# A Novel Variant of Combined Granular-Lattice Corneal Dystrophy Associated With the Met619Lys Mutation in the *TGFBI* Gene

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## Abstract

**Objective**—To report a novel mutation in *TGFBI* (Gen-Bank NM_000358), p.Met619Lys, associated with a variant of combined granular-lattice corneal dystrophy.

**Methods**—Slitlamp examination and DNA collection from the proband and affected and unaffected relatives. All 17 exons of *TGFBI* were amplified and sequenced in the proband. Exon 14 was amplified and sequenced in the proband's family members and in 100 controls. Histopathologic examination of the excised corneal buttons from the proband and 3 family members was also performed.

**Results**—Affected individuals demonstrated an age-dependent phenotype, with the progression from central subepithelial needlelike deposits in younger individuals to polymorphic anterior stromal opacities in older family members. Screening of *TGFBI* in the proband demonstrated a novel mutation, p.Met619Lys, which was also present in all affected family members. Histopathologic examination revealed stromal deposits that stained with the Congo red and Masson trichrome stains as well as an antibody to the protein product of *TGFBI*.

**Conclusions**—We present a unique corneal dystrophy phenotype associated with the novel p.Met619Lys mutation in *TGFBI*.

Iт наs вееn 10 years since Munier and colleagues[1] published their seminal article that identified pathogenic mutations in the transforming growth factor β–induced gene (*TGFBI*) in families with a number of autosomal dominant corneal dystrophies, including lattice, granular, combined granular-lattice, and corneal dystrophy of the Bowman layer, type I. While a significant number of mutations have been identified in *TGFBI* during the last 10 years, many of which are associated with an atypical phenotype, most affected patients demonstrate a conserved mutation in either codon 124 or 555.[2] Thus, a well-conserved genotype-phenotype correlation exists for the classic forms of the TGFBI dystrophies, with an atypical phenotype suggesting a less commonly encountered or novel underlying mutation.[3-6]

If one includes histopathologic with clinical findings in defining the affected phenotype, in no other TGFBI corneal dystrophy is the genotype-phenotype correlation as invariant as with combined granular-lattice corneal dystrophy. Dystrophic corneal stromal amyloid deposition is most commonly associated with the p.Arg124Cys mutation in the *TGFBI* gene, which defines the classic form of lattice corneal dystrophy. However, 26 other mutations in *TGFBI* have also been associated with variants of the lattice corneal dystrophy phenotype, [3-5,7-39] 18 of which have been confirmed histopathologically to be associated with stromal amyloid deposition.[*] Similarly, dystrophic stromal hyaline deposition in the absence of amyloid deposition is most commonly associated with the p.Arg555Trp mutation in the *TGFBI* gene, which defines the classic form of granular corneal dystrophy. However, 3 other mutations in *TGFBI* have been associated with variants of the granular corneal dystrophy phenotype,[24-26,37,41,42] 2 of which have been confirmed histopathologically to be associated with stromal hyaline deposition.[24,25,37] In each of the 25 families reported to date in which histopathologic examination demonstrated corneal stromal amyloid and hyaline deposition and *TGFBI* screening has been performed, the p.Arg124His mutation has been identified.[25,34,43-47] None of the other 34 reported mutations in *TGFBI* have been associated with corneal stromal amyloid and hyaline deposition, suggesting that the nature and location of the p.Arg124His mutation has a unique effect on the structure and function of the TGFBI protein (TGFBIp). Against the backdrop of this invariant phenotype-genotype relationship, we present a family with an atypical variant of lattice corneal dystrophy associated with a novel missense mutation in *TGFBI*, p.Met619Lys. The distinct clinical phenotype in several affected members, suggestive of an uncommon or novel underlying mutation, was confirmed to be associated with a novel *TGFBI* mutation through screening of the proband and affected and unaffected family members. However, the demonstration of both amyloid and hyaline deposits on histopathologic examination of several affected individuals violates the previously absolute phenotype-genotype correlation that had existed for combined granular-lattice corneal dystrophy. Thus, this article highlights that clinical and histopathologic features alone cannot be relied on to accurately diagnose and categorize the corneal dystrophies.

## METHODS

### REPORT OF CASES

**Proband**—The proband (II-4, Figure 1) is a 52-year-old Hispanic woman who was referred to one of the authors (A.J.A.) for corneal transplantation following an aborted corneal transplant in the left eye that had been associated with spontaneous expulsion of the crystalline lens. The patient stated that she initially noted reduced corrected visual acuity approximately 7 years before initial examination but denied a history of recurrent corneal erosions. Two of her siblings had previously undergone corneal transplantation as had her mother. Additionally, her maternal grandfather had "died blind," and each of his 4 brothers had had poor eyesight that was not correctable with glasses.

Uncorrected visual acuity measured 20/40 OD and hand motions OS. Slitlamp examination of the right eye revealed spine-like anterior stromal opacities characterized by a linear deposit with fine extensions along its length. In addition, polymorphic, semiconfluent opacities were axially distributed in the anterior corneal stroma and midcorneal stroma (Figure 2). A penetrating keratoplasty was successfully performed in the patient's left eye, followed by a secondary intraocular lens placement 1 year later. Twenty months following corneal transplantation, no evidence of recurrent dystrophic deposition was noted in the

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[*]*References* 5, 9, 13, 15, 17, 19, 21-23, 25, 26, 29-32, 34, 36, 38-40.

corneal transplant, and the patient's uncorrected visual acuity was 20/30 OS. The patient subsequently underwent an uneventful penetrating keratoplasty in the right eye as well.

**Additional Cases**—Ten of the proband's family members were examined (Figure 1). The proband's 75-year-old mother (I-2) underwent a penetrating keratoplasty in her right eye at age 73 years. Slitlamp examination of her right eye demonstrated a few branching linear opacities in the peripheral host cornea but a clear graft, with no evidence of recurrent dystrophic deposits. Examination of the left cornea demonstrated axially distributed, linear branching anterior and midstromal deposits that appeared gray-white on direct illumination and translucent on retroillumination. In addition, discrete and semiconfluent anterior and midstromal polymorphic deposits, reminiscent of the stromal deposits associated with polymorphic amyloid degeneration, were noted (Figure 2).

The proband's 59-year-old brother (II-1) developed decreased vision in each eye at approximately age 48 years without associated recurrent corneal erosions. He underwent corneal transplantation 5 (left eye) and 6 (right eye) years later, with no evidence of recurrence of dystrophic deposits in either corneal graft noted 5 years after the corneal transplant was performed in the right eye. The peripheral host corneal tissue demonstrated linear branching opacities that appeared similar to those characteristic of classic lattice corneal dystrophy (Figure 2).

The proband's 55-year-old sister (II-3) underwent a penetrating keratoplasty in her right eye less than 1 year before examination. She denied a history of recurrent corneal erosions but did complain of significantly reduced vision in her left eye. Slitlamp examination demonstrated a clear graft in the right eye and dense, confluent, gray-while anterior and midstromal deposits involving the central 6 to 8 mm of the left cornea. Although a few linear extensions of the central opacity were noted superiorly, most of the deposits appeared in clumps and not in linear forms as noted in the proband. Superficial vascularization of the inferior cornea was also noted, giving an overall appearance reminiscent of gelatinous droplike corneal dystrophy (Figure 2). The patient subsequently underwent a penetrating keratoplasty in the left eye as well.

The proband's 46-year-old sister (II-8) denied any history of ocular complaints, such as decreased vision, previous ocular trauma or surgery, and recurrent corneal erosions. Slitlamp examination of her right eye revealed a single fine stellate opacity in the cornea, located at the level of the Bowman layer, overlying the inferior pupillary border. Two similar-appearing opacities were noted in the subepithelial region of the left cornea, with an otherwise unremarkable anterior segment examination (Figure 2).

The proband's eldest child (III-1), a 26-year-old man, denied a history of recurrent corneal erosions and decreased vision. On examination of the right cornea, a small, stellate-shaped subepithelial opacity was noted (Figure 2); no opacities were noted in the left cornea. The proband's other 2 male off-spring, aged 25 (III-2) and 21 (III-4) years, had no ocular complaints and had normal corneal evaluations. The proband's 25-year-old daughter (III-3) had no ocular complaints and no corneal abnormalities in her right eye. Careful evaluation of the left cornea revealed a single translucent, needle-shaped central subepithelial deposit shorter than 0.5 mm, which was sufficient to classify her as affected.

## PATIENT IDENTIFICATION AND SPECIMEN COLLECTION

The researchers followed the tenets of the Declaration of Helsinki in the treatment of the patients described here. After institutional review board approval was granted, informed consent was obtained from the proband as well as affected and unaffected family members.

A slitlamp examination was performed to determine each individual's disease status (affected or unaffected).

## DNA COLLECTION AND ANALYSIS

Buccal epithelial swab (CytoSoft Cytology Brush; Medical Packaging Corp, Camarillo, California) samples were collected from the proband and family members. Genomic DNA was prepared from the buccal epithelial cells using the QIAamp DNA Mini Kit spin protocol (Qiagen, Valencia, California). DNA previously collected from 100 healthy individuals served as control samples.

## POLYMERASE CHAIN REACTION AMPLIFICATION AND DNA SEQUENCING

All 17 exons of *TGFBI* were amplified in the proband using previously reported primers and conditions.[3] Exon 14 was amplified in the proband's family members to screen for the p.Met619Lys mutation. Purification of the polymerase chain reaction products and automated DNA sequencing were then performed using the reagents and settings that have been reported previously.[3] Nucleotide sequences, read manually and with Mutation Surveyor, version 2.2 (Softgenetics, State College, Pennsylvania), were compared with the published *TGFBI* complementary DNA sequence.

## HISTOPATHOLOGIC EXAMINATION

The corneal buttons excised from the proband at the time of penetrating keratoplasty were fixed in neutral buffered formaldehyde, 10%. They were analyzed with light microscopy after staining with hematoxylin and eosin, Congo red, periodic acid–Schiff, and Masson trichrome.

## IMMUNOHISTOCHEMICAL EXAMINATION

Immunohistochemistry was performed on slides prepared from paraffin-embedded sections of the corneal buttons from the proband using a goat polyclonal antibody directed against keratoepithelin (R&D Systems Inc, Minneapolis, Minnesota) at a concentration of 0.5 μg/mL. Antibody binding was detected using an RTU Vectastain Universal Quick Kit (Vector Laboratories, Burlingame, California) and a 3′3-diaminobenzidine hydrochloride peroxidase substrate kit (Vector Laboratories). Slides were then counterstained with Hematoxylin QS (Vector Laboratories).

# RESULTS

## *TGFBI* MUTATION ANALYSIS

Sequencing of all 17 exons of *TGFBI* in the proband revealed an unreported nucleotide substitution, c.1903T>A, in the heterozygous state, predicted to result in a novel missense mutation, p.Met619Lys, in exon 14 (Figure 3). In addition, 4 previously reported synonymous substitutions were identified, each in the heterozygous state: c.698C>G (p.Leu217Leu; rs1442); c.1028A>G (p.Val327Val; rs1054124); c.1463C>T (p.Leu472Leu; rs1133170); and c.1667T>C (p.Phe540Phe; rs4669).

Screening of *TGFBI* exon 14 in the proband's family members revealed the c.1903T>A nucleotide substitution in the heterozygous state in each of the 6 clinically affected individuals (Figure 1). The c.1903T>A substitution was also identified in 1 of the 4 clinically unaffected family members screened, the 25-year-old son (III-2) of the proband. None of the 100 samples from the controls demonstrated the c.1903T>A sequence variant.

## LIGHT MICROSCOPY

Light microscopic examination of both excised corneal buttons from the proband revealed numerous large stromal deposits that appeared eosinophilic with the hematoxylineosin stain (Figure 4). Periodic acid–Schiff and Congo red stains also highlighted deposition of material at the level of the Bowman layer as well as the stromal deposits that stained with hematoxylin-eosin. The Congo red–stained deposits demonstrated birefringence and dichroism under polarized light with the use of polarizing filters characteristic of amyloid fibrils (Figure 4). The stromal deposits also demonstrated significant staining with Masson trichrome, characteristic of the granular or hyaline deposits in the TGFBI dystrophies.

Histopathologic examination of the excised corneal buttons from the other family members who underwent penetrating keratoplasty also had findings consistent with combined granular-lattice corneal dystrophy. In both of the corneal buttons from the proband's 55-year-old sister (II-3) and the corneal button from the proband's mother (I-2), eosinophilic stromal deposits were noted, which demonstrated staining with Congo red as well as birefringence and dichroism when viewed with a polarized light source. As observed in the proband's cornea, these Congo red–stained deposits also demonstrated staining with Masson trichrome. However, in the corneal button from the proband's 55-year-old sister (Figure 2), Masson trichrome revealed intensely bright staining deposits surrounding the amyloid deposits. Immunostaining of both corneal buttons from the proband with an antibody to TGFBIp showed intense reactivity of the Congo red– and Masson trichrome–staining stromal deposits, confirming that the stromal deposits consist of TGFBIp (Figure 5).

## COMMENT

The corneal dystrophy associated with the p.Met619Lys mutation in *TGFBI* is best classified as a variant of combined granular-lattice corneal dystrophy. The clinical features in affected individuals are sufficiently similar to those of the previously reported classic and variant forms of lattice corneal dystrophy that the presumptive clinical diagnosis was a dystrophy associated with a *TGBFI* mutation. However, the clinical features were sufficiently atypical that a rare or novel *TGFBI* mutation was suspected and later confirmed. The presence of only a single focal corneal deposit in the 25- and 26-year-old affected children of the proband is not surprising, given that the TGFBI dystrophies are characterized by a progressive increase in the number and size of dystrophic deposits over time. Additionally, the identification of the p.Met619Lys mutation in the clinically unaffected 25-year-old son (III-2) of the proband is not surprising, given plausible explanations such as incomplete penetrance or a delayed onset of the affected phenotype. The development of a delayed-onset variant of lattice corneal dystrophy has been reported numerous times, most commonly in association with a mutation in exon 14 of *TGFBI*[4,35,39,48] (as was identified in the family we report). Unexpected findings were that the proband's 46-year-old sibling (II-8) demonstrated only very subtle corneal deposits and the proband's 55-year-old sibling (II-3) demonstrated significantly greater corneal involvement than her 75-year-old mother (I-2). We previously reported[4] the intrafamilial clinical variability observed in this pedigree in another family with a TGFBI dystrophy; this strengthens the contention that though the phenotypic expression is primarily determined by the effect of the identified mutation on the structure and function of the encoded protein, the genetic background of each individual as well as environmental factors likely influence the manner and degree of expression.

The p.Met619Lys mutation likely produces dystrophic corneal deposition through interference with an essential function of TGFBIp: cell adhesion. The TGFBIp contains 4 domains of high-sequence similarity that are also highly conserved across several species.[49] Each of these domains, known as fas-1 domains owing to the original description of homologous domains in the insect cell-adhesion molecule fasciclin I,[50] contains highly

conserved sequences that are thought to be essential for cell adhesion.[51] Kim and colleagues[51] have identified 2 such conserved peptide sequences in TGFBIp, 1 of which is glutamic acid, proline, aspartic acid, isoleucine, and methionine (amino acids 615-619) in the fourth fas-1 domain, as the essential motifs for mediating cell adhesion. Mutation of either amino acid 617 (aspartic acid) or 618 (isoleucine) results in a loss of cell adhesion mediated by the fourth fas-1 domain. Thus, it is quite plausible that a mutation involving amino acid 619 (methionine), located in the curved β strand β6,[52] would also result in loss of the cell adhesion mediated by the fourth fas-1 domain and thus in a partial or complete loss of function of TGFBIp.

What remains to be elucidated is the mechanism through which the p.Met619Lys mutation results in the clinical and histopathologic features observed in the family that we describe. It is inaccurate to assume that identification of the mutated amino acid residue in *TGFBI* is sufficient to predict the morphology and nature of the resultant corneal deposits, as is exemplified by the association of various missense substitutions involving the arginine residue at codon 124 with morphologically distinct dystrophies: p.Arg124Cys with classic lattice corneal dystrophy; p.Arg124His with combined granular-lattice corneal dystrophy; p.Arg124Leu with corneal dystrophy of the Bowman layer type I; and p.Arg124Ser with a variant of granular corneal dystrophy.[26,37] Although the extracellular dystrophic deposits in each of these dystrophies contain mutant TGFBIp,[44,53-55] each is characterized by clinically and histopathologically distinct amyloid or hyaline deposition, or both in the case of combined granular-lattice corneal dystrophy. Clout and Hohenester[52] have proposed that mutations at codons 124 and 555 may produce dystrophic deposition through interference with protein-protein interactions, while the less commonly identified mutations in the fourth fas-1 domain of *TGBFI* may cause protein misfolding, resulting in abolition of secretion or significant protein destabilization. However, the novel p.Met619Lys mutation that we report, located in the fourth fas-1 domain of *TGFBI*, results in the deposition of both corneal stromal amyloid and hyaline, which has previously been associated only with the arginine to histidine mutation at codon 124. Thus, dystrophies that are produced by mutations in different domains of *TGFBI*, presumably through different mechanisms of inducing protein dysfunction, may have more clinical and histopathologic features in common than dystrophies that result from different substitutions of the same nucleotide. A complete understanding of the TGFBI dystrophies, therefore, involves not only an appreciation of the clinical and histopathologic features associated with the classic and variant forms of each but also knowledge of the underlying molecular genetic basis for the development of the dystrophic TGFBIp deposits.

### Clinical Relevance

The atypical and variable phenotype and the demonstration of both hyaline and amyloid stromal deposits indicate that neither clinical nor histopathologic features may be relied on to accurately diagnose and classify the corneal dystrophies.

## Acknowledgments

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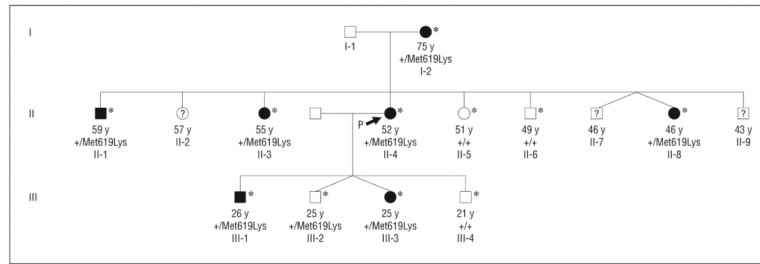
**Figure 1.**
Pedigree of a 3-generation Hispanic family with a unique transforming growth factor β–induced (TGFBI)–associated corneal dystrophy. Presence of the wild-type allele (+) or the mutant allele is shown. * indicates members of the family who underwent examination, DNA collection, and *TGFBI* screening.
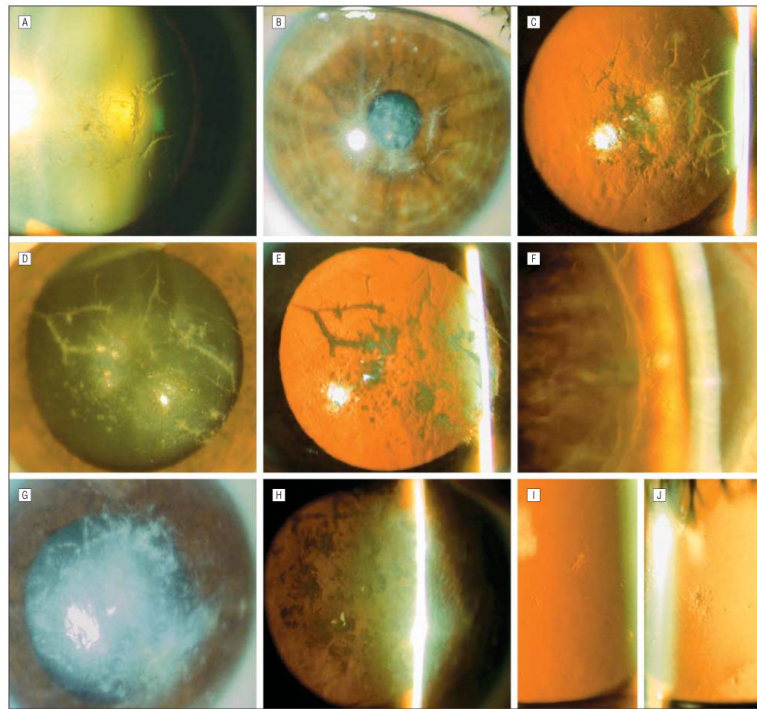
**Figure 2.**
Slitlamp photomicrographs. The 52-year-old proband's right cornea demonstrates spinelike and polymorphic central stromal opacities seen in retroillumination against the lens (A), with direct illumination (B), and against the red reflex (C). The proband's 75-year-old mother has axially distributed branching linear opacities in the left cornea, seen with direct (D) and indirect (E) illumination. F, The proband's 59-year-old brother, demonstrating thin branching anterior stromal lines in the host cornea adjacent to the edge of the corneal transplant in the right eye. G, The proband's 55-year-old sister demonstrates confluent gray-white central stromal opacities seen with direct illumination. H, Retroillumination against the red reflex demonstrates that the deposits are in clumps, not linear aggregates, and cause significant diminution of the red reflex owing to the confluence of the deposits. The proband's 46-year-old sister (I) and 26-year-old son (J) demonstrate focal stellate subepithelial opacities.
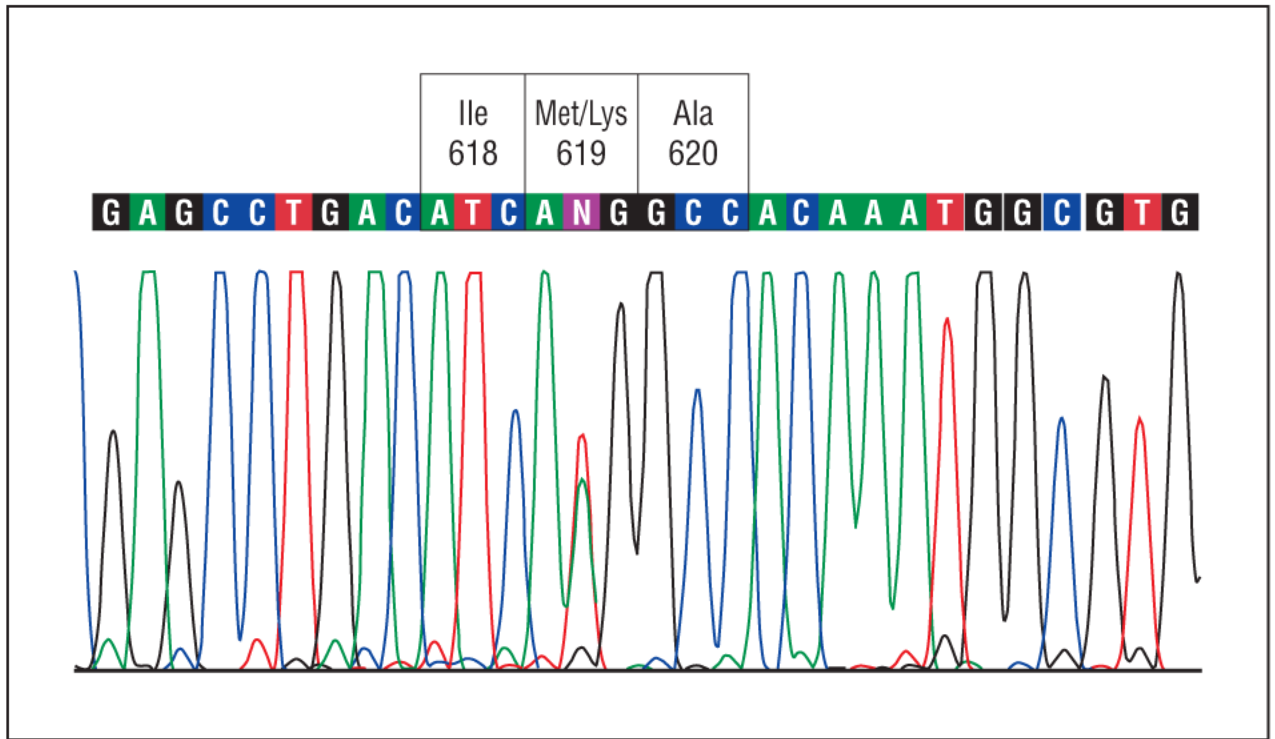
**Figure 3.**
Portion of coding region of *TGFBI* exon 14 around codon 619 in the proband demonstrating heterozygous T to A transition at nucleotide 1903, resulting in a change in the encoded amino acid residue at codon 619 from methionine (Met) to lysine (Lys). Ala indicates alanine; Ile, isoleucine.
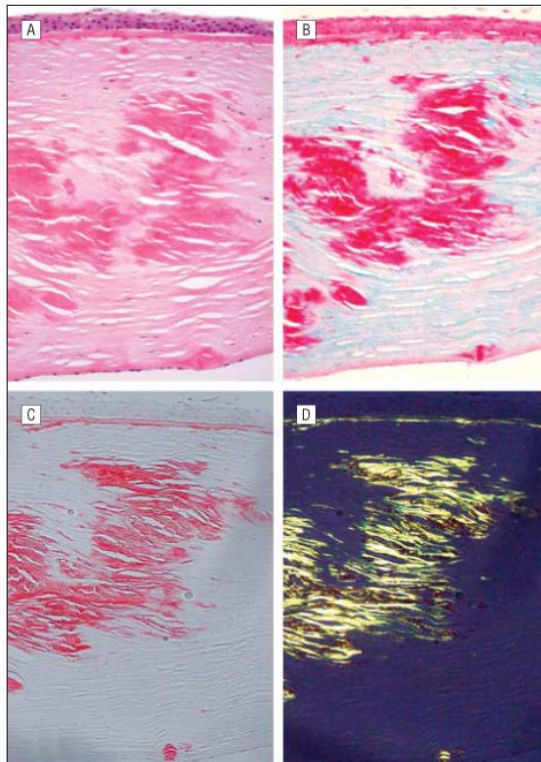
**Figure 4.**
Histopathologic examination of the excised corneal button from the right eye of the proband. Large eosinophilic deposits are noted in the anterior, mid, and posterior corneal stroma (A, hematoxylin-eosin, original magnification ×200). The deposits noted stain brightly with Masson trichrome (B, original magnification ×200) and Congo red (C, original magnification ×250). The Congo red-stained section demonstrates dichroism with the use of polarizing filters (D), produced by the presence of amyloid fibrils in the stromal deposits.
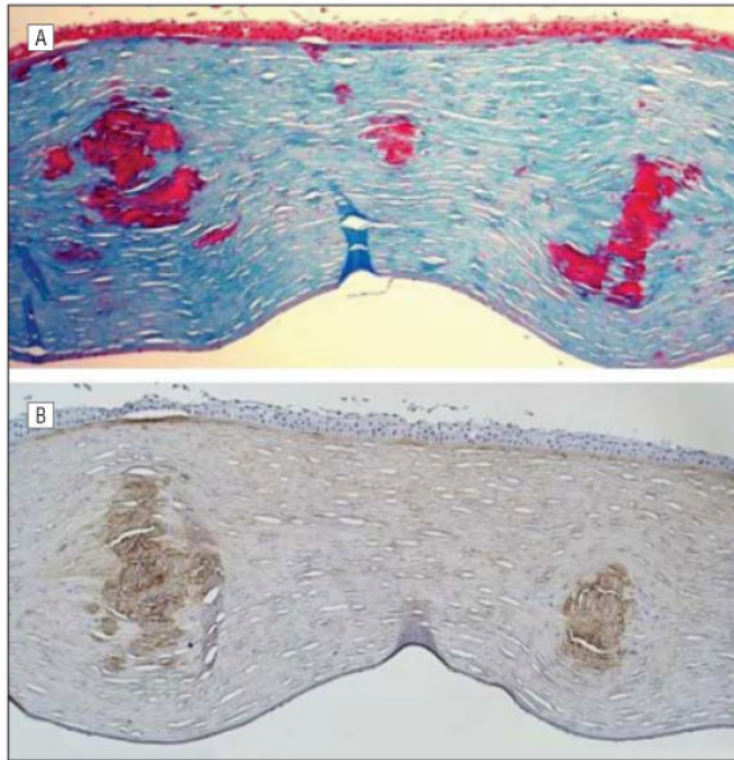
**Figure 5.**
Histopathologic and immunohistochemical analysis of the excised corneal button from the left eye of the proband. A, Large stromal deposits stain bright red with Masson trichrome (original magnification ×200). B, The same deposits also stain with an antibody to transforming growth factor β–induced (TGFBI) protein, indicating that the deposits represent focal aggregations of the protein product of the *TGFBI* gene (original magnification ×200).