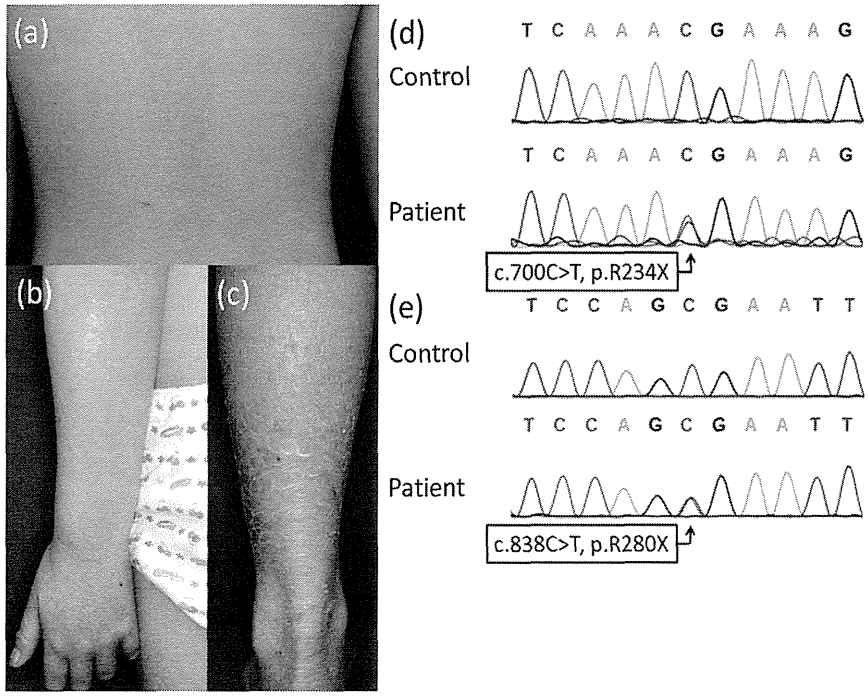

Fig. 1. Clinical and molecular characterization of the patient with DCS. (a–c) The patient shows thin, whitish scales with mild erythema on the back (a), the left arm (b) and the lower leg (c). (d, e) Direct sequencing reveals compound heterozygous mutations in *ABHD5*, c.700C > T (p.Arg234*) (d) and c.838C > T (p.Arg280*) (e), in the patient, but not in normal control DNA.

Tunisian patient with DCS caused by a homozygous *ABHD5* splice site mutation, p.Ser258Argfs*21 [8]. In that case, the main abnormalities were CIE and liver dysfunction but without any neurologic symptoms. Another DCS patient with compound heterozygous truncation mutations in *ABHD5*, c.616_646insGGG in exon 4 and c.934C > T (p.Arg312*) in exon 6, also showed an ichthyosis phenotype mimicking erythrokeratoderma variabilis, and liver abnormalities [9]. This case also had no signs of abnormalities in other organs [9]. Collectively, it is possible that a nonsense mutation near the C-terminal domain, that leads to lack of the HXXXXD motif (amino acids 327–332), but retention of upstream motifs in *ABHD5* [3], is associated with a mild DCS phenotype mostly restricted to skin and liver abnormalities (Fig. 2). Aggarwal et al. have previously reported that a DCSfamily with a homozygous nonsense mutation p.E336* [10]. The mutation was at the position closer to the C-terminus of ABHD5 protein as compared to those identified in the present study. However, the patients unexpectedly showed more than mild phenotype to various degrees. The authors concluded that genotype–phenotype correlations have not been possible in DCS [10]. However, the patients in the family had several atypical complications of DCS, and there was an intrafamilial phenotypic heterogeneity in three alive affected individuals. Therefore, we hypothesized that mutations in other genes might have affected their phenotypes through modifier effects in the reported family. Further accumulation of DCS cases with mutation data is needed to confirm the genotype/phenotype correlations in *ABHD5* mutations.

In summary, this study has identified a novel *ABHD5* mutation, c.838C > T (p.Arg280*), in trans with p.Arg234* in a Chinese patient with very mild DCS. Our observations demonstrate the clinical heterogeneity of DCS and highlight possible new insight about genotype–phenotype correlation for some extra-cutaneous features of this syndromic disorder.

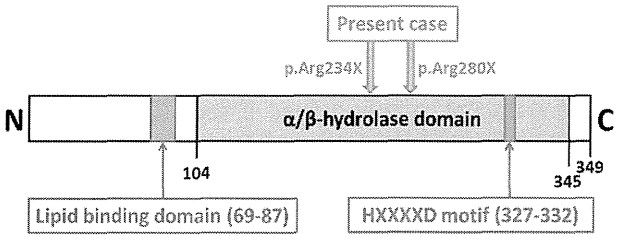

Fig. 2. Schematic model of ABHD5 domain structure. The blue area marks the putative lipid-binding domain (amino acids 69–87). The α/β-hydrolase domain (gray area, amino acids 104–345) is containing the HXXXXD motif (red box, amino acids 327–332). The mutations in the DCS patient are marked by green arrows.

Funding statement

None.

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.10.015.

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Letter to the Editor

Percutaneous exposure to high-dose hapten induces systemic immunosuppression through the inhibition of dendritic cell migration

While sensitization with the optimal dose of an antigen induces antigen-specific T-cell responses, the immune response to a supraoptimal dose of antigen is suppressed [1]. In addition, high-dose antigen exposure under certain conditions suppresses subsequent immune response to the antigen [2,3]. The mechanisms underlying high-dose antigen-induced immunosuppression appear to vary according to the administration route of the high-dose antigen: intravenous injection of high-dose hapten induces suppressor cells [2], while oral administration of high-dose hapten induces anergy or deletion of antigen-specific T cells [3].

Percutaneous sensitization of mice with an optimal dose of haptens such as dinitrofluorobenzene (DNFB), trinitrochlorobenzene (TNCB), and oxazolone induces hapten-bearing dendritic cell (DC) migration from sensitized skin into the draining lymph node (dLN), leading to the proliferation and differentiation of the hapten-specific interferon (IFN)-γ-producing $CD8^+$ effector T (Tc1) cells. Re-exposure to the relevant hapten five days after sensitization elicits allergic contact hypersensitivity (CHS) response by antigen-specific Tc1 cells [4]. A previous report has shown that topical high-dose hapten application induces dysfunction of DCs at hapten-applied sites, resulting in the impaired capacity of hapten-applied skin to support subsequent CHS induction by an optimal sensitizing dose of another hapten [5]. However, it remains unclear whether and how percutaneous exposure to high-dose antigen inhibits subsequent immune responses systemically. In this study, we investigated the systemic effect of high-dose hapten exposure on subsequent sensitization with an optimal dose of hapten.

Mice sensitized with a high dose (3%) of DNFB showed significantly attenuated CHS responses after elicitation compared to mice sensitized with an optimal dose (0.5%) of DNFB (Fig. 1A), which was consistent with a previous report [1]. In addition, CHS responses induced by an optimal dose of DNFB were significantly suppressed in mice pretreated with high-dose DNFB on the abdominal skin one day before sensitization (Fig. 1B–D). To confirm that high-dose DNFB pretreatment inhibited subsequent sensitization with the optimal dose of hapten, CHS transferred via dLN cells of sensitized mice with or without DNFB pretreatment was assessed. Mice subjected to adoptive transfer of dLN cells that had been collected from vehicle-pretreated mice five days after sensitization exhibited substantial CHS responses after elicitation. Mice subjected to adoptive transfer of dLN cells that had been

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REVIEW ARTICLE

Inherited ichthyosis: Non-syndromic forms

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ABSTRACT

Inherited ichthyoses are a group of genetic disorders characterized by generalized dry skin, scaling and hyperkeratosis, and often associated with erythroderma. These manifestations are due to mutations in genes mostly involved in skin barrier formation. Inherited ichthyoses consist of non-syndromic ichthyoses and ichthyosis syndromes. Non-syndromic ichthyoses are characterized by the phenotypic expression of the disorder being seen only in the skin. Non-syndromic ichthyoses include ichthyosis vulgaris, recessive X-linked ichthyosis, autosomal recessive congenital ichthyosis, keratinopathic ichthyosis and other forms. This review focuses on updates for each type of non-syndromic ichthyosis, highlighting molecular mechanisms and phenotype/genotype correlations. Included in autosomal recessive congenital ichthyosis are three of the major phenotypes (harlequin ichthyosis, lamellar ichthyosis and congenital ichthyosiform erythroderma) and three of the minor subtypes (self-healing collodion baby, acral self-healing collodion baby and bathing suit ichthyosis). Keratinopathic ichthyosis is proposed as an umbrella term for ichthyoses caused by mutations in keratin genes. Next-generation sequencing technologies have become powerful tools for the diagnosis of inherited ichthyoses and the discovery of their genetic causes. This article reviews the current understanding of molecular pathomechanisms for non-syndromic ichthyoses and explores future perspectives.

Key words: autosomal recessive congenital ichthyosis, ichthyosis vulgaris, inherited ichthyoses, keratinopathic ichthyosis, recessive X-linked ichthyosis.

INTRODUCTION

Inherited ichthyoses are genetic disorders characterized by generalized dry skin, scaling and hyperkeratosis, and often associated with erythroderma. These manifestations are due to mutations in genes mostly involved in skin barrier formation. Clinically, inherited ichthyoses were divided into two groups at the first Ichthyosis Consensus Conference of 2009: non-syndromic ichthyoses and syndromic forms.[1] Non-syndromic ichthyoses are characterized by the disorder expressing phenotypically only in the skin. Non-syndromic ichthyoses include common ichthyoses, autosomal recessive congenital ichthyosis (ARCI), keratinopathic ichthyosis and other forms (Table 1).[1] Recently, the pathomechanisms and underling genetic defects of non-syndromic ichthyoses have been elucidated in rapid succession. In addition, significant progress has been made for a decade in our understanding of the molecular basis of human barrier processes. For example, the number of genes identified and demonstrated to cause ARCI in human patients has reached nine.[2]

This review focuses on updates for each non-syndromic ichthyosis, highlighting molecular mechanisms and phenotype/genotype correlations. Additionally, we briefly describe promising new technologies for identifying the causative genes of ichthyoses.

COMMON ICHTHYOSES

Oji *et al.*[1] classified ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (RXLI) as common ichthyoses, based on their high prevalence. IV and RXLI often have a delayed onset compared with the other inherited non-syndromic ichthyoses.

Ichthyosis vulgaris

Ichthyosis vulgaris is the most common ichthyosis, with an incidence of 1:250 to 1:1000.[1] IV is the mildest form of hereditary non-syndromic ichthyosis, characterized by xerosis, scaling, pruritus and eczema, and it is strongly associated with atopic manifestations.[1,3] The phenotypic manifestations tend to appear from the age of 2 months and often improve in summer. Characteristically, the extensor sides of the lower legs and the back are most commonly affected; the chest and the abdomen tend not to be involved (Fig. 1a). Keratosis pilaris and palmoplantar hyperlinearity are often complications of IV (Fig. 1b).

Profilaggrin is a precursor protein of filaggrin, and in 2006, null mutations in the gene coding profilaggrin (*FLG*) were detected as the causative defects leading to IV.[4] Filaggrin is a key protein that facilitates terminal differentiation of the epidermis and the formation of the protective skin barrier. In the outer granular layer of the epidermis, filaggrin is associated with keratin intermediate filaments and it aids their packing into

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Table 1. Subtypes of inherited ichthyoses: non-syndromic forms

Phenotypes	Causative genes
Common ichthyosis	
Ichthyosis vulgaris	*FLG*
Recessive X-linked ichthyosis	*STS*
Autosomal recessive congenital ichthyosis	
Major types	
Harlequin ichthyosis	*ABCA12*
Lamellar ichthyosis	*ABCA12*, *ALOXE3*, *ALOX12B*, *CERS3*, *CYP4F22*, *NIPAL4/ICHTHYIN*, *PNPLA1*, *TGM1*
Congenital ichthyosiform erythroderma	*ABCA12*, *ALOXE3*, *ALOX12B*, *CERS3*, *CYP4F22*, *LIPN*, *NIPAL4/ICHTHYIN*, *PNPLA1*, *TGM1*
Minor types	
Self-healing collodion baby	*ALOXE3*, *ALOX12B*, *TGM1*
Acral self-healing collodion baby	*TGM1*
Bathing suit ichthyosis	*TGM1*
Keratinopathic ichthyosis	
Major types	
Epidermolytic ichthyosis	*KRT1*, *KRT10*
Superficial epidermolytic ichthyosis	*KRT2*
Minor types	
Annular epidermolytic ichthyosis	*KRT1*, *KRT10*
Ichthyosis Curth-Macklin	*KRT1*
Autosomal recessive epidermolytic ichthyosis	*KRT10*
Epidermolytic nevi	*KRT1*, *KRT10*
Congenital reticular ichthyosiform erythroderma	*KRT1*, *KRT10*
Other forms	
Loricrin keratoderma	*LOR*
Erythrokeratoderma variabilis	*GJB3*, *GJB4*
Peeling skin disease	*CDSN*
Keratosis linearis with ichthyosis congenita and sclerosing keratoderma	*POMP*

bundles. In terminal differentiation, filaggrin is cross-linked to the cornified cell envelope, which constitutes an insoluble barrier in the stratum corneum to protect the organism against environmental agents and to prevent epidermal water loss.[5] Two months after the report of *FLG* mutations as a cause of IV, Palmer *et al.*[6] demonstrated unequivocally that two prevalent loss-of-function mutations in *FLG* are a major primary predisposing risk factor for atopic dermatitis (AD). The presence of population-specific *FLG* mutations of IV/AD has been reported in Europeans, Asians and Africans.[7,8] We have reported that eight *FLG* null variants of IV/AD have been found in the Japanese population.[9] More recently, we established a high-throughput *FLG* mutation detection system that uses real-time polymerase chain reaction (PCR) with a set of two double-dye probes, and we conducted a comprehensive screening for almost all of the Japanese population-specific *FLG* mutations (10 *FLG* mutations).[10]

Recessive X-linked ichthyosis

Recessive X-linked ichthyosis is clinically characterized by widespread, dark brown, polygonal scales and generalized dryness. RXLI is the second most common ichthyosis, with a prevalence of 1:2000 to 1:6000.[1] Cutaneous manifestations are present soon after birth and usually tend not to improve with age. Phenotypically, RXLI is a more severe ichthyotic form than IV, as large, dark brown scales form and the lesion spreads to the whole body (Fig. 1c,d). The histopathology of RXLI typically shows compact hyperkeratosis and slight acanthosis with a normal granular layer. With these features are non-specific, they can help to exclude IV, which instead displays decreased keratohyalin granules.[11]

With respect to the phenotypic spectrum of RXLI, Hand *et al.*[12] recently suggested that *STS* gross deletions may cause milder skin abnormalities than most classic forms of RXLI, in that those cases incidentally found to have an *STS* deletion by whole-genome chromosomal microarray (CMA) typically lacked the polygonal or "dirty" scales considered a hallmark of RXLI. In such cases, the milder findings consisted of dry or peeling skin and eczema. More recently, we reported that a patient with the small indel mutation in *STS* showed a unique "self-healing" phenotype of RXLI.[13] Some mild and severe phenotypes of RXLI may be clinically challenging to differentiate from IV and ARCI, respectively. Because roughly 90% of RXLI patients have large deletions involving *STS* and adjacent DNA, in some instances with contiguous gene loss, fluorescence *in situ* hybridization (FISH) analysis is a useful technique for identifying patients and carriers of RXLI who have such deletions. Nevertheless, although FISH is helpful in diagnosing these cases, it is not helpful for diagnosing other individuals with partial deletions or point mutations.[13]

AUTOSOMAL RECESSIVE CONGENITAL ICHTHYOSIS

Autosomal recessive congenital ichthyosis is a comprehensive definition used to represent a generic phenotype of erythrodermic, scaly skin presenting over almost the entire body surface at birth.[1] ARCI is clinically divided into three major phenotypes and three minor subtypes, although it has 11 genetic subtypes in the Online Mendelian Inheritance in Man (OMIM) database.

Harlequin ichthyosis

Harlequin ichthyosis (HI) is the most phenotypically severe inherited ichthyosis. It is occasionally fatal. The clinical features include thick, plate-like scales with severe ectropion, eclabium and flattening of the ears (Fig. 1e).[14] Skin development is altered *in utero*; hyperkeratosis of the hair canal occurs in the second trimester and characteristic ultrastructural abnormalities including abnormal lamellar granules are present in the

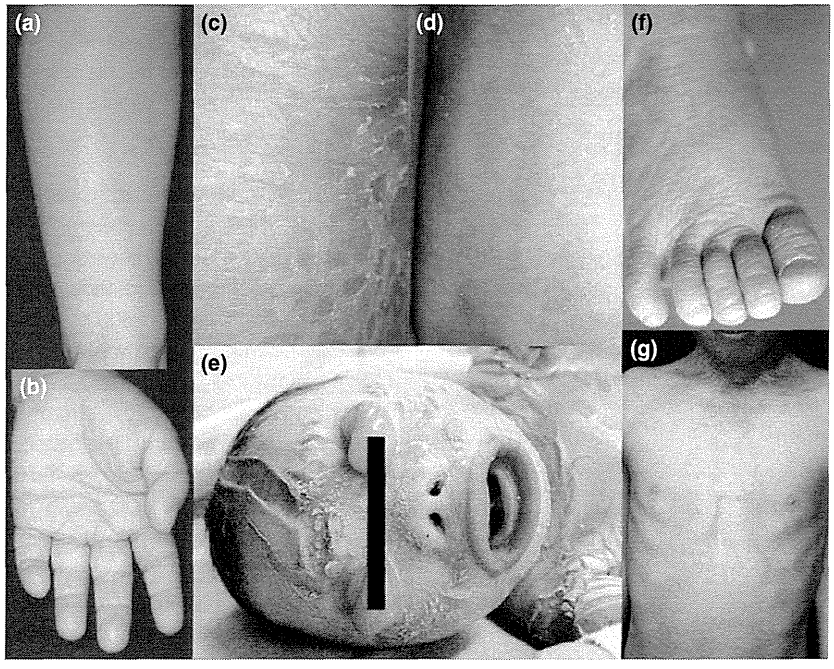

Figure 1. Clinical features of ichthyosis vulgaris (IV), recessive X-linked ichthyosis (RXLI) and autosomal recessive congenital ichthyosis. (a,b) A 1-year-old IV boy with a *FLG* mutation.[9] (a) Fine scaling visible on his leg and (b) marked hyperlinearity on his palm. (c,d) A 1-month-old RXLI boy with an *STS* mutation.[13] Large, fine, whitish scales with dry skin are seen on his (c) abdomen and (d) right thigh. (e) A harlequin ichthyosis neonate with *ABCA12* mutations shows thick plate-like scales with deep fissuring overlying erythrodermic skin.[25] Severe eclabium and ectropion are seen. (f) A lamellar ichthyosis patient with *CYP4F22* mutations.[32] Large scales with mild erythema are seen on the dorsal foot. (g) A patient with congenital ichthyosiform erythroderma caused by *ABCA12* mutations. This 11-year-old male has generalized fine white scales on his entire body and mild erythroderma on his neck.

affected fetal epidermis.[15,16] In 2005, we and other groups reported that loss-of-function mutations in the adenosine triphosphate (ATP)-binding cassette subfamily A member 12 (ABCA12) gene underlie HI.[17,18] Lack of ABCA12 function leads to disruption of lamellar granule lipid transport in keratinizing keratinocytes of the upper epidermis.[17]

Diagnosing HI is generally not difficult, because of its characteristic phenotype, although finding the pathogenic mutations in *ABCA12* is the most important diagnostic confirmation. An accurate prenatal diagnosis is also necessary. Until the identification of *ABCA12* as the causative gene, prenatal diagnosis for HI had been performed by electron microscopy of fetal skin biopsy samples.[16,19–21] We achieved the first DNA-based prenatal diagnosis of HI by direct sequence analysis of the *ABCA12* mutation from amniotic fluid cells and showed the efficiency of early DNA-based prenatal diagnosis of HI.[22] This DNA-based diagnostic method is becoming a standard technique for earlier and accurate diagnosis of HI. Furthermore, pre-implantation genetic diagnosis (PGD) is a novel technique developed to prevent the transmission of inherited diseases to the offspring.[23] The goal of PGD is to select and transfer embryos that have been determined by genetic analysis to be unaffected, before a clinical pregnancy has been established. This avoids the very difficult dilemma of whether or not to terminate a pregnancy or deliver a sick child.[23] Since the *ABCA12* has been identified as a causative gene for HI, known carriers have been able to undergo PGD testing.

In addition to the appropriate therapy in the neonatal intensive care unit (NICU), early systemic retinoid treatment has been shown to increase survival in patients with HI.[24,25] We also reported that systemic retinoids and administration in the NICU are considered to contribute to relatively good outcomes and high survival rates for HI patients in the Japanese population.[26] Furthermore, Cottle *et al.*[27] revealed that the *in utero* suppression of inflammatory chemokines promotes the improvement of keratinocyte differentiation in *Abca12*-deficient mice. Their observations suggest that anti-inflammatory therapies for HI may serve to correct defects in keratinocyte differentiation. Serum chemokine levels in an HI-affected fetus with *ABCA12* mutations are potential targets for intrauterine therapies.

Lamellar ichthyosis

Lamellar ichthyosis (LI) is a milder phenotype than HI. The severity of the skin symptoms, including hyperkeratosis and scales, varies from patient to patient. The characteristic scales covering most of the body surface in LI are large, thickened and dark gray or brown (Fig. 1f). Generally, LI does not include erythroderma, although several cases with very mild erythema have been reported.[28] Patients who show the classic LI phenotype later in life were frequently born as collodion babies. Palmoplantar keratoderma is frequently seen in LI.[28]

Light microscopy of affected skin from LI patients shows marked hyperkeratosis with only a small number of parakeratotic

cells. The granular layer is normal or mildly thickened.[29–31] The stratum corneum is usually thicker in LI than in congenital ichthyosiform erythroderma (CIE).

Eight genes have been reported as causative genes of LI: *ABCA12*; lipoxygenase-3 (*ALOXE3*); 12R-lipoxygenase (*ALOX12B*), ceramide synthase 3 (*CERS3*); cytochrome P450, family 4, subfamily F, polypeptide 22 (*CYP4F22*); NIPA-like domain containing 4 (*NIPAL4/ICHTHYIN*); patatin-like phospholipase domain-containing protein 1 (*PNPLA1*); and transglutaminase 1 (*TGM1*) (Table 1). Of these, mutations in *TGM1* and *ABCA12* have been reported frequently, even in Asia. Mutations in *ALOXE3*, *ALOX12B*, *CERS3*, *CYP4F22*, *NIPAL4/ICHTHYIN* and *PNPLA1* have been found mainly in families of Mediterranean and Middle Eastern descent. Recently, we described an LI patient with compound heterozygous *CYP4F22* mutations including a novel frame-shift mutation in a non-consanguineous family outside the Mediterranean region.[32] In addition, we reported the first case of LI with *ALOXE3* mutations in a family from East Asia.[33] These findings expand our knowledge of the genetic background of LI and suggest that we should screen LI patients for all the possible causative genes for an accurate diagnosis, even though the most common underlying gene defect is in *TGM1*.

Congenital ichthyosiform erythroderma

A CIE-affected child is frequently born as a collodion baby. After the collodion membrane is dropped, erythroderma and scaling appear. In CIE, the scales are typically fine and white or light gray (Fig. 1g). In severe cases of CIE, the erythroderma is systemic and persistent. However, especially in milder cases, the erythroderma improves in infancy.[28,34] Light microscopy of skin biopsy samples from patients shows marked to moderate hyperkeratosis, a normal or moderately thickened granular cell layer, slight acanthosis and variable parakeratosis.[30] Notably, parakeratosis and inflammatory cell infiltration in the upper dermis are more frequent in CIE than in LI.[28]

Mutations of a member of causative genes have been identified for CIE: *ABCA12*, *ALOX12B*, *ALOXE3*, *CERS3*, *CYP4F22*, lipase N (*LIPN*), *NIPAL4/ICHTHYIN*, *PNPLA1* and *TGM1*.[35] Of these, *CERS3* was identified most recently as the ninth causative gene of CIE from two different groups.[36,37] The groups revealed the synthesis of very long chain ceramides by ceramide synthase 3 as a crucial early step for skin barrier formation and they linked disorders presenting congenital ichthyosis to defects in sphingolipid metabolism and the epidermal lipid architecture.

Self-healing collodion baby

Self-healing collodion baby (SHCB) (also called "self-improving collodion baby") is a minor variant of ARCI and is defined as a collodion baby with nearly complete resolution of scaling within the first 3 months of life.[1,38] SHCB accounts for approximately 10% of all ARCI cases.[1] Raghunath *et al.*[39] showed that the phenotype at birth is possibly due to a hydrostatic pressure-sensitive phenotype from *TGM1* mutations. SHCB has been reported to be associated with *ALOX12B* and *ALOXE3* mutations.[40]

Acral self-healing collodion baby

A unique case of acral self-healing collodion baby associated with the compound heterozygous mutation, p.Val359Met and p.Arg396His, in the *TGM1* was reported in 2009.[41] The transglutaminase 1 (TGM1) activity of both the mutant proteins was reduced: p.Val359Met showed only 12.8% of the activity seen in the wild type, and p.Arg396His activity was almost abolished (only 3.3% of the activity seen in the wild type). In this family, the sister showed classic LI and was found to carry the mutation p.Arg396His in association with a third c.1922_1926+2delGGCCTGT mutation. Additionally, the structure modeling of p.Val359Met suggested a minor disruption of the protein structure for this mutation.[41] The p.Val359Met mutation may be responsible for the healing phenotype, as the other mutation was present in the elder sister, who developed generalized LI with a later onset. Their observation suggests that the healing of this limited neonatal condition is due to TGM1 enzymatic activity that is too low *in utero* to build up a normal epidermis, but is high enough later in life. This is the only such case to have been reported.

Bathing suit ichthyosis

Bathing suit ichthyosis (BSI) is another minor variant of ARCI. It is characterized by a unique distribution of lesions on the trunk, the most proximal parts of the upper limbs, the scalp and the neck, but not the central face and extremities.[42] The term "bathing suit" ichthyosis for this unusual phenotype of lamellar ichthyosis was proposed in South Africa.[42,43] Oji *et al.*[44] showed that BSI is caused by TGM1 deficiency with heat-dependent TGM1 dysfunction. There have been several reports of BSI from different ethnic backgrounds.[45,46] Twenty missense mutations of *TGM1* have been reported in BSI patients.[45] Of these 20 missense mutations, nine occurred only in patients with the BSI phenotype, and the remaining 11 were common to BSI and the other types of ARCI. Until recently, no genotype/phenotype correlation had been known. Therefore, the same mutation of *TGM1* could result in either generalized ARCI or BSI. This probably reflects a suspected interaction between the environment and the gene, leading to a differential expression of the same mutation.[45,47] Recently, Washio *et al.*[48] demonstrated that hypohidrosis plays a crucial role in the heating spiral of BSI cases with summer exacerbation. In TGM1-deficient patients with BSI exhibiting seasonal exacerbation, they speculated that hypohidrosis following excessive keratinization results in heat accumulation, which possibly causes additional hyperkeratosis in the summer.

KERATINOPATHIC ICHTHYOSIS

Keratinopathic ichthyosis is proposed as an umbrella term for epidermolytic ichthyosis (EI), superficial epidermolytic ichthyosis (SEI), annular epidermolytic ichthyosis (AEI), ichthyosis Curth-Macklin (ICM), autosomal recessive epidermolytic ichthyosis (AREI), epidermolytic nevi (EN) and congenital reticular ichthyosiform erythroderma (CRIE). All types of keratinopathic ichthyosis are caused by mutations in the keratin family genes *KRT1*, *KRT2* and *KRT10*.

Epidermolytic ichthyosis

The most prevalent keratinopathic ichthyotic phenotype, EI is characterized by generalized blister formation and multiple erosions with erythroderma. The patients show blistering and erythema at birth, which diminishes with age, and generalized epidermolytic hyperkeratosis in adulthood (Fig. 2a,b).[49] Skin biopsy in EI shows marked epidermal acanthosis and hyperkeratosis. Histologically, granular degeneration is the most characteristic feature in EI.[50] There is extensive phenotypic variation in EI; therefore, infant patients may be clinically challenging to differentially diagnose from other skin disorders.[51–54] Mutations in *KRT1* or *KRT10* underlie EI.[1] Palmoplantar keratoderma (PPK) is a more prominent feature of patients with EI with mutations in *KRT1* than in those with mutations in *KRT10*.[55] However, a few exceptions exist where *KRT10* mutations have been identified in patients with severe EI and PPK.[56] With regard to the genotype/phenotype correlations in EI, Haruna *et al.*[57] reviewed the published work on the highly conserved amino acid codon 156 in *keratin 10*, as it could be a mutational hotspot. In reviewing cases of EI, they investigated the clinical severity of EI resulting from a *KRT10* mutation at codon 156 in 31 patients. Of these 31 patients, 23 were described as severe or typical cases and six as mild cases. (The clinical features were not described for the remaining two cases.) In seven patients harboring an R156C mutation in *KRT10*, however, the EI was severe or typical.[57]

Recently, Palombo *et al.*[58] presented two unique sporadic patients showing a mild diffuse ichthyosis with PPK. Interestingly, one of them showed significant hyperkeratosis of the palms and soles similar to those present in mal de Meleda (also called Meleda disease, OMIM no. 248300). Both patients carry the *KRT1* p.I479T substitution; however, in the palmoplantar areas of the more severe of these cases, only the mutated allele is expressed (the somatic loss of the wild-type allele).[58] We reported the successful treatment of facial lesions in an adolescent case of EI by topical adapalene. Our observations suggest that topical adapalene may be greatly superior to systemic retinoids in improving facial lesions and quality of life for pediatric patients with EI.[59]

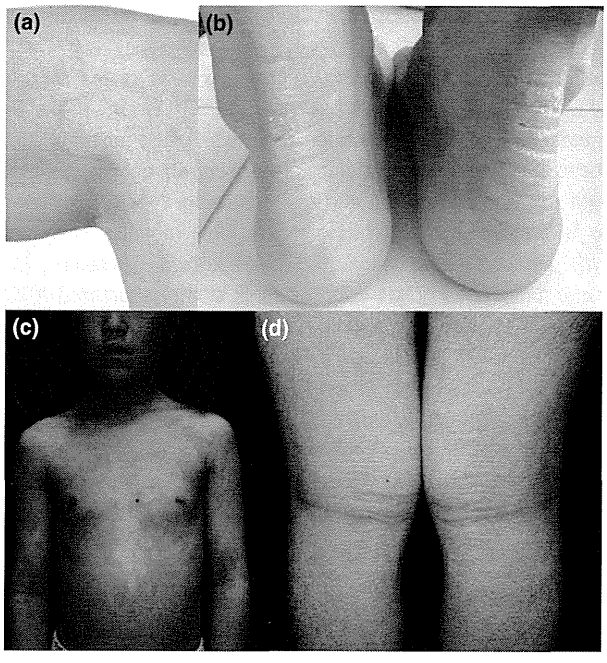

Figure 2. Clinical features of epidermolytic ichthyosis (EI) and erythrokeratoderma variabilis (EKV). (a,b) An EI patient with *KRT10* mutation.[54] This 25-year-old woman presents scaly, annular, erythematous patches on the (a) right armpit and (b) ankles. (c,d) An 8-year-old girl with EKV caused by *GJB3* mutation. (c) Geographic erythematous lesions of various sizes are present on the cheeks, chest and upper arms. (d) Generalized scaly hyperkeratosis with erythroderma is noted on the legs.

Superficial epidermolytic ichthyosis

The term "superficial epidermolytic ichthyosis" had been proposed for the well-defined entity ichthyosis bullosa Siemens, which in contrast to EI, shows a more superficial pattern of epidermolysis and is caused by mutations in *KRT2*, rather than in *KRT1* or *KRT10*.[1] SEI is clinically characterized by mild epidermal hyperkeratosis over flexural areas, blister formation and the development of superficially denuded areas of hyperkeratotic skin.[60] Histologically, the epidermolytic hyperkeratosis in SEI is confined to the granular and upper spinous layers, with intracorneal blister formation.[61] This distribution pattern of the granular degeneration is consistent with the expression site of keratin 2. To date, 15 pathogenic missense mutations in *KRT2* have been reported in SEI (www.hgmd.cf.ac.uk). All substitutions are in the 1A and 2B domains of keratin 2.

Annular epidermolytic ichthyosis

Annular epidermolytic ichthyosis is a distinct phenotypic variant of EI characterized by the intermittent development of annular, polycyclic, erythematous, scaly plaques over the proximal extremities and the trunk.[62] The histological features are similar to those of EI: hyperkeratosis, acanthosis and degeneration of the granular layer.[62,63] Yang *et al.*[64] identified a unique mutation in *KRT10* that results in an alanine to proline mutation at residue 12 (p.A12P) of the 1A helical segment. This residue is predicted to occupy the *b* position of the heptad repeat, so that it lies on the outside of a coiled-coil where it interacts with neighboring molecules within the keratin intermediate filament superstructure.[64,65]

Ichthyosis Curth-Macklin

Ichthyosis Curth-Macklin is a rare autosomal dominant disorder characterized by extensive, spiky or verrucous hyperkeratosis that affects the large joints and the trunk, with or without palmoplantar keratoderma.[66,67] Histologically, acanthosis of the epidermis and papillomatosis with severe hyperkeratosis are seen and are accompanied by some vacuolated or binucleated cells in the granular and suprabasal layers, although no granular degeneration is seen.[67,68]

Characteristically, the electron microscopic study of both normal-looking and lesional skin revealed disordered

keratinization characterized by tonofilament shells, perinuclear vacuoles and binuclear keratinocytes.[67] Four distinct germ line mutations of the V2 tail domain of the *KRT1* (OMIM no. 139350) have been identified in four patients with ICM.[68–71] The V2 domain of keratin 1 consists of 10 glycine loops. All four reported mutations in ICM are deletions and/or insertions causing frame-shifts in the V2 tail domain. Aberrant keratin 1 tails may contribute to abnormal keratinization in ICM, partially because the tail domain is involved in the interaction between the keratin intermediate filament network and other proteins such as loricrin.[69] However, two different mutations in the V2 tail domain are associated with patients of striate palmoplantar keratoderma and an atypical form of epidermolytic hyperkeratosis.[72,73] Further investigations are needed to clarify why mutations in the identical V2 tail domain result in different phenotypes.

Autosomal recessive epidermolytic ichthyosis

Autosomal recessive epidermolytic ichthyosis is caused by rare autosomal recessive mutations in *KRT10*.[74–78] AREI cases have been reported to be due to nonsense mutations resulting in premature termination codon of *KRT10*, leading to a complete absence of the protein. Recently, Gutierrez *et al.*[78] reported a novel homozygous nonsense mutation in the 1B domain of keratin 10, although four other reported mutations affected the 2B domain. AREI has been found to have phenotypic variability, from a lethal form to a milder form than dominant EI.[75,77] None of the AREI patients has shown involvement of palmoplantar areas.[78] The presence of clinically unaffected heterozygous carriers suggests that one allele of *KRT10* is sufficient to retain a normal phenotype. There have been no reports of AREI caused by *KRT1* mutations. It is possible that a homozygous nonsense mutation of *KRT1* may lead to embryonic lethality in humans.

Epidermolytic nevus

The clinical features of EN include circumscribed verrucous lesions of any size presenting singly or multiply, and EN can occur at any site, frequently following Blaschko's lines.[79,80] Histologically, papilloma-like proliferation and granular degeneration occur in the epidermis.

In 1994, *KRT10* mutations were reported in EN.[80] Since then, EN has been thought to reflect a genetic mosaicism of EI. Additionally, we reported the first case of EN caused by a *KRT1* mutation.[81] Interestingly, in our case, the *KRT1* mutation that had been reported to cause PPK led to epidermal nevi with an EI phenotype distributed on the trunk and the extremities. In the previous PPK patient with an identical mutation, only mild hyperkeratosis was found and it was on limited body areas.[81,82]

Congenital reticular ichthyosiform erythroderma (also called "ichthyosis with confetti")

Congenital reticular ichthyosiform erythroderma is a very rare skin disease, and affected subjects are born with erythroderma on almost the entire body surface from defective skin barrier function, prominent scales and palmoplantar keratoderma.[83–85] Early in life, hundreds to thousands of pale confetti-like spots appear across the body surface and increase in number and size with age. The histological findings are epidermal thickening in the ichthyotic skin and a disturbed differentiation of keratinocytes with parakeratosis.[83] Ultrastructurally, perinuclear shells are seen in the cytoplasm of epidermal keratinocytes.

In 2010, the disease was demonstrated to be caused by heterozygous frame-shift mutations of ichthyosis lesion typically localized in the C terminus of *KRT10*, leading to an arginine-rich tail of the protein.[86] Additionally, CRIE is remarkable for its high frequency of spontaneous reversion, with more than a thousand revertants in many subjects. Interestingly, the loss of heterozygosity of the mutant allele in normal-appearing spots was observed.[86] Spoerri *et al.*[87] proposed major and minor criteria, including several ectodermal malformations, based on the clinical variability of CRIE in six patients with confirmed mutations in *KRT10*. Very recently, a causal de novo mutation in *KRT1* was identified in CRIE patients. Similar to CRIE-causing *KRT10* mutations, this mutation in *KRT1* resulted in a C-terminal frame-shift, replacing 22 C-terminal amino acids with an alternate 30-residue peptide.[88]

OTHER FORMS

Loricrin keratoderma

Loricrin keratoderma (LK) is an autosomal dominant genodermatosis showing mild ichthyosis and honeycomb PPK.[89,90] LK is often associated with pseudoainhum, and the affected individuals are sometimes born as collodion babies.[89,91] LK is caused by heterozygous mutation in *LOR*, which encodes loricrin, a major component of the cornified cell envelope found in terminally differentiated epidermal keratinocytes. To date, at least eight mutations in *LOR* have been reported in pedigrees from various ethnic back grounds.

Recently, Yoneda *et al.*[92] showed that the profilaggrin N-terminal domain interacts with loricrin and keratin 10 *in vivo* and that these interactions are likely to be relevant to cornified cell envelope assembly and subsequent epidermal barrier formation. Their group also reported that filaggrin deficiency accompanied with subsequent loricrin deficiency leads to reductions in the expression of epidermal growth factor receptor, E-cadherin and occludin and to disruption of the SIRT1 pathway in the skin of flaky tail mice.[93]

Erythrokeratoderma variabilis

Erythrokeratoderma variabilis (EKV) is usually an autosomal dominant disorder that presents in the first year of life. There are two characteristic skin manifestations: localized, sharply circumscribed keratotic lesions, and migratory erythematous areas (Fig. 2c,d).[94,95] Mutations in *GJB3* and *GJB4* encoding connexin 31 and connexin 30.3, respectively, have been reported in EKV.[94,95] To date, three recessive cases, both caused by homozygous mutations in *GJB3*, have been reported from the Middle East.[96–98] Although several dominant *GJB3* mutations causing EKV have been shown to be gain-of-function mutants that lead to increased sensitivity to apoptosis

of keratinocytes or that interfere with gap junction conductance,[99,100] different mechanisms have been shown to mediate the pathogenic effects of disease-causing mutations in connexin genes, including impaired protein trafficking to the plasma membrane, a dominant-negative effect on co-expressed connexins, constitutive activation of functional channels, and cell death due to endoplasmic reticulum stress and unfolded protein response.[98,101] Further accumulation of recessive EKV cases with mutation data is needed to confirm the pathomechanisms in *GJB3* mutations.

No specific therapy for EKV is currently available. Several investigators have used topical and oral retinoids (vitamin A, etretinate, isotretinoin and acitretin) with good to excellent results.[102–104] Recently, Singh *et al.*[105] reported the successful treatment of EKV by low-dose isotretinoin.

Peeling skin disease

Generalized peeling skin syndrome (PSS) has been classified into a non-inflammatory type, designated type A, and an inflammatory type, designated type B.[1] Inflammatory peeling skin disease refers to PSS type B (OMIM no. 270300) and results from homozygous mutations in *CDSN*, the gene encoding corneodesmosin, an extracellular adhesion glycoprotein located in desmosomes and corneodesmosomes.[103] Clinically, it presents as CIE with continual inflammatory skin peeling leading to migratory exfoliative patches. Consequently, defects in epidermal barrier function lead to pruritus, a susceptibility to skin infections and AD (often with elevation of total immunoglobulin E levels and eosinophilia).[106] Histologically, it manifests as psoriasiform dermatitis with cleavage above the stratum granulosum, or above an acanthotic epidermis lacking a stratum granulosum. Ultrastructural examination shows intercellular detachment of corneocytes.

Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome

Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome (KLICK syndrome) is an autosomal recessive skin disorder characterized by palmoplantar keratoderma, linear hyperkeratotic plaques, ichthyosiform scaling, circular constrictions around fingers and numerous papules in a linear pattern in the arm folds and on the wrists.[107] In 2010, a single nucleotide deletion of the 5′ untranslated region of *POMP* in 12 KLICK patients was identified by using whole-genome single nucleotide polymorphism analysis, though their six available parents were heterozygous and the five available healthy siblings were either heterozygous or non-carriers for the deletion.[108] There have been no other reported mutations in the *POMP*. Histological examination of the skin of affected individuals shows hyperplasia and hypertrophy of the spinous, granular and horny layers of the epidermis.[107] Recently, Dahlqvist *et al.*[109] reported that KLICK was caused by reduced levels of proteasome maturation protein, leading to proteasome insufficiency in differentiating keratinocytes. Proteasome insufficiency disturbs terminal epidermal differentiation, presumably by increased endoplasmic reticulum stress, and leads to perturbed processing of profilaggrin.[109]

DETECTION OF CAUSATIVE GENES BY NEW TECHNOLOGIES

We currently use whole-exome sequencing (WES) to identify causative genes in most cases of genodermatosis, following our own recent positive experience in using WES to diagnose genetically heterogeneous inherited skin diseases.[110–112] These days, in cases with suspected non-syndromic congenital ichthyoses, WES is cheaper than conventional Sanger sequencing for all the reported candidate genes in inherited ichthyoses; for example, *ABCA12* contains 53 exons, and more than 50 PCR primer pairs are necessary for amplification of all exons and flanking introns.[25] As mentioned above, we reported that a patient with the small indel mutation in *STS* showed a unique "self-healing" phenotype of RXLI, as identified by WES. In addition, Hand *et al.* expanded the phenotypic spectrum of RXLI by CMA. CMA analysis identifies chromosomal imbalances and is commonly used as a first-line test for congenital anomalies, rapidly replacing traditional karyotype analysis.[113] Collectively, next-generation sequencing technologies represent powerful tools for the diagnosis and discovery of inherited ichthyoses. As access to accurate and efficient genetic diagnosis improves, the need for invasive hospital investigations of the patients will decrease.

CLOSING REMARKS

The burdens of inherited ichthyoses were described in a French national survey.[114] The negative impact of ichthyoses was obvious in domestic life, educational/professional life and leisure/sports activities. To reduce patient distress, further clinical and laboratory investigations for treatment and diagnosis are needed. Fortunately, technologies have rapidly advanced in the past decade.

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Intrafamilial phenotypic heterogeneity of epidermolytic ichthyosis associated with a new missense mutation in keratin 10

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Summary

Mutations in the keratin 10 gene (*KRT10*) have been shown to underlie several forms of epidermolytic ichthyosis (EI), including generalized, annular and naevoid variants. We investigated an autosomal dominant pedigree with ichthyosis in which there was intrafamilial clinical heterogeneity, with the affected individual family members presenting with features of either erythrokeratoderma progressiva, annular EI, localized or superficial EI, or more generalized EI. Sanger sequencing identified a new heterozygous missense mutation (c.457C>A; p.Leu153Met) in *KRT10* in all affected individuals. No additional mutations were identified in the genes for keratin 1 (*KRT1*) keratin 2 (*KRT2*), connexin 31 (*GJB3*) or connexin 30.3 (*GJB4*) that might account for the clinical heterogeneity seen in this family. Our findings illustrate the intrafamilial variability in phenotype and diverse clinical presentations that can occur in EI resulting from a single mutation in *KRT10*.

The keratinopathic ichthyoses represent a clinically diverse group of mostly autosomal dominant disorders that result from mutations in the genes for keratin 1 (*KRT1*) keratin 2 (*KRT2*) or keratin 10 (*KRT10*).[1] Mutations in *KRT1* or *KRT10* underlie most cases of epidermolytic ichthyosis (EI), whereas mutations in *KRT2* lead to a relatively milder condition, superficial EI. Cases of EI may be clinically diverse;[2] as well as erythroderma and blistering in early life followed by generalized ichthyosis, the spectrum of EI also includes annular, autosomal recessive and naevoid variants, ichthyosis with confetti, and some cases of Curth–Macklin ichthyosis.[1–3] We present a new heterozygous missense mutation in *KRT10* that resulted in intrafamilial phenotypic heterogeneity in a UK family with an autosomal dominant ichthyosis.

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Report

A 25-year-old white woman presented with a 1-year history of spontaneously appearing, multiple, migratory, erythematous patches over her arms, legs and trunk (Fig. 1a). Each lesion started as a small inflamed papule and then spread in an annular configuration. The lesions were initially painful but not pruritic, and resolved with peeling after a few days. The onset of the inflammatory skin lesions occurred within a few months of the patient starting the oral contraceptive pill, but no other exacerbating factors were noted. Some improvement was noted during the summer months or after sun exposure. The patient had a history of ichthyosis during her infancy and early childhood particularly over the knees, neck and skin creases, which resolved in adulthood. There was no history of erythoderma or blistering at birth. There was an autosomal dominant family history of an ichthyosis that affected at least seven individuals over five generations (Fig. 1b; proband is individual IV-3) although no other affected individual reported similar skin lesions. By contrast, the proband's 60-year-old

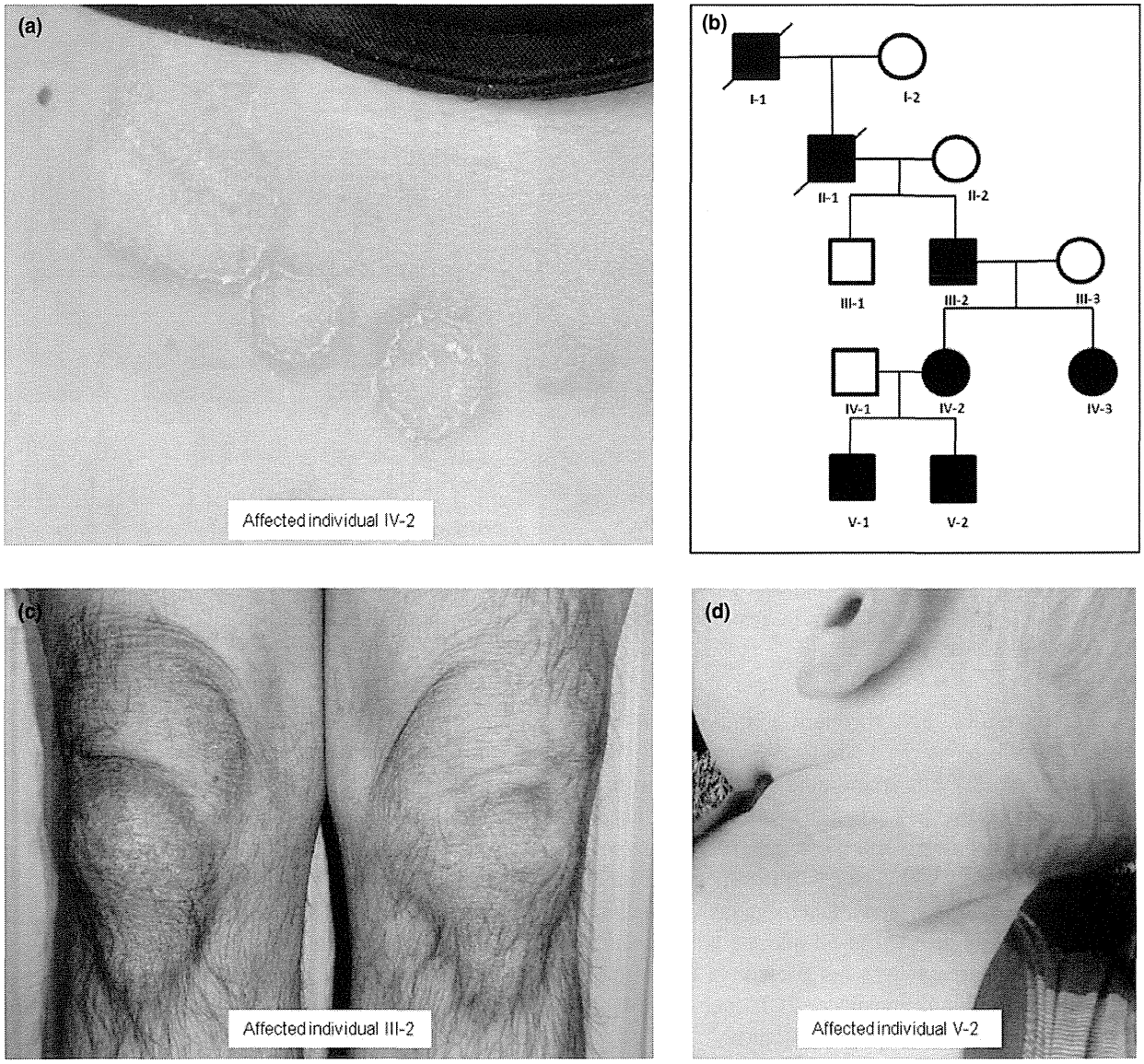

Figure 1 Variable clinical features were seen in this autosomal dominant ichthyosis. (a) Superficial erythematous annular plaques in the inframammary region in the proband (individual IV-3). (b) The family pedigree. (c) Lichenified, hyperkeratotic plaques on both knees in the proband's father (III-2). (d) Mild skin thickening and discolouration on the neck in the proband's nephew (V-2).

father (individual III-2) had a history of generalized ichthyosis with accentuation over the knees (Fig. 1c) and elbows, although details of his skin (e.g. blistering) as a neonate were not known. The proband's 18-month-old nephew (individual V-2 in the pedigree) had minor skin thickening and brown discolouration on his neck (Fig. 1d), and to a lesser extent on his knees, elbows and heels. No individual had any palmoplantar keratoderma.

Following informed consent, a skin biopsy was obtained from the left knee of the father (III-2), and light microscopy showed features of epidermolytic hyperkeratosis (Fig. 2a). Genetic screening for a keratinopathic ichthyosis was then performed, specifically focusing on *KRT10* in view of the lack of palmoplantar keratoderma. Genomic DNA from the proband (IV-2) was extracted from peripheral blood, and mutation analysis was performed using bidirectional Sanger sequencing of PCR products spanning the exons and flanking introns of *KRT10*. A heterozygous single nucleotide substitution c.457C>A was found in exon 1, which converts leucine (CTG) to methionine (ATG), designated p.Leu153Met. This missense change occurs within the helix initiation motif of keratin 10, a highly conserved region critical to keratin filament assembly through interactions with the 2B segment of neigh-

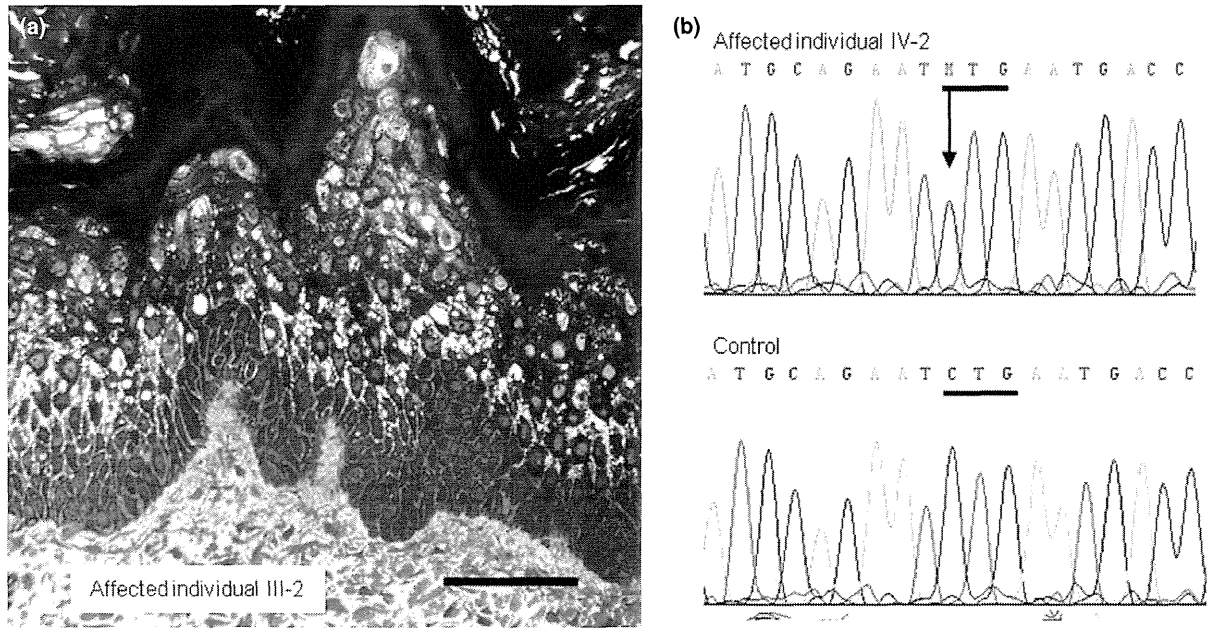

Figure 2 Skin and molecular pathology. (a) Epidermolytic hyperkeratosis in skin from the knee (scale bar = 50 μm); (b) heterozygous missense mutation in keratin 10.

bouring molecules, and therefore mutations in this region are likely to lead to a deleterious clinical phenotype.[2] A different amino acid substitution in the same residue (c.457C>G; p.Leu153Val) has been identified previously in two unrelated Japanese pedigrees with generalized EI,[4,5] but substitution to methionine is a new finding. The heterozygous mutation p.Leu153Met was detected in all affected family members in our pedigree; it was not present in DNA from the unaffected family member III-1, nor in 1400 ethnically matched control chromosomes.

Predicting the precise phenotype from an underlying mutation in *KRT10* is difficult.[6] Most reported pathogenic mutations occur within the 1A or 2B domains of the protein, but diverse clinical consequences are often seen for mutations in the same residue. For example, > 20 pathogenic mutations in p.Arg156 have been reported in cases of EI, although the phenotype can be generalized, localized or annular in different families.[7] In most cases of generalized EI there is a lessening of blistering with time, but increasing age provides only a partial explanation for individual phenotypic differences. The most clear genotype–phenotype correlation is for frameshift mutations in the tail of keratin 10 in ichthyosis with confetti.[8]

Clinically, the skin lesions in the proband in our study (IV-2) seemed somewhat milder and of shorter duration than most other reported cases of annular EI, and more closely resembled a form of erythrokeratoderma, yet her father (III-2) had the more typical adult features of generalized EI that would be expected from a dominant-negative missense mutation in the helix initiation peptide of keratin 10. The proband's nephew (V-2) did not have erythroderma or blistering at birth, and the mild skin thickening became apparent only at the age of 9 months. What might account for the intrafamilial variation in disease expression is not clear. It is possible that the oral contraceptive pill may have triggered the inflammatory lesions in the proband, as oestrogens may influence keratin 10 expression, and reported cases of annular EI can appear or worsen during pregnancy.[9] Nevertheless, the precise environmental or genetic triggers are currently unknown.

We therefore used Sanger sequencing on the genomic DNA from family members for underlying mutations in *GJB3* and *GJB4*, encoding the gap junction proteins connexins 31 and 30.3, respectively, which are mutated in erythrokeratoderma,[10] as well as *KRT1* and *KRT2*, the other two genes implicated in keratinopathic ichthyoses.[1,2] No potentially pathogenic mutations were found in any of these genes. The reason for the disparity in phenotype therefore remains currently unresolved. The proband responded extremely well to low-dose isotretinoin, with almost complete resolution of symptoms. The mechanism of action of retinoids in EI is not completely understood, although a reduction in the formation of keratin aggregates in keratinocytes

in vitro has been demonstrated.[11] None of the other family members has received retinoids.

In summary, we present an autosomal dominant pedigree with EI resulting from a new heterozygous missense mutation in keratin 10. This family shows intrafamilial variation in phenotype, emphasizing the protean nature of clinical pathology for *KRT10* mutations and the key importance of other, currently unknown, regulatory or modifying factors that influence disease expression.

Learning points

- Keratinopathic ichthyoses are usually autosomal dominant, and caused by mutations in *KRT1*, *KRT2* or *KRT10*.
- Mutations in *KRT10* can result in diverse epidermolytic ichthyosis phenotypes, including erythroderma and blistering in early life followed by generalized ichthyosis, as well as annular, autosomal recessive, and naevoid variants, ichthyosis with confetti, and some cases of Curth–Macklin ichthyosis.
- Mutations in *KRT10* can also lead to intrafamilial variability in the clinical features.

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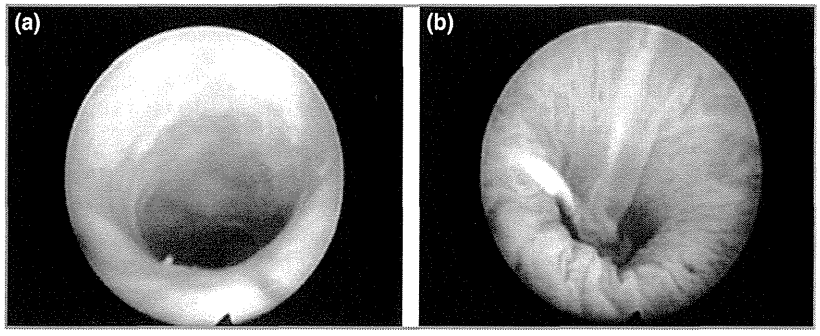

Fig 2. Cystoscopy. An evaluation of the urethral mucosa with cystoscopy included successful serial catheter urethral dilation to 24 French, after which the cystoscope could pass. After 15 months of adalimumab treatment, cystoscopy showing (a) the entire urethra with pale whitish mucosa and (b) transition to normal mucosa at the external sphincter with normal prostatic urethra.

with LS should be considered. Other limitations are financial in nature; adalimumab is costly, and at this phase authorization is dependent on the insurance provider. Yet, the outcome of our patient on therapy is extremely promising and offers another line of therapy for recalcitrant, advanced pathology.

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Noteworthy clinical findings of harlequin ichthyosis: digital autoamputation caused by cutaneous constriction bands in a case with novel *ABCA12* mutations

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Dear Editor, Harlequin ichthyosis (HI) (OMIM 242500) is the most severe form of autosomal recessive congenital ichthyosis caused by mutations in the ATP-binding cassette, sub-family A (ABCA), member 12 (ABCA12) gene (*ABCA12*). The clinical features at birth include ectropion, eclabium, flattened ears and large, thick plate-like scales over the entire body.[1]

Although HI is occasionally fatal even today, the survival rate has improved due to the early introduction of oral retinoids and intensive neonatal care.[2] Long-term HI survivors report improvement of the dermatological symptoms. It is important to prevent irreversible after-effects associated with HI in order to improve long-term quality of life in affected patients.

Here, we report a case of HI survival with digital autoamputation caused by cutaneous constriction bands. We also describe novel *ABCA12* mutations detected in the case.

A girl was born by scheduled Caesarean section because of breech presentation. She is the first child of healthy, unrelated Japanese parents. There was no remarkable family history of any related disorders. Apgar scores were 4 and 8. The skin revealed hard, thick plate-like scales with deep fis-

suring overlying erythrodermic skin. Severe eclabium and ectropion were observed. These clinical features are typical of HI. She was brought to the neonatal intensive care unit due to the skin abnormality. She was treated with local application of emollient ointment and systemic retinoids (1 mg kg^{-1}) from postnatal day 6 under respiratory management, nutrition management and infection control. The digital necrosis caused by cutaneous constriction bands appeared from postnatal day 2 to day 3, and resulted in the autoamputation of all the fingers and toes at the distal middle phalanx, except the left forefinger, the thumbs and the first toes (Fig. 1a,b). Now at the age of 2·5 years, she shows generalized erythroderma with desquamative scaling, persistent mild ectropion and flattened ears. She has normal mental and physical development.

The ethics committee of the Nagoya University Graduate School of Medicine approved the present studies, which were conducted according to the principles of the Declaration of Helsinki. The participants provided written informed consent. We searched for mutations in the *ABCA12* gene in the patient and both parents. Direct sequencing of the patient's polymerase chain reaction (PCR) amplification products revealed that the patient had the following compound heterozygous *ABCA12* gene mutations: c.1194_1221del28 (p.Ile398Metfs15X) in exon 11 and c.5985G>A (p.Gly1996Asp) in exon 41 (Fig. 2a, b). The patient's father was heterozygous for c.1194_1221del28 and her mother heterozygous for c.5985G>A. To the best of our knowledge, both mutations are novel. The c.5985G>A mutation was not detected in 100 control alleles (50 individuals; data not shown), and the resulting mutation occurs at the amino acid residue Gly^{1996}, which is highly conserved across different species (Fig. 2c). p.Gly1996Asp was analysed using SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). The SIFT score was 0·002 and the PolyPhen-2 score was 1·000; both scores predicted that the p.Gly1996Asp substitution was a damaging aberration. The p.Ile398Metfs15X mutation is located in the N-terminal domain of the protein, while the p.Gly1996Asp mutation is located within the second cluster of the transmembrane domains (Fig. 2d). Because the two mutations are considered to lead to severe loss-of-function, we predicted that these mutations were causative mutations for HI.

In the literature, seven cases of HI with digital autoamputation or necrosis due to cutaneous constriction bands have been reported previously.[3–5] Of these, both cases with an established outcome died of sepsis within 15 days after birth. Our case survived thanks to thorough treatment, even though her fingers and toes were necrotic just after birth. Resolution of digital ischaemia by relaxation incision was reported in one HI case.[6] Relaxation incision is thought to be a conceivable therapy for digital ischaemia. Even the incision of a superficial, thick stratum corneum may be effective for treatment of digital necrosis in HI cases.

In conclusion, we report a digital autoamputation caused by cutaneous constriction bands in a long-term survivor of HI due to two novel *ABCA12* mutations. The present case suggests that the digital necrosis observed could occur despite the application of appropriate general treatments. It is important to inform family members of patients affected by HI about the possibility of digital necrosis.

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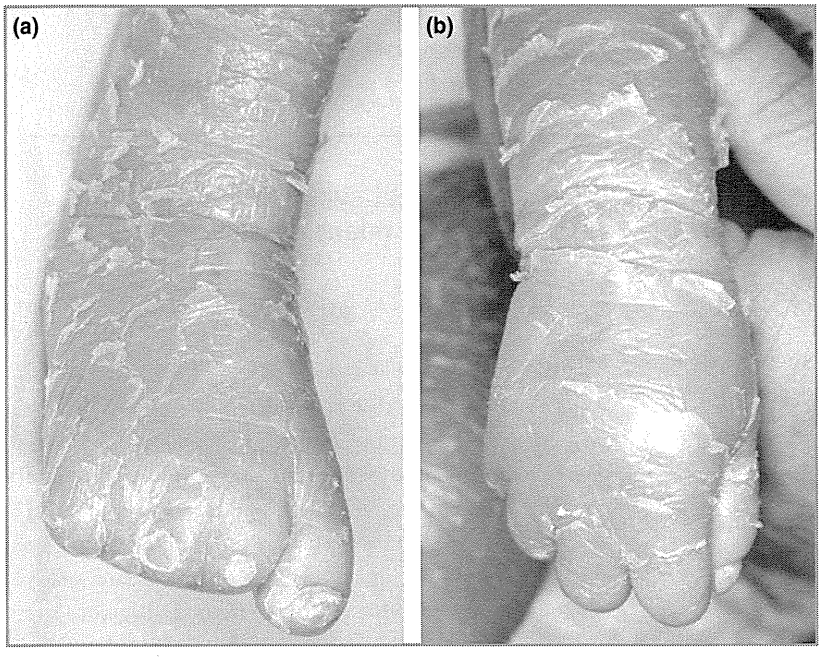

Fig 1. Clinical features of the patient's foot and hand. The toes (a) and the fingers (b) were autoamputated at the distal middle phalanx, except the thumbs and the first toes.

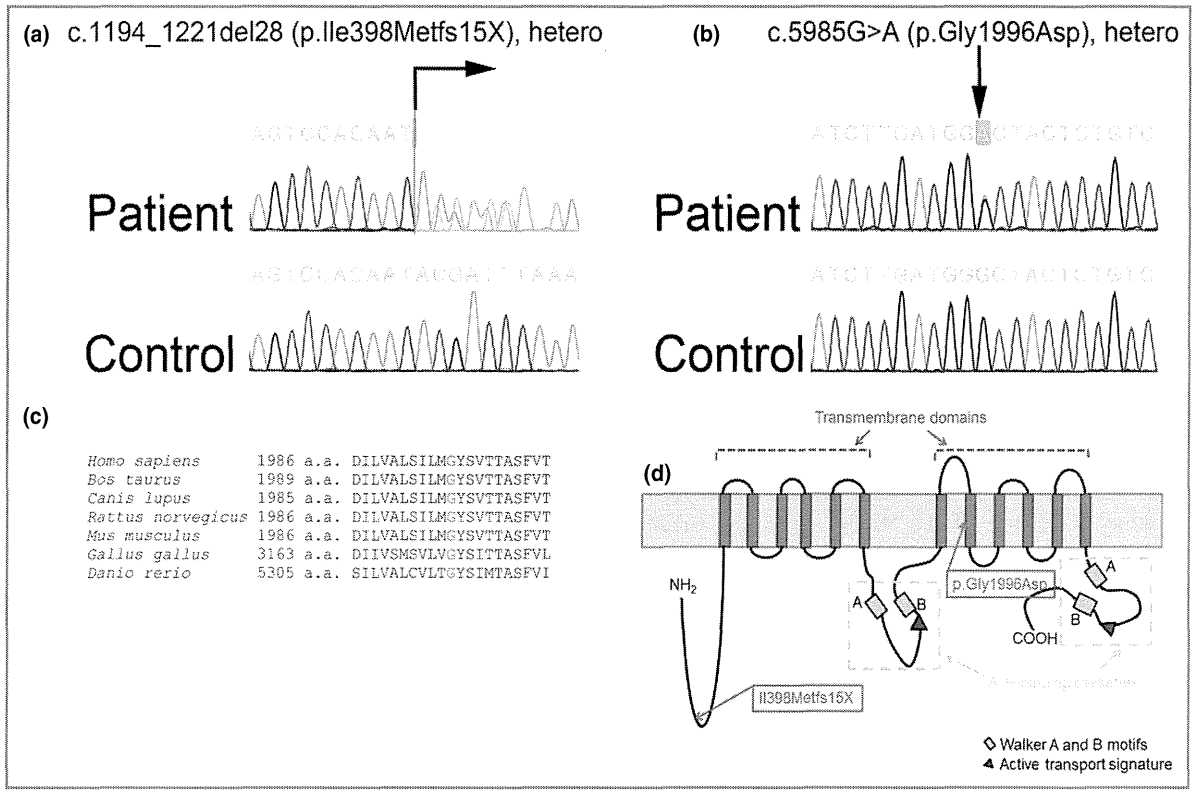

Fig 2. Mutational analysis and localization of the *ABCA12* gene mutations. (a) Direct sequencing revealed a heterozygous c.1194_1221del28 (p.Ile398Metfs15X) mutation in exon 11 of the *ABCA12* gene in the patient and her father, but not in normal control samples. (b) A heterozygous c.5985G>A (p.Gly1996Asp) mutation was identified in exon 41 of the *ABCA12* gene in the patient and her mother, but was absent in normal control samples. (c) Partial *ABCA12* amino acid sequence alignment of diverse species shows a high conservation of the Gly^{1996} residue (red characters). (d) The p.Ile398Metfs15X and p.Gly1996Asp mutations (red arrows) identified in the patient with harlequin ichthyosis.

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Histiocytoid Sweet syndrome: a novel association with relapsing polychondritis

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Dear Editor, Histiocytoid sweet syndrome (HSS) is a variant of Sweet syndrome (SS) that is histologically characterized by a predominant inflammatory infiltrate of mononuclear histiocytoid cells intermingled with neutrophils.[1,2] Although the predominant inflammatory cells have been speculated to be M2-like macrophages, their exact nature and origin has not been elucidated.[3] Relapsing polychondritis (RP) is a rare systemic autoimmune disorder that primarily affects cartilaginous struc-

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Original Article

Gradual tapering of desmopressin leads to better outcome in nocturnal enuresis

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Abstract ***Background***: Although desmopressin therapy is effective in treating polyuric monosymptomatic nocturnal enuresis (MNE), the relatively high rates of recurrence are problematic. To date, the treatment protocol on the discontinuation of oral desmopressin melt (ODM) tablet, MinirinMelt, has not been established. We tested two protocols of tapering ODM when the patients achieved full response on ODM, and compared the treatment outcomes.

Methods: One hundred and fifty-seven polyuric MNE children were newly treated with ODM at the authors' outpatient clinics (Juntendo Nerima Hospital and Musashi-Murayama Hospital). When the patients did not respond to the 8 week ODM therapy, we added another options such as alarm, anti-cholinergics, and imipramine (92 patients; 58.6%). Sixty-five patients (41.4%) achieved full response on ODM alone, and 49 of them accepted gradual tapering of ODM: group B (n = 25), 240 μg ODM per day → 120 μg ODM per day → 120 μg ODM per alternate day → cessation; and group C (n = 24), 240 μg ODM per day → 120 μg ODM per day → 60 μg ODM per day → 60 μg ODM per alternate day → cessation.

Results: Fourteen patients in group B (56%) and four in group C (17%) had relapses of enuresis after the discontinuation of ODM ($P = 0.026$).

Conclusions: Gradual tapering of ODM therapy in MNE patients leads to better outcome.

Key words: nocternal enuresis, desmopressin.

Nocturnal enuresis is involuntary nocturnal urination, as defined by the International Children's Continence Society (ICCS) standardization document, and a heterogeneous condition that includes a spectrum of disorders with different underlying pathophysiological mechanisms.[1] Nocturnal enuresis in children without other lower urinary tract symptoms (LUTS) and without a history of bladder dysfunction is defined as monosymptomatic nocturnal enuresis. Currently enuresis may be divided into two main subtypes: diuresis dependent and detrusor dependent.[2]

For the patients with diuresis-dependent nocturnal enuresis, desmopressin, a synthetic analog of arginine vasopressin, the naturally occurring anti-diuretic hormone, is considered as the first-line therapy.[3]

Approximately 30% of children with enuresis are full responders and 40% have a partial response,[4] whereas the relapse rate after discontinuation is high (60–70%).[5] Although initial treatment guidelines for enuretic patients have been established recently,[3] and commonly used all over the world, the procedures by which therapy is terminated have not been fixed to date.

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A few clinical studies have compared treatment outcomes between two groups: structured withdrawal or direct cessation of desmopressin therapy after response, but the results are still controversial.[6–8] We performed retrospective analysis of patients with polyuric monosymptomatic nocturnal enuresis (MNE), who were treated with two different withdrawal protocols of oral desmopressin melt (ODM) tablet, MinirinMelt (Ferring Parmaceuticals, Saint-Prex, Switzerland), after they responded well to it.

Methods

A total of 157 patients were given ODM as the treatment for PMNE between May 2012 and October 2013 at the outpatient clinics of Juntendo University Nerima Hospital (Tokyo, Japan), Juntendo University Juntendo Hospital (Tokyo, Japan) and Musashimurayama Hospital (Tokyo, Japan). Fourteen of them were enrolled in our previous study,[9] and only children 6–15 years old with enuresis without daytime LUTS were included in the study. Any child with neuropathic bladder, spinal dysraphism, anatomical abnormalities (e.g. posterior urethral valves), or a known history of treatment with desmopressin was excluded.

Before starting treatment, a detailed history, physical examination and 14 day bladder diary were obtained from each patient. Maximum voided volume was recorded in the bladder diary and expected bladder capacity was calculated using the following