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Novel mutations in the genes *TGM1* and *ALOXE3* underlying autosomal recessive congenital ichthyosis

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Abstract

Background—Ichthyoses are clinically characterized by scaling or hyperkeratosis of the skin or both. It can be an isolated condition limited to the skin or appear secondarily with involvement of other cutaneous or systemic abnormalities.

Methods—The present study investigated clinical and molecular characterization of three consanguineous families (A, B, C) segregating two different forms of autosomal recessive congenital ichthyosis (ARCI). Linkage in three consanguineous families (A, B, C) segregating two different forms of ARCI was searched by typing microsatellite and single nucleotide polymorphism marker analysis. Sequencing of the two genes *TGM1* and *ALOXE3* was performed by the dideoxy chain termination method.

Results—Genome-wide linkage analysis established linkage in family A to *TGM1* gene on chromosome 14q11 and in families B and C to *ALOXE3* gene on chromosome 17p13. Subsequently, sequencing of these genes using samples from affected family members led to the identification of three novel mutations: a missense variant p.Trp455Arg in *TGM1* (family A); a nonsense variant p.Arg140* in *ALOXE3* (family B); and a complex rearrangement in *ALOXE3* (family C).

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Conflicts of interest: None.

Conclusion—The present study further extends the spectrum of mutations in the two genes involved in causing ARCI. Characterizing the clinical spectrum resulting from mutations in the *TGM1* and *ALOXE3* genes will improve diagnosis and may direct clinical care of the family members.

Introduction

Autosomal recessive congenital ichthyosis (ARCI) is a rare, heterogeneous keratinization disorder of the skin. Classically, it is divided into lamellar ichthyosis (LI), congenital ichthyosiform erythroderma (CIE), and harlequin ichthyosis (HI).[1] Patients with LI are often born encased in collodion membrane that later changes into large, dark-brown plate-like scales.[2] Keratoderma is often also found on the palms and soles of patients affected with LI. Patients with CIE show variable erythroderma and generalized fine white scaling; additionally they may be born as collodion babies.[3–5] Newborns with HI often show large, thick, plate-like scales with pronounced ectropion/eclabium, which is the most severe and lethal form among congenital ichthyoses.[6] To date, mutations in nine genes have been implicated in ARCI; including five LI associated genes; *TGM1* (MIM 242300), *CYP4F22* (MIM 604777), *NIPAL4* (MIM 612281), *LIPN* (MIM 613924), and *PNPLA1* (MIM 612121); three CIE associated genes; *ALOX12B* (MIM 603741), *ALOXE3* (MIM 607206), and *CERS3* (MIM 615276); and a single HI associated gene; *ABCA12* (MIM 601277).[7–17] However, few studies also reported *TGM1* mutations in patients with CIE.[18,19]

In the present study, we have investigated three unrelated consanguineous Pakistani families segregating LI and CIE phenotypes. Genotyping and DNA sequencing was used to identify sequence variants in the two genes (*TGM1*, *ALOXE3*) in these families.

Materials and methods

Subjects

For the study presented here, three consanguineous families (A, B, C), segregating LI and CIE phenotypes, were located in remote regions of Pakistan (Fig. 1). Approval of the study was obtained from the Institutional Review Board of Quaid-i-Azam University, Islamabad, Pakistan, and Baylor College of Medicine and Affiliated Hospitals, Houston, TX, USA. Informed consent was obtained from all family members who participated in the study. Pedigree drawings of the families were based upon detailed question/answer sessions conducted with elders of the families.

Peripheral blood samples were collected from 18 pedigree members including two affected (IV-1, IV-2) and three unaffected (III-4, III-5, IV-3) individuals from family A two affected (VI-1, VI-3) and two unaffected (V-3, V-4) from family B, and six affected (III-1, III-2, IV-2, IV-4, IV-5, IV-7) and three unaffected (IV-1, IV-3, IV-6) members from family C (Fig. 1). Genomic DNA was extracted from peripheral blood samples using the GenElute™ blood genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). DNA was quantified by Nanodrop-1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA) measuring its optical density at 260 nm and diluted to 40–50 ng/μL for amplification by polymerase chain reaction (PCR).

Genotyping and mutation analysis

Based upon the clinical features observed in the affected members and autosomal recessive mode of inheritance, homozygosity mapping in two families (A, B) was performed by genotyping several microsatellite markers flanking known ARCI genes. PCR amplification of microsatellite markers[20] was performed by following standard procedures in a total volume of 25 μL. The amplified products were resolved on 8% non-denaturing polyacrylamide gel, stained with ethidium bromide, and genotypes were assigned by visual inspection.

DNA samples from available members of family C were submitted to the University of Washington Center for Mendelian Genomics to perform genome scan using Infinium® Human core exome chip (Illumina, San Diego, CA, USA), which consists of more than 500,000 single nucleotide polymorphism (SNP) markers. The genotype data were analyzed by homozygosity mapper to find common homozygous by descent regions,[21] and linkage analysis was performed using Superlink.[22] Analyses were performed by assuming a disease frequency 0.001 and recessive mode of inheritance. Allele frequencies for SNP markers were estimated using founders and reconstructed founders from additional Pakistani families genotyped along with family C. The genetic map positions were based on the Rutgers combined linkage-physical maps (build 37).[20]

For mutation identification, *TGM1* (NM_000359) and *ALOXE3* (NM_021628) genes were sequenced using Big Dye Terminator v3.1 Cycle Sequencing Kit on ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA, USA). Bioedit sequence alignment tool (editor version 6.0.7; Ibis Biosciences Inc., Carlsbad, CA, USA) was used to align the sequence of each amplicon with reference sequence of both genes.

Deletion breakpoint mapping

In family C, *ALOXE3* deletion breakpoint was identified using a PCR-based assay consisting of eight overlapping set of primers specifically designed to cover both sides of a putatively deleted genomic region. After several PCR reactions, a primer pair (5′-TGCTTGAACCCAGGAAGTG-3′; 5′-TCTTCCACACCCGTCACTTA-3′) was selected to amplify and sequence the deletion breakpoint. To determine the deletion coordinates, sequence data was mapped against reference human genome using BLAT tool from UCSC genome browser.[23]

Results

Clinical features

Affected individuals of the three families (A, B, C) were clinically investigated by dermatologists at the local government hospitals. In family A, the entire body surface of the affected members was covered with thick, large, dark-brown scales (Fig. 2a,b). Palms and soles showed severe keratoderma. Hairs were sparse and dry, and eyebrows were scanty. Ectropion, eclabium, and reduced sweating ability (hypohidrosis) were observed in the affected members. In family B, affected members displayed finer scales on arms, legs, and abdomen (Fig. 2c,d). In family C, affected individuals exhibited slightly thick dark-brown

scales all over the body (Fig. 2e,f). No signs of ectropion, eclabium, or alopecia were observed in the affected individuals of family B and C. They had problems of minor sweating, severe heat intolerance, and bleeding from scaling skin, which occurs mostly in severe cold conditions. The clinical presentation of family A is compatible to LI, whereas milder phenotype of patients from family B and C is suggestive of CIE.

Affected members in all the three families were of normal height, growth, and mental health. Association of the phenotype with other ectodermal appendages such as nail and sebaceous glands was not observed in any of the affected members. Heterozygous carrier individuals had normal skin and were clinically indistinguishable from unaffected individuals of the respective families who are homozygous wild type.

Genotyping and mutation analysis

Homozygosity mapping in two families (A and B) was performed by using microsatellite markers flanking genes *ABCA12* (2q34–q35), *NIPAL4* (5q13), *TGM1* (14q11), *ALOX12B* (17p13), *ALOXE3* (17p13), and *CYP4F22* (19p12–q12). Haplotype analysis showed mapping of family A to *TGM1*and family B to*ALOXE3* gene. In family C, data analysis with Homozygosity mapper[21] indicated a 2.16 Mb homozygous region on chromosomes 17, which was delineated by markers rs14309 (6.91 Mb) and rs9906162 (9.08 Mb). The 2.16 Mb region of homozygosity was shared by all the affected individuals of family C and contains two known ARCI genes, *ALOX12B* and *ALOXE3*. Two-point linkage analyses yielded LOD score of 3.6 for several markers within the region of homozygosity. A maximum multipoint LOD score of 5.1 was obtained for the region containing the *ALOXE3* gene.

In family A, sequence analysis of exon 9 of the *TGM1* gene revealed a novel homozygous missense mutation involving T to C transition at nucleotide position 1363 (c.1363T > C). This resulted in substitution of a codon for tryptophan at amino acid position 455 with arginine (p.Trp455Arg) (Fig. 3a). The missense mutation created a restriction site for the enzyme Ssi1 (Aci1) in exon 9 of the *TGM1* gene. This was verified by Ssi1 (Aci1) restriction analysis of 448 bp PCR product encompassing exon 9 of *TGM1* gene. Restriction enzyme analysis of PCR-amplified products produced two DNA fragments of 300 and 148 bp in affected members (IV-1, IV-2), three fragments of 448, 300, and 148 bp in carriers (III-4, III-5), and a single fragment of 448 bp in an unaffected (IV-3) member (Fig. 3b).

In family B, the sequence analysis of exon 4 of the *ALOXE3* gene revealed a novel homozygous nonsense mutation involving a C to T transition at nucleotide position 418 (c. 418C > T). This resulted in substitution of a codon for arginine at amino acid position 140 with a stop codon (p.Arg140*) (Fig. 3c).

In family C, a careful analysis of genotypes revealed missing data in all affected individuals for six consecutive SNP markers, from rs147383866 to rs3027232, within the mapped homozygous region. This corresponds to a large genomic region and is flanked by intact SNP markers rs3027213 (8.01 Mb) and rs1442849 (8.02 Mb) (Fig. 3d). To determine the exact breakpoint of the underlying deletion, long range DNA polymerase (Clontech Laboratories, Mountain View, CA, USA) was used to obtain 832 and 6130 bp products from

DNA samples of affected and normal individuals, respectively (Fig. 3e). Sequencing analysis of the amplified PCR product revealed a complex rearrangement involving a 5299 bp deletion and a single nucleotide insertion (Fig. 3d). BLAT search of the sequence obtained from an affected individual indicates that the deletion occurs between chr17:8,017,292 and 8,022,592 (hg19), spanning a region of 5299 bp (hg19:g.chr17:8017293_8022591del). All affected individuals of this family were homozygous for cytosine insertion and 5299 bp deletion (hg19: g.chr17:8017293_8022591delinsC), which spans the first six exons of the *ALOXE3* gene (Fig. 3d).

The novel sequence variants, identified in family A and B, were predicted as disease causing and damaging by mutation taster, Provean, SIFT, and polyphen-2 and segregate with disease in the respective families.[24–26] The missense mutation (p.Trp455Arg), identified in family A, nonsense mutation (p.Arg140*), identified in family B, and gross deletion, identified in family C, were not found in panels of 300, 50, and 50 unaffected unrelated ethnically matched control individuals, respectively. These variants were also not found in the public variant database dbSNP[27] and 1000 genomes,[28] but p.Arg140* exists in the heterozygous state in the Exome Variant Server.[29]

Discussion

In the present investigation, we have described three consanguineous families segregating two different forms of ARCI. Most of the features associated with LI[3,4] have been observed in affected members of family A. This included dark-brown plate-like scales on the trunk, face, scalp, and flexor areas, ectropion of eyelids, alopecia, reduced sweating ability (hypohidrosis), and erythema. However, photophobia,[30] mixed dentition,[31] and blepharitis[32] reported in a few patients of LI were not found in our patients. Affected members in the other two families, B and C, exhibited erythroderma, hypohidrosis, and severe heat intolerance. However, ichthyotic scales were fine white on the skin of the affected members of family B, and slightly thick and dark brown on those of family C.

DNA sequence analysis led to the identification of a novel missense mutation (p.Trp455Arg) in the *TGM1* gene in family A and two novel mutations (p.Arg140*, delEx1_6InsC) in the *ALOXE3* gene in family B and C.

The *TGM1* gene contains 15 exons, spanning 14.3 kb of genomic DNA on chromosome 14q11.2. The gene encodes calcium-dependent transglutaminase-1 (TGase-1) containing 817 amino acids. The TGase-1 contains N-terminal domain, a catalytic core domain and C-terminal domain, which is further divided into beta-barrel 1 and beta-barrel 2 (C-terminal end domain).[33] To date several different mutations have been reported in the *TGM1* gene, including missense, splicing, small and gross deletions, small insertions, and regulatory.[34] The tryptophan at the 455th position is conserved in all vertebrates and is located in the catalytic core domain of TGase-1 enzyme. PolyPhen-2 analysis predicted that the p.Trp454Arg mutation is damaging with a probability score of 0.998. Transglutaminase-1 is a catalytic membrane-bound enzyme that functions in the formation of the cornified cell envelope. The cornified cell envelope is made up of different proteins, the cross-linking of

which is facilitated by transglutaminase-1. The cornified cell envelope surrounds the skin cells and protects water loss and infection.[32] Most of the ARCI causative *TGM1* mutations are located in the catalytic core domain or its upstream part.[17] All such mutations result in the defective intercellular lipid layers of the stratum corneum. This leads to the defective barrier function of stratum corneum resulting in ichthyotic skin phenotypes.[35]

In the present study the other two novel mutations (p.Arg140*, delEx1_6InsC) were identified in the *ALOXE3* gene which contains 15 exons, spanning 22 kb of genomic DNA on chromosome 17p13.1. These two mutations are predicted to result in loss of function of the ALOXE3 protein either through nonsense-mediated mRNA decay (NMD) or transcription initiation failures. So far, only 13 mutations in the *ALOXE3* gene, which cause CIE, have been reported. This included eight missense, three splicing, and two small deletions.[34] The 843 amino acids lipoxygenase-3, encoded by *ALOXE3* gene, contains the PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain, the lipoxygenase homology 2 domain and a large lipoxygenase domain. The mutation (p.Arg140*) identified in family B is located in the lipoxygenase domain and could either lead to NMD or production of a truncated lipoxygenase-3 protein. This potentially shortened protein (p.Arg140*) should lack the major part of lipoxygenase domain that is required for lipid metabolism. The exome variant server[29] has reported presence of the variant p.Arg140* in the heterozygous state only in a single African-American (AA = 0; AG = 1; GG = 6502). However, the absence of ARCI phenotype in individuals heterozygous for p.Arg140* variant, in our family, clearly ruled out its pathogenicity in the heterozygous state. It is highly likely that large deletion (delEx1_6InsC), identified in the family C, would result in loss of function mutation through NMD. However, in the case of the formation of a truncated protein PLAT, lipoxygenase homology 2 and a part of lipoxygenase domain would be missing.

Lipoxygenase-3 is non-heme iron-containing dioxygenase highly expressed in a suprabasal epidermis. This enzyme is involved in lipid metabolism of lamellar granule content or intercellular lipid layer by acting as hydroperoxide isomerase (epoxyalcohol synthase) using 12R-HPETE product of *ALOX12B* into a specific epoxy alcohol product 8R-hydroxy-11R, 12R-epoxyeicosa-5Z, 9E,14Z-trienoic acid.[30,36] The pathomechanism involved behind the mutations in the *ALOXE3* gene is the disruption of the normal permeability barrier of the skin, which might be due to the abnormal lipid metabolism in the keratinocytes.[36]

In summary, we have identified three novel sequence variants, one in *TGM1* and two in *ALOXE3*, in three consanguineous families segregating LI and CIE types of ARCI. The mutations in *ALOXE3* probably represent loss of function mutations, while the other mutation in the *TGM1* may impair enzyme activity, supporting the crucial role played by these two genes during epidermal barrier formation.

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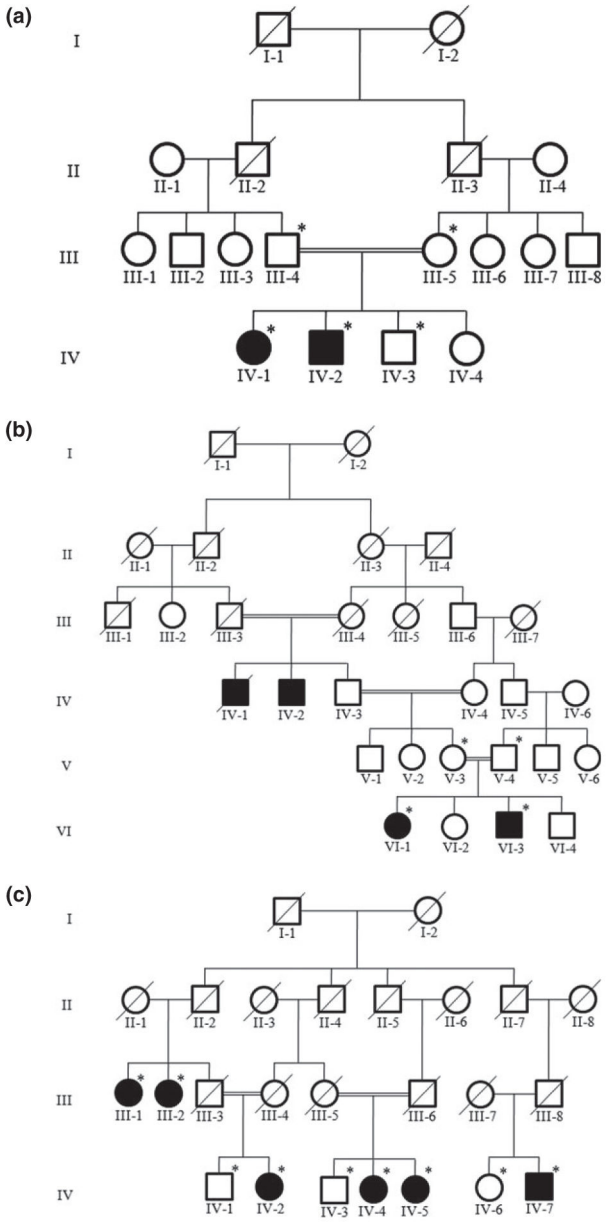

Figure 1.

Pedigree drawings of three consanguineous Pakistani families segregating lamellar ichthyosis (a) and congenital ichthyosiform erythroderma (b,c). Filled symbols represent affected individuals. Symbols with crossed lines represent deceased individuals. Symbols with a star denote those individuals with an available DNA sample

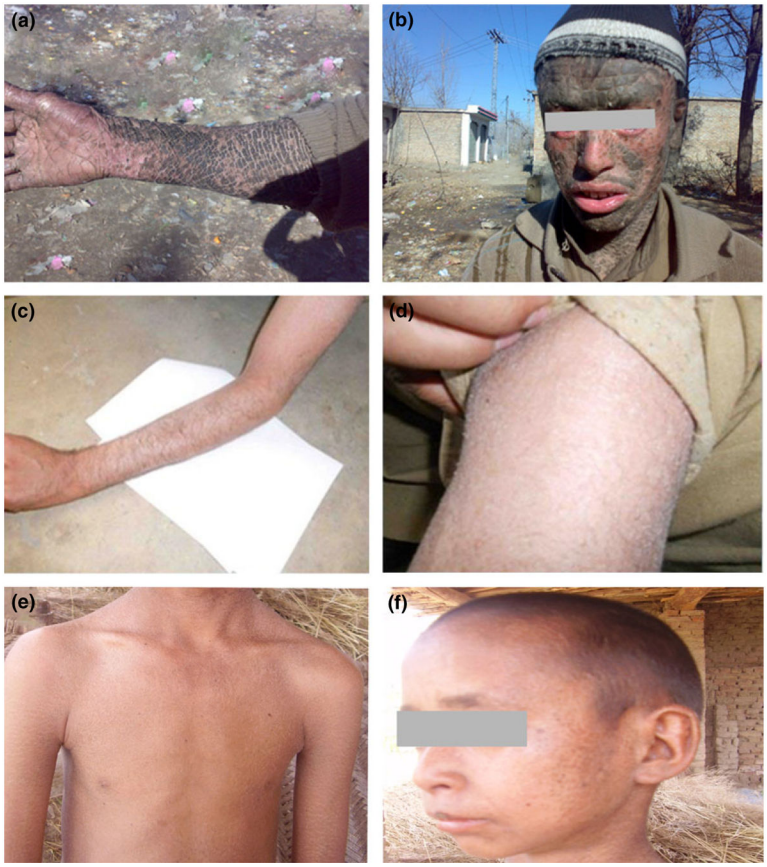

Figure 2.

Clinical presentation of ARCI in families A, B, and C. (a,b) Thick, large dark-brown scales on the arm and face in 21-year-old affected individual (IV-2) in family A. (c,d) Fine white ichthyotic scales on the arm of a 14-year-old affected individual (VI-3) in family B. (e,f) Slightly thick dark-brown scales on the neck, arms, chest, and face of a 16-year-old affected individual (IV-7) in family C

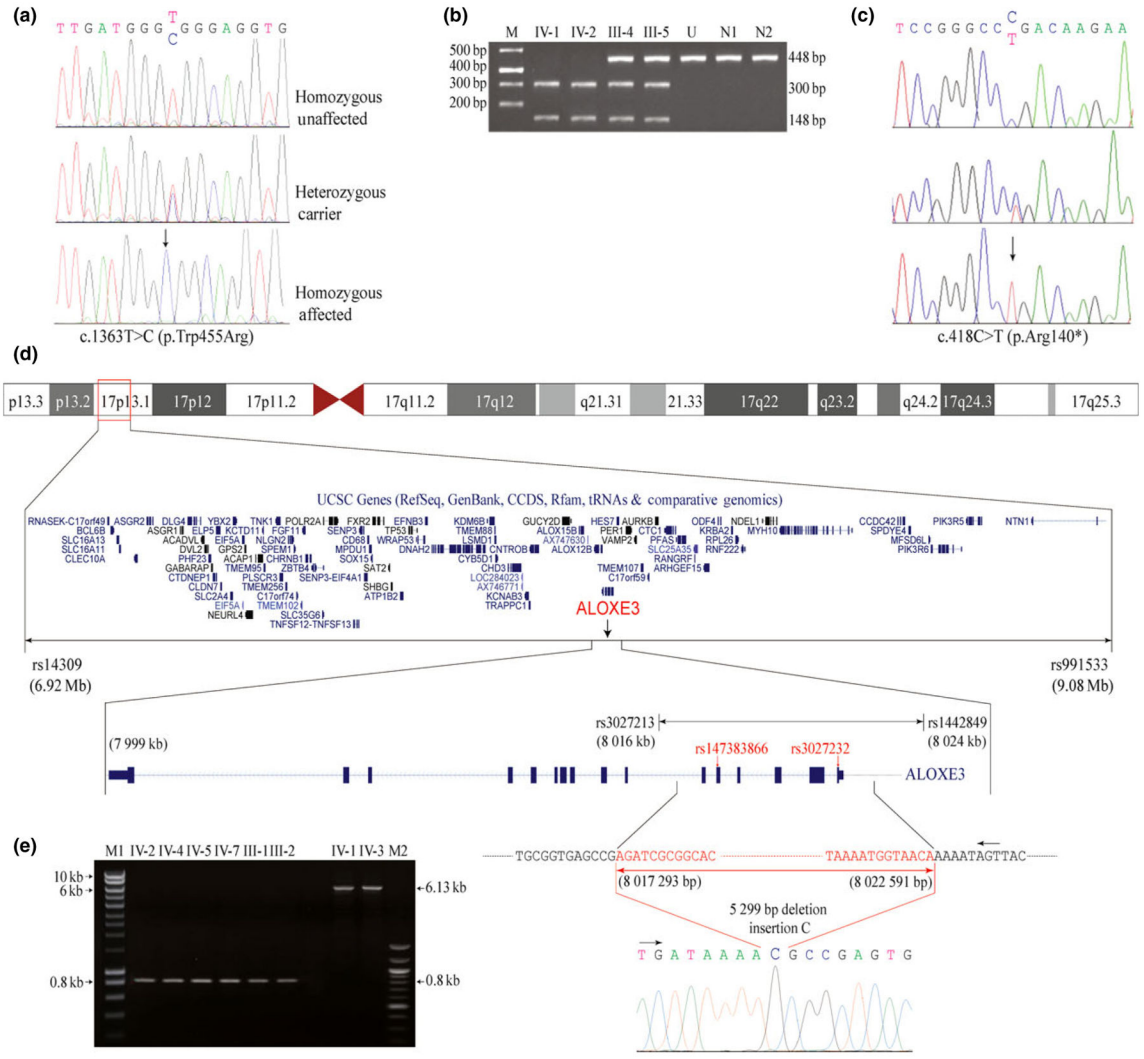

Figure 3.

Sequence analysis of *TGM1* and *ALOXE3* genes. a: a novel homozygous missense mutation (c.1363T > C; p.Trp455Arg) in *TGM1* gene in family A. (b) Restriction enzyme *Ssi*1 (*Aci*1) analysis of 448 bp polymerase chain reaction (PCR) products in the family members IV-1 and IV-2 (affected), III-4 and III-5 (carrier), N1 and N2 (controls), and U (undigested PCR product of IV-1). (c) Sequence analysis of a novel nonsense mutation (c.418C > T; p.Arg140*) in *ALOXE3* gene in family B. Arrows indicate position of mutations in the affected individuals. (d) Ideogram depicts a 2.1 Mb homozygous by descent region identified in family C. Deletion flanking single nucleotide polymorphism markers are indicated in red. (e) Amplified PCR product of *ALOXE3* deletion flanking region of affected and normal individuals of family C. M1, 10,000 bp molecular marker and M2 represents 100 bp molecular marker