A Clinical and Histopathologic Examination of Accelerated TGFBIp Deposition After LASIK in Combined Granular-Lattice Corneal Dystrophy

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• PURPOSE: **To report the clinical and histopathologic features of accelerated TGFBI protein (TGFBIp) deposition after lamellar keratorefractive surgery in a patient with combined granular-lattice corneal dystrophy (CGLCD) who underwent bilateral corneal transplantation.**

• DESIGN: **Interventional case report.**

• METHODS: **A 28-year-old woman with a presumed TGFBI corneal dystrophy, but who retained best corrected visual acuity of 20/20 in each eye, underwent myopic laser-assisted in-situ keratomileusis (LASIK) both eyes (OU). For definitive diagnosis of the corneal dystrophy, buccal epithelial cells were collected as a source of genomic DNA and screening of *TGFBI* exons 4 and 12 was performed.**

• RESULTS: **Four months after the performance of an uncomplicated LASIK procedure, the patient's uncorrected visual acuity was 20/15 OU. Over the following two years, the appearance of confluent white stromal deposits at the LASIK flap interface resulted in disabling glare and a reduced best-corrected visual acuity of 20/40 OU. Corneal transplantation was performed in each eye, and histopathologic examination of the excised corneal buttons was performed. Eosinophilic material that stained positively with the Masson trichrome stain was present in the LASIK flap interface, as well as in the stroma of the flap and the anterior portion of the stromal bed. No amyloid deposits were identified with the Congo red stain. Screening of *TGFBI* exons 4 and 12 revealed the Arg124His mutation associated with CGLCD.**

• CONCLUSIONS: **Accelerated deposition of TGFBIp may occur after lamellar corneal surgery in patients with CGLCD. Therefore, LASIK surgery should be avoided in patients with any of the TGFBI dystrophies, and surgeons should be aware of the potential for rapid interface TGFBIp deposition after lamellar corneal surgery. (Am J Ophthalmol 2007;143:416–419. © 2007 by Elsevier Inc. All rights reserved.)**

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COMBINED GRANULAR-LATTICE CORNEAL DYSTROphy (CGLCD, MIM no. 607541) is an autosomal dominant disorder associated with the highly conserved Arg124His mutation in the transforming growth factor, beta-induced gene (*TGFBI*, MIM no. 601692). As is the case with each of the TGFBI corneal dystrophies, the mutated protein product of *TGFBI* (TGFBIp) is a major component of the corneal deposits that characterize CGLCD.[1–4] Although affected patients will often demonstrate discrete granular opacities in the first several decades of life, stromal lattice deposits may not appear until later in life, and often do not present as characteristic stromal lattice lines, leading to these patients being misdiagnosed as having granular corneal dystrophy.[5,6] Because patients with CGLCD may retain excellent corrected visual acuity for decades after the initial appearance of corneal deposits, affected patients have been considered candidates for keratorefractive surgery. However, exacerbation of CGLCD has been reported after laser-assisted in-situ keratomileusis (LASIK) in nine Korean patients, who developed decreased vision one to six years after uncomplicated LASIK surgery, and one Caucasian North American patient, who developed decreased vision 14 months after uneventful LASIK surgery.[7–10] Although light and scanning electron microscopic analysis of the surgically amputated corneal flap was preformed in three patients, because none of the reported patients required a penetrating keratoplasty for visual rehabilitation, histopathologic analysis of the corneal flap, interface, and posterior stroma has not been reported previously. In this report, we present the clinical and histopathologic features of accelerated TGFBIp deposition after LASIK in a patient with confirmed CGLCD who underwent bilateral corneal transplantation.

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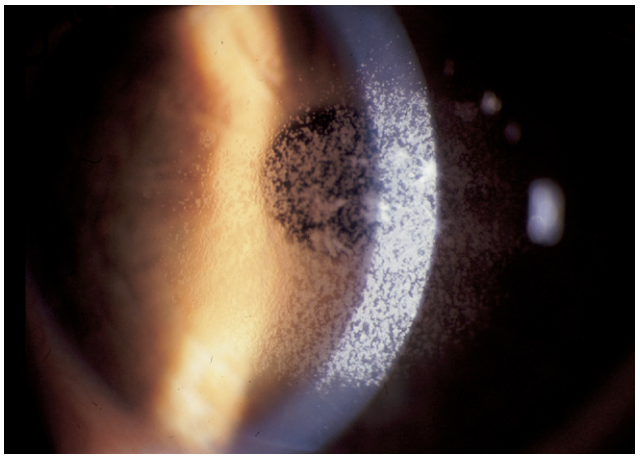

FIGURE 1. Slit-lamp photomicrograph of the right cornea of a patient with combined granular-lattice corneal dystrophy (CGLCD), taken two years after laser-assisted in-situ keratomileusis (LASIK) surgery. Confluent central corneal opacities are seen, some of which demonstrate stellate configurations, characteristic of CGLCD.

METHODS

THE RESEARCHERS FOLLOWED THE TENETS OF THE DECLARation of Helsinki in the treatment of the subject reported herein. After Institutional Review Board (IRB) approval was granted (UCLA IRB no. 94-07-243-23), informed consent was obtained from the patient.

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

• **DNA COLLECTION AND ANALYSIS:** Buccal epithelial swabs were collected from the patient using CytoSoft CP-5B brushes (Medical Packaging Corporation, Camarillo, California, USA). Genomic DNA was prepared from the buccal epithelial cells using the QIAamp DNA Mini Kit spin protocol (Qiagen, Valencia, California, USA). Polymerase chain reaction amplification and automated sequencing of exons 4 and 12 of the *TGFBI* gene was performed using previously described primer sequences and conditions.[11] Nucleotide sequences were compared with the published *TGFBI* cDNA sequence (GenBank accession no. NM_000358).

RESULTS

A 28-YEAR-OLD WOMAN WITH MODERATE MYOPIA AND A presumed TGFBI corneal dystrophy presented to one of the authors (S.L.F.) for a refractive surgery evaluation. The

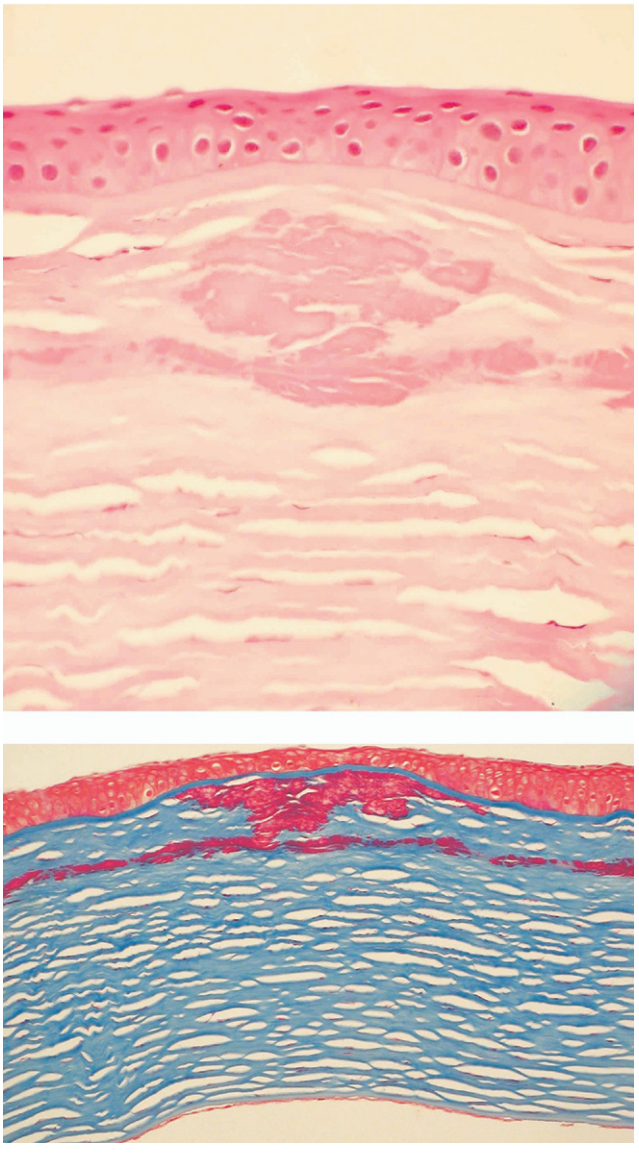

FIGURE 2. Histopathologic examination of the excised corneal button from a patient with combined granular-lattice corneal dystrophy [CGLCD]. (Top) Eosinophilic deposits are noted beneath the Bowman layer and in a linear pattern in the anterior stroma (corresponding to the laser-assisted in-situ keratomileusis [LASIK] flap interface), (hematoxylin and eosin stain, original magnification ×250). (Bottom) The anterior stromal deposits stain brightly with the Masson trichrome stain, consistent with the diagnosis of CGLCD (Masson trichrome stain, original magnification ×200).

patient's family history was significant for her Korean father having been diagnosed with an inherited corneal disorder, although no history of corneal abnormalities was present in her mother's family, which was Caucasian. Because the scattered, discrete, white anterior stromal corneal deposits were not felt to be visually significant, and the patient's spectacle-corrected visual acuity was 20/20 in both eyes, she underwent an uncomplicated LASIK procedure in each eye, obtaining uncorrected visual acuity of

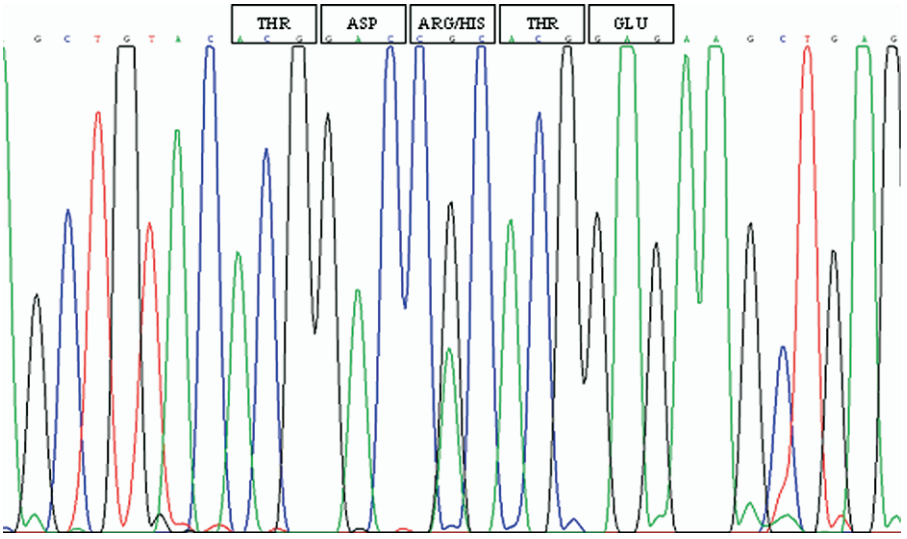

FIGURE 3. Sequence chromatogram from exon 4 of *TGFBI* in a patient with combined granular-lattice corneal dystrophy (CGLCD), demonstrating the region around codon 124. A single heterozygous nucleotide change in this codon results in a change in the encoded amino acid from arginine to histidine.

20/15 in each eye four months after the procedure. Over the following two years, however, the patient developed incapacitating glare, and her visual acuity decreased to 20/40 in each eye secondary to the progressive deposition of confluent white deposits in the anterior corneal stroma of each eye (Figure 1).

Bilateral penetrating keratoplasties were performed and the excised corneal specimens were submitted for histopathologic examination. Hematoxylin and eosin staining of the excised corneal buttons revealed eosinophilic deposits in the stroma of each LASIK flap, the majority of which were contained within the stroma of the flap, but some of which extended from the Bowman layer to the flap interface. The flap interface was clearly defined by the presence of a lamellar, continuous eosinophilic deposition, although it was not possible to determine whether the deposits were located within the potential space of the flap interface, in the stroma on either side of the interface, or both (Figure 2, Top). Only a single eosinophilic deposit was identified with the corneal stroma beneath, but not contiguous with, the flap interface. Each of these deposits stained positively with the Masson trichrome stain, indicating that the deposits were of the "granular phenotype" (Figure 2, Bottom). Use of the Congo red stain did not reveal the presence of any amyloid deposits associated with the "lattice phenotype" in either corneal button.

Screening of exons 4 and 12 of the *TGFBI* gene in the patient reveled a heterozygous c.418G>A nucleotide substitution in exon 4, resulting in the substitution of histidine for arginine at codon 124, confirming the diagnosis of CGLCD (Figure 3).

DISCUSSION

SIMILAR TO THE MAJORITY OF THE PREVIOUSLY REPORTED cases of the exacerbation of CGLCD after LASIK, the patient who we report developed a rapid, visually significant increase in the number and density of stromal deposits within two years of LASIK surgery.[7–10] Interestingly, the patient we report is also of Korean ancestry, which is not surprising because CGLCD is a common corneal dystrophy in the Korean population.[6] Unique to this report, however, are the results of histopathologic examination of an excised corneal button after LASIK in a patient with CGLCD. It is not surprising that the Congo red–stained sections did not reveal the presence of amyloid deposits, which may not be detected clinically or histopathologically in patients with confirmed CGLCD.[12,13] The demonstration of Masson trichrome–stained dystrophic deposits in the flap interface indicates that the deposition is a result of the lamellar corneal incision; accordingly, immunohistochemical analysis of human corneas after perforating corneal injuries has demonstrated an increased production of TGFBIp by keratocytes in the region of the corneal injury.[14] Other authors have hypothesized that the excimer laser ablation of the corneal stroma during LASIK surgery in patients with CGLCD may be primarily responsible for accelerated dystrophic deposition, pointing out that the axially distributed deposits are present only in the region of tissue ablation.[8] Although the deposits in the case that we report were also confined to the central 6 to 8 mm of each cornea, and did not extend to the margins of the lamellar interface, this is the typical distribution of the deposits in each of the TGFBI dystrophies, and does not necessarily imply a significant pathogenic role for the

excimer laser ablation. Additionally, that such rapid acceleration of TGFBIp deposition is not observed after laser phototherapeutic keratectomy in patients with CGLCD[15] indicates that the creation of the lamellar corneal incision is necessary for the phenomenon that we describe to occur. Therefore, because the material deposited in each of the TGFBI dystrophies consists of a mutated form of TGFBIp,[1–4] such an accelerated deposition would be expected in patients with any of the TGFBI dystrophies, and thus these patients should not be considered candidates for LASIK surgery. Additionally, surgeons should be aware of the potential for rapid TGFBIp deposition in the stromal interface following any form of lamellar corneal surgery in these patients, and should limit the depth of lamellar incisions as much as possible to allow repeat lamellar or photoablative procedures to be performed.

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