, multiply by 0.0167). Serum concentration of immunoglobulin, complement titer, CD4/CD8 ratio, and lymphocyte stimulation test results were normal for her age. Serum anti-CMV IgM findings were negative, and anti-CMV IgG findings were positive at age 3 months. There was no serologic evidence of infection by herpes simplex virus (HSV), herpes zoster virus (HZV), or Epstein-Barr virus. Results of the pp65 antigenemia assay for circulating CMV antigen were positive (226 positive cells of 41 000 total cells). Real-time polymerase chain reaction (PCR) CMV findings were positive in urine and blood plasma samples. Cytomegalovirus DNA findings were negative on a Guthrie card, which meant that congenital CMV infection was excluded. No bacteria or HSV-1, HSV-2, or HZV specimens were isolated by cultures from the blister fluid. Findings of real-time PCR analyses for HSV and HZV sequences were negative for the blister fluid. The patient and her mother both tested negative for human immunodeficiency virus infection.

The bullae, hepatitis, and copy numbers of CMV were improved by anti-CMV hyperimmune gammaglobulin and intravenous ganciclovir treatment. The skin lesions healed with scarring (Figure 1C). Her physical and mental development was normal.

Comment. Congenital CMV infection or perinatal symptomatic CMV infection in immunocompromised or low-birth-weight infants occasionally shows systemic manifestations (eg, neurologic and developmental problems, hepatitis, pneumonia, retinitis, enterocolitis) and skin manifestations, including indurated

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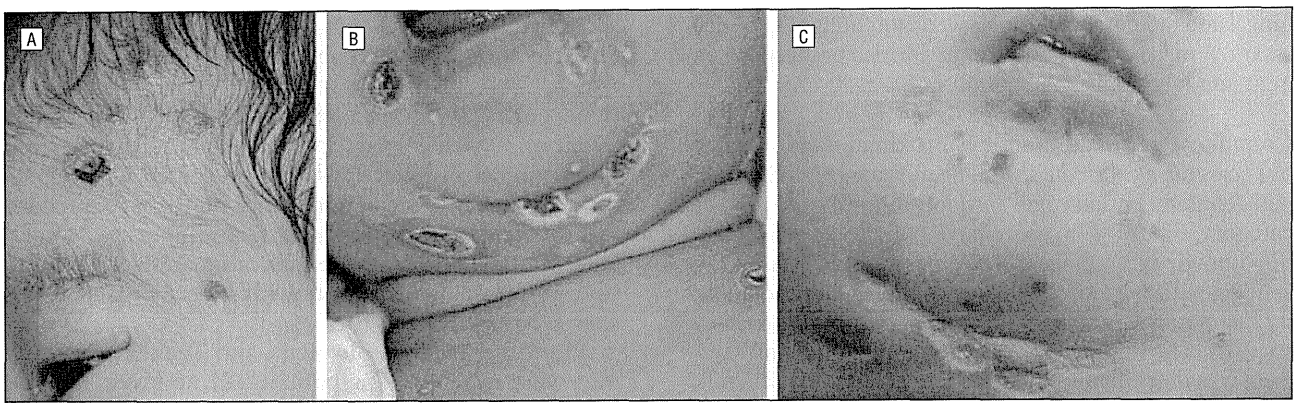

Figure 1. Skin manifestations of the patient. A and B, On the day of admission at patient age 1 month, pustules and erosions with crusts are scattered on the forehead (A) and on the chin and the chest (B). C, After administration of anticytomegalovirus agents, the skin lesions on the chin healed with scarring.

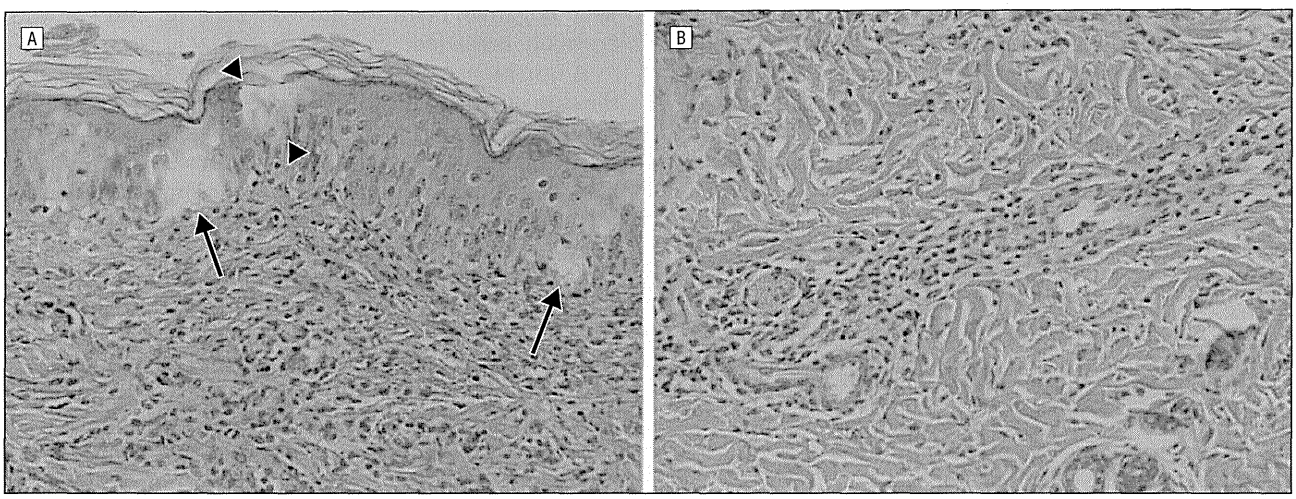

Figure 2. Histopathologic findings from the bulla on the lower jaw. A, Microblisters (arrows) and reticular degeneration (between triangles) of the epidermis are seen (hematoxylin-eosin, original magnification ×400). B, Perivascular inflammatory cell infiltration and thickening of the blood vessel wall are seen in the superficial dermis (hematoxylin-eosin, original magnification ×400).

hyperpigmented nodules and papules, papular and pruritic eruptions, vesiculobullous lesions, and perianal and perineal ulcers.[1-3] To our knowledge, symptomatic CMV-associated skin manifestations have not been previously reported in immunocompetent infants,[4] but CMV-associated erythema multiforme (EM) has been reported in immunocompetent adults.[5] The present case suggests that symptomatic CMV skin infection can occur not only in immunocompromised hosts and preterm infants but also in immunocompetent infants.

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Financial Disclosure: None reported.
Funding/Support: This study was supported in part by Grant-in-Aid for Scientific Research (C) 23591617 from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Dr Sugiura).

1. Bale JF Jr, Reiley TT, Bray PF, Kelsey DK. Cytomegalovirus and dual infection in infants. *Arch Neurol.* 1980;37(4):236-238.
2. Sandler A, Snedeker JD. Cytomegalovirus infection in an infant presenting with cutaneous vasculitis. *Pediatr Infect Dis J.* 1987;6(4):422-423.
3. Hancox JG, Shetty AK, Sangueza OP, Yosipovitch G. Perineal ulcers in an infant: an unusual presentation of postnatal cytomegalovirus infection. *J Am Acad Dermatol.* 2006;54(3):536-539.

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4. Tajiri H, Kozaiwa K, Tanaka-Taya K, et al. Cytomegalovirus hepatitis confirmed by in situ hybridization in 3 immunocompetent infants. *Scand J Infect Dis*. 2001;33(10):790-793.
5. Koga T, Kubota Y, Nakayama J. Erythema multiforme-like eruptions induced by cytomegalovirus infection in an immunocompetent adult. *Acta Derm Venereol*. 1999;79(2):166.

Chinese Fortune-Telling Based on Face and Body Mole Positions: A Hidden Agenda Regarding Mole Removal

We were asked to remove moles from the faces of 2 Chinese children whose parents were born in mainland China. It was only after learning about their belief in Chinese mole reading did we understand their insistence. According to this belief, mole position, shape, size, and color have fortune-telling weight. The Thai of Chinese descent call this type of fortune-telling *Ngao Heng*.

Report of Cases. *Case 1*. A 12-year-old Chinese boy presented with an asymptomatic acquired nevus on his right nasolabial fold (**Figure 1**). Dermoscopy revealed a brown, bland-appearing nevocellular nevus with a globular pattern. The boy's father insisted on having the nevus removed even after he was assured that it was not necessary and the outlines of the scar were drawn on the boy's face.

Case 2. A 7-year-old Chinese girl (unrelated to the first patient) presented with a similar nevus on her cheek, just lateral to the nasolabial fold. Gross and dermoscopic examination revealed that the nevus was approximately 4 mm in diameter, bland in color, and normal in shape. The girl's mother insisted on removal. No amount of reassurance as to the benign nature of the nevus could dissuade her.

Comment. Chinese face and body mole reading is an ancient philosophy, recorded as early as 700 BC.[1] Other beliefs revolve around the shape and size of various facial parts. For example, if a person's ears stick out, they are called "wind-catching ears" and are equated to a self-serving personality. If a person has a prominent rounded nose, it means that he or she lacks support from other people. And if a person has a receding chin, it means that he or she will be poor.[1]

Chinese mole reading teaches that mole placement reflects on character and/or personality traits[2] (**Figure 2**). Mole placement can predict everything from good luck to bad luck, a healthy life vs a sickly life, success in business and marriage, and compatibility with people at home and work to the many fears, phobias, and wishes of everyday life.[3] Most people who follow Chinese mole reading check the "meaning" before making a decision about removal. Although removing the mole might not change the person's fate, it is often done to make the person feel better and to boost his or her confidence.

In our cases, the readings were "Tend to have problems related to diet or food," "Tend to have foot problems," and/or "Need to prevent water-related accidents." The philosophy of fortune-telling according to the location, color, and number of moles has been extensively written about and can be found in many sources. Currently, there are numerous Web sites for Chinese face and body mole predictions,[2,3] such as http://www.chinesefortunecalendar.com/FaceMoleReading.htm and http://www.weirdasianews.com/2010/01/11/chinese-face-readers-observe-moles/.

In conclusion, Asians brought up with Chinese physiognomy fortune-telling beliefs have dispersed all over the world. Many of them know and follow these

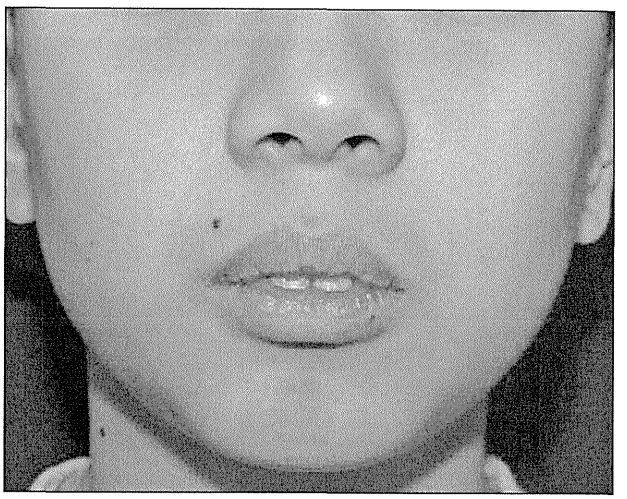

Figure 1. A 12-year-old Chinese boy with a nevocellular nevus on the right nasolabial fold.

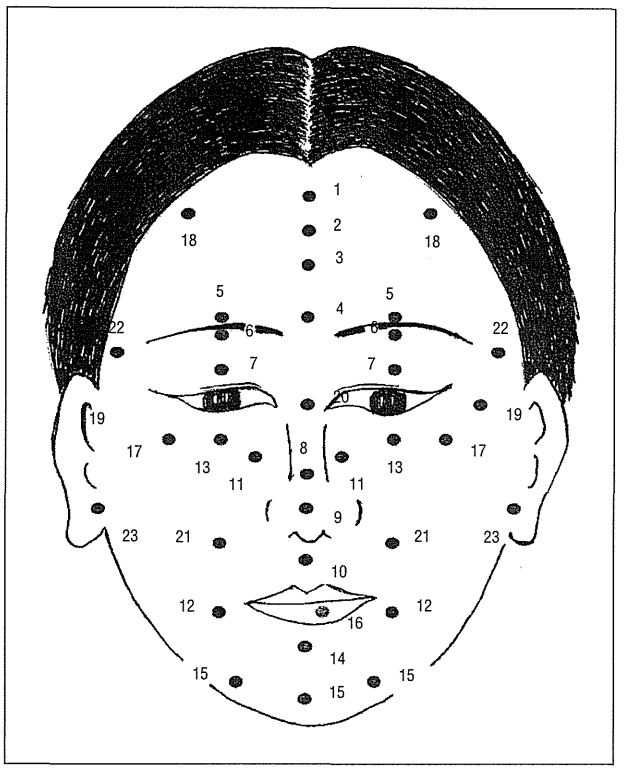

Figure 2. A facial mole–reading map (our own) based on http://www.chinesefortunecalendar.com/FaceMoleReading.htm. Our cases could be interpreted as "Tend to have problems related to diet or food," "Tend to have foot problems," and/or "Need to prevent water-related accidents."

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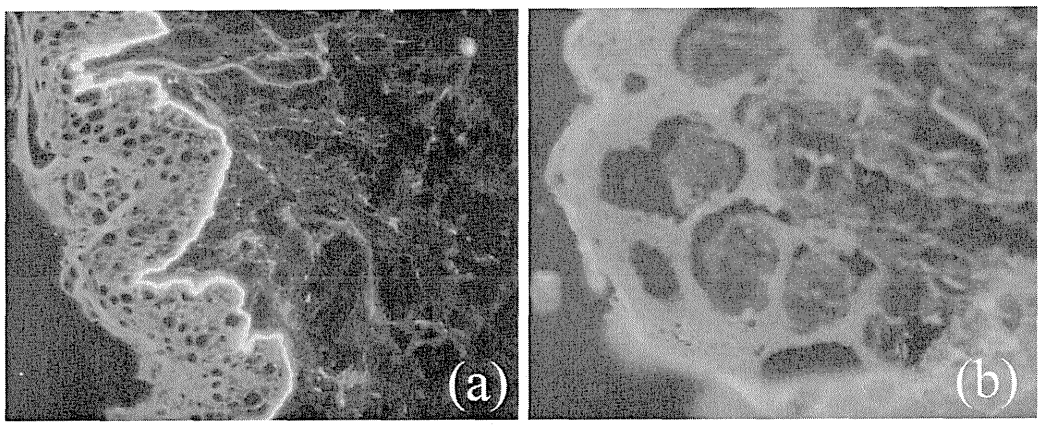

Fig. 2. Indirect immunofluoresence for collagen VII autoantibodies on normal skin (a) and collagen VII deficient skin (b) with serum from EBA patient, 200×.

We agree with the authors that more studies are indicated to determine the use of this test for monitoring disease activity in EBA patients. Similar studies in pemphigus patients with recombinant desmoglein 1 and 3 ELISA's reveal that the sera with identical titers of antibodies by IIF give variable results with ELISA [7]. Unless high titer sera are diluted, saturation of antibody–antigen reactions in ELISA may lead to false low positive ELISA index values to begin with. Such sera may not appear to show a decline in ELISA index values with treatment response [8]. We also have observed, in some pemphigus sera, that even though the IIF titers show a decline, ELISA index values still remain high. Therefore, we may have to use this ELISA with caution to monitor the disease.

References

[1] Saleh MA, Ishii K, Kim YJ, Murakami A, Ishii N, Hashimoto T, et al. Development of NC1 and NC2 domains of Type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. J Dermatol Sci 2011;62(3):169–75.
[2] Gammon WR, Briggaman RA, Inman III AO, Queen LL, Wheeler CE. Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0 M sodium chloride-separated skin. J Invest Dermatol 1984;82(2):139–44.
[3] Vodegel RM, de Jong MC, Pas HH, Yancey KB, Jonkman MF. Anti-epiligrin cicatricial pemphigoid and epidermolysis bullosa acquisita: differentiation by use of indirect immunofluorescence microscopy. J Am Acad Dermatol 2003;48(4):542–7.
[4] Parker SR, MacKelfresh J. Autoimmune blistering diseases in the elderly. Clin Dermatol 2011;29(1):69–79.
[5] Ishii N, Yoshida M, Hisamatsu Y, Ishida-Yamamoto A, Nakane H, Iizuka H, et al. Epidermolysis bullosa acquisita sera react with distinct epitopes on the NC1 and NC2 domains of type VII collagen: study using immunoblotting of domain-specific recombinant proteins and postembedding immunoelectron microscopy. Br J Dermatol 2004;150(5):843–51.
[6] Ishii N, Yoshida M, Ishida-Yamamoto A, Fritsch A, Elfert S, Bruckner-Tuderman L, et al. Some epidermolysis bullosa acquisita sera react with epitopes within the triple-helical collagenous domain as indicated by immunoelectron microscopy. Br J Dermatol 2009;160(5):1090–3.
[7] Bystryn JC, Akman A, Jiao D. Limitations in enzyme-linked immunosorbent assays for antibodies against desmogleins 1 and 3 in patients with pemphigus. Arch Dermatol 2002;138(9):1252–3.
[8] Cheng SW, Kobayashi M, Kinoshita-Kuroda K, Tanikawa A, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002; 147(2):261–5.

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27 July 2011

doi:10.1016/j.jdermsci.2011.12.004

Letter to the Editor

CYP4F22 is highly expressed at the site and timing of onset of keratinization during skin development

Keywords:
Ichthyosis;
Keratinization;
Skin barrier

Autosomal recessive congenital ichthyoses (ARCI) include several subtypes: harlequin ichthyosis (HI), lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). To date, six causative genes have been identified in ARCI patients: *ABCA12*, *TGM1*, *NIPAL4*, *CYP4F22*, *ALOXE3* and *ALOX12B* [1]. The localization of transglutaminase 1, ABCA12 and 12R-lipoxygenase have been analyzed using samples from patients and model mice [1]. However, as for NIPAL4, CYP4F22, and lipoxygenase-3, neither localization nor function has been fully clarified yet. Herein, we investigate the expression pattern and localization of NIPAL4, CYP4F22 and lipoxygenase-3 in developing human epidermis and primary cultured normal human keratinocytes.

By quantitative reverse transcription (RT)-PCR analysis, at 10 and 14 weeks EGA, mRNA of *NIPAL4*, *CYP4F22* and *ALOXE3* was hardly expressed (Fig. 1A). The *CYP4F22* mRNA expression at 18

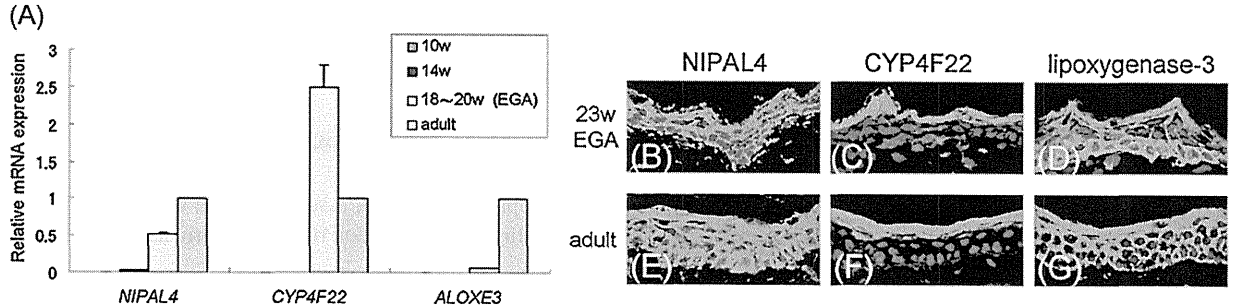

Fig. 1. NIPAL4, CYP4F22 and lipoxygenase-3 expression in developing human skin. (A) mRNA expression in developing human skin. The mRNA expression of *NIPAL4*, *CYP4F22* and *ALOXE3* in fetal human whole skin was studied by quantitative RT-PCR analysis, normalized by *GAPDH* [Applied Biosystems: Hs00398027_m1*, Hs00403446_m1*, Hs00222134_m1*, Hs03929097_ gl*]. At 10 and 14 weeks EGA, *NIPAL4*, *CYP4F22* and *ALOXE3* mRNA are hardly expressed. At 18–20 weeks EGA, the rate of *CYP4F22* mRNA expression is higher than in adult human whole skin ($n = 3$, mean ± SD). (B–G) Immunofluorescence staining of NIPAL4, CYP4F22 and lipoxygenase-3 in developing human skin. Fetal skin samples at 10–23 weeks EGA and adult skin samples were stained for NIPAL4 [Rabbit polyclonal anti-NIPAL4 antibody against a 16-amino acid sequence synthetic peptide (residues 445–461)], CYP4F22 [B01; Abnova, Taipei City, Taiwan], and lipoxygenase-3 [T-14; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.] (Supplementary Fig. S1). For the 23 weeks EGA sample and the adult skin, CYP4F22 (C and F) is expressed in the upper layer of the epidermis, mainly in the granular layers. NIPAL4 (B and E) and lipoxygenase-3 (D and G) are expressed at the cell periphery throughout the epidermis. NIPAL4 expression is seen evenly from the basal cell layer to the granular layers, although lipoxygenase-3 expression is slightly stronger towards the granular layers. NIPAL4, CYP4F22 and lipoxygenase-3 green (FITC), nuclear stain, red (PI solution) (original magnification 40×). Data are presented as representative of triplicate experiments.

and 20 weeks EGA was higher than that in adult human skin. At 18 and 20 weeks EGA, *NIPAL4* mRNA expression was approximately half of that in adult skin, and only a tiny amount of *ALOXE3* mRNA was expressed.

We investigated protein localization by immunofluorescence staining (Fig. 1B–G). For the 10 weeks EGA sample, NIPAL4, CYP4F22 and lipoxygenase-3 were not detected. A similar pattern was obtained for the 14 weeks EGA sample. For the 23 weeks EGA sample, CYP4F22 was expressed in the upper layer of epidermis, mainly in the granular layers, and NIPAL4 and lipoxygenase-3 were expressed at the cell periphery in the entire epidermis. Staining patterns of NIPAL4, CYP4F22 and lipoxygenase-3 in the adult skin were similar to those at 23 weeks EGA. Lipoxygenase-3 is usually considered to be a partner with 12R-LOX. 12R-LOX has been visualized at the cell periphery only in the upper epidermis [2]. In our results, lipoxygenase-3 was distributed at the cell periphery in the entire epidermis. Concerning to lipoxygenase-3 in the upper epidermis, lipoxygenase-3 is thought to work with 12R-LOX, although function of lipoxygenase-3 in the lower epidermis is unknown.

In cultured keratinocytes, RT-PCR analysis (Fig. 2A) and immunoblot analysis (Fig. 2B and C) confirmed that mRNA and protein expression of CYP4F22 were increased under the high Ca^{2+} condition (1.2 mmol/L for 48 h). In contrast, there was no significant increase in the mRNA or protein expression of NIPAL4 or ALOXE3 under the high Ca^{2+} condition.

The present study of the adult human epidermis clarified that NIPAL4 and lipoxygenase-3 were expressed at the cell periphery in the entire epidermis of adult human skin. CYP4F22 was expressed in the cytoplasm of keratinocytes in the upper layer of adult human epidermis, mainly in the granular layers. One previous report [3] noted that, inconsistent with our present observations, *NIPAL4* mRNA is highly expressed in the granular layers of the epidermis with *in situ* hybridization analysis. The cause of this discrepancy is unclear, but it might be due to difference in sensitivity between *in situ* hybridization and immunostaining.

We have demonstrated that the mRNAs of *NIPAL4*, *CYP4F22* and *ALOXE3* are not expressed in the early stages of fetal development, at 10 weeks EGA or at 14 weeks EGA. At 18 and 20 weeks EGA, *NIPAL4* mRNA expression was about half that in adult skin, although *ALOXE3* mRNA was only weakly expressed. Among the keratinization-associated genes, the mRNA expression pattern of *NIPAL4* is similar to that of *ABCA12*, and the pattern of *ALOXE3* resembles those of other keratinization-related molecules, such as *TGM1*, *LOR* and *KLK7* [4].

NIPAL4 encodes a putative transmembrane protein of 404 amino acids with a molecular weight of 44 kDa [6]. The NIPAL4 protein is highly expressed in the brain, lung and stomach, and in

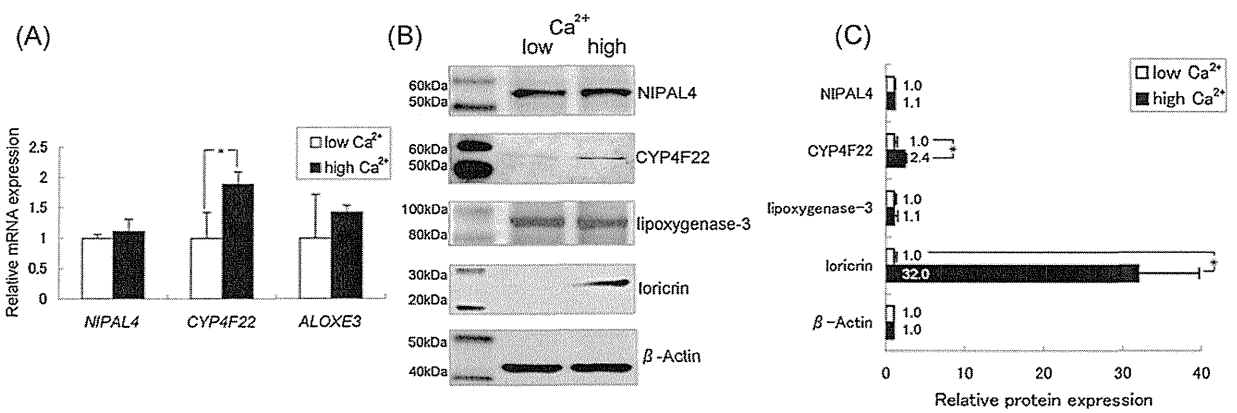

Fig. 2. mRNA and protein expression of NIPAL4, CYP4F22 and ALOXE3 in developing human skin and NHEK. (A) mRNA expression in NHEK. mRNA expression of *CYP4F22* is significantly higher in the NHEK under the high Ca^{2+} condition than in those under the low Ca^{2+} condition. There are no significant differences between the high and low Ca^{2+} conditions in terms of the mRNA expression of *NIPAL4* and *ALOXE3* ($n = 3$, mean ± SD, $^*p < 0.05$). (B) Protein expression assessed by Western blot analysis. The expression of CYP4F22 is higher in the NHEK raised under the high Ca^{2+} condition than in those raised under the low Ca^{2+} condition. However, neither NIPAL4 nor lipoxygenase-3 is increased under high Ca^{2+} condition. Anti-ALOXE3 antibody for immunoblotting: NBP1-32533; Novus Biologicals, LLC, U.S.A. (C) Quantitative analysis by Image J software revealed that the protein expression of CYP4F22 was significantly increased under the high Ca^{2+} condition. Data are presented as representative of triplicate experiments.

leukocytes and keratinocytes. The protein product of the *ALOXE3* gene, lipoxygenase-3, is thought to function as a hydroperoxide isomerase to generate epoxy alcohol [5]. CYP4F22 is a member of the cytochrome P450 family 4, subfamily F. The gene includes 12 coding exons and the cDNA spans 2.6 kb in length. All *CYP4F22* mutations reported to date are predicted to abolish the function of the encoded CYP protein and to compromise the 12(R)-lipoxygenase (hepoxilin) pathway.

Human epidermis contains 15S-lipoxygenase type 1, 12S-lipoxygenase and 12R-lipoxygenase [6]. Skin also contains cytochrome 450, and members of the CYP4 family with unknown epidermal function [3]. 12R-lipoxygenase has attracted great medical interest. 12R-lipoxygenase is expressed only in the epidermis and the tonsils [6,7] and is upregulated in psoriatic lesions [8]. It transforms 20:4n-6 to 12R-hydroperoxyeicosatetraenoic acid (12R-HPETE), which is important for the development of the water permeability barrier function in the epidermis [2]. 12R-LOX and eLOX3 play a crucial role in releasing ω-hydroxyceramide for construction of the corneocyte lipid envelope which is essential for intact skin barrier [9]. O-linoleoyl-ω-hydroxyceramide is oxygenated by the consecutive actions of 12R-LOX and eLOX3 and the products are covalently attached to protein *via* the free ω-hydroxyl of the ceramide, forming the corneocyte lipid envelope [9].

It is hypothesized that CYP4F22 may be linked to the 12R-lipoxygenase and lipoxygenase-3 pathway. Hydroxyeicosatetraenoic acids (HEETs) can be hydrolyzed to triols by epoxide hydrolases, and these products might be substrates of CYP4F members. Thus, it is possible that CYP4F22 might be involved in a downstream step in the 12R-lipoxygenase/lipoxygenase-3 pathway. CYP4F22 could be involved in the oxidation of 8R,11R,12R-HEET. However, from a systemic study of MS/MS spectra of HEETs derived from 12- and 15-HPETE, CYP4F22 did not appear to oxidize 8R,11R,12R-HEET [10]. Nilsson et al. [10] reported that recombinant CYP4F22 catalyzed the omega-3 hydroxylation of 20:4n-6; however, oxygenation of 8R,11R,12R-HEET was not detected. An additional function of CYP4F22 is to synthesize the omega-hydroxy fatty acids in the ceramide [10].

Our study revealed CYP4F22 to be highly expressed at the site and the onset of keratinization during skin development. From this it is speculated that CYP4F22 is involved in the metabolism of lipid substrates that are important to differentiation/keratinization of epidermal keratinocytes, at least during the fetal period. Further studies of the function of CYP4F22 would be needed to elucidate its function in development of the epidermis and keratinocytes.

Acknowledgments

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (Kiban A 23249058 to M. Akiyama), a grant from the Ministry of Health, Labor and Welfare of Japan (Health and Labor Sciences Research grants; Research on Intractable Diseases: H22-177 to M. Akiyama) and the Health and Labor Sciences Research Grant (Research on Allergic Diseases and Immunology; H21-Meneki- Ippan-003 to H. Shimizu). We thank Sapporo Maternity Women's Hospital (Sapporo, Japan) for providing fetal skin samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2011.12.006.

References

[1] Akiyama M. Updated molecular genetics and pathogenesis of ichthyoses. Nagoya J Med Sci 2011;73:79–90.
[2] Epp N, Fürstenberger G, Müller K, de Juanes S, Leitges M, Hausser I, et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. J Cell Biol 2007;177:173–82.
[3] Wajid M, Kurban M, Shimomura Y, Christiano AM. NIPAL4/ichthyin is expressed in the granular layer of human epidermis and mutated in two Pakistani families with autosomal recessive ichthyosis. Dermatology 2010;220:8–14.
[4] Yamanaka Y, Akiyama M, Sugiyama-Nakagiri Y, Sakai K, Goto M, McMillan JR, et al. Expression of keratinocyte lipid transporter ABCA12 in developing and reconstituted human epidermis. Am J Pathol 2007;171:43–52.
[5] Eckl KM, Krieg P, Küster W, Traupe H, André F, Wittstruck N, et al. Mutation spectrum and functional analysis of epidermis-type lipoxygenases in patients with autosomal recessive congenital ichthyosis. Hum Mutat 2005;26:351–61.
[6] Holtzman MJ, Turk J, Pentland A. A regiospecific monooxygenase with novel stereopreference is the major pathway for arachidonic acid oxygenation in isolated epidermal cells. J Clin Invest 1989;84:1446–53.
[7] Boeglin WE, Kim RB, Brash AR. A 12R-lipoxygenase in human skin: mechanistic evidence, molecular cloning, and expression. Proc Natl Acad Sci USA 1998;95:6744–9.
[8] Woollard PM. Stereochemical difference between 12-hydroxy-5,8,10,14-eicosatetraenoic acid in platelets and psoriatic lesions. Biochem Biophys Res Commun 1986;136:169–76.
[9] Zheng Y, Yin H, Boeglin WE, Elias PM, Crumrine D, Beier DR, et al. Lipoxygenases mediate the effect of essential fatty acid in skin barrier formation. A proposed role in releasing omega-hydroxyceramide for construction of the corneocyte lipid envelope. J Biol Chem 2011;286:24046–5.
[10] Nilsson T, Ivanov IV, Oliw EH. LC–MS/MS analysis of epoxyalcohols and epoxides of arachidonic acid and their oxygenation by recombinant CYP4F8 and CYP4F22. Arch Biochem Biophys 2010;494:64–71.

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1 August 2011

doi:10.1016/j.jdermsci.2011.12.006

often unnecessary as lesions typically develop rapidly and resolve spontaneously. Systemic steroids are the most helpful therapeutic modality. Reported pediatric dosages of prednisone therapy for Wells syndrome range from 1 to 2 mg/kg/d.[4]

To our knowledge only one report associates Wells syndrome to parvovirus B19 as a potential trigger factor.[5] This was an 8-year-old boy who developed firm, flesh-colored micropapular lesions of both arms and a slapped-cheek appearance on the face. Our patient could possibly be the first case with the association Wells syndrome and parvovirus B19 in a child younger than 24 months. Fortuitous associations cannot be excluded and we lacked the detection of the parvovirus B19 genome by polymerase chain reaction in the biopsy specimen to confirm the diagnosis.

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Funding sources: None.

Conflicts of interest: None declared.

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REFERENCES

1. Wells GC. Recurrent granulomatous dermatitis with eosinophilia. Trans St Johns Hosp Dermatol Soc 1971;57:46-56.
2. Gilliam AE, Bruckner AL, Howard RM, Lee BP, Wu S, Frieden IJ. Bullous "cellulitis" with eosinophilia: case report and review of Wells' syndrome in childhood. Pediatrics 2005;116:149-55.
3. Moon HS, Park K, Lee JH, Son SJ. Eosinophilic cellulitis in an infant. Int J Dermatol 2010;49:592-3.
4. Anderson CR, Jenkins D, Tron V, Prendiville JS. Wells' syndrome in childhood: case report and review of the literature. J Am Acad Dermatol 1995;33:857-64.
5. Toulon A, Bourdon-Lanoy E, Hamel D, Fraitag S, Leruez-Ville M, de Prost Y, et al. Wells' syndrome after primoinfection by parvovirus B19 in a child. J Am Acad Dermatol 2007;56(Suppl):S50-1.

http://dx.doi.org/10.1016/j.jaad.2012.01.012

Autoantibodies to nuclear matrix protein 2/MJ in adult-onset dermatomyositis with severe calcinosis

To the Editor: A 73-year-old woman presented with erythematous eruptions on the face, eyelids, and front of chest. The patient also showed multiple subcutaneous nodules on the lower aspect of her back, the buttocks (Fig 1), and the front of her right thigh. She had concurrent proximal weakness of muscles in the thigh. She did not have skin sclerosis anywhere on the body. Laboratory investigations revealed increased levels of serum creatine kinase and serum myoglobin, and the existence of circulating antinuclear antibodies. Circulating anticentromere antibodies were detected in the patient's serum by anti-centromeric protein-B enzyme-linked immunosorbent assay. Histopathological examinations of a biopsy sample from the right quadriceps femoris muscle revealed infiltration of lymphocytes in the muscle. Lung function tests revealed no apparent abnormalities. The patient underwent a series of investigations to exclude underlying malignancy, including computed tomography imaging of the chest and pelvis, and esophagogastroduodenoscopy. No internal malignancy was detected, although computed tomography confirmed marked soft-tissue calcification. Histopathologically, one of the calcification nodules showed infiltration of mixed inflammatory cells and focal calcification within the fat. The diagnosis was dermatomyositis (DM) with subcutaneous calcinosis. She was initially treated with oral betamethasone at 1.5 mg/d, which improved the DM symptoms dramatically.

Nuclear matrix protein 2 (NXP-2), also called MJ, is an autoantigen reported in juvenile DM (JDM).[1] We developed an immunoprecipitation assay for anti-NXP-2 autoantibodies using transcription and translation products of recombinant NXP-2. By this newly developed system, anti-NXP-2 autoantibodies were detected in our patient's serum (Fig 2).

In this study, the 73-year-old patient with adult-onset DM had anti-NXP-2 antibodies that had been thought to be JDM specific. Gunawardena et al[1] reported that sera from 37 (23%) of 162 patients with JDM were positive for anti-NXP-2 autoantibodies. To our knowledge, this is the first reported case of adult-onset DM with anti-NXP-2 antibodies.

Calcium deposits develop in 20% to 40% of children with JDM.[2] In contrast, calcinosis is a rare feature in adult-onset DM.[3,4] Anti-NXP-2 antibodies are significantly associated with calcinosis in patients with JDM.[1] Interestingly, the current patient with adult-onset DM and anti-NXP-2 antibodies also showed severe calcinosis, although calcinosis tends to be a rare feature in adult-onset DM, as mentioned above.

Gunawardena et al[1] reported that anti-NXP-2 antibodies were detected exclusively in patients with JDM and not in patients with JDM overlap syndrome or control subjects.[1] No patients who were anti-NXP-2 antibody positive had other myositis-specific autoantibodies.[1] In contrast, it is noteworthy

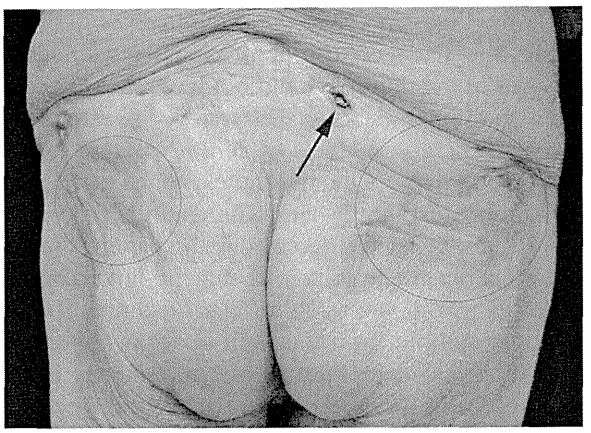

Fig 1. Dermatomyositis with calcinosis. Cutaneous findings of buttocks of patient: ulcers (*arrow*) and subcutaneous calcification (*circles*) are observed.

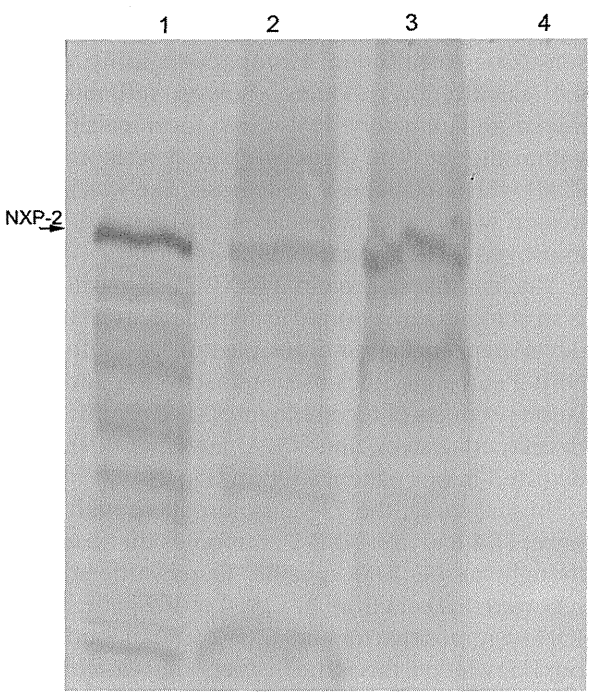

Fig 2. Immunoprecipitation assay for nuclear matrix protein 2 (*NXP-2*). Lane 1: recombinant NXP-2 used for assay; lane 2: positive control serum (our manuscript, in preparation); lane 3: patient's serum; lane 4: negative control serum.

that anticentromere antibodies, which are also associated with calcinosis, were concomitantly detected in the current patient. She had none of the other symptoms of CREST syndrome.

This case suggests that anti-NXP-2 antibodies might be a useful marker for calcinosis not only in JDM, but also in adult-onset DM, although we do not have any information on the prevalence of anti-NXP-2 autoantibodies in adult-onset DM to date. Correlations between the anti-NXP-2 antibodies and calcinosis or other clinical manifestations, including interstitial pneumonia and internal malignancies, in DM should be clarified in the near future.

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Supported in part by two grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, (C) 23591617 (Dr Sugiura) and (A) 23249058 (Dr Akiyama), and by a grant from the Ministry of Health, Labor and Welfare of Japan (Dr Muro).

Conflicts of interest: None declared.

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REFERENCES

1. Gunawardena H, Wedderburn LR, Chinoy H, Betteridge ZE, North J, Ollier WE, et al. Autoantibodies to a 140-kd protein in juvenile dermatomyositis are associated with calcinosis. Arthritis Rheum 2009;60:1807-14.
2. Pachman LM, Veis A, Stock S, Abbott K, Vicari F, Patel P, et al. Composition of calcifications in children with juvenile dermatomyositis: association with chronic cutaneous inflammation. Arthritis Rheum 2006;54:3345-50.
3. Weinel S, Callen JP. Calcinosis cutis complicating adult-onset dermatomyositis. Arch Dermatol 2004;140:365-6.
4. Kavala M, Sudogan S, Can B, Zindanci I, Kuru I, Beyhan S, et al. An extremely severe case of cutaneous calcinosis complicating adult dermatomyositis. Clin Exp Dermatol 2009;34:115-6.

http://dx.doi.org/10.1016/j.jaad.2012.01.015

Giant nevus lipomatosus cutaneous superficialis with intramuscular lipomatosis

To the Editor: Nevus lipomatosus cutaneous superficialis (NLCS) was first described by Hoffmann and Zurhelle[1] in 1921. Clinically it is classified into two clinical subtypes: a multiple form and a solitary form. The lesions are usually found in the flank, buttocks, and upper part of the posterior aspect of the thigh, but they can occur on the abdomen, chest, and face.[2] We report a case of giant NLCS on the buttock associated with diffuse intramuscular lipomatosis within the entire gluteal muscle.

A 38-year-old man presented with an asymptomatic, slowly growing, huge mass on the right buttock

5 Quintanilla-Martinez L, Kimura H, Jaffe E. EBV-positive T-cell lymphoproliferative disorders of childhood. In: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (Swerdlow S, Campo E, Harris N, Jaffe E, Pileri S, Stein H *et al.*, eds), 4th edn. Lyon: IARC Press, 2008; 278–80.
6 Koyama M, Takeshita Y, Sakata A *et al.* Cytotoxic chemotherapy successfully induces durable complete remission in 2 patients with mosquito allergy resulting from Epstein–Barr virus-associated T-/natural killer cell lymphoproliferative disease. *Int J Hematol* 2005; **82**:437–40.
7 Kimura H, Miyake K, Yamauchi Y *et al.* Identification of Epstein–Barr virus (EBV)-infected lymphocyte subtypes by flow cytometric *in situ* hybridization in EBV-associated lymphoproliferative diseases. *J Infect Dis* 2009; **200**:1078–87.
8 Iwatsuki K, Satoh M, Yamamoto T *et al.* Pathogenic link between hydroa vacciniforme and Epstein–Barr virus-associated hematologic disorders. *Arch Dermatol* 2006; **142**:587–95.

Funding sources: None.

Conflicts of interest: None declared.

Novel adenosine triphosphate (ATP)-binding cassette, subfamily A, member 12 (*ABCA12*) mutations associated with congenital ichthyosiform erythroderma

DOI: 10.1111/j.1365-2133.2011.10516.x

MADAM, Autosomal recessive congenital ichthyosis (ARCI) is a keratinization disorder, characterized by general desquamation. ARCI is a heterogeneous entity, including harlequin ichthyosis (HI, MIM 242500), lamellar ichthyosis type 2 (LI2, MIM 601277) and congenital ichthyosiform erythroderma (CIE, MIM 242100). The reported mutations in CIE include adenosine triphosphate (ATP)-binding cassette, subfamily A, member 12 (*ABCA12*),[1] transglutaminase 1 (*TGM1*),[2] lipoxygenase-3, 12(R)-lipoxygenase,[3] *NIPAL4*[4] and *CYP4F22*.[5] Mutations in *ABCA12* also result in LI2 and HI.[6,7] We report *ABCA12* mutations in four unrelated Japanese patients with CIE and identified five unreported and two recurrent mutations.

Patient 1 is a 3-year-old girl with generalized scales on erythroderma, ectropion, eclabium, severely deformed ears and alopecia (Fig. 1a–c). Her elder sister displayed similar symptoms and died after dehydration and infection. Patient 2 is a 9-year-old girl with generalized scales on an erythrodermic skin, mild ectropion, alopecia of the forehead and mild auricular malformation. Her younger sister died after severe skin symptoms and subsequent complications. Patient 3 is a 4-month-old boy, born as a collodion baby, with systemic whitish scales and generalized erythrodermic skin. There is no family history. Patient 4 is a 3-month-old boy, born as a collodion baby, with generalized whitish scales on a mild erythrodermic skin (Fig. 1d,e). Ectropion, eclabium and auricular malformation were not seen. There is no family history. Pathological findings of all patients revealed hyperkeratosis, mild acanthosis and perivascular lymphocytic infiltration.

We initially examined for *ABCA12* mutation, because *ABCA12* mutations have been found frequently in Japanese patients with CIE. For analysis of the *ABCA12* gene, polymerase chain reaction (PCR) fragments were amplified with 53 primer pairs, as previously reported.[6] We identified five unreported and two recurrent mutations (Table 1). Patient 1 had compound heterozygosity of missense/small deletion mutations [(p.Thr1575Pro)+(c.6031delG)]. Patients 2 and 3 had compound heterozygosity of missense/splice-site mutations [(p.Arg986Trp)+(c.5940–1G>C), (p.Asn1380Ser)+(c.5128+3A>G), respectively]. Patient 4 had compound heterozygosity

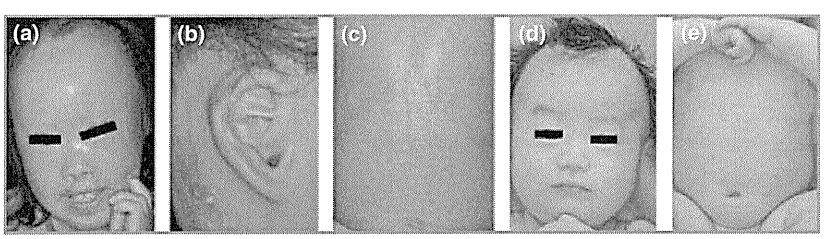

Fig 1. (a–c) Clinical features of patient 1. The whole body was covered with whitish scales on the erythrodermic skin. Ectropion, eclabium and alopecia of the forehead were seen. (d,e) Clinical features of patient 4. Whitish scales and generalized erythrodermic skin were seen.

Table 1 Summary of mutation analysis of *ABCA12* in the present study

Patient	Age, sex	Mutation	Maternal	Paternal
1	3 years, girl	Compound heterozygous	p.Thr1575Pro (c.4723A>C)	c.6031delG
2	9 years, girl	Compound heterozygous	p.Arg986Trp (c.2956C>T)	c.5940–1G>C
3	4 months, boy	Compound heterozygous	p.Asn1380Ser (c.4139A>G)	c.5128+3A>G
4	3 months, boy	Compound heterozygous	p.Thr1575Pro (c.4723A>C)	p.Gly1651Ser (c.4951G>A)

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of missense mutations [(p.Thr1575Pro)+(p.Gly1651Ser)]. Each of the parents was a heterozygous carrier. Five mutations (p.Thr1575Pro, c.6031delG, p.Arg986Trp, c.5940–1G>C and c.5128+3A>G) have not been reported previously. Two recurrent mutations (p.Asn1380Ser and p.Gly1651Ser) have been reported previously in LI2.[6] These mutations were not found in 200 normal, unrelated Japanese alleles.

In cDNA from the skin of patient 2, reverse transcriptase-PCR (RT-PCR) across the c.5940–1G>C mutation site showed a single band of 526 bp. Subcloning and direct sequencing

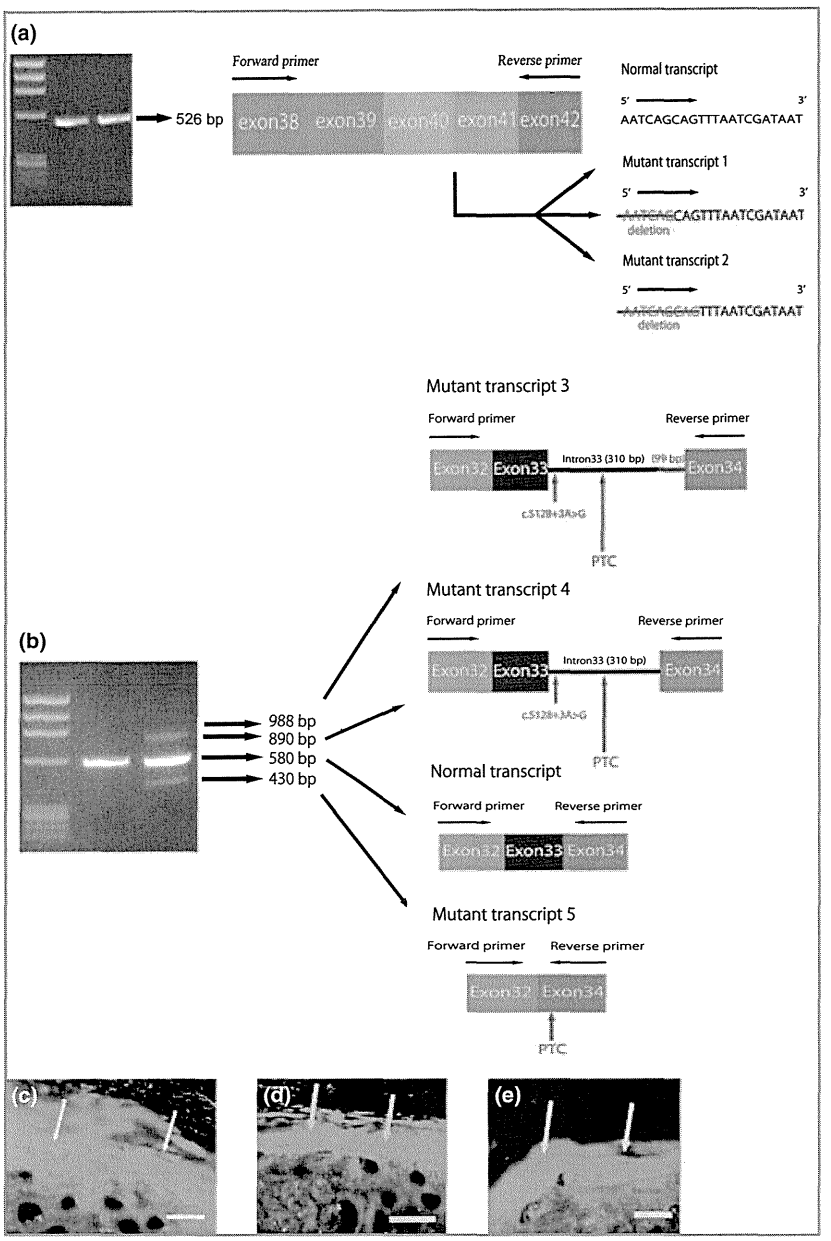

Fig 2. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of mRNA fragments around the splice-site mutations and immunofluorescent analysis. (a) In patient 2, RT-PCR, subcloning and direct sequencing through the exon 40–41 boundary revealed two mutant transcripts as well as a normal transcript. Mutant transcript 1 had lost 6-bp nucleotides from exon 41, which resulted in a 2-amino acid deletion (Ile1981_Ser1982del). Mutant transcript 2 had lost 9-bp nucleotides from exon 41, which resulted in a 3-amino acid deletion (Ile1981_Ser1983del). Both mutant transcripts were within-frame deletions. (b) In patient 3, three aberrant mutant transcripts, all of which led to a premature termination codon, were identified by RT-PCR, subcloning and direct sequencing through the exon 33–34 boundary. Mutant transcript 3 was 988 bp in length with the inclusion of 310 bp and another 99 bp of intron 33. Mutant transcript 4 was 890 bp in length with the inclusion of 310 bp of intron 33. Mutant transcript 5 had exon 33 skipping. (c–e) Immunofluorescent labelling of ABCA12 in the skin. (c,d) A dot-like pattern of ABCA12 staining was seen in the cytoplasm of keratinocytes in the upper epidermis in patient 1 (c) and patient 2 (d). (e) In the normal control epidermis, ABCA12 staining was relatively strong in the granular layers and seemed to be dominant at the cell periphery. Bar = 5 μm.

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revealed two mutant transcripts with in-frame deletions (Fig. 2a). In cDNA from the skin of patient 3, RT-PCR across the c.5128+3A>G mutation site identified four bands of 988, 890, 580 and 430 bp, with a single 580-bp band in the control sample (Fig. 2b). Subcloning and direct sequencing revealed three aberrant mutant transcripts, all of which led to premature termination codons. Immunofluorescence using anti-ABCA12 antibody revealed a diffuse staining of ABCA12 in the granular layers of control skin (Fig. 2e) and of the non-ABCA12 form (TGM1) from patient CIE (data not shown), while a dot-like staining in the cytoplasm was observed in patients 1 and 2 (Fig. 2c,d).

ABCA12 is a membrane lipid transporter that functions in the lipid transport from the *trans*-Golgi network to lamellar granules.[8] *ABCA12* mutations result in heterogeneity, including LI2, HI and CIE.[1,6,7] LI2 is characterized by generalized scales without serious erythroderma, and caused by either homozygote or compound heterozygote for missense mutations within the first nucleotide-binding folds of *ABCA12*.[6] HI is the severest form of ARCI, characterized by generalized large, plate-like scales with ectropion, eclabium and flattened ears.[7] HI is usually caused by homozygous or compound heterozygous truncation mutations in *ABCA12*.[7] In contrast, CIE with *ABCA12* mutation clinically shows milder manifestations.[1] Thus far, 17 different mutations in *ABCA12* have been reported in 12 cases of CIE. Eleven of 12 cases have at least one missense mutation. Only three of 17 mutations (p.Asn1380Ser, p.Ile1494Thr and p.Arg1514His) were located in the first nucleotide-binding folds. Other mutations were located outside ABCA12 active transporter sites: two nucleotide-binding folds and 12 transmembrane domains. The mutation p.Thr1575Pro was identified in two unrelated patients with different clinical severity. Patient 1 with severer features had a heterozygous truncation mutation (c.6031delG) on another allele, while patient 4, with a milder phenotype, had another heterozygous missense mutation (p.Gly1651Ser). We suggest that the phenotypic variability in these two patients was caused by different mutations.

We identified two *ABCA12* splice-site mutations, which were not reported in CIE: c.5128+3A>G and c.5940−1G>C. RT-PCR analysis across the site of the c.5940−1G>C mutation in patient 2 revealed two mutant transcripts. These findings demonstrate expression of the in-frame shorter transcript lacking two or three amino acids due to this splice-site mutation, which may account for the mild phenotype. In contrast, RT-PCR analysis across the site of the c.5128+3A>G mutation in patient 3 revealed three aberrant mutant transcripts, all of which led to premature termination codons. Therefore, patient 3 had a compound heterozygosity for missense/truncated combinations of mutations.

Using high-throughput sequencing analyses, screening of all ARCI-related genes is currently possible, but the cost is still expensive.[9] Once this is overcome, the elucidation of the pathogenesis of ARCI will greatly progress in the near future.

Acknowledgments

We thank the patients for their participation. We also thank Ms Takako Ishikawa and Ms Ayumi Suzuki for their fine technical assistance, and Ms Akiko Tanaka, Ms Yasuko Nakayama and Ms Hanako Tomita for secretarial work.

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References

1 Natsuga K, Akiyama M, Kato N *et al.* Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. *J Invest Dermatol* 2007; **127**:2669–73.

2 Laiho E, Ignatius J, Mikkola H *et al.* Transglutaminase 1 mutations in autosomal recessive congenital ichthyosis: private and recurrent mutations in an isolated population. *Am J Hum Genet* 1997; **61**:529–38.

3 Jobard F, Lefèvre C, Karaduman A *et al.* Lipoxygenase-3 (ALOXE3) and 12(R)-lipoxygenase (ALOX12B) are mutated in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. *Hum Mol Genet* 2002; **11**:107–13.

4 Lefèvre C, Bouadjar B, Karaduman A *et al.* Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. *Hum Mol Genet* 2004; **13**:2473–82.

5 Lefèvre C, Bouadjar B, Ferrand V *et al.* Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006; **15**:767–76.

6 Lefèvre C, Audebert S, Jobard F *et al.* Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. *Hum Mol Genet* 2003; **12**:2369–78.

7 Akiyama M, Sugiyama-Nakagiri Y, Sakai K *et al.* Mutations in ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest* 2005; **115**:1777–84.

8 Sakai K, Akiyama M, Sugiyama-Nakagiri Y *et al.* Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes. *Exp Dermatol* 2007; **16**:920–6.

9 Pastinen T. Genome-wide allele-specific analysis: insights into regulatory variation. *Nat Rev Genet* 2010; **11**:533–8.

Funding sources: This study was supported by Grants-in-Aid for Scientific Research and Strategic Research Basis Formation Supporting Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Health and Labour Sciences Research Grants and grants for Research on Measures for Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan. The study was also supported by grants from the Uehara Memorial Foundation, the Nakatomi Foundation, the Kaibara

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Morikazu Medical Science Promotion Foundation, the Japan Lydia O'Leary Memorial Foundation, the Cosmetology Research Foundation, the Japanese Dermatological Association (Shiseido Award), the Fukuoka Foundation for Sound Health, and Galderma K.K. (Galderma Award).

Conflicts of interest: None declared.

Iatrogenic androgenetic alopecia in a male phenotype 46XX true hermaphrodite

DOI: 10.1111/j.1365-2133.2011.10511.x

MADAM, Androgenetic alopecia (AGA) is a term that describes the androgen-dependent and genetically determined nature of the disease.[1] However, although it is known that androgen replacement therapy can induce AGA, no report has previously been issued regarding the development of iatrogenic AGA in a hermaphrodite undergoing androgen therapy. Herein, we describe a unique case of a castrated male phenotype 46XX true hermaphrodite receiving exogenous androgen supplementation who developed male-type hair loss.

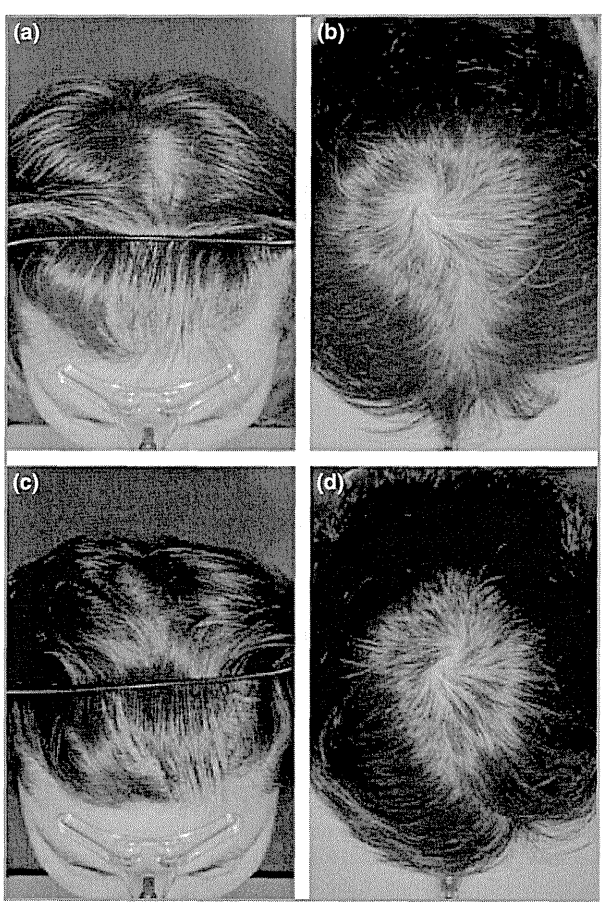

Fig 1. Iatrogenic androgenetic alopecia in a male phenotype 46XX true hermaphrodite showed a great improvement compared with baseline (a, b) after 4 months of finasteride treatment (c, d).

A 21-year-old male phenotype 46XX true hermaphrodite presented with a 3-year history of progressive hair loss. At the age of 16 years he was diagnosed as a 46XX true hermaphrodite with bilateral ovotestis, and subsequently underwent bilateral orchiectomy and testis prosthesis insertion. In addition, he was then given testosterone replacement therapy (testosterone enanthate, Jenasteron®; Jenapharm, Jena, Germany) for surgically induced andropausal status, which halted the development of secondary sexual characteristics. After 3 years of androgen therapy, progressive hair thinning developed on the scalp. Hair examination revealed nonscarring Norwood–Hamilton type III vertex alopecia with frontotemporal recession or BASP classification M1V2 alopecia (Fig. 1a, b).[2] Digital microscopy (Folliscope®; LeadM Corporation, Seoul, Korea) showed miniaturized hair shafts, and hair shaft size variation over the vertex scalp (Fig. 2). Serum testosterone, at the time, was 4·1 ng mL^{-1} (normal 2·7–10·7) and serum dehydroepiandrosterone sulphate was 1845 ng mL^{-1} (normal 800–5600). Under a diagnosis of iatrogenic androgen-induced alopecia, finasteride (1 mg daily) therapy was started. After 4 months of treatment, the hair loss stabilized and scalp hair regrowth was observed, despite the continuance of testosterone replacement therapy (Fig. 1c, d).

True hermaphroditism is an extremely rare disorder, which is defined as the coexistence of testicular and ovarian tissue in the same subject. The most frequent karyotype of true hermaphrodites is 46XX.[3] Gender assignments for hermaphrodites are made according to genetic, gonadal, social and psychologically determined sex, and the requests of patients and their relatives.[4] To be reared as male or female, surgical correction of ambiguous external genitalia, surgical removal of dysgenetic gonads, and sex hormone replacement for the surgically induced andropausal or menopausal state are required. The unwanted dermatological side-effects of testosterone replacement therapy include acne, excessive hair growth and male pattern baldness. As in our case, to be reared

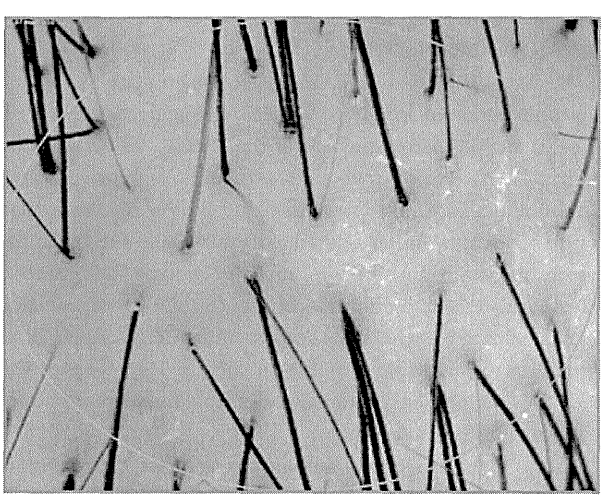

Fig 2. Photomicrograph showing miniaturized hair shafts, and variations in hair shaft size over the vertex scalp (original magnification ×50).

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