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A Novel Transglutaminase-1 Missense Mutation in a Palestinian Family with Autosomal Recessive Congenital Ichthyosis: A Case Report

Mohammed J. Ashour[1], Shadi F. Al-Ashi[1] and Fadel A. Sharif[1*]

[1]Department of Medical Laboratory Sciences, Islamic University, Gaza, Palestine.

Authors' contributions

The authors contributed equally to this work.

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Case Study

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ABSTRACT

This work presents the molecular genetics investigation of a male neonate referred to our genetics laboratory with the diagnosis of classical lamellar ichthyosis (one form of autosomal recessive congenital ichthyosis). The neonate was born as a "collodion-baby" and he is the product of a maternal first cousin marriage. DNA sequencing of the coding exons of transglutiminase-1 (TGM1) gene revealed a novel missense (c.A1621C) mutation in exon 11. The mutation altered codon 541 from ACC into CCC thus changing the amino acid threonine into proline (p.T541P) and was predicted to be pathogenic. The presence of the mutation in both parents in heterozygous form and in the patient in homozygous form was further confirmed by PCR-restriction fragment length polymorphism (PCR-RFLP) designed specifically for the identified mutation. It is concluded that the T541P mutation is the cause of the congenital ichthyosis in the presented case and the parents were advised to undergo a PGD-IVF for embryo selection prior to their next pregnancy.

Keywords: Autosomal recessive congenital ichthyosis; TGM1gene; T541P; novel mutation.

**Corresponding author: Email: fsharif@iugaza.edu.ps;*

1. INTRODUCTION

Autosomal recessive congenital ichthyosis-1 "ARCI-1" (OMIM: 242300) is a rare panethnic keratinization disorder with an estimated incidence of about 1 per 200,000. Individuals with ARCI-1 (lamellar ichthyosis) present with severe and generalized ichthyosis and they may have ectropion, eclabium, scarring alopecia and palmoplantar keratoderma [1-3].

Mutations in *TGM1* gene (GenBank NM_000359.2) account for 90% or more of ARCI-1. The gene is located on chromosome 14q11.2 and consists of 15 exons. TGM1 encodes 817 amino acid enzyme called transglutaminase-1"TGase-1", one of the enzymes responsible for cross-linking epidermal proteins during formation of the stratum corneum [4,5]. *TGM1* is characterized by a wide spectrum of mutations with over 130 unique mutations affecting all the 14 coding exons (Exon 2 through 14) of the gene [6-10].

Here we report for the first time in our area the identification of a novel *TGM1* mutation in a Palestinian patient with classic lamellar ichthyosis phenotype.

2. CASE REPORT

A two-weeks "collodion baby" male born to maternal first cousin consanguineous parents at 39 weeks gestation presented to the Genetics lab at the Islamic University of Gaza for genetics study. The coding region (exons 2 through 14) of *TGM1* was amplified from patient genomic DNA by PCR using the primers described previously [8]. Direct sequencing of the patient's PCR products revealed that the patient had a missense p.T541P (c.A1621C) mutation in homozygous form in exon 11 Fig. 1, a novel mutation that has not been reported before. The presence of the mutation in heterozygous form in the parents was also confirmed by direct sequencing.

The identified mutation effect was evaluated *in silico* using Sorting Intolerant FROM Tolerant (SIFT) (http://sift.jcvi.org/www/SIFT_BLink_submit.html) [11] and PolyPhen: prediction of functional effect of human nsSNPs; (http://genetics.bwh.harvard.edu/pph) [12] programs. The threonine residue mutated into proline is located in a motif of the catalytic core domain [9] of the enzyme and both programs predicted that the mutation is functionally damaging. This threonine residue was confirmed to be highly conserved across many primate and non-primate species, a sample of which is illustrated in Fig. 2.

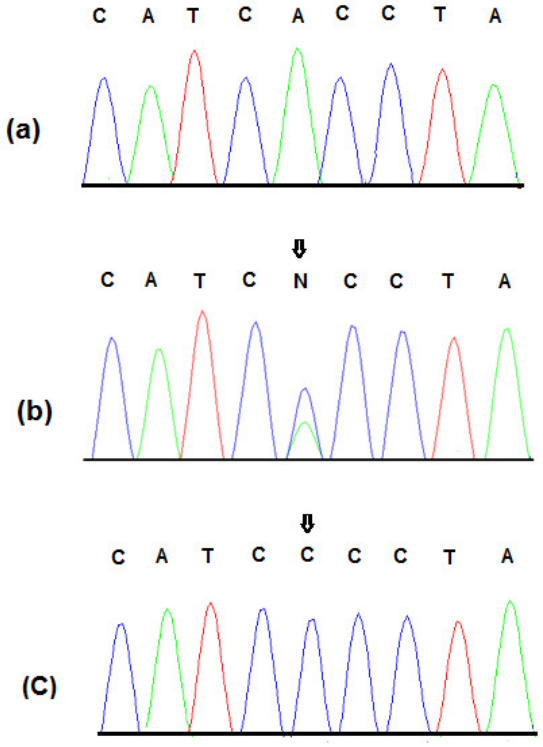

Fig. 1. The detected TGM1 exon 11 missense mutation is illustrated on the partial chromatograms above, (a) the normal sequence from a healthy individual (b) one of the heterozygous parents (c) the homozygous c.A1621C mutation. The arrow indicates the mutation site

The c.A1621C mutation thus identified abolishes the cutting site of *HphI* restriction enzyme therefore, the following PCR primers (TGMF: AGTGACAAGGTGTACTGGCA and TGMR: GAGG TTCCAATTCCCACGTG) were designed to amplify a 238bp fragment encompassing the mutation. *HphI* digests the normal allele into two fragments (124 and 114 bp fragments) whereas the mutant allele remains uncut. Fig. 3 shows a photo of an agarose gel where the patient and his parents' genotypes are indicated. The parents' genotypes confirm their heterozygosity for the mutation (as illustrated by having 3 fragments).

Human	GTLIVTKAISSNMREDITYLYKHPEGSDAERKAVETAAAHGSKPNVYANRG
Chimpanzee	GTLIVTKAISSNMREDI**T**YLYKHPEGSDAERKAVETAAAHGSKPNVYANRG
Macaca	GTLIVTKAI-SNMREDI**T**YLYKHPEGSDAERKAVETAAAHGSKPNVYANRG
Guinea pig	GTLIVTKAISSNMREDI**T**++YKHPEGS+AERKAVETAAAHGSKPNVYA-R-
Mouse	GTLIVTKAI-SN-REDI**T**++YKHPEGS+AER+AVE-AAAHGSKPNVYA-R-
Dog	GTLIVTKA+-SNM++D+**T**++YKHPEGS+AERKAVETAAAHGSKPNVY-NR-
Rat	GTLIVTKAI+SNMREDI**T**++YKHPEGS+AERKAVE-AAAHGSKPNVYA-R-

Fig. 2. TGase-1 partial amino acid sequence (residues 524 to 574) alignments from a motif of the catalytic core domain, Comparisons include human, common chimpanzee, macaca, guine pig, mouse, dog and rat. Shaded amino acid (T) is the amino acid altered by the mutation, The amino acid sequence alignment was obtained by using the NCBI protein Blast analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

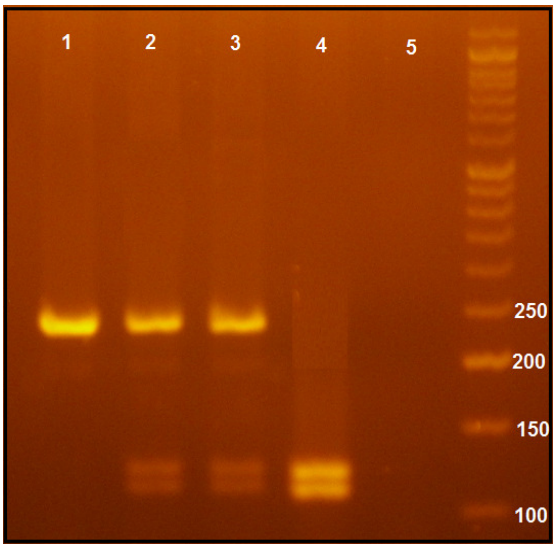

Fig. 3. A photo of ethidium bromide stained gel for PCR-RFLP results of the patient (lane 1), his mother and father (lanes 2 and 3, resepcetively), one homozygous normal control subject (lane 4) and a negative (no genomic DNA) control (lane 5), The size in base pairs is shown on some of the ladder bands on the last lane

3. DISCUSSION

Classic lamellar itchthyosis (ARCI-1) is a rare disorder that is present worldwide [7]. Deleterious mutations in *TGM1* gene have been shown to be responsible for most of the cases reported so far [8]. The case presented here is the first genetically characterized one in Gaza-Palestine. The novel mutation identified here is located in one of the exons coding for part of the catalytic core domain of transglutaminase-1 enzyme. Most of the previously reported mutations also affect this domain of the enzyme [13].

The A to C transversion at codon 541 detected in our patient is expected to lead to an amino acid change from threonine to proline.

Several lines of evidence suggest the this mutation is pathogenic: (1) The mutation is present in homozygous form in the patient, in heterozygous form in the proband parents and absent in healthy control subjects Figs. 1 and 3, (2) Amino acid sequence alignment Fig. 2 showed that the mutated threonine residue is highly conserved across distant species i.e., it is important for the enzyme activity and, (3) The altered amino acid is located in a critical region of the enzyme (the catalytic core domain) and *in silico* analysis using SIFT and PolyPhen programs predicted that the amino acid change is damaging for the enzyme function.

Additionally, threonine and proline have quite different characteristics: threonine is non-cyclic and polar, proline is cyclic and hydrophobic therefore, the two amino acids serve entirely different structural roles during polypeptide folding. While threonine fits into regular secondary structures (particularly β-strand conformation) proline behaves as a structural disruptor in the middle of regular secondary structural elements. Hence it is believed that proline disrupts the proper folding of TGase-1 enzyme and its catalytic function.

4. CONCLUSION

The ARCI-1 in the present case is attributed to a novel p.T541P mutation (RCV accession: RCV000114750) in the catalytic core domain of

the TGase-1 enzyme. This finding expands the mutations spectrum of TGM-1 gene and is important for delivering proper counseling and diagnosis opportunities for the proband's family.

CONSENT

The objective of the study was fully explained to the parents and their written informed consent was taken to carry out the investigation. Moreover, the authors declare that 'written informed consent was obtained from the patient's parents for publication of this case report.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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