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CASE REPORT

Phenotypic diversity of the recurrent p.Val379Leu missense mutation of the *TGM1* gene

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ABSTRACT

Autosomal recessive congenital ichthyosis type 1 (ARCI1), a clinically heterogeneous group of keratinization disorders, develops due to mutations in the *transglutaminase 1* (*TGM1*) gene. Here we report a Hungarian pedigree affected by the lamellar ichthyosis clinical form of the ARCI1 phenotype. Direct sequencing revealed two recurrent heterozygous mutations: a splice site (c.877-2A > G) and a missense (c.1135G > C, p.Val379Leu) mutation. This splice site mutation is the most frequently observed in ARCI1 worldwide. The missense mutation is relatively rare and has been reported in only 13 Scandinavian patients. Comparison of the clinical phenotypes of our Hungarian patients and the Scandinavian patients demonstrates great phenotypic diversity associated with the p.Val379Leu genotype.

Introduction

Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogenic group of rare monogenic diseases characterized by abnormal skin scaling over the whole body.[1] The main skin phenotypes are lamellar ichthyosis and congenital ichthyosiform erythroderma, although phenotypic overlap in the same patient or in patients of the same family can occur.[1]

ARCI type 1 (ARCI1, OMIM 242300) is a common form of ARCI caused by mutations in the *transglutaminase 1* (TGM1, OMIM 190195) gene on chromosome 14q12.[2] The *TGM1* gene encodes transglutaminase 1 (*TGM1*), which is responsible for crosslinking epidermal proteins during formation of the stratum corneum.[2] *TGM1* mutations have been linked to several clinical variants of ARCI1, such as the well-known lamellar ichthyosis phenotype[3] and the self-healing collodion baby phenotype, in which the condition is present at birth but spontaneously improves.[4] A very rare form of this latter clinical variant is the acral self-healing collodion baby, in which the membrane is located on the extremities only.[5] *TGM1* mutations can also lead to development of the bathing suit ichthyosis, another clinical form of ARCI1, in which scaling is pronounced on the bathing suit area and is less pronounced on the extremities.[6]

In this study, we report a Hungarian family with two members affected by ARCI1. Our genetic investigation identified that these members carried two recurrent heterozygous mutations in a compound heterozygous state.

Case report

A Hungarian family with two affected siblings was investigated. The affected individuals were 18 (Patient II/1) and 13 (Patient II/3) years old at the time of investigation. Patient II/1 had dark brown thick scales on her chest (Fig. 1a) and back (Fig. 1b). Her elbows and knees (Fig. 1c) and her flexural regions (Fig. 1d) were affected. The back of her hands were also covered by thick scales (Fig. 1e), but her palms (Fig. 1f) and soles were unaffected. Patient II/3, whose symptoms were milder than Patient II/1, had light brown thick scales covering his chest (Fig. 1g) and back (Fig. 1h). His elbows and knees (Fig. 1i) and his flexural regions (Fig. 1j) were also affected. The back of his hands were also scaly (Fig. 1k), but his palms (Fig. 1l) and soles were unaffected. Ectropion was present in both patients, whereas alopecia was not observed. Both Patients II/1 and II/3 were

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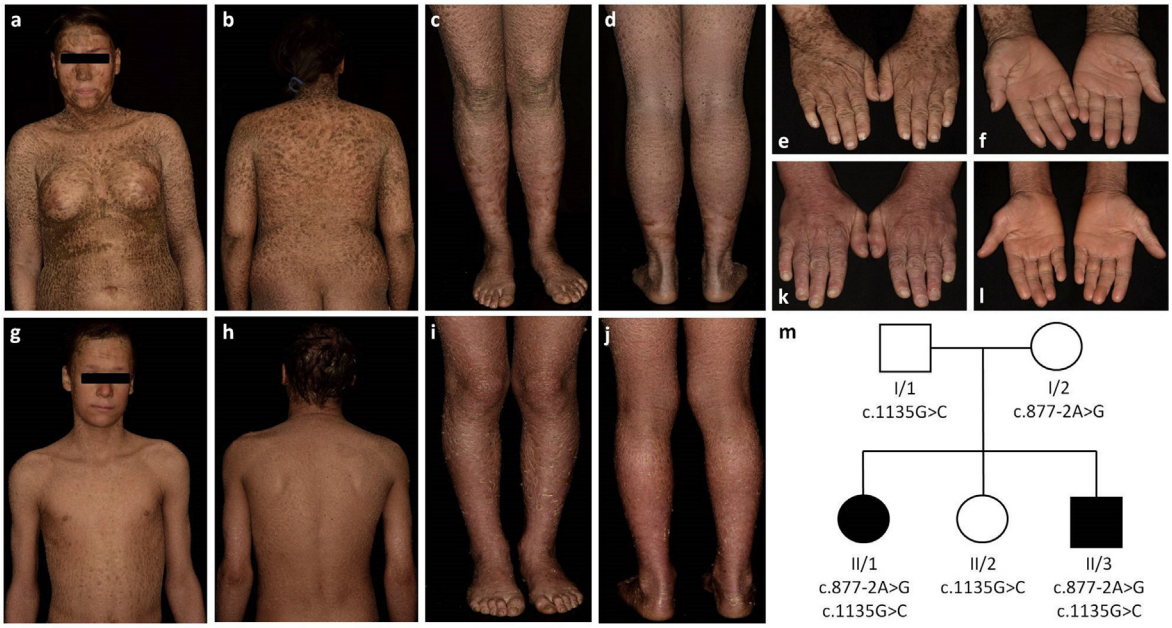

Fig. 1 Clinical symptoms of the affected patients and pedigree of the Hungarian family. Squares and circles denote males and females; filled symbols indicate clinically affected family members.

born with a collodion membrane encasing their bodies. The parents (I/1 and I/2) and the third sibling (II/2) were clinically unaffected by ARCI1 (Fig. 1m).

The patients have been treated with the repeated course of isotretinoin, which could reduce scaling successfully. Besides systemic treatments, the patients are continuously using topical creams both with keratolytic agents such as urea, lactic acid or salicylic acid and both with moisturizers. The treatments can reduce the symptoms of the patients, but if the treatments are stopped the symptoms are coming back, therefore they need regular dermatological care.

We aimed to identify the disease-causing mutation of the *TGM1* gene in this Hungarian family. For genetic investigation, peripheral blood samples were taken from the affected patients and their clinically unaffected family members as well as from unrelated, healthy Hungarian individuals (n = 30), and genomic DNA was isolated using a BioRobot EZ1 DSP Workstation (QIAGEN; Godollo, Hungary). The coding regions and the flanking introns of the *TGM1* gene were amplified and sequenced (primer sequences used were taken from the UCSC Genome Browser www.genome.ucsc.edu). The investigation was approved by the Internal Review Board of the University of Szeged, Szeged, Hungary. Written informed consent was obtained all the investigated subjects. The study was conducted according to the Principles of the Declaration of Helsinki.

Direct sequencing of the coding regions and the flanking introns of the *TGM1* gene revealed two heterozygous mutations, one splice site mutation (c.877-2A > G) 5′ of the sixth exon and one missense mutation (c.1135G > C p.Val379Leu) in the seventh exon. Both affected siblings (II/1 and II/3) carried these mutations, suggesting a compound heterozygous state. The patients' mother (I/2) carried the splice site mutation and the patients' father (I/1) carried the missense mutation. The clinically unaffected sibling (II/2) carried the paternal missense mutation in heterozygous form, but did not carry the maternal splice site mutation (Fig. 1m). These results suggest that the splice site mutation is of maternal origin and the missense mutation is of paternal origin. All the unrelated healthy Hungarian controls (n = 30) carried the wild type sequence.

Discussion

The investigated two Hungarian ARCI1 patients carried two recurrent mutations of the *TGM1* gene: a missense mutation (p.Val379Leu) and a splice site mutation (c.877-2A > G). Both mutations were located in the catalytic core domain of the TGM1 enzyme. Previous functional studies have demonstrated that mutations affecting this domain might severely diminish TGM1 activity due to the less stable structure of the catalytic core.[7] Others proved that the domain lowers the specific activity of the TGM1 protein due to misfolding or by resulting in an excessively stable protein that cannot be processed.[8] Mutations of the catalytic core domain mainly cause the development of the lamellar ichthyosis phenotype of ARCI1, but some of mutations, including p.Arg264Gln, p.Arg264TRp, p.Tyr276Asn, p.Arg307Gly, p.Arg315Cys and p.Arg315His, have been linked with the bathing suit ichthyosis phenotype.[6] Note, arginine is the most frequently mutated amino acid in *TGM1*, possibly due to methyl-induced deamination of CpG dinucleotides.[9]

The splice site mutation (c.877-2A > G) can lead to the formation of two different splice variants, both of which result in a premature stop codon.[10] One variant results in an insertion of a G before nucleotide c.877T.[11] The other causes the inclusion of intron 5 in between exon 5 and 6 in transcribed mRNA.[12] This mutation has been reported in approximately 28% of ARCI1 patients, making it the most common *TGM1* gene mutation reported worldwide, and affects Caucasian Americans, African-Americans, Germans, Norwegians and Egyptians.[9,13] This mutation probably originated in Northern Germany (Westphalia) and later spread worldwide with immigration.[10] However, our investigated Hungarian family was not aware of any German ancestry; although, the family name suggests Bulgarian origin.

The p.Val379Leu missense mutation has only been reported previously in 13 ARCI1 patients of Scandinavian origin.[12,14,15] None of these patients carried this mutation in a homozygous form (Table 1). In 12 cases of the total 15 patients (including the Hungarian individuals), a compound heterozygous state for two

Table 1 Comparison of the clinical phenotypes of ARCI1 patients carrying the heterozygous p.Val379Leu mutation.

Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Mutation 1	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L	p.V379L
Mutation 2	c.877-2A > G	c.877-2A > G	p.R143C	p.R143C	Not known	p.R143C	p.R143C	p.R143C	p.R143C	p.R143C	p.R142H	p.R396L	p.D430V	p.S358R	p.S358R
Reference	This study	This study	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Laiho et al., 1999	Pigg et al., 2000	Huber et al., 1997	Huber et al., 1997
Nationality (H = Hungarian, F = Finnish, N = Norwegian, S = Swedish)	H	H	F	F	F	F	F	F	F	F	F	F	N	S	S
Sex (F = female, M = male)	F	M	F	M	F	M	M	F	M	M	M	M	F	F	F
Skin at birth (C = collodion, I = ichthyotic)	C	C	C	C	I	C	C	C	C	C	C	C	I	C	C
Scales (BK = brown, thick WN = whitish, thin)	BK	BK	WN	WN	WN	BK	BK	BK	BK	BK	BK	BK	BK	WN	WN
Ectropion	+	+	+	+	+	+	+	+	+	+	+	+	NA	+	+
Alopecia	–	–	+	+	+	–	+	–	+	–	–	–	NA	–	–
Flexures	+	+	+	+	+	+	+	+	+	+	+	+	NA	+	+
Palms and soles	–	–	+	+	+	+	+	+	+	+	+	+	NA	+	+
Knees and elbows	+	+	–	–	–	–	–	–	–	–	–	–	NA	–	–

NA = not available.

missense mutations were present.[12,14,15] Of these mutations, p.Val379Leu was most frequently (n = 7 patients, 58%) associated with the p.Arg143Cys mutation and was reported in Finnish patients.[14] In two Swedish patients, the p.Val379Leu was combined with the p.Ser358Arg mutation.[12] The combinations of the p.Val379Leu mutation with p.Arg142His, p.Arg396Leu and p.Asp430Val were detected only once in Finnish and Norwegian patients.[14,15] The investigated Hungarian family was not aware of any Scandinavian ancestry. It would be interesting to use haplotype analysis to investigate whether the same mutation (p.Val379Leu) carried by the Scandinavian and the Hungarian patients is the result of the same founder event or it is the consequence of independent mutation events.

The detailed comparison of the clinical symptoms of the reported 15 ARCI1 patients who all carry the p.Val379Leu mutation give further insight into the genotype–phenotype correlations of this mutation (Table 1). Regarding the skin symptoms, the majority of these patients clearly show the typical lamellar ichthyosis phenotype with brown, thick scales (n = 9, 60%); however, four of them developed thin, whitish scales.[12,14,15] The most common symptom associated with the p.Val379Leu-phenotype is the presence of ectropion and the degree of severity on flexural areas, which were present in nearly all of the patients (n = 14, 93%).[12,14,15] The palms and soles are also frequently affected, detected in 80% of the patients (n = 12).[12,14,15] Alopecia was present only in one third of the patients (n = 5, 33%), all of whom are Finnish.[7] It is interesting to note that the knees and elbows were affected only in our Hungarian patients (n = 2, 13%). Regarding the skin at birth, in 13 cases (86%) the patients were born with collodion membrane encasing their body.[14,15] Two patients showed ichthyosiform erythroderma at birth. One of these patients (P13) subsequently developed lamellar ichthyosis phenotype at the age of two years.[15]

The observed differences in the clinical symptoms of the 15 ARCI patients carrying the heterozygous p.Val379Leu missense mutation clearly demonstrate the wide phenotypic diversity and the variable expressivity of the disease. In general, ectropion and the affectedness of the flexural areas and unaffectedness of the palms and soles are the hallmarks of this phenotype. Further studies are needed to identify putative genetic, environmental or lifestyle factors, which might be responsible for the observed wide phenotypic diversity. The availability of the extended clinical findings for the carriers of the p.Val379Leu mutation – as provided by this study – is critical for promoting our understanding of the disease and might enhance the development of causative new therapeutic modalities for ARCI1 patients. One recent example for this attempt is the development of the enzyme-replacement therapy for individuals suffering from TGM1-deficient ARCI1.[16] It has been used successfully in a mouse model for the restoration of the TGM1 activity and for the rearrangement of the epidermal integrity and thus barrier function.[16] This approach is a promising putative modality for the treatment of this extremely stigmatizing disease and hopefully more effective than the current symptomatic therapies available for ARCI1 patients.

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