The TGFBI A546D mutation causes an atypical type of lattice corneal dystrophy

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Purpose: To report the clinical, molecular, and histopathological features of a distinct transforming growth factor-β-induced (TGFBI) gene-linked amyloidotic corneal dystrophy exhibiting an unusual lattice pattern.

Methods: A complete ophthalmologic examination was performed in 10 individuals of a Mexican family in which autosomal dominant transmission of the disease was observed. DNA was obtained from peripheral blood leukocytes of each participating subject. Genetic analyses included TGFBI polymerase chain reaction (PCR) amplification and automated nucleotidic sequencing of exons 4, 11, 12, 13, and 14 from genomic DNA. Histological analysis of corneal tissue from an affected individual who underwent a penetrating keratoplasty was also performed.

Results: The corneal phenotype in this pedigree was characterized by multiple bilateral round opacities in the central part of the cornea combined with a conspicuous central and peripheral lattice pattern. TGFBI analysis revealed a heterozygous point mutation at exon 12 (1637 C>A) in all affected individuals, predicting an A546D missense change.

Conclusions: The lattice phenotype resulting from the TGFBI A546D mutation in this family is distinct from that observed in a previously described pedigree carrying the A546D mutation and exhibiting a phenotype designated “polymorphic corneal amyloidosis”. We propose this particular disorder to be classified as an atypical type of lattice stromal corneal dystrophy.

Corneal dystrophies (CDs) are hereditary diseases involving the formation of corneal opacities on different layers of the cornea, which lead to significant impairment of corneal transparency and refraction [1]. CDs are primary corneal diseases which, in general, are not associated to prior inflammation or trauma or to systemic diseases. Most CDs are inherited as autosomal dominant traits with great intrafamilial and interfamilial variation on clinical expressivity and with a high degree of penetrance [2]. CDs are classified upon both the layer of the cornea that is affected and the biomicroscopic characteristics of the deposits [3]. In recent years, several autosomal dominant CDs have demonstrated to be the result of heterozygous mutations in the transforming growth factor-β-induced gene, TGFBI, located at chromosome 5q31 [4-9]. Characteristically, inherited CDs caused by mutations in TGFBI are associated with an extracellular corneal deposition of insoluble protein aggregates, which can be amyloid (as seen in CD lattice type [LCD]), granular/non-amyloid (in CD granular types I and III), or a mixture of both (in CD Avellino type) [10].

To date, approximately 35 distinct TGFBI gene mutations have been demonstrated in patients from diverse countries that are suffering from four distinct autosomal dominant Bowman’s layer/stromal corneal dystrophies: granular CD type I, granular CD type II or Avellino type, granular CD type III (Reis-Bücklers dystrophy), and Lattice CD of types I, IIIA, IIIIA, IIIB, and IV [11, 12]. A phenotype-genotype correlation has been established in this group of CDs as specific TGFBI amino acid changes result in defined corneal phenotypes (reviewed in [4]). However, exceptions to this correlation are not uncommon [13, 14].

In 2004, Eifrig et al. [15] identified a novel type of corneal dystrophy characterized by the presence of polymorphic corneal opacifications due to stromal amyloid deposits but without formation of a characteristic lattice pattern. The disorder was originated by the A546D mutation in TGFBI and it was named “polymorphic corneal amyloidosis” to differentiate it from the other classical lattice-forming CDs [15]. In this report, we describe the clinical, molecular, and histopathological features of a novel family with an amyloidotic CD originated by the A546D mutation and with an unusual pattern of lattice deposits on the corneal stroma. This lattice phenotype is different from that observed in the A546D-caused “polymorphic corneal amyloidosis” and we propose this particular disorder to be considered a separate variant of lattice corneal dystrophy.

METHODS

Study approval was obtained from the Institutional Review Board at the Institute of Ophthalmology “Conde de Valenciana”, in Mexico City. Patients pertained to a five-generation Mexican mestizo family (Figure 1) with ages ranging from 1 to 74 years.
Clinical Examination: To determine the status of the corneas (affected or unaffected), detailed slit-lamp examination was performed in each participating individual. In addition, all patients underwent a more profound ophthalmologic evaluation, including Best Corrected Visual Acuity determination and dilated fundus examination. The possible occurrence of extraocular somatic defects and/or developmental abnormalities was investigated by a clinical geneticist in all relatives.

Genetic Analysis: TGFBI was analyzed in a total of 10 subjects. DNA was obtained from peripheral blood leukocytes according to standard procedures. Polymerase chain reaction (PCR) amplification of exons 4, 11, 12, 13, and 14 of TGFBI was performed using the following pairs of primers: Exon 4: 5’-TCC CTC CTT CTG TCT TCT GC-3’ and 5’-AGA CTC CCA TTC ATC ATG CC-3’; Exon 11: 5’-CTC GTG GGA GTA TAA CCA GT-3’ and 5’-TGG GCA GAA GCT CCA CCC GG-3’; Exon 12: 5’-AAA TAC CTC TCA GCG TGG TG-3’ and 5’-TCA TTC AGT AAA CAC TTG CT-3’; Exon 13: 5’-CTT GCC AAG CAC GAC ACC ACC A-3’ and 5’-TGG CTC AGT AAT CAC TTG AAT AAC T-3’. Each 25 µl PCR amplification reaction contained 1X PCR buffer, 100-200 ng of genomic DNA, 0.2 mM of each dNTP, 1 unit Taq polymerase, 1 mM of forward and reverse primers, and 1.5 mM MgCl₂. PCR products were analyzed in 1.5% agarose gels from which the bands with the amplified templates were excised, and the DNA was subsequently purified with the help of the Qiaex II kit (Qiagen, Carlsbad, CA). Direct nucleotide sequencing was performed with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) adding 10 ng of template DNA to 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 4 min. All samples were analyzed in an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Nucleotidic sequences obtained from patient’s DNA were manually compared with wild type TGFBI sequence (Ensemble Database Transcript ID ENST00000305126).

Histologic Analysis: Corneal tissue, excised at the time of a penetrating keratoplasty in one affected patient (a 51-year-old female), was available for histopathological examination. The corneal button was fixed in 10% neutral buffered formaldehyde and analyzed with light microscopy after staining with congo red, periodic acid-Schiff (PAS), and Masson’s trichrome stains.

RESULTS
Clinical findings: The proband was a 29-year-old Mexican male who came to our institution because of long-term bilateral decrease of visual acuity. A total of nine additional members of his family were examined (Figure 1). All patients denied past history of ocular surgery, trauma, or infection. No systemic diseases, extraocular congenital defects, or developmental delay were apparent in any subject. The onset of the disease was in the third decade of life with blurry vision, foreign body sensation, and photophobia. No recurrent corneal epithelial erosions were reported by any subject. After slit-lamp biomicroscopy, 5 of out 10 members of the family (aged 74, 51, 46, 32, and 25 years) showed bilateral, symmetric, polymorphic opacities in the central part of the corneal stroma.

![Figure 1. Genealogy of the family with corneal dystrophy. Autosomal dominant transmission of the disease is evident. The asterisk indicate subjects who underwent clinical and molecular analyses. Black symbols represent affected subjects while gray symbols correspond to asymptomatic young relatives aged 18 (IV-5), 10 (IV-6), and 1 (V-2) years of age. The arrow indicates the propositus.](http://www.molvis.org/molvis/v13/a189/)
(Figure 2A,B) combined with conspicuous interdigitating, branching, filamentous opacities and multiple lattice opacities in the corneal periphery. Small opacities of granular aspects were also observed in the peripheral cornea (Figure 2C,D).

**TGFBI analysis:** After sequencing the PCR products spanning *TGFBI* exons 4, 11, 12, 13, and 14 in DNA from the proband, we identified a heterozygous point mutation consisting of a C>A transversion at nucleotide position 1637 in exon 12 (1637C>A; Figure 3), predicting an amino acid replacement from alanine (GCC) to aspartic acid (GAC) at residue 546 (A546D). This residue is situated within an alpha helix motif of the Fasciclin 4 domain of the TGFBI protein. The same missense change in *TGFBI* exon 12 was demonstrated in seven out of nine additional members of the family, three of them (aged 18 [IV-5], 10 [IV-6], and 1 [V-2] years of age, see Figure 1) without clinical manifestations of corneal disease. The 1637C>A mutation was confirmed by sequencing the antisense DNA strand. No additional pathogenetic nucleotide changes were detected in the remaining four *TGFBI* exons analyzed.

**Histologic evaluation:** Histologic analysis of the corneal button that was excised during a penetrating keratoplasty from one of the affected patients demonstrated variably sized and irregularly shaped round eosinophilic deposits, situated predominantly in the mid and posterior corneal stroma. These deposits stained positively with congo red demonstrating to be amyloid in nature (Figure 4). The corneal epithelium as well as Bowman’s and Descemet’s membranes appeared normal.

Figure 2. Corneal phenotype associated with the A546D TGFBI mutation. Slit lamp photographs of the proband show significant stromal opacification in the central part of the cornea (A and B). Corneal retroillumination demonstrated numerous central lesions combined with multiple linear opacities in central and peripheral cornea producing a typical lattice pattern (C). Small granular opacities are also evident in the periphery (D).
DISCUSSION

To date, about 35 different CD-causing mutations have been identified in TGFBI [1,4] and more than half of them affect arginine residues 124 and 555. Although in general, particular mutations in TGFBI are linked to specific corneal dystrophies independently of ethnic origin, exceptions to this genotype-phenotype correlation do occur [13,14].

Lattice CDs manifest biomicroscopically as linear, radially oriented, branching opacities with deposits that are fusiform in shape. At the histopathological level, they are characterized by deposits of amyloid material that stain positively with congo red in the corneal stroma. To date, four types of LCD have been established according to the stromal location of deposits, the characteristics of lattice lines, and the age of onset and these include LCD type I, LCD type II, LCD type IIIA, and LCD type IV. In addition, several atypical or “intermediate” forms of LCD have been observed associated to at least eight distinct TGFBI mutations [1,4]. The phenotype in these uncommon forms cannot be classified as LCD I, III, IIIA, or IV.

In 2004, a novel form of stromal amyloidosis in which affected corneas did not form a lattice pattern was described by Eifrig et al. [15] This phenotypically distinct disorder results from the A546D mutation in TGFBI and was designated “primary polymorphic amyloidosis” to differentiate it from the ordinary lattice-forming amyloidoses. In this paper, we demonstrate that the TGFBI A546D mutation causes a distinct lattice corneal dystrophy which has differences at the biomicroscopic level from the polymorphic corneal amyloidosis phenotype caused by the same missense mutation. However, it should be noted that some similarities exist between the phenotype described by Eifrig et al. [15] and the cases reported here including similar ages of onset, polymorphic appearance of the opacities, and mid to deep stromal location of the deposits.

The North American pedigree carrying the A546D allele studied by Eifrig et al. [15] originated from Germany while the Mexican family we described here has no history of ancestors from other countries, indicating that both families are unrelated.

Two additional families carrying the TGFBI A546D mutation have been described previously [16,17]. However, in these two pedigrees, an accompanying P551Q mutation was demonstrated in the same TGFBI allele (in cis mutations). In

Figure 3. Partial nucleotide sequence of TGFBI exon 12. A heterozygous C>A transversion was demonstrated (indicated by the arrow) at nucleotide position 1637 (c.1637C>A), predicting a replacement from alanine (GCC) to aspartic acid (GAC) at protein residue 546 (A546D).

Figure 4. Histopathological features of an affected cornea carrying the A546D TGFBI mutation. Light microscopy of excised corneal tissue stained with congo red revealed variably-sized stromal deposits of amyloid material, predominantly in the mid and posterior stroma. A: 10X; B: 40X.
the proband of the family described by Aldave et al. [16], bilateral, symmetric, radially arranged, branching refractile lines within and surrounding an area of central anterior stromal haze were noted. In addition, multiple polymorphic, refractile deposits were noted in the mid and posterior stroma in both the proband and her daughter. Deposits of amyloid material were demonstrated on histologic examination of diseased corneas and the disorder was cautiously classified as LCD intermediate type I/IIIA. In the case described by Klintworth et al. [17], affected members had several refractile lattice-like stromal deposits characterized by branching and nonbranching lattice figures resembling pipe stems. Delicate filamentous and discrete, short irregularly shaped stromal deposits along with a corneal haze were also evident and the disorder was classified as an atypical LCD type I [17]. Recurrent corneal erosions developed in affected individuals from these two families which sharply contrast with affected individuals in the two families carrying the A546D allele whom did not develop corneal erosions (present report and [15]). These observations suggest that the P551Q allele could exert a phenotypic modifier effect over the A546D mutation phenotype. Recurrent corneal erosions are a common finding in most LCDs. Analysis of microsatellite markers flanking the TGFBI region at 5q31 revealed that both families carrying the A546D/P551Q allele share the same haplotype, indicative of a common origin of this allele (founder effect mutation) [16].

The four families described to date with the A546D mutation, either as a unique molecular defect (Eifrig et al. [15], and present report) or in combination with the P551Q mutation in cis [16,17], have exhibited an atypical phenotype characterized by polymorphic amyloid stromal opacities (which are characteristic of lattice dystrophies) and inconstant formation of lattice lines. This combination of features is clearly different from the established types of LCD (reviewed in [4]). Considering these aspects, we propose to classify the polymorphic corneal amyloidoses caused by the A546D mutation (either alone or in combination with the P551Q mutation) as a separate lattice CD.

It is clear that missense mutations at residue Ala546 are invariably associated with amyloid deposits within the corneal stroma. In fact, another mutation involving codon 546 (A546T), has been demonstrated to cause a distinct type of LCD characterized by thick lattice lines located predominantly in the central cornea, some nodular opacities, a diffuse hazy- ness between the lines, and a history of recurrent epithelial erosions. The disorder was named French lattice corneal dystrophy type IIIA [18].

In summary, we provide clinical and molecular evidence supporting the occurrence of a distinct lattice CD attributable to the TGFBI A546D mutation. Given the variable phenotypic features of this polymorphic corneal amyloidosis, we propose to classify those CD cases caused by this particular mutation as a separate lattice type CD.

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