



Unilateral lattice corneal dystrophy associated with the novel His572del mutation in the *TGFBI* gene

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Purpose: To report a novel mutation in the *TGFBI* gene, c.1761_1763del (p.His572del), associated with a unilateral variant of lattice corneal dystrophy (LCD).

Methods: A 63-year-old man presenting with the complaint of decreased vision in one eye was noted to have a unilateral lattice corneal dystrophy. Examination of the patient's wife and two sons, ages 20 and 27 years old, failed to reveal the presence of any corneal opacities. Following the collection of DNA from the patient and his family members, the *TGFBI* gene was screened for mutations previously associated with lattice corneal dystrophy and any novel coding region changes.

Results: In the affected patient, none of the mutations previously associated with the classic and variant forms of LCD were identified. However, a novel mutation, c.1761_1763del (p.His572del), was identified in exon 13 of *TGFBI* in the patient and his sons. This mutation was not identified in the patient's wife or in 200 control chromosomes.

Conclusions: The novel *TGFBI* gene mutation (p.His572del) is associated with a unilateral, late-onset variant of lattice corneal dystrophy. This case highlights the utility of molecular genetic analysis in differentiating corneal dystrophies associated with an atypical phenotype from nondystrophic conditions.

Lattice corneal dystrophy type I (CDL1, LCD; OMIM 122200) has been associated with a large number of missense mutations in the transforming growth factor beta induced (*TGFBI*) gene. While all corneal dystrophies, including the *TGFBI* dystrophies, typically present with bilateral, symmetric involvement, unilateral and markedly asymmetric variants of LCD have been reported [1-3]. We report the case of a patient with decreased vision but no history of recurrent corneal erosions who presented with a unilateral, late-onset variant of LCD. Screening of all 17 exons of *TGFBI* revealed a novel mutation, c.1761_1763del, in exon 13 that resulted in a deletion of the amino acid histidine at codon 572. This case represents the first report of this novel mutation in the *TGFBI* gene, the second report of a pathogenic mutation in exon 13 of *TGFBI*, and adds to the number of mutations associated with the late-onset, unilateral LCD phenotype.

METHODS

Report of a case: A 63-year-old man was referred for evaluation of unilateral LCD in the right eye. He complained of a gradual decline in the vision in the right eye for several years and was noted to have branching stromal opacities in the right cornea three years previously. The patient denied previous contact lens wear, ocular trauma, or a personal or family history of recurrent corneal erosions, and he was unaware of a family history of corneal disease (Figure 1).

Corrected visual acuity measured 20/30 OD and 20/15 OS. The right cornea demonstrated linear, branching, anterior

and midstromal opacifications with clear intervening stroma (Figure 2A,B). The left cornea was devoid of any opacities (Figure 3).

Neither the patient's wife nor either of his two sons, ages 20 and 27 years old, demonstrated any corneal deposits. As both parents and one of the patient's three siblings were deceased, and his other two siblings lived in Germany, none were available for examination or DNA analysis.

DNA collection and preparation: After institutional review board approval was granted (UCLA IRB 94-07-243-22B), informed consent was obtained from the patient, his wife, and two sons. Buccal epithelial swabs were collected from each using CytoSoft CP-5B brushes (Medical Packaging Corporation, Camarillo, CA). Genomic DNA was prepared from the buccal epithelial cells using the QIAamp DNA Mini Kit spin protocol (Qiagen, Valencia, CA). DNA collected from 100 unaffected healthy volunteers served as controls.

PCR amplification: All 17 exons of the *TGFBI* gene were amplified from the patient's DNA using custom-designed oligonucleotide primers (Table 1). After identification of the c.1761_1763del in the patient, exon 13 of the *TGFBI* gene was amplified from his family members' DNA. Each reaction was carried out in a 25 μ l mixture containing 12.5 μ l of MasterAmp PCR 2X PreMix "D" (100 mM Tris-HCl pH 8.3, 100 mM KCl, 400 μ M of each dNTP, proprietary concentrations of MgCl₂, and MasterAmp PCR Enhancer; Epicentre, Madison, WI), 0.12 μ M of each primer, 1.5 units of RedTaq Genomic DNA polymerase (Sigma-Aldrich Corp., St. Louis, MO), and approximately 100 ng of genomic DNA. Thermal cycling was performed in an iCycler Thermal Cycler (Bio-Rad, Hercules, CA).

DNA sequencing: Purification of the PCR products was achieved by incubating 15-30 ng DNA with 5 units of Exonu-

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clease I and 0.5 units of Shrimp Alkaline Phosphatase (USB Corp., Cleveland, OH) for 15 min at 37 °C. After inactivation of the nucleases (80 °C for 15 min), sequencing reactions were performed by the addition of 2 µl BigDye Terminator Mix version 3.1 (Applied Biosystems, Foster City, CA), 2 µl of SeqSaver (Sigma-Aldrich) and 0.2 µM primer. Samples were denatured at 96 °C for 2 min, then cycled 25 times at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Unincorporated nucleotides were removed using the CleanSeq reagent and a SPRI

plate (Agencourt Bioscience Corporation, Beverly, MA) following the manufacturer's instructions and then analyzed on an ABI-3100 Genetic Analyzer (Applied Biosystems) after resuspension in 0.1 mM EDTA. Nucleotide sequences were compared with the published *TGFBI* cDNA sequence (GenBank NM_000358).

Polymerase chain reaction restriction fragment length polymorphism: After identification of the c.1761_1763del in the patient, a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was performed to screen for the deletion in 100 control individuals. This mutation abolishes a site recognized by the restriction enzyme *Msi*I (NEB, Beverly, MA), permitting distinction of sequence variants from the wild type. PCR products of the control DNAs were digested with the restriction enzyme and resolved on a 2% agarose gel. The products were then visualized with SYBR

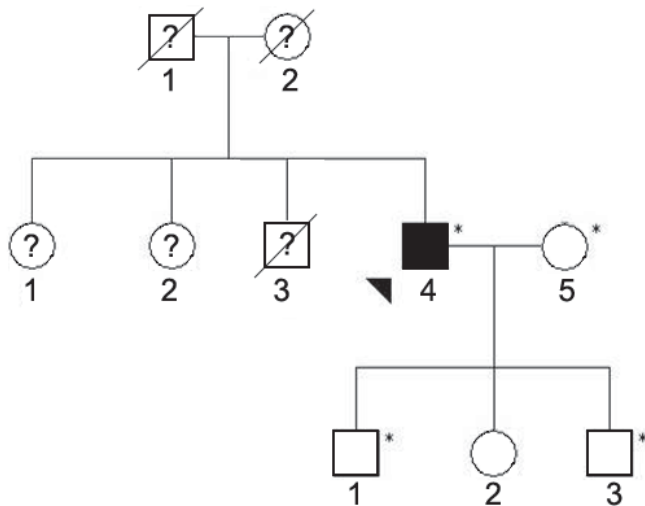


Figure 1. Pedigree of family with unilateral lattice corneal dystrophy. The arrowhead indicates the proband. The black symbol indicates the affected individual, unfilled symbols represent unaffected individuals, question marks denote those individuals who were not examined, but were considered unaffected based on history. Asterisks indicate those individuals who were examined and in whom DNA analysis was performed.

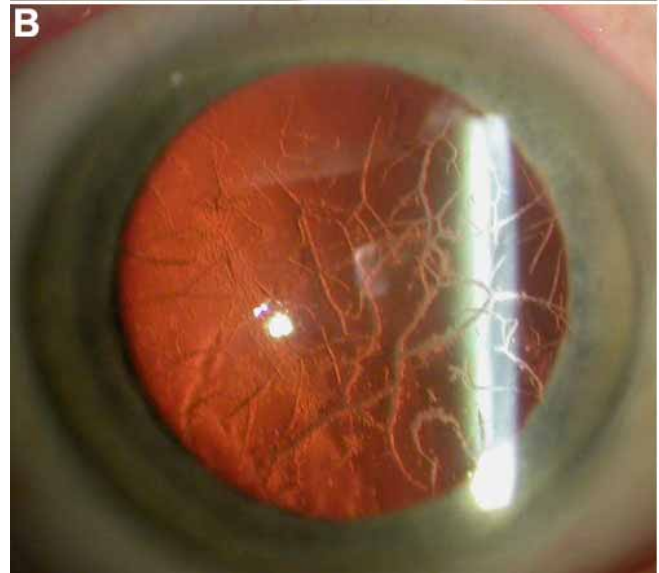
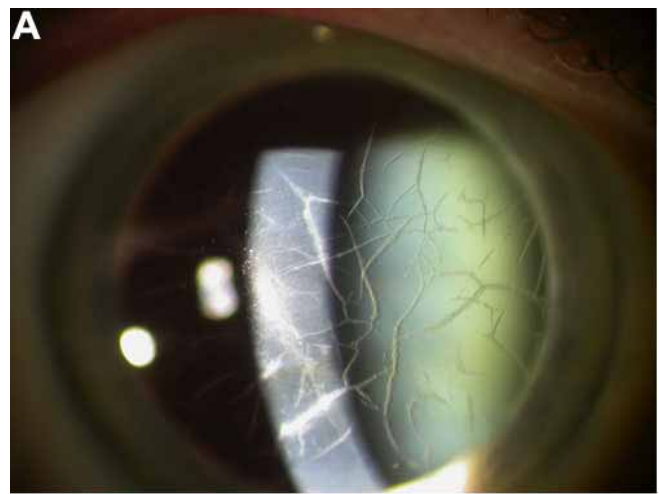


Figure 2. Unilateral lattice corneal dystrophy. Shown in this slit lamp photomicrograph of the right cornea are thin, branching, diffusely distributed anterior stromal lattice lines, seen with both direct (A) and indirect (B) illumination. This pattern is consistent with the corneal changes in lattice corneal dystrophy type 1.

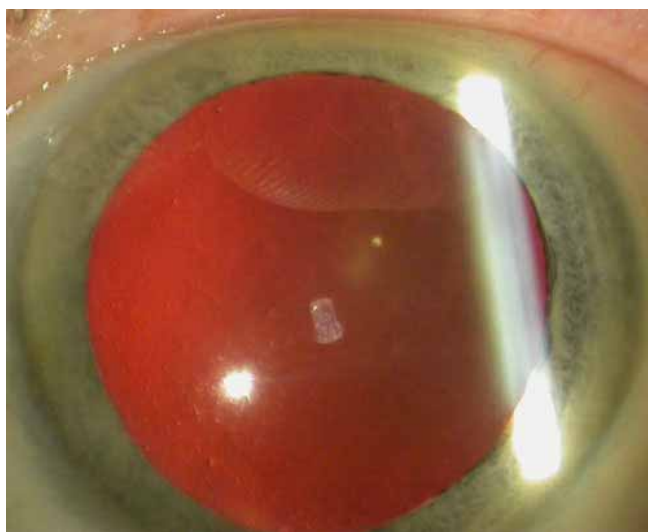


Figure 3. Clear cornea in patient with contralateral lattice corneal dystrophy. Evident in this slit lamp photomicrograph of the left cornea, seen in retroillumination, is an absence of any corneal opacities.

Green 1 staining. (Molecular Probes, Eugene, OR)

RESULTS

None of the mutations previously associated with either the classic or variant forms of LCD were identified in the patient. However, a novel deletion, c.1761_1763del, which resulted in an in-frame deletion of a single amino acid (p.His572del), was identified in the heterozygous state in exon 13 (Figure 4). This deletion was not identified in the patient’s wife, but was identified in both of the patient’s sons. The only other allelic variant noted in the patient was a previously identified polymorphism, Leu217Leu (refSNP ID: rs1442), in exon 6. The c.1761_1763del mutation was not identified in any of 200 control chromosomes.

DISCUSSION

The identification of the *TGFBI* gene and characterization of the mutations associated with both the classic and variant forms

of LCD have given clinicians the ability to definitively differentiate between the various manifestations of LCD and the myriad presentations of pseudolattice-corneal dystrophy [4-6]. In the case we report, initial screening of the *TGFBI* gene did not reveal any pathogenic mutations previously associated with classic and variant forms of LCD [7,8]. However, complete screening of all 17 exons of the *TGFBI* gene revealed a presumed pathogenic mutation in exon 13, resulting in the deletion of histidine at codon 572. The only other mutation previously reported in exon 13 of the *TGFBI* gene was a single base pair substitution (c.1753T>G) resulting in an amino acid substitution at codon 569 (p.Leu569Arg) that was also associated with lattice corneal dystrophy, albeit the classic CDL1 phenotype [8]. The association of the p.Leu569Arg mutation with the classic LCD type I phenotype, which is typically associated with the p.Arg124Cys mutation [7,8], and the association of the p.His572del mutation with the late onset, unilateral LCD phenotype, which has been previously identi-

TABLE 1. PRIMERS SEQUENCES USED FOR *TGFBI* AMPLIFICATION

Exon	Forward primer	Reverse primer	Product size (bp)	Exon (bp)
1	CGGAGGCGCTCTCACTTCC	CGAGCCCCGACTACCTGACC	265	181
2	GGGAGTCATTAAGTGGGGTGA	AGCTTGGTCTCCTGGCTGGTTAC	196	99
3	CAACTTAGTGAGAGGGGCCAGA	CTCTCTCCCACCATTCCCTTCC	206	65
4	GCCATCCCTCCTTCTGTCTCTG	CCGGGCAGACGGAGGTCATC	217	161
5	ACTGACACCTGTCTTCTCCT	AGCCACACATGGAACAGAAATG	261	165
6	CTGCTCATCTTGCTGCTTCTCT	AGAGTTCCTGCTAGGCCCTTCT	249	147
7	TCTGTGGGGAGTGCCAGAGTC	CAAATGAGGCAGCAGCAGGA	234	142
8	TGGACCTGACTTGACCTGAGTC	AAAGGATGGCAGAAGAGATGGTG	311	213
9	CCCTGGGGTGGATGAATGATAAA	GCCTCCAGGGACAATCTAACAGG	251	138
10	ATTGCAGGAGCACATCTCTTGG	GCTTCCCAGGAGCATGATTTAGG	230	146
11	GCCCTCGTGAAGTATAACCAG	ATCCCACTCCAGCATGACCACT	248	137
12	TGACAGGTGACATTTTCTGTGTG	GGGCCCTGAGGGATCACTACTTT	224	131
13	TGACCAGGCTAATTACCATTCTTGG	CAGCCTTTGATTTGCAGGACACT	210	125
14	TGCTCTACTTTCAACCACTACTCTG	CCAACCTGCCACATGAAGAAAAGG	198	103
15	TCACTCTGGTCAAACCTGCCTTT	CCTCTATGGCCAAACAGAGGAC	183	80
16	GCCATTGCATAAGCAGTTGCAG	ATACAGCAGATGGCAGGCTTGG	176	25
17	TGGGGAGATCTGCACCTATTTGA	GGTCAGCACACTGTACCATGCAC	710	633

All 17 exons of the *TGFBI* gene were amplified from the patient’s DNA using custom-designed oligonucleotide primers.

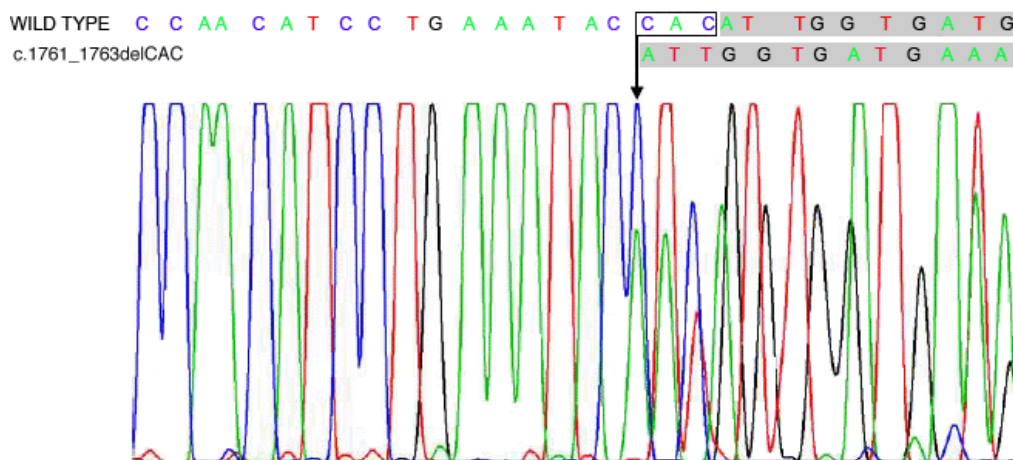


Figure 4. Sequence chromatogram from exon 13 of the *TGFBI* gene, demonstrating the region around codon 572. The top row of letters represents the wild type nucleotide sequence; codon 572 is designated by the three boxed nucleotides CAC. The bottom row represents the mutated strand, with codon 572 deleted. Shading of both rows highlights the nucleotide sequence 3’ of the deleted codon, which is identical on each strand.

fied with the Leu527Arg, Asn622His, and His626Arg mutations [1,3], underscore the need to screen the entire coding region prior to excluding a *TGFBI* corneal dystrophy.

Evidence to support the pathogenicity of the 3 base pair in-frame deletion, c.1761_1763del, is provided by previous descriptions of two other in-frame deletions in the *TGFBI* gene associated with variants of granular (p.Thr125_Glu126del) [9] and lattice (p.Val624_Val625del) [10] corneal dystrophies. In contrast to the novel mutation we report, these deletions involved two adjacent amino acids, and although both were associated with an atypical phenotype, neither was associated with unilateral involvement. However, these reports demonstrate that in-frame deletions in the *TGFBI* gene, in addition to the more commonly described missense mutations, may be associated with the development of lattice and granular corneal dystrophies. Additionally, the absence of the c.1761_1763del allelic variant in 200 control chromosomes provides further evidence that this mutation is associated with the observed unilateral LCD phenotype. The identification of the same deletion in the patient's unaffected 20- and 27-year-old sons is not surprising given the late onset of the disease phenotype in the patient. Unfortunately, the siblings and parents of the patient were not available for examination to perhaps provide a more complete characterization of the clinical features associated with the c.1761_1763del mutation.

The discovery of this novel mutation, of course, does not explain why the dystrophy that we describe, or any corneal dystrophy for that matter, is associated with unilateral involvement when the epithelial cells and keratocytes of the contralateral cornea harbor the same *TGFBI* gene mutation. The answer likely resides in the influence of modifier genes and environmental effects. The wide variation in the affected phenotype that has been demonstrated both between families sharing a common mutation in the *TGFBI* gene and between members of a single family with similar environmental influences suggests a role for both environmental factors and modifier genes [11-14]. Stewart and colleagues have proposed that factors such as the development of recurrent corneal erosions may explain the development of unilateral LCD, as they have noted the development of corneal amyloid deposition shortly after the onset of recurrent corneal erosions or other forms of corneal trauma [3]. Thus, a unilateral corneal abrasion or the development of unilateral or markedly asymmetric corneal erosions may precipitate the development of corneal amyloid deposition, with an uninvolved contralateral eye. The authors have also noted an association between disruption of the corneal epithelium, in association with recurrent corneal erosions and trichiasis, and nondystrophic, unilateral corneal stromal amyloid deposition [4,5]. Others have also noted an association between corneal injury in the form of LASIK surgery and a dramatic acceleration in the number and density of dystrophic corneal deposits, concentrated in the interface between the flap and residual stroma, in a series of patients with combined granular-lattice corneal dystrophy [15,16].

The patient that we report denied a history of recurrent corneal erosions, previous ocular surgery, or ocular trauma. Thus, the explanation for the unilateral nature of the corneal

changes remains enigmatic. The identification of modifier genes that may alter the expression of the *TGFBI* corneal dystrophies would provide not only a greater understanding of the factors that determine age-dependent expression and phenotype variability, but also a target for gene-based therapeutic interventions.

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REFERENCES

- Hirano K, Hotta Y, Nakamura M, Fujiki K, Kanai A, Yamamoto N. Late-onset form of lattice corneal dystrophy caused by leu527Arg mutation of the *TGFBI* gene. *Cornea* 2001; 20:525-9.
- Sridhar MS, Laibson PR, Eagle RC Jr, Rapuano CJ, Cohen EJ. Unilateral corneal lattice dystrophy. *Cornea* 2001; 20:850-2.
- Stewart H, Black GC, Donnai D, Bonshek RE, McCarthy J, Morgan S, Dixon MJ, Ridgway AA. A mutation within exon 14 of the *TGFBI* (*BIGH3*) gene on chromosome 5q31 causes an asymmetric, late-onset form of lattice corneal dystrophy. *Ophthalmology* 1999; 106:964-70.
- Aldave AJ, Lin DY, Principe AH, Yellore VS, Weissman BA. Anterior basement membrane corneal dystrophy and pseudo-unilateral lattice corneal dystrophy in a patient with recurrent corneal erosions. *Am J Ophthalmol* 2004; 137:1124-7.
- Aldave AJ, Principe AH, Lin DY, Yellore VS, Small KW. Lattice dystrophy-like localized amyloidosis of the cornea secondary to trichiasis. *Cornea* 2005; 24:112-5.
- Yoshida S, Yoshida A, Nakao S, Emori A, Nakamura T, Fujisawa K, Kumano Y, Ishibashi T. Lattice corneal dystrophy type I without typical lattice lines: role of mutational analysis. *Am J Ophthalmol* 2004; 137:586-8.
- Klintworth GK. The molecular genetics of the corneal dystrophies—current status. *Front Biosci* 2003; 8:d687-713.
- Warren JF, Abbott RL, Yoon MK, Crawford JB, Spencer WH, Margolis TP. A new mutation (Leu569Arg) within exon 13 of the *TGFBI* (*BIGH3*) gene causes lattice corneal dystrophy type I. *Am J Ophthalmol* 2003; 136:872-8.
- Dighiero P, Drunat S, D'Hermies F, Renard G, Delpech M, Valleix S. A novel variant of granular corneal dystrophy caused by association of 2 mutations in the *TGFBI* gene-R124L and DeltaT125-DeltaE126. *Arch Ophthalmol* 2000; 118:814-8.
- Chakravarthi SV, Kannabiran C, Sridhar MS, Vemuganti GK. *TGFBI* gene mutations causing lattice and granular corneal dystrophies in Indian patients. *Invest Ophthalmol Vis Sci* 2005; 46:121-5.
- Aldave AJ, Rayner SA, King JA, Affeldt JA, Yellore VS. A unique corneal dystrophy of Bowman's layer and stroma associated with the Gly623Asp mutation in the transforming growth factor beta-induced (*TGFBI*) gene. *Ophthalmology* 2005; 112:1017-22.
- Konishi M, Yamada M, Nakamura Y, Mashima Y. Varied appearance of cornea of patients with corneal dystrophy associated with R124H mutation in the *BIGH3* gene. *Cornea* 1999; 18:424-9.
- Rosenwasser GO, Sucheski BM, Rosa N, Pastena B, Sebastiani A, Sassani JW, Perry HD. Phenotypic variation in combined granular-lattice (Avellino) corneal dystrophy. *Arch Ophthalmol* 1993; 111:1546-52.
- Hellenbroich Y, Tzivras G, Neppert B, Schwinger E, Zuhlke C. R124C mutation of the betaIGH3 gene leads to remarkable phe-

- notypic variability in a Greek four-generation family with lattice corneal dystrophy type 1. *Ophthalmologica* 2001; 215:444-7.
15. Wan XH, Lee HC, Stulting RD, Kim T, Jung SE, Kim MJ, Kim EK. Exacerbation of Avellino corneal dystrophy after laser in situ keratomileusis. *Cornea* 2002; 21:223-6.
16. Jun RM, Tchah H, Kim TI, Stulting RD, Jung SE, Seo KY, Lee DH, Kim EK. Avellino corneal dystrophy after LASIK. *Ophthalmology* 2004; 111:463-8.