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Case Report

Triallelic Inheritance of TGM1 and ALOXE3 Mutations Associated with Severe Phenotype of Ichtyosis in an Iranian Family - A Case Report

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Abstract

Lamellar ichthyosis is one form of congenital autosomal recessive ichthyosis. To date, seven causative genes for ARCI have been identified. To understand further the genetic spectrum of the disease, we analyzed a four-generation Iranian family with ARCI that had observable inheritance. Exome sequencing data for one of the affected individuals with ichthyosis from a consanguineous Iranian family was analyzed. Potential candidate mutations were analyzed in additional family members to determine if the putative mutation segregated with disease status. A novel homozygous mutation (p.D414V) in TGM1 and rs3027232 in ALOXE3 gene in heterozygous form were identified which segregated with disease status in the family. Bioinformatic studies with Polyphen-2 and SIFT showed that these variants are damaging. We identified a possible triallelic inheritance in this study. Moreover, this paper illustrates how advances in genome sequencing technologies could be utilized to rapidly elucidate the molecular basis of inherited skin diseases which can be caused by mutations in multiple disease genes.

Keywords: ARCI, Lamellar ichthyosis, TGM1, ALOXE3, Iran

Introduction

Mutation of key genes involved in different phases of keratinocyte differentiation and thereinafter cornification, process leads to congenital skin disorders such as ichthyosis. Autosomal recessive form of ichthyosis is a rare heterogeneous genetic disease.

Up to now, seven causative genes for autosomal recessive congenital ichthyosis (ARCI) have been identified, including TGM1, ABCA12, ALOX12B, ALOXE3, NIPAL4, CYP4F22, and PNPLA (1). About half of ARCI cases show complete or partial transglutaminase 1 (TGase1) deficiency due to germline mutations in the TGM1 gene (2).

The transglutaminase 1 is a member of a class of enzymes that form Ne-(g-glutamyl) lysine or mono- or bis (g-glutamyl) spermidineiso peptide bond cross-links between proteins, and is a calcium-dependent enzyme (3). To date, more than 130 disease causing mutations associated with divers forms of LI/CI have been reported in different populations (1).

Several kinds of mutations have been identified in TGM1 gene but single base changes and INDELs are the most frequent and the rarest, respectively. Nevertheless, severe phenotypes are more frequently caused by truncating mutations (4).

The ALOXE3 encodes the epidermal LOXs eLOX-3 and is involved in advanced stages of epidermal differentiation through its role in the processing of lamellar bodies. This enzyme partici-

pates in the hepoxilin pathway by converting 12R-hydroxyeicosatetraenoic acid in to an epoxyalcohol isomer. Apparently, keratinocytes differentiation is induced by products of this pathway (5). Approximately 10 mutations have been reported in ALOXE3 up to now which are responsible for 17% of ARCIs (6).

Case Report

Family description

This study was conducted in 2015 in Tehran Medical Genetics Laboratory, Tehran, Iran. The family has two female patients affected with ARCI. The pedigree, including all family members is depicted in Fig. 1A. The proband is a 28 years old pregnant woman. She is married to her cousin. She has two nieces whom both are affected with ichthyosis from consanguineous parents.

TheIV-3individualisa9years old girl and the IV-4 individual is 12 yr old girl who both is affected withLI. They both have scarring alopecia, brownish lamellar desquamation and plantar hyperkeratosis (Fig.1B).

This study was approved by Tehran Medical Genetics Laboratory Ethical Committee. Written informed consent was obtained from all participants.

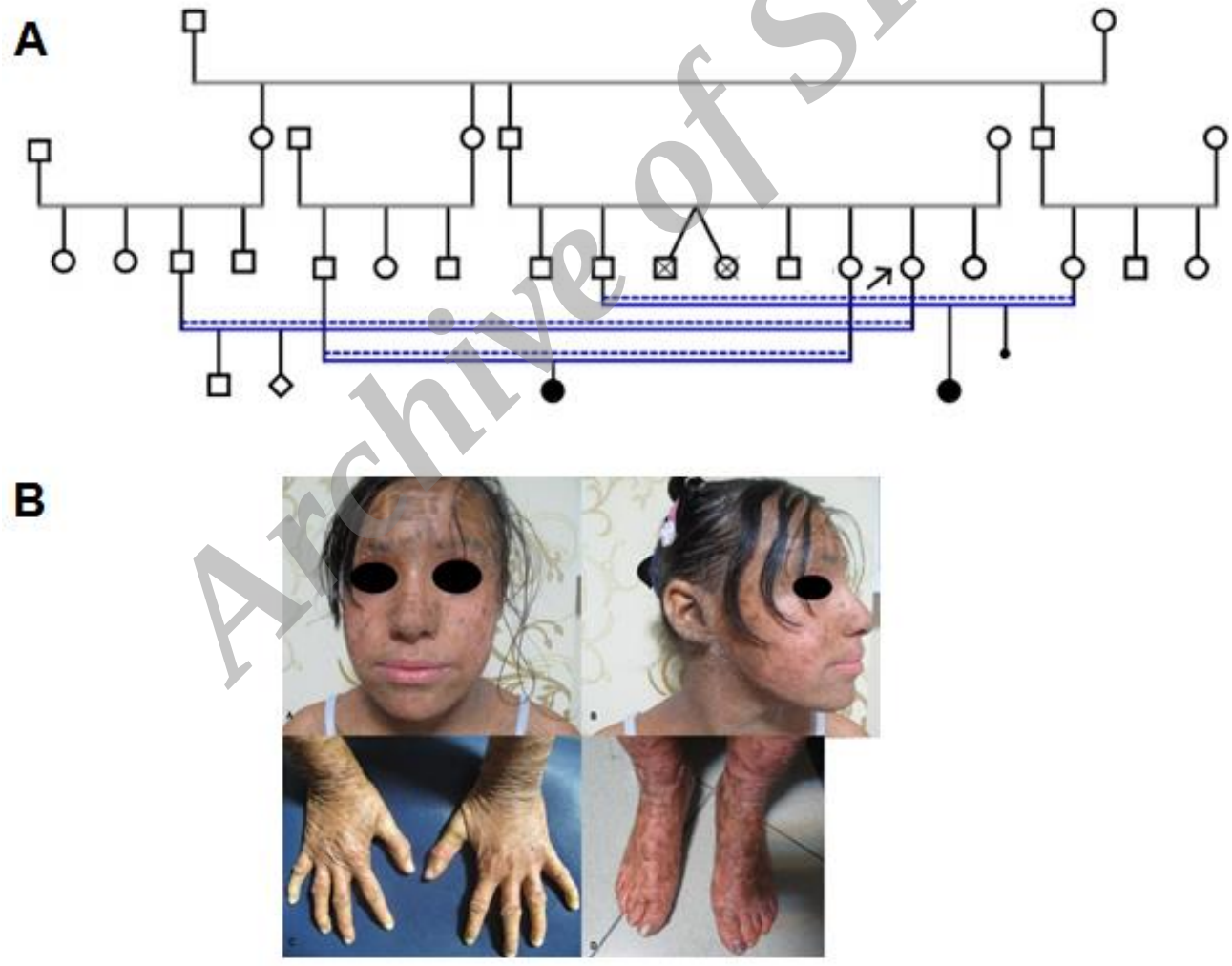

Fig. 1: A: family pedigree B: affected individual IV-4 with scarring alopecia and brownish lamellar desquamation

Sample preparation

Peripheral blood sampling was performed from two affected, their parents and the proband. DNA was extracted using the conventional salting-out method.

Whole exome sequencing

Whole exome sequencing of the subject IV-3 was performed at BGI China. Briefly, 3 µg of genomic DNA was used to perform exome capture with Agilent SureSelect kit following the manufacturer's instructions. Paired-end sequencing of100bp was performed on IlluminaHiSeq2000 platform.

Bioinformatic analysis

Sequencing reads were aligned to hg19 reference genome using the BWA software (7). Variant discovery and genotype calling of multi-allelic substitutions, insertions and deletions was performed on tested individual globally using the Unified Genotyper module from Genome Analysis Toolkit (GATK) (8) with the minimum call quality parameter set to 30. SAMtools and Annovar were used to call and annotate variants (9, 10). Variants with an allele frequency higher than 5% in the 1000 genomes database (http://www.1000genomes.org), higher than 5% in the NHLBI exomes (Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, WA; URL: http://evs.gs.washington.edu/EVS/, v.0.0.14, June 20, 2012) were filtered out. We applied a recessive genetic model which required the variant to be present in tested individual in homozygous form. We used computational tools including PolyPhen-2, SIFT andMutationTaster to predict the potential impact of sequence variants on protein function. Wealso obtained conservation scores using GERP and PhastCons to predict mutation impact based on evolutionary constraint analyses.

Validation of mutations and Segregation analysis

To validate the causal mutations and segregation of the mutations with the disease in the family, direct Sanger sequencing was performed in family members (affected and unaffected) from whom DNA was available. Primers were designed using GENERUNR v3.4.0.0 to surround the candidate mutation and the amplified targets (forward and reverse) were sequenced by standard Sanger's sequencing technique using BigDye® Terminator (Invitrogen, ABI, Foster City, CA). Primer sequences are available upon request.

Discussion

In this family with LI we identified novel missense homozygous mutation p.D414V in TGM1 gene. Direct sequencing confirmed that the patient had the homozygous TGM1 mutation and her affected cousin and her parents had homozygous and heterozygous status, respectively (Fig. 2).

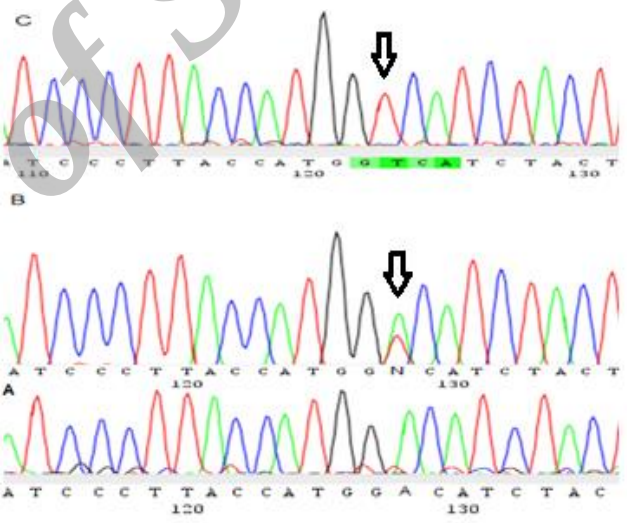

Fig. 2: A: Sanger sequencing of normal homozygous proband for TGM1 mutation B: Sanger sequencing of heterozygous parents for TGM1 mutation C: Sanger sequencing of homozygous affected individuals for TGM1 mutation

Bioinformatic studies with Polyphen-2 and SIFT showed that this variant is damaging. This mutation has been located in catalytic core domain of this gene where the majority of causative previously reported TGM1 mutations harbored. The proband was tested for the mutation but she was not a carrier.

In addition to TGM1 homozygous mutation, a number of variants in other genes involved in ARCI were identified which are as follows: rs7560008 in ABCA12 gene, rs6860507 in NIPAL4 gene and rs3027232 in ALOXE3 gene. The first two SNPs were homozygous and are already identified as benign variants, whereas rs3027232 in ALOXE3 gene was heterozygous and has been rated as damaging by SIFT.

As mentioned before, severe forms of the disease are frequently associated with truncating mutations. Moreover previously reported missense mutations of TGM1 gene are mostly substitution of Arg residues, whereas the detected mutation for our family is a novel missense one with the substitution of Asp residue. Therefore, we postulate that severe phenotype in our patients is due to the presence of mutation in ALOXE3, simultaneously, suggesting triallelic inheritance phenomenon.

Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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