Novel mutation in the *TGFBI* gene in a Moroccan family with atypical corneal dystrophy

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Research article

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Abstract

Background: Corneal dystrophy (CDs) is a heterogeneous group disease, genetically determined non-inflammatory bilateral corneal diseases (usually limited to the cornea). CD is characterized by a large variability in the age of onset, evolution and visual impact and the accumulation of insoluble deposits at different depths of the cornea. Clinical symptoms revealed bilaterally multiple superficial, epithelial, and stromal anterior granular opacities, in different stages of severity among three patients of this family. 99 genes are involved in (CDs).

The aim of this study is to identify pathogenic variant caused atypical corneal dystrophy in a large Moroccan family and to describe the clinical phenotype with their severe different stages of evolution.

Methods: In this study, we report a large Moroccan family with fourteen individuals affected by corneal dystrophy. Whole Exome Sequencing (WES) was performed in the propositus **(IV-7)** which had corneal pain since the age of 18, associated with a decrease in visual acuity with anterior epithelial and stromal corneal dystrophy, in the form of microvacuole and poorly individualized anterior opacities, with fuzzy edges and an unevenness of the epithelial layers taking the sawtooth appearance. The familial segregation was done by Sanger sequencing

Results: Whole exome sequencing showed a novel heterozygous mutation (c.1772C>A; p.Ser591Tyr) in *TGFBI* gene. Clinical examinations demonstrated bilaterally multiple superficial, epithelial and stromal anterior granular opacities; in different stages of severity among three patients of this family.

Conclusions: This report presents a novel mutation in *TGFBI* gene, found in all family members affects with different phenotypic aspects. This mutation is associated with Thiel-Behnke corneal dystrophy and therefore, it could be considered as a novel phenotype genotype correlation, which will help in genetic counseling for this family

Background

Corneal dystrophy (CDs) are hereditary bilateral primary alterations of the cornea that are not related with prior inflammation or systemic diseases; they mostly appear in the late second decade of progressive evolution (1). Theses alterations have no systemic manifestations and they present with variable shaped corneal opacities impair visual acuity to varying degrees and often have varying results in damage of corneal transparency and serious visual impairment (2). Most of the corneal dystrophy tend to be autosomal dominant in inheritance, with a high degree of penetrance; only one case was inherited as an autosomal recessive trait (3) The most used classification (The IC3D: International Committee for Classification of Corneal Dystrophy), divides CDs into epithelial and subepithelial dystrophy, epithelial-stromal *TGFBI* dystrophy, stromal dystrophy, and endothelial dystrophy with new genetic, clinical and pathologic information(4, 5). Mutations in some genes have been reported as being the cause for various CDs. Pathogenic mutations in the transforming growth factor beta-induced gene (*TGFBI*, OMIM 601692)

are the most frequent (6-13). The IC3D classification describes *TGFBI*-linked dystrophies by the recognition that they affect multiple layers rather than being confined to one corneal layer.

To our knowledge, 69 disease-associated variants in *TGFBI* have been described as involved in different subtypes of CDs in patients from different ethnic groups in the locus specific database LOVD v.3.0 (https://databases.lovd.nl/shared/genes/ TGFBI).

Several phenotypes have been described according to the corneal layer alteration and the Investigation of pathogenic mutations in the *TGFBI* gene, with the exception of metabolic affections. Pathogenic mutations in the *TGFBI* gene have been implicated in various phenotypes depending on the degree of damage to the corneal layer such as Thiel-Behnke corneal dystrophy (TBCD), Reis-Bucklers corneal dystrophy (RBCD), Groenouw granular corneal dystrophy type I (CDGG1), type of corneal dystrophy I and IIIA (LCD1, OMIM 122200 and LCD3A, OMIM 608471) and corneal dystrophy with epithelial basement membrane (EBMD, OMIM 121820) (14, 15). Such dystrophy are called variant LCD by IC3D conventions (4).Here, we present a novel heterozygous mutation (c.1772C>A; p.Ser591Tyr) in *TGFBI* gene, using whole exome sequencing within a Moroccan family with CD.

Methods

Patients recruitment, pedigree construction, and DNA extraction

Three patients of a consanguineous family from the south of Morocco were referred to our Department of Medical Genetics at the National of Health in Rabat for Corneal dystrophy. After obtaining written consent from all participants involved in the study, we collected blood samples from family members. A five generational pedigree diagram was constructed after a thorough interview of the affected mother (III-2). An autosomal dominant mode of inheritance (Fig. 1).Blood samples were collected from five members of the family, the available three family members affected (IV-7, IV-8 and III-2) including two phenotypically unaffected (IV-1, IV-3). The extraction of genomic DNA from whole peripheral blood by using QIAamp DNA Blood Mini Kit (Qiagen Valencia, CA), strictly following the manufacturer's protocol

Genetic analysis

Whole-exome sequencing and Sanger sequencing

WES was performed; 500ng of fragmented DNA by enzymatic fragmentation with Kapa Hyper Plus Kit (KapaBiosystems Inc. Wilmington, MA, USA) was amplified according to the manufacturer instructions and was subjected to enrichment with SeqCap EZ Human Exome v3.0 Roche Nimblegen (Roche, Basel, Switzerland).

The Illumina HiSeq 2500 system was used to sequence 64 enriched megabases in a fast-running double-ended mode (2x100bp). Use bcl2fastq v1.8.4 (Illumina) to convert the original data (bcl file) into a fastq file. The sequence was analyzed according to GATK best practice recommendations; BWA-MEM was

used for mapping, and GATK (haplotype calling program) was used for variant calling. Adopt Variant Studio (Illumina) for annotation and filtering steps.

Candidate variant selection: Candidate variants were selected using an autosomal dominant mode of inheritance according to the following criteria: i) heterozygous variants, ii) non-synonymous variants, iii) Variants predicted pathogenic or likely pathogenic, iv) Variants with Minor allele frequency (MAF) of <0.01 were selected from the 1000 Genome Project (http://www.1000genomes.org/) and ESP6500 exome project (https://evs.gs.washington.edu/EVS/) and iv) segregation analysis. Candidate variants in favor of clinical manifestations and passing the criteria were validated by Sanger sequencing.

The established variants were cross-checked with the 1000 genomes database (http://www.1000genomes.org/), with the Exome Variant Server (http://evs.gs.washington. edu/EVS/), HGMD (http://www.biobase-international. com/product/hgmd) and with the « clinvar » database (http://www.ncbi.nlm.nih.gov/clinvar/).

Variant validation: To confirm the mutation detected by exome sequencing, standard PCR to amplify the exon 10 of *TGFBI* gene was carried by using the *TGFBI*_F:5'-GACCAGGCTAATTACCATTCTTG-3' and *TGFBI*_R:5'-TGAGATATGTCCTGGAGCCC-3' primer pair. Amplification products were electrophoresed on 1% agarose gel. Sanger sequencing was performed with dye terminator chemistry (ABI Prism BigDye v3.1) and run on automated sequencer Using 3130 Genetic Analyzer (Thermo Fisher Scientific). The results obtained were aligned with the reference genome (GRCh37 / hg19) and then analyzed by DNA variant analysis software (Mutation Surveyor® software).

Results

Genetics and clinical investigations: The transmission form of this family was consistent with an autosomal dominant inheritance (Figure 1).

Clinical examinations demonstrated bilaterally multiple superficial, epithelial and stromal anterior granular opacities; in different stages of severity among three patients of this family. The three patients on the same mutation who shared a mixed phenotype with a superficial form of granular corneal dystrophy type 1 and Thiel-Behnke corneal dystrophy patterns: Clinical features of patients are given below.

The patient 1 (IV-7) was a 34-year-old woman who complained of recurrent episodes of corneal pain since the age of 18, associated with a decrease in visual acuity. Her vision was 4/10 OD and 6/10 OS. Slit-lamp examinations revealed, in the two eyes, anterior epithelial and stromal corneal dystrophy, in the form of spaced microvacuoles by a heterogeneous thickening of the epithelium due to thickening of an abnormal subepithelial fibrous layer and poorly individualized anterior opacities, with fuzzy edges (Figure 2). The rest of the examination of the anterior segment is normal, including a normal iris, a clear lens, and an intraocular pressure at 17mmHg in ODG. The Optical Coherence Tomography (OCT) scan shows an unevenness of the epithelial layers by a homogeneous confluent layer of hyper-reflected deposits with a

serrated anterior border taking the sawtooth appearance, replacing the Bowman layer and reaching the anterior stroma (Figure 3 (A , B, C, D)). It is thicker and becomes thinner on the periphery and disappears towards the limb. The pachymetry is 512 µm in OD and 523 µm in OS.

The patient 2 (IV-8) is a woman 44 years old; she presents episodes of recurrent keratitis with a decrease in visual acuity of progressive installation since the age of 20 years. Visual acuity in OD shows that she counts the fingers at 3m / OS at 2/10 (irremovable). Examination with the slit lamp shows in OD and OS anterior epithelial and stromal corneal dystrophy in the form of microvacuoles, especially in the periphery, with a heterogeneous thickening of the epithelium, more pronounced in the center giving a large central opacity with fuzzy edges at both eyes (Figure 4), Clear lens; ocular tone at 15 mmHg in OD 14 mmHg in OS, and normal fundus. The OCT of the cornea shows an unevenness of the anterior epithelial and stromal layers with thicker hyper-reflective deposits and a clear central opacity in both eyes (Figure 5: A, B, C, D), As well as a pachymetry at 538 µm in OD, 543 µm in OS.

The patient 3 (III-2) is a woman 72 years old, she has bilateral osteoarthritis, and she reports episodes of recurrent corneal pain. As for her visual acuity, in far vision OD, she can barely see the movement of the fingers / OS: (she counts the fingers at 3m). The Slit lamp examination showed in the right eye, central and paracentric yellowish and gelatinous central and paracentric deposition associated with a corneal opacity deeper than the previous one and affecting the epithelial layers of the Bowman's membrane and the anterior stroma in the form of an epithelial fibrous layer, taking almost the entire corneal surface (Figure: 6). The rest of the examination is hampered by the very important dystrophy of the right eye, (i.e., ineclairable fundus). In the left eye, the slit lamp examination showed corneal dystrophy involving the epithelial layers of the Bowman's membrane and the anterior stroma in the form of a heterogeneous thickening more important at the center, associated with opacity with fuzzy boundaries and a corticonuclear cataract (Figure 6). In the Fundus, the pupillary glow with flattened retina was noted. The OCT of the cornea shows in the right eye: the form of microvacuoles as thin patches (blue arrows) associated with a significant loss of epithelial cells with disorganization of the epithelial layers, the absence of Bowman's membrane, and an anterior intrastromal bubble (white arrows). The Pachymetry is at 586 µm (Figure 7). In the Left eye, the OCT shows an irregularity of the corneal surface with disorganization of the epithelial layers, a discontinuous Bowman’s membrane, and anterior stromal reshaping especially in the center (Figure 7). The process of corneal transplantation, in this patient, is ongoing.

Whole Exome Sequencing and Variant Validation**:** WES was performed on three family members (IV-7, IV-8 and III-2). The analysis of the expression of the three patients shows 30887 variants in 19 712 genes. Filtering of variants is illustrated in Figure 9. After filtering, this number was reduced to a single allelic variant in the *TGFBI* gene. Only a heterozygous (c.1772C>A; p.Ser591Tyr) mutation in the TGFBI gene, identified in these three patients, was proposed as the potential pathogenic mutation within this family.

Bioinformatics analysis using Polyphen-2 and SIFT suggested that this mutation was a disease causing mutation and was predicted to be probably damaging., By Sanger sequencing, the mutation was

confirmed in all patients and absent among the unaffected family.

The genomic and clinical data both supported a diagnosis of Thiel-Behnke corneal dystrophy (TBCD) in this family.

Discussion And Conclusion

Thiel-Behnke corneal dystrophy (TBCD, OMIM 602082) was first described in 1967, it is characterized by sub-epithelial honeycomb-shaped corneal opacities in the superficial cornea, progressive visual impairment and autosomal dominant inheritance (16) . Symptoms begin with recurrent corneal erosion during childhood (first and second decade of life) and visual acuity is affected later in life. TBCD can be particularly confused with Reis-Bücklers corneal dystrophy (RBCD, OMIM 608470) during the first two decades of life.

TBCD is in the form of bilaterally cross-linked epithelial opacities in honeycomb in remarkable symmetry with the accumulation of reticular flecks at the level of the Bowman membrane.

Peripheral cornea is typically not involved but can be affected with time in older patients. While RBCD has a geographic-like phenotype, TBCD has a honeycomb-like phenotype. Histopathologically, TBCD is featured by alternating irregular thickening and thinning, and a focal absence of the epithelial basement membrane.

The Bowman layer is replaced by a superficial fibrocellular scar with a pathognomonic wavy sawtoothed pattern or “curly” fibers. The prevalence of TBCD is unknown. Only few Cases have been reported in European population, the United States, Japanese's and other ethnicities, but none in North African population (17, 18).To our knowledge, in the *TGFBI* gene and in the same position, the mutations (p.Arg555Gln; p.Arg555Trp) were correlated with TBCD (6, 19, 20), have been also reported by Xiang et al and Yu y et al (21, 22). In this study, we identified a novel mutation in TGFBI gene (c.1772C>A; p.Ser591Tyr) .The association of p.Ser591Tyr mutation with TBCD has never been, to our knowledge, reported previously and it is not listed in LOVD v.3.0 (Leiden open variation database). The *TGFBI* gene is located on chromosome 5q31.1, which codes for a protein of 17 exons composed of 683 amino acids. (23, 24). This protein is used for both cell adhesion and the integrin recognition sequence. However that extracellular matrix protein *(TGFBIp)* contains an EMILIN-like domain rich in N-terminal cysteine, four consecutive and highly homologous domains of fascilin 1 (FAS1), and a C-terminal arginine-glycine-aspartic, acid motif (25, 26). All pathogenic variants caused in accumulation of insoluble extracellular material in the cornea (27). Accumulation of full length and of fragments of aberrant *TGFBI*p has been demonstrated in corneal deposits (28). It appears that mutations are also likely to change protein degradation pathways and impact structure and stability of aggregated proteins (23). Most gene *TGFBI* mutations associated with CD are heterozygous, and some patients with homozygous mutations have a more severe phenotype, indicating that the potential toxic effect has a dose response effect (29).The p.S591Y mutation is in the fourth domain (Fasc domain 4), causing misfold in the protein leading, in our case, to TBCD all mutations unrelated to R124C are located at the border or in the Fasc 4 domain which is

localized between residues 502 and 632 of the domain. This domain is suspected to have a specific action directly or by interaction with an unknown protein (30). The two hot spot in the gene *TGFBI* have been in granular corneal dystrophy type I (GCD1) and granular corneal dystrophy type II (GCD2) respectively. The p.Arg124Cys reported associated with the most common corneal dystrophies, which in the codon 124 and the codon 555, which include p.Arg555Trp and p.Arg124His. The p.Arg124Cys mutation was reported in lattice corneal dystrophy type 1 (LCD1), whereas the anterior stromal CD, Reis-Bücklers corneal dystrophy and TBCD are caused by the *TGFBI* mutations p.Arg124Leu and p.Arg555Gln respectively (31).

The management of the corneal dystrophy varies with the specific disease. For the treatment of this pathology. Corneal transplantation was the recommended treatment, a major limitation being the recurrence of post-transplant corneal deposits, however, some patients are treated with drugs, others use methods that suggest removing the abnormal layer. Like phototherapeutic keratectomy or DALK.(26). Regarding our family case, the chronological evolution of this new dystrophy indicates an early treatment by photokeratectomy in an earlier stage with a promising postoperative result. The particularity in this family is the evolution and the preservation of the posterior layer, which will imply an adapted surgery.

To sum up .We reported in this paper a novel pathogenic mutation of *TGFBI* gene, which could be a novel phenotype genotype correlation. The clinical and genetic results of our study may further expand the spectrum of *TGFBI*-linked CDs and may be of great help in genetic counseling for this family.

List Of Abbreviations

CDs: Corneal dystrophies

IC3D: The International Committee for Classification of Corneal Dystrophies

GCD: Granular corneal dystrophies

ACD: Avellino corneal dystrophies

LCD: Lattice corneal dystrophies

RBCD: Reis-Bücklers corneal dystrophies

CDGG1: Groenouw granular corneal dystrophy type I

TGFBI: Transforming growth factor beta induced

TBCD: Thiel Behnke corneal dystrophy

EBMD: Corneal dystrophy with epithelial basement membrane

OCT: Optical Coherence Tomography

FAS1: Fasciclin1

WES: Whole Exome Sequencing

Declarations

Ethics approval and consent to participate.

Ethical approval is considered unnecessary according to national provisions. This report was not presented as a research study as all family members were seen in a medical consultation for diagnostic purposes and they gave their written consent to participate and benefit from the molecular analysis

Consent for publication

This family gave written consent for clinical data to be published

Availability of data and materials

All data is contained in the manuscript

Competing interests

The authors declare that there are no conflicts of interest

Funding

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Authors' contributions

YB and IC drafted the manuscript and carried out the Sanger study. HT; HO and AB carried diagnostic study and revising the work critically for important intellectual content clinic.MO: drafted the manuscript. AZ and JL participated of design of the study and the exome analysis and revised the manuscript. KS and AS participated in the design of the study and in the draft of the manuscript. All authors read and approved the final manuscript.

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Figures

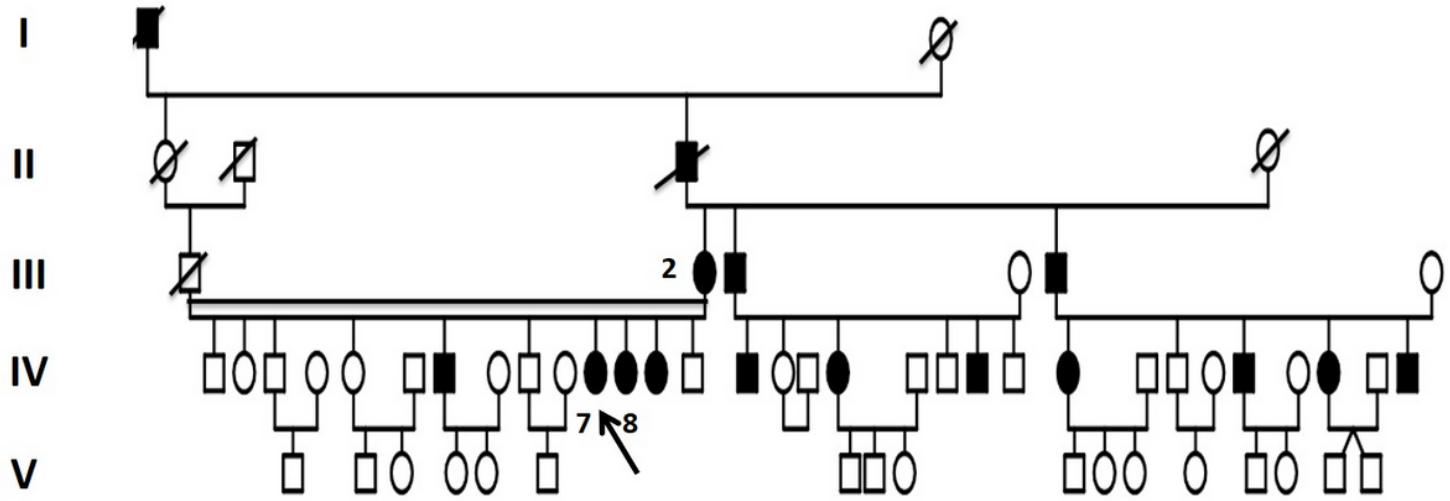

Figure 1

Pedigree a four-generation family affected by Thiel-Behnke corneal dystrophy. The pedigree shows an autosomal dominant transmission of the disease. The arrow indicates the proband and the filled symbols indicate affected subjects and open symbols represent unaffected individuals. Squares represent males and circles represent females. A slash mark through the square or circle indicates deceased patients.

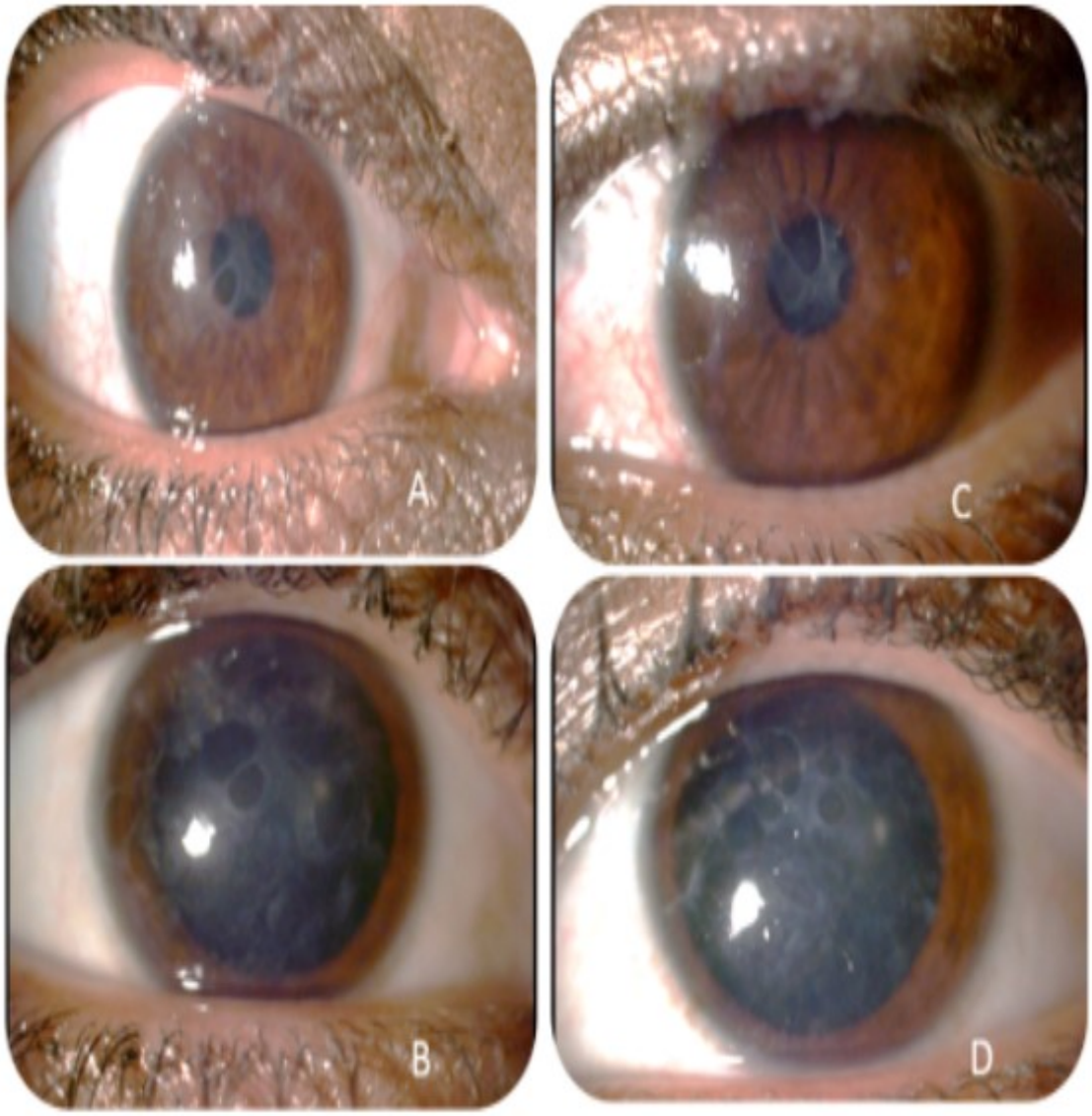

Figure 2

A: Right eye without dilatations, B: The appearance of the anterior previously poorly individualized with fuzzy edges of the right eye in the form of microvacuoles, C: Left eye without dilation, D: The appearance of the opacities previously poorly individualized with edges blurred left eye in the form of microvacuoles well visible after dilatation.

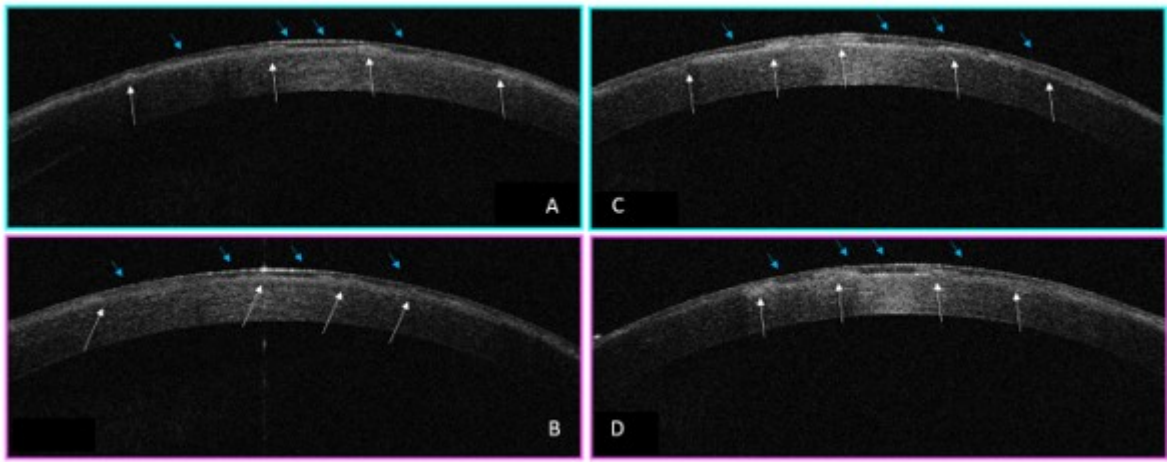

Figure 3

A and B. Two OCT sections show the form of microvacuoles as thin patches (blue arrows) and the sawtooth deposits on Bowman's membrane (white arrows). C and D: The same aspect is seen in the controlateral eye.

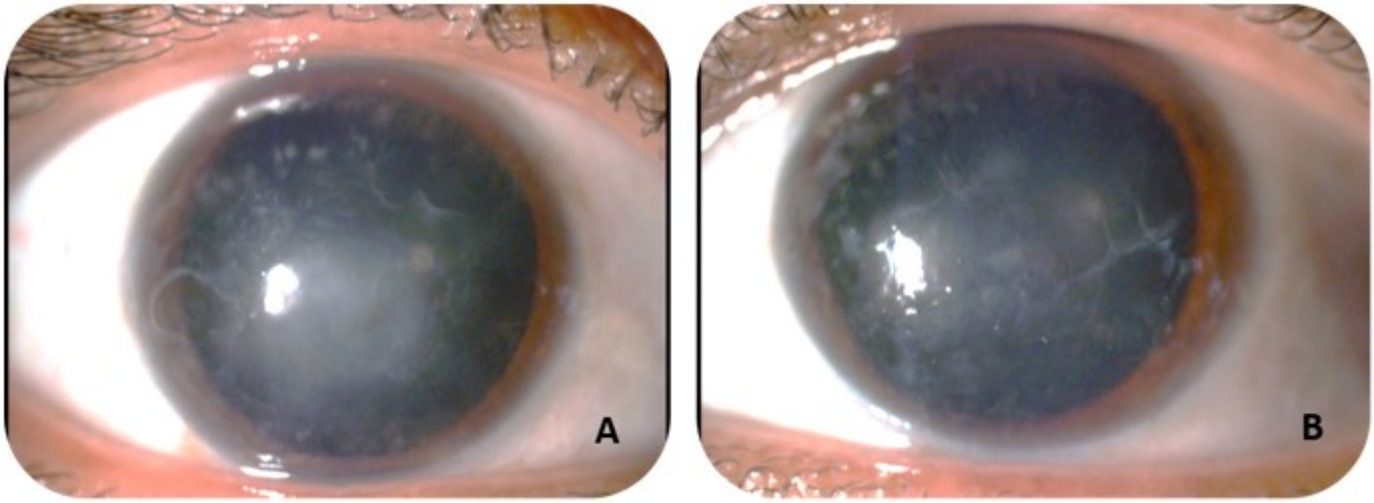

Figure 4

A. Undistinctive appearance of the anterior opacities, with blurred edges of the right and left eye clearly visible in the form of microvacuoles.

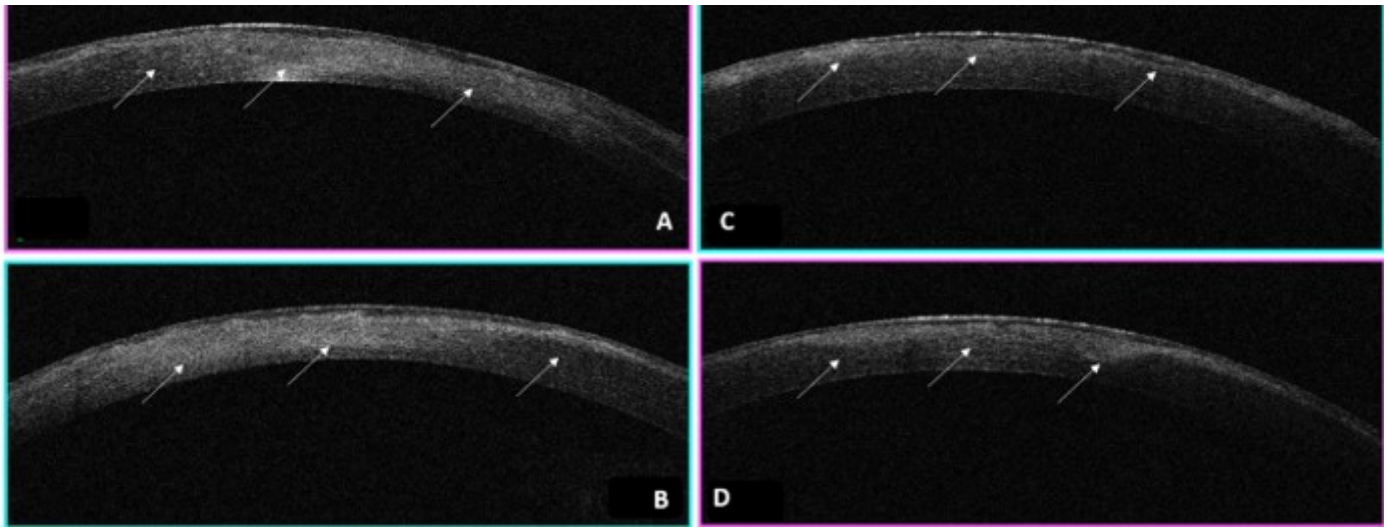

Figure 5

A and B. Two cuts of corneal OCT of the OD. Irregularity of the anterior layers with thicker hyperreflective deposits of an apparent central opacity (arrows). C and D. Two sections of OCT, irregularity of anterior epithelial and anterior stromal layers with thicker hyperreflective deposits (arrows).

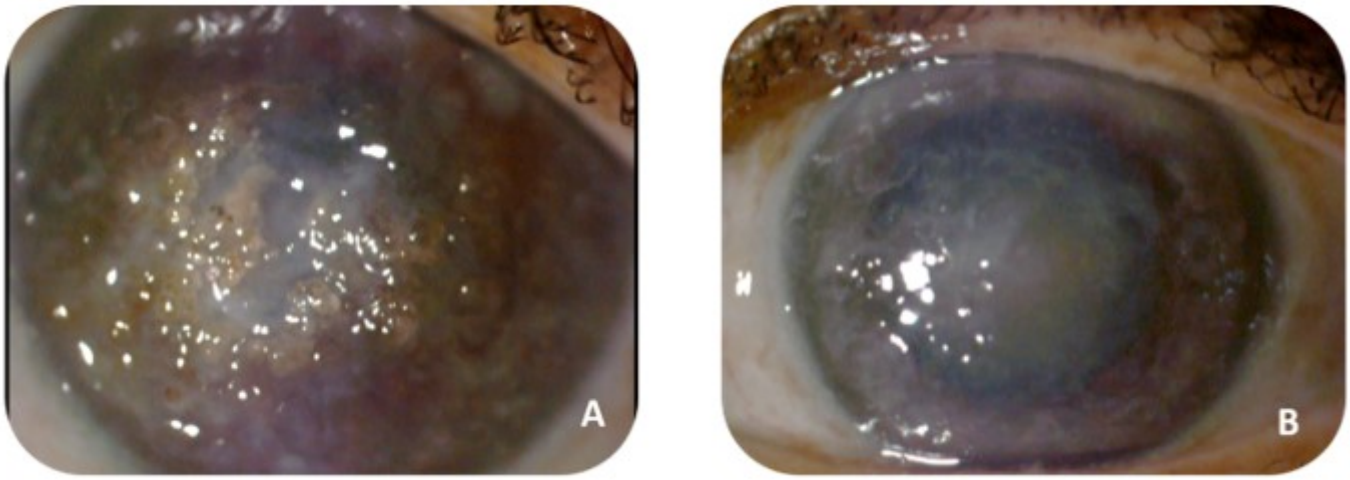

Figure 6

A. The right eye has white, yellowish, gelatinous central and paracentric epithelial along with corneal opacity. B. Central heterogeneous thickening and marks in the left eye, and an obvious central opacity of blurred edges.

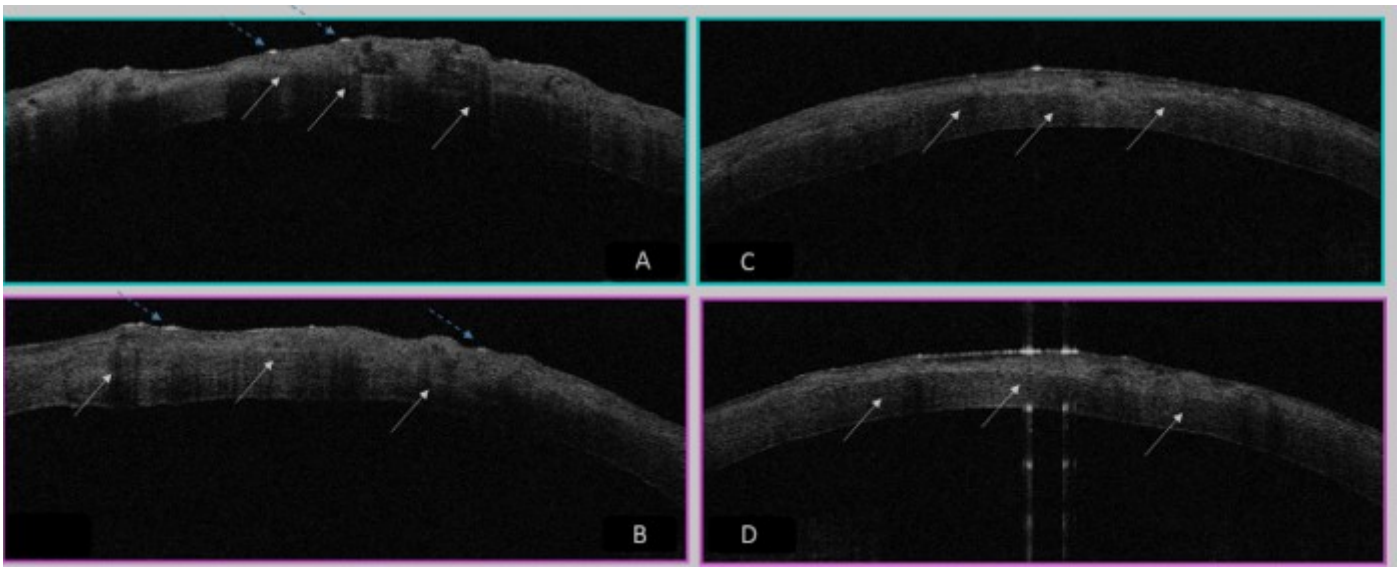

Figure 7

A and B. Two sectionsof corneal OCT of OD.Deposits(white arrows); a significant reshapingof the cornea. Disorganization of the epithelial layer along, absence of Bowman's membraneand blebs in the anterior intrastromal cornea (white arrows). C and D. Two sections of corneal OCT of left eye.irregularity of the corneal surface and Disorganization of the epithelial layer, absence of Bowman's membrane in different sites and the presence of a thick deposit layer of intrastromal to the center (arrows).

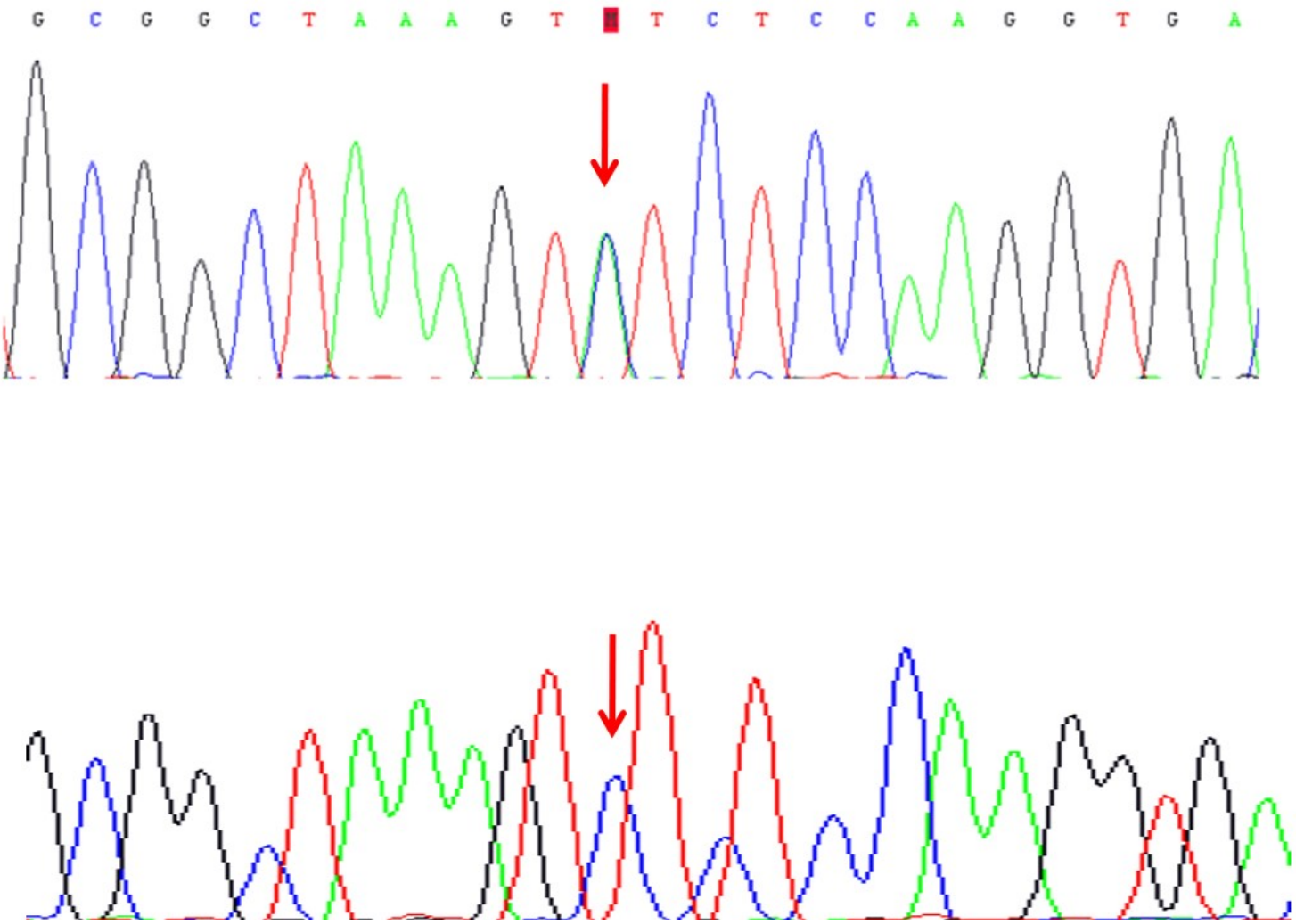

Figure 8

Electropherograms showing the c.1772C>A heterozygous mutation in the patients (IV-7, IV-8 and III-2) and the absence of mutation in the unaffected individual (IV-6). Arrows indicate the region of the mutation.

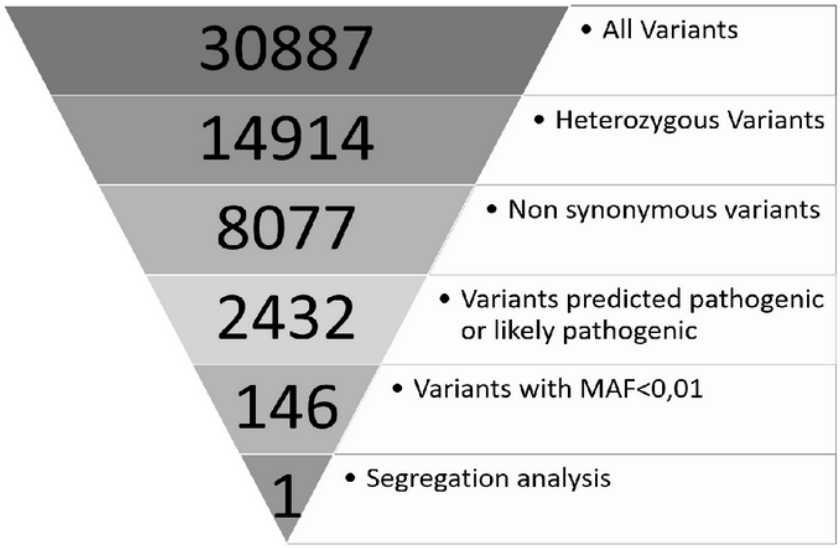

Figure 9

Bioinformatic analyzes of whole exome sequencing in IV.7.IV.8, and III.2 patients