



Expanding the mutational spectrum in *TGFBI*-linked corneal dystrophies: Identification of a novel and unusual mutation (Val113Ile) in a family with granular dystrophy

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Purpose: To report the clinical and molecular study of a family with an autosomal dominant stromal granular dystrophy of the cornea caused by a novel and unusual *TGFBI* gene mutation.

Methods: A complete ophthalmological examination, corneal dystrophy phenotype characterization, PCR amplification, and automated nucleotide sequencing of exons 4, 11, 12, 13, and 14 of the *TGFBI* gene was carried out on the family. DNA from 40 unrelated ethnically matched healthy individuals were analyzed as controls.

Results: Corneal dystrophy in two sisters was characterized by multiple grayish-white lesions located in the anterior and mid-stroma. Numerous small sized non-coalescent opacities were observed in the peripheral cornea while fewer larger lesions were apparent towards the central part of the cornea. A heterozygous missense mutation, consisting of a G to A transition at nucleotide position 384 in *TGFBI* exon 4 that predicts a valine (GTT) to isoleucine (ATT) replacement in residue 113 (Val113Ile) of the TGFBI protein was identified.

Conclusions: This is the most 5' located mutation detected so far in subjects with *TGFBI*-linked corneal dystrophy. Valine 113 is strictly conserved in TGFBI from several species and we suggest that the phenotype observed in these patients is related to the unusual location of the mutation. Our results expand the mutational spectrum in the group of *TGFBI*-linked corneal dystrophies.

Corneal dystrophies (CDs) are a group of hereditary conditions characterized by the progressive accumulation of deposits on different layers of the cornea, with the resulting loss of refractive index and transparency. They are clinically and genetically heterogeneous disorders that typically initiate in the first or second decades of life, affect the central part of the cornea, and are not associated with inflammatory processes [1]. Over time, the deposits can lead to visual impairment. Surgical procedures are frequently required to restore visual acuity. Present classifications of CDs are based upon both the layer of the cornea that is affected and the biomicroscopic characteristics of the deposits [2]. Most CDs are inherited as autosomal dominant traits with intra- and interfamilial variable clinical expressivity and a high degree of penetrance [3].

The molecular genetics approach to subjects and families with different types of CDs has allowed the recognition of a group of autosomal dominant dystrophies that arise from a common pathogenetic mechanism represented by recurrent mutations in the 5q31-located (transforming growth factor- β induced *TGFBI*, also known as *BIGH3*) gene [2,4-8]. Over the past few years, it has been evident that specific *TGFBI* mutations strongly correlate to particular forms of CDs. To date, approximately 30 distinct *TGFBI* gene mutations have

been demonstrated in patients of several ethnic groups with four distinct autosomal dominant Bowman's layer/stromal corneal dystrophies: granular CD type I [6-8], granular CD type II or Avellino type [6,8,9], granular CD type III (Reis-Bücklers dystrophy) [8,10,11], and Lattice CD of types I, IIIA, I/IIIA, IIIB, and IV [12-14]. Extracellular deposition of insoluble protein aggregates within the cornea is the hallmark of inherited corneal disorders caused by mutations in *TGFBI* [15,16].

The most common type of granular dystrophy is type I, also known as classic granular corneal dystrophy or Groenouw CD, which is characterized by multiple discrete crumb-like corneal opacities [17]. The condition usually becomes apparent in the first decade of life or in puberty with grayish-white opacities that involve the superficial stromal layers of the cornea [18,19]. The lesions tend to aggregate, expand, and increase in number, spreading both peripherally and more deeply although a clear zone around the corneoescleral limbus remains typically present. Central disk-shaped opacities are formed after the third or fourth decade of life. Visual acuity gradually decreases and affected subjects may maintain unaffected vision for a long time [19]. A striking genotype-phenotype correlation is evident in granular CD type I as almost all subjects suffering this disease have a C to T transition at nucleotide position 1,710 of *TGFBI* exon 12, predicting an Arg555Trp change in the protein [2,4,6-8]. However, in at least two unrelated subjects, the granular CD type I phenotype has been shown to be the result of a C to A transversion at nucle-

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otide 417 of exon 4, causing a Arg124Ser substitution in TGFBI [6,20]

In this paper we describe the clinical and molecular data of a Mexican family with an autosomal dominant granular dystrophy in which a novel TGFBI Val113Ile mutation was identified. This is the most 5' mutation described so far in the group of *TGFBI*-linked CDs, and our data suggest that the phenotypic peculiarities observed are related to the unusual location of the mutation.

METHODS

Clinical examination: The initial patient was a Mexican female aged 26 years who presented at the Institute of Ophthalmology "Conde de Valenciana" in Mexico City asking for refractive surgery due to previously diagnosed myopia. Ophthalmologic examination revealed best-corrected visual acuity of 20/20, intraocular pressure of 16 mm Hg, no anatomic defects of the anterior segment, transparent lenses, and normal funduscopy in both eyes. Biomicroscopic inspection of the cornea revealed bilateral, symmetric, sharply demarcated grayish-white opacities localized to the corneal stroma. The opacities showed a "centrifuge" distribution with numerous small (less than 2 mm in diameter) non-coalescent opacities located at the periphery and few larger lesions toward the most central part of the cornea (Figure 1, Figure 2). The intervening stroma between the opacities was clear. The lesions were located at the anterior and midstroma (Figure 3) while the overlying epithelium and underlying endothelium were both unaffected. No epithelial erosions nor stromal haze were noted at this age. A 23-year-old sister (visual acuity of 20/25 in both eyes) exhibited identical findings in both corneas. No accompanying somatic anomalies were noted in either patient. Their father, aged 55 years, was unavailable for clinical and molecular study but he was reported as having been diagnosed with a bilateral "degenerative corneal disease" which was evident by the third decade of life.



Figure 1. The corneal phenotype of the proband is illustrated by this photograph. Numerous small non-coalescent opacities are evident in the peripheral cornea while a few larger lesions are apparent toward the central part of the cornea.

DNA analysis: After obtaining local Institutional Ethics Committee approval and informed consent from patients, DNA was extracted from peripheral blood leukocytes of the proband and her affected sister, according to standard procedures. PCR amplification of exons 4, 11, 12, 13, and 14 of the *TGFBI* gene was achieved using four pairs of primers previously described [6]. Each 25 μ l PCR amplification reaction contained 1X PCR buffer, 100-200 ngs of genomic DNA, 0.2 mM of each dNTP, 1 unit Taq polymerase, 1 mM of forward and reverse primers, and 1.5 mM $MgCl_2$. PCR products were analyzed in 1.5% agarose gels from which the bands with the amplified templates were excised and the DNA subsequently purified with the help of the Qiaex II kit (Qiagen, Hilden, Germany). Direct sequencing was performed with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) adding about 10 ng of template DNA in each reaction and using a temperature program which included 25 cycles of denaturation at 97 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for four min. All samples were analyzed in an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequence variations were confirmed in both sense and antisense DNA strands using a newly PCR amplified fragment. Forty DNA samples (80 *TGFBI* alleles) from normal Mexican people were analyzed as controls. Sequences were compared with the nucleotide and deduced amino acid sequence of published *TGFBI* cDNA (Ensembl ENST00000305126).

RESULTS

After sequencing the PCR product spanning exon 4 of the *TGFBI* gene in DNA from the proband, we identified a heterozygous missense mutation consisting of a G to A transition at nucleotide position 384 in exon 4 that predicts a valine (GTT) to isoleucine (ATT) replacement in TGFBI protein residue 113

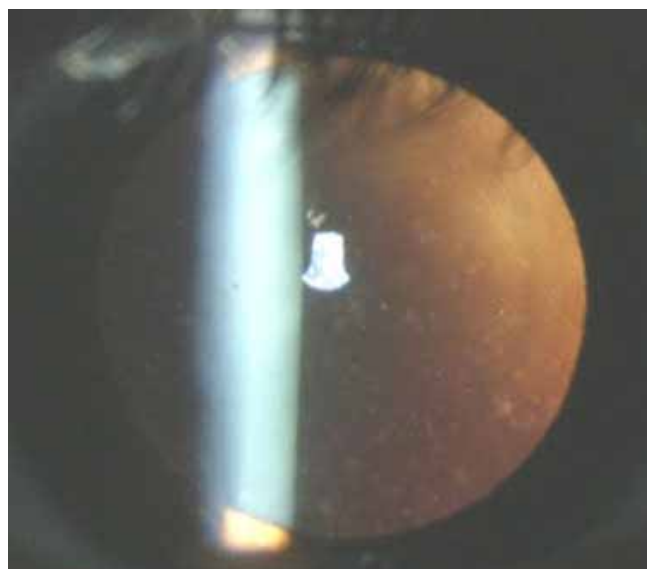


Figure 2. With retroillumination, the corneal opacities are sharply demarcated with a clear intervening stroma.

(Val113Ile; Figure 4). This change is located in the region coding for the TGFBI FAS1-1 helix $\alpha 1$ domain, and it was absent in DNA from 80 control chromosomes, excluding the possibility of being a rare polymorphism. As this particular mutation does not create or abolish a restriction enzyme site, all samples were directly sequenced. The same mutation was identified in genomic DNA from the affected sister. The change was confirmed in both DNA strands in the patients. No additional pathogenetic nucleotide changes were detected in the examined *TGFBI* exons. A previously reported inconsequential polymorphic change (Phe540Phe) was also identified in our two patients.

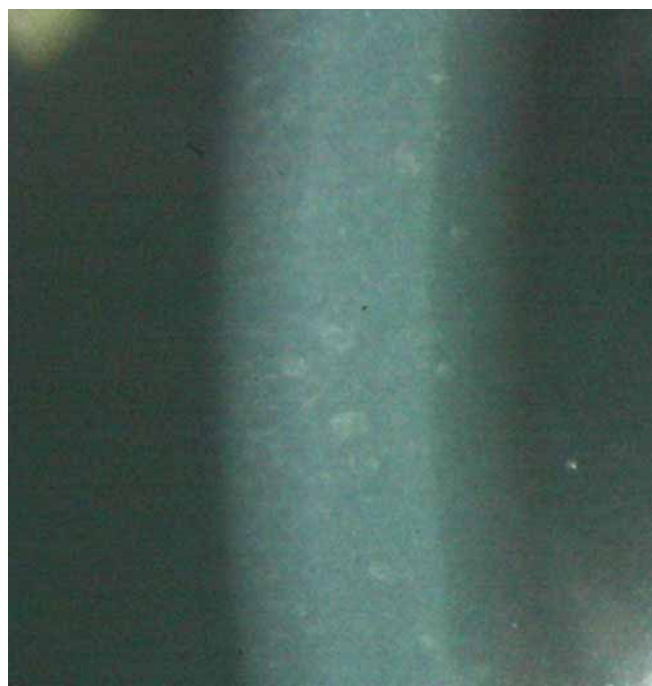


Figure 3. Photograph of the proband's cornea showing granular opacities in the anterior and midstroma. Note that the deposits tend to be more numerous in the anterior corneal stroma.

DISCUSSION

Mutations in the *TGFBI* gene result in a variety of dominantly inherited dystrophies of the cornea that are classified according to the biomicroscopic features of the deposits and to its location within the corneal layers. At least four variants of granular CDs have been recognized (for a detailed review see reference [2]). Granular CD type I or classic granular dystrophy is distinguished by multiple discrete crumb-like corneal opacities in the anterior and mid-stroma commonly associated with painful epithelial erosions and almost exclusively caused by the R555W mutation in TGFBI. Granular CD type II or Avellino CD is characterized by stromal opacities shaped like rings, disks, stars, or snowflakes combined with linear opacities resembling some features of lattice CD. Epithelial erosions occur less often than in granular CD type I, and virtually all cases studied presented a TGFBI R124H mutation. Granular CD type III or superficial granular CD exhibits deposits that are morphologically identical from those observed in granular CD type I but are limited to the subepithelial region or the epithelium (most subjects have had the R124L mutation in TGFBI). Granular CD type IV, which is intermediate in severity between the classical and superficial variants, has been observed in a French family where it displayed round snowflake-shaped opacities in the subepithelial and most anterior stroma along with recurrent erosions that began in childhood. Notably, granular CD type IV affected patients carried two TGFBI mutations: R124L and a deletion of amino acids 125 and 126 [21]. The phenotype found in our patients was not easily categorized under any of these four granular types, because a "centrifuge" pattern of opacities was observed with apparently most significant involvement of the peripheral than the central cornea. We consider that this phenotypic variation is related to the unusual location of the mutation in TGFBI. Unfortunately, the affected father was not examined. It is possible that in this family the corneal phenotype at an older age will be similar to the common types of granular dystrophies.

The *TGFBI*-expressed polypeptide is a 683 amino acids-long secreted protein containing four tandemly repeated do-

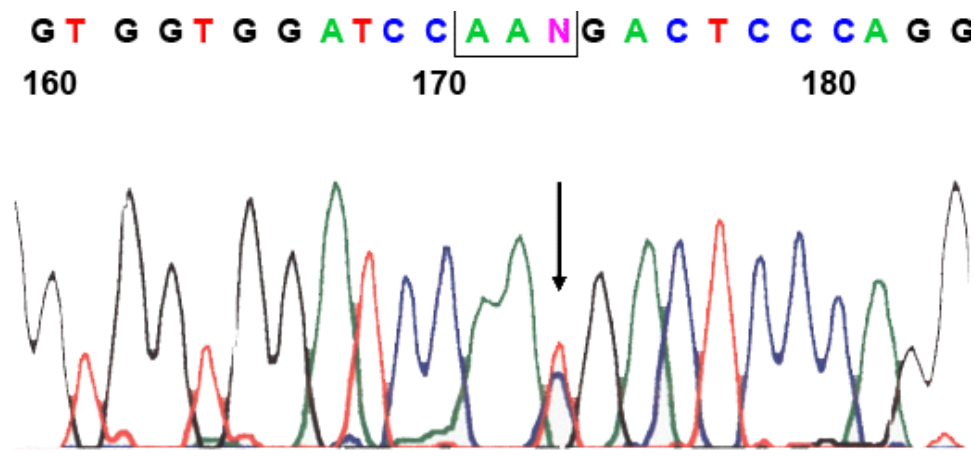


Figure 4. The *TGFBI* gene partial nucleotide sequence analysis illustrates a heterozygous G to A transition (arrow) at nucleotide position 384 in *TGFBI* exon 4. This change predicts a valine (GTT) to isoleucine (ATT) replacement in residue 113 (Val113Ile). The antisense strand is shown; codon 113 is boxed.

main of 140 residues which have a marked sequence similarity to the *Drosophila melanogaster* axonal guidance molecule fasciclin 1 (FAS1) [22]. The four FAS1 domains in human TGFBI protein correspond to amino acids 134-236 (FAS1-1), 242-372 (FAS1-2), 373-501 (FAS1-3), and 502-632 (FAS1-4). To date, all mutations described in TGFBI associated to diverse types of CDs are located between residues 124 and 631 with the only two mutations described so far outside the fourth FAS1 domain (residues 502-632) being those affecting residues 501 (third FAS1 domain) and 124 (on the NH₂-terminal side of FAS1 first domain). The mutation described in this paper is located in residue 113, which is 11 residues away from arginine 124, the most NH₂-terminus located mutated residue in CDs [6]. A BLASTP search revealed that valine residue at position 113 is strictly conserved in TGFBI proteins from different species as *Pan troglodytes*, *Canis familiaris*, *Mus musculus*, *Bos Taurus*, and *Gallus gallus*, suggesting that this amino acid is important for normal protein function. Valine 113 is located in the α 1 helix of TGFBI FAS1-1 domain [23]. Although there is indirect in vitro evidence that the presence of valine-valine at positions 112-113 may be important for blocking fibril formation [24], the role of valine 113 in TGFBI protein folding awaits further experimental investigations.

Inherited corneal disorders caused by mutations in *TGFBI* are associated with an extracellular deposition of insoluble protein aggregates within the cornea. These deposits can be amyloid (as seen in CD lattice type), granular/non-amyloid (in granular CD type I and CD Reiss-Bücklers), or a mixture of both (CD Avellino type) [23]. The reason that amyloid deposition is a prominent manifestation of certain, but not all, TGFBI mutations remains to be established [16]. Granular phenotypes in CDs result from missense mutations in three specific TGFBI amino acids (for a review see [2] and references therein); 555 (R555W mutation in almost all granular CD type I cases, R555Q in some cases of granular CD type III), 623 (in some cases of granular CD type III), and 124 (R124H in all cases of granular CD type II), R124L in most cases of granular CD type III and R124S in two unrelated subjects with a granular CD type I phenotype [6,20]. Thus, valine 113 is the fourth TGFBI residue whose missense mutations can result in a corneal granular dystrophy. Genetic analysis on more numerous groups of CD patients from different ethnic backgrounds will result in the recognition of nonclassic mutations, ultimately allowing a more accurate clinical-molecular classification of *TGFBI*-linked CDs.

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