A REPORT OF TWO CASES OF TGM1 MUTATIONS IN IRANIAN PATIENTS WITH LAMELLAR ICHTHYOSIS

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Abstract

Objective

Autosomal Recessive Congenital Ichthyosis (ARCI) is a rare, heterogenous keratinization disorder of the skin, classically divided into two clinical subtypes, Lamellar Ichthyosis (LI) and Nonbullous Congenital Ichthyosi-formis Erythroderma (NCIE). Lamellar Ichtyosis is caused by mutations in the TGM1 gene that encodes transglutaminase 1 enzyme, which is critical for the assembly of the cornified cell envelope in terminally differentiating keratinocytes. TGM1 is a complex enzyme existing as both cytosolic and membrane-bound forms. Moreover, TGM1 is proteolytically processed, and the major functionally active form consists of a membrane-bound 67/33/10-kDa complex with a myristoylated and palmitoylated amino-terminal 10-kDa membrane anchorage fragment. In this study, all 14 coding exons of TGM1 gene were investigated using PCR-sequencing method in three Iranian patients with different phenotypes which are often caused by homozygote or compound heterozygote mutations and a homozygote mutation (G218S) in exon 4 and three heterozygote mutations (R37K, D58N, D86N) in exon 2 were observed. The mutation (D86N) was seen in two patients simultaneously.

Key words: TGM1gene, mutation, ARCI, lamellar, ichthyosis, sequencing.

Introduction

Autosomal Recessive Congenital Ichthyosis (ARCI) is characterized by epidermal hyperkeratosis with widespread scaling and a variable degree of erythema. In contrast, the more common types of non-bullous ichthyosis, autosomal-dominant ichthyosis vulgaris and X-linked recessive ichthyosis are clinically less severe and hardly ever present at birth. Although most neonates with autosomal recessive congenital ichthyosis (ARCI) are collodion babies, the clinical presentation and severity of ARCI may vary significantly. LI and NCIE are seemingly distinct phenotypes: classic severe lamellar ichthyosis (LI) with dark brown, plate-like scaling without erythroderma and NCIE with finer whiter scaling and underlying generalized redness of the skin. Patients with LI are typically born encased in a translucent collodion membrane, which
is replaced over the first month of life with generalized scaling that is accentuated in flexural areas as well as on the forehead and lower extremities. Eclabium, ectropion, and scarring alopecia at the periphery of the scalp are often observed as a sequelae of excessively taut skin, and heat intolerance frequently occurs due to obstruction of the sweat ducts by plates of scale. Patients may also develop palmoplantar keratoderma,

pseudoainhum, and nail dystrophy. Lamellar Ichthyosis (LI) is a genetically heterogeneous group of disorders of keratinization that are inherited in an autosomal recessive fashion. LI has an equal incidence in male and female individuals and is estimated to occur in approximately 1 in 150.000-300.000 live births.

Mutations occur in the transglutaminase-1 (TGM1) gene on chromosome 14q11 which has 15 exons with exon 1 being non-coding. TGM1 is a member of a class of enzymes that form Ne-lysine or mono- or bis-spermidine isopeptide bond cross-links between proteins, thereby forming stable, insoluble macromolecular assemblies accounting for approximately half of the cases of LI and a minority of cases of NCIE. The TGM1 mutations are heterogeneous (including point mutations, deletions, truncations, and splice-site mutations), and consistent genotype-phenotype correlations have not been observed. The protein product of the TGM1 gene has 817 amino acid residues with a molecular weight of 89.3 KD. Lack of transglutaminase 1 activity impairs cross-linking of proteins and lipids in the cornified cell envelope of the upper epidermis and leads to defective cornification and desquamation.

Material and Mthods

Patients' Phenotype:

One male and two female patients were studied for TGM1 gene mutations. The male patient had very mild signs of LI but the female patients had more significant signs. The patients were born in a translucent collodion membrane with generalized scaling. Also, eclabium and ectropion were observed as a result of taut skin.

DNA Extraction:

Blood samples of the patients were collected in EDTA contained blood tubes. DNA was extracted using GPP genomic DNA extraction kit from whole blood (Gen Pajoohan Pouya, Tehran, Iran). Extracted DNA was checked for quality by running on agarose 1%, staining with ethidium bromide and using U.V transilluminator.

Mutation Detection:

Specific primers for all 14 coding exons of TGM1 gene were designed with primer blast software in NCBI (Table1). Designed primers were synthesized by 1st base company (Singapore). Synthesized primers were diluted to 10 µM before use in PCR tests. PCR tests were carried out by MWG-BIOTECH thermocycler under this program: denaturation in 95°c for 3 min and 35 cycles for denaturation in 95°c for 55 sec, annealing in 59°c for 55 sec and extension in 72°c for 55 sec; final extension was done in 72°c for 8 min. PCR components for 25 reactions were H2O:18µl, 10X PCR buffer:2.5µl, MgCl2(50mM):0.7µl, dNTP(10mM):0.5µl, Primer(10µM): 0.8µl (for each forward and reverse), DNA:200ng and Taq DNA pol:0.2µl (5U/µl). After amplification, electrophoresis was done on 1.5% agarose gel for 40 minutes by an electrical power supplier. Then, the gel was stained with ethidium bromide and was seen by U.V transilluminator. The PCR products that had a good quality were sent to sequencing. The size of PCR products is shown in Table1.

Sequencing:

Because sequencing is the best method to analyze sequences, all PCR products were sent for sequencing after amplifying and electrophoresis. Sequencing was done by First Base laboratories (Malaysia) using ABI (3730). The sequencing results were analyzed by Finch TV software and variations were studied with NCBI BLAST database.

Results

After analysis according to NCBI database, these results were observed: one homozygote mutation (G218S) (Fig.1) in exon 4 and a heterozygote mutation (D86N) (Fig.2) in exon 2 in one of the female patients (Patient 1) and three heterozygote mutations (R37K) (Fig.3), (D58N) (Fig.4) and (D86N) (Fig.2) in the other female patient (Patient 2). The D86N mutation that changes GAC=>AAC was seen in both female patients (Patients 1 & 2) simultaneously who had no family relationships. No mutation in TGM1 gene was seen in the male patient. The mutations are under lined in the figures.

Discussion

Previously, missense mutations in the ABCA12 gene on chromosome 2q35 have been found to account for the LI phenotype in nine families from Northwest Africa. Large deletions in ABCA12 were recently shown to

cause Harlequin Ichthyosis (HI), a severe congenital skin disease that is usually lethal. ABCA12 encodes a transmembrane transporter in the ATP-binding cassette family that is thought to play a critical role in keratinocyte lipid trafficking and the formation of lamellar granules, which would explain the barrier defects observed in both LI and HI. Mutations in the genes encoding ichthyin, lipoxygenase 3 (ALOXE3), and 12 (R)-lipoxygenase (ALOX12B) have also been shown to underlie both NBCIE and LI phenotypes. According to the above-mentioned studies, Lamellar Ichtyosis is not restricted to mutations that directly affect TGM1 gene. Based on the studies, TGM1 mutations are majorly responsible for Lamellar Ichtyosis but there are other genes that can cause LI. In this study, we evaluated three Iranian patients that were susceptible to LI. We used PCR-sequencing method that is a very good method with high accuracy to analyze the whole sequence of the target gene. After analyzing of all 14 coding exons of TGM1 gene, we only found one homozygote mutation (G218S) in one of the patients while other patients did not show any homozygote mutations. Recently, some compound heterozygote states are reported that cause Lamellar Ichthyosis. Based on these observations, a patient that has 3 different heterozygotes mutations in different locus is recognized to have a compound heterozygote pattern. Regarding our male patient, no mutation was seen in the TGM1 gene coding region; therefore it can be concluded that other genes may cause the LI phenotype in the male patient.

Table1: Primer sequences of human TGM1 gene

Exon	Primer Sequence 5`=>3`		Product Size (bp)
2-3	F: TCAACTGGCTGGGACTACCT	R:CACCAAACATAGGGCCTTCA	759
4-5	F:CTCCATCCCCTCTCCTCAG	R:CCAGCTCCTCTGGGTGTATG	860
6-7	F:GTGCTGGCCTAGGGTTCAG	R:CCTGGCTTTCCTCCCTTC	851
8-9	F:GACACGATGCCTCACTTGC	R:CTGTGTTAATCAGGTGGGGG	512
10-11	F:CCTCTCCGCCTTCTCAGAT	R:TTGGCAGGAACACTTGTTGT	818
12-13	F:GGAATTGGAACCTCACCCTT	R:CTGGCCTTCACTCTCTGACC	676
14	F:CTCTGGTGCAGTGTACGGTC	R:GGGAAGGCCAGAGTGGAA	259
15	F:ACTCCACCCCCAATTACTCC	R:CTCCCCACCTGAGCTCCT	378

TGM1 gene has 14 coding exons that designed primers (F:Forward and R:Reverse) and PCR products size are as Table1. Exon 1 of TGM1 gene is not coding exon.

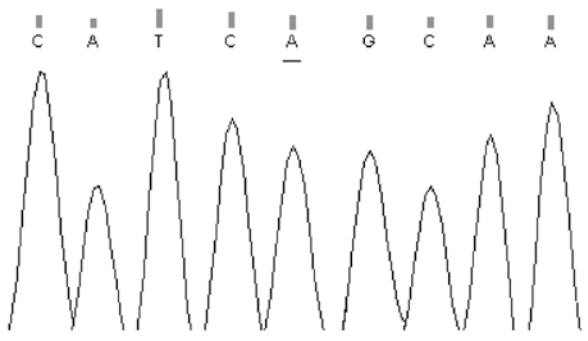

Fig1. Shows the sequence for the homozygote mutation G218S that changes GGC=>AGC in exon 4 of TGM1 gene.

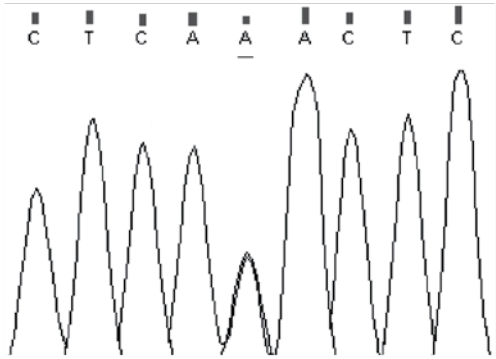

Fig2. Shows the sequence for the heterozygote mutation D86N that changes GAC=>AAC in exon 2 of TGM1 gene.

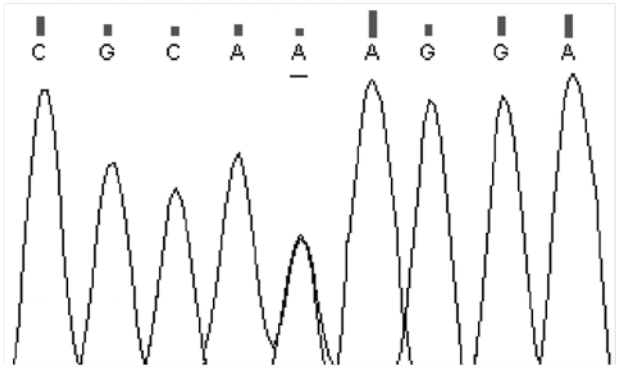

Fig3. Shows the sequence for the heterozygote mutation R37K that changes AGA=>AAA in exon 2 of TGM1 gene.

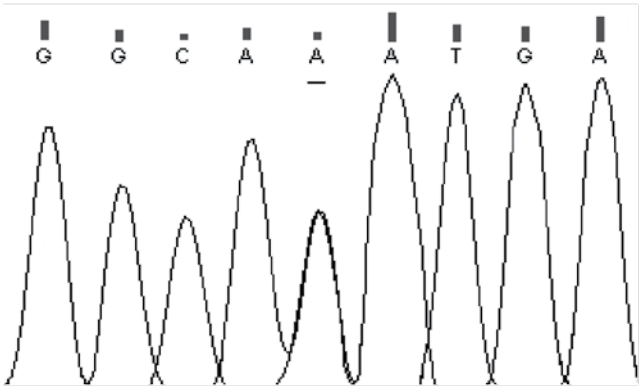

Fig4. Shows the sequence for the heterozygote mutation D58N that changes GAT=>AAT in exon 2 of TGM1 gene.

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