·Clinical Research·

TGFBI gene mutation analysis in a Chinese pedigree of Avellino corneal dystrophy

Ai-Rui Xie[1,2], Su-Ping Cai[1], Yin Yang[3], Yin-Chuan Fan[3], Wen-Han Yu[1], Li-Heng Guo[1], Qiao-Na Yang[1], Jin Zhu[2], Xu-Yang Liu[1]

Foundation item: Supported by National Natural Science Foundation of China(No.81000370)

[1]Ophthalmic Laboratories & Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
[2]The 363 Hospital, Chengdu 610041, Sichuan Province, China
[3]Department of Ophthalmology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610072, Sichuan Province, China
The first three authors contributed equally to this paper
Correspondence to: Xu-Yang Liu. Ophthalmic Laboratories and Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. xliu1213@yahoo.com.cn

Received:2011-05-04 Accepted:2011-05-19

Abstract

• AIM: To analyze phenotype and genotype of a Chinese pedigree with Avellino corneal dystrophy (ACD).

• METHODS: Complete ophthalmic examinations were performed on all the family members. Exons of TGFBI were amplified by polymerase chain reaction, sequenced, and compared with a reference database.

• RESULTS: A single heterozygous G>A(R124H) point mutation was identified in exon 4 of TGFBI in three affected members and two unaffected children who were offsprings of the affected members, but not in the other family members.

• CONCLUSION: Mutation R124H in TGFBI was identified in this pedigree and appeared to be the disease causing mutation. Atypical phenotype and low penetrance was observed in this pedigree.

• KEYWORDS: corneal dystrophy; corneal opacity; genetics; keratomileusis; LASIK
DOI:10.3980/j.issn.2222-3959.2011.03.13

Xie AR, Cai SP, Yang Y, Fan YC, Yu WH, Guo LH, Yang QN, Zhu J, Liu XY. TGFBI gene mutation analysis in a Chinese pedigree of Avellino corneal dystrophy. *Int J Ophthalmol* 2011;4(3):275-279

INTRODUCTION

Bilateral corneal dystrophy includes a group of genetically determined, non-inflammatory corneal diseases, which result in loss of corneal transparency and visual impairment [1,2]. Identified genes responsible for corneal dystrophies include TGFBI, GSN, K12, K3, M1S1, CHST6, COL8A2 and SLC4A11 [2]. Up to date, besides symptomatic treatment, cornea transplantation is still the most common surgical therapy [3]. Munier *et al*[4] identified the transforming growth factor beta-induced gene (TGFBI, OMIM 601692, formerly called BIGH3) on chromosome 5q31 responsible for these autosomal dominant corneal dystrophies. Sharing a common genetic origin, the hereditary corneal dystrophies linked to chromosome 5q31 and TGFBI gene include Reis-Bücklers corneal dystrophy (RBCD, also called Granular corneal dystrophy, type 3 [GCD3]), Thiel-Behnke corneal dystrophy (TBCD), Classic Lattice corneal dystrophy (LCD1), Granular corneal dystrophy, type 1 (GCD1) and type 2 (GCD2) [1,4].

Avellino corneal dystrophy (ACD), known as GCD2, is one of the TGFBI associated corneal dystrophies, of which the clinical aspect is the coexistence of granular and amyloid deposits in the cornea [5]. It is characterized clinically by corneal opacities that are shaped like rings, disks, stars, and snowflakes. Some studies showed that mutations in the BIGH3 gene resulted in abnormal keratoepithelin[6]. In 5q31 linked corneal dystrophies, corneal keratocytes and/or epithelial cells expressed abnormal keratoepithelin, which interacted with proteoglycans, keratin, and other extracellular proteins leading to various stromal deposits[7]. Early symptoms of ACD occur during the first or second decade of life. Linear opacities may be present [8,9], but the typical lines of LCD are usually absent. Compared to GCD-I, the progression of ACD is delayed and slower and the visual acuity is less impaired. In the absence of a histopathologic evaluation or an examination of the molecular genetic defect, ACD can be difficult to be distinguished from GCD-I. In Japanese individuals, ACD is the most common hereditary corneal dystrophy, responsible for 72% of corneal dystrophies associated with TGFBI [10]. Its homozygous phenotype is more severe than that of heterozygous mutation. The most frequently reported sites of mutations are at positions 124 and 555 of the transforming growth

Table 1 Primers used in Polymerase Chain Reaction for amplification of TGFBI

Exon		Sequence (5'→3')	Annealing temperature(℃)
1	Forward:	GCTTGCCCGTCGGTCGCTA	62
	Reverse:	TCCGAGCCCCGACTACCTGA	
2	Forward:	AGGCAAACACGATGGGAGTCA	60
	Reverse:	TAGCACGCAGGTCCCAGACA	
3	Forward:	CCAGATGACCTGTGAGGAACAGTGA	60
	Reverse:	CCTTTTATGTGGGTACTCCTCTCT	
4	Forward:	TCCTCGTCCTCTCCACCTGT	58
	Reverse:	CTCCCATTCATCATGCCCAC	
5 and 6	Forward:	CCTGGGCTCACGAGGGCTGAGAACAT	64
	Reverse:	GCCCCTCTTGGGAGGCAATGTGTCCC	
7	Forward:	GTGAGCTTGGGTTTGGCTTC	63
	Reverse:	ACCTCATGGCAGGTGGTATG	
8	Forward:	TGAGGTTATCGTGGAGTG	53
	Reverse:	CACATCAGTCTGGTCACA	
9	Forward:	ACTCACGAGATGACATTCCT	60
	Reverse:	TCCAGGGACAATCTAACAGG	
10	Forward:	TAGAAGATACCAGATGTTAAGG	56
	Reverse:	TGTCAGCAACCAGTTCTCAT	
11	Forward:	CCTGCTACATGCTCTGAACAA	58
	Reverse:	GAATCCCCAAGGTAGAAGAAAG	
12	Forward:	GACTCTACTATCCTCAGTGGTG	58
	Reverse:	ATGTGCCAACTGTTTGCTGCT	
13	Forward:	CATTAGACAGATTGTGGGTCA	60
	Reverse:	GGGCTGCAACTTGAAGGTT	
14	Forward:	GCGACAAGATTGAAACTCCAT	58
	Reverse:	CTCTCCACCAACTGCCACAT	
15	Forward:	CCCTCAGTCACGGTTGTT	58
	Reverse:	GGAGTTGCCTTGGTTCTT	
16	Forward:	CTTGCACAACTTATGTCTGC	58
	Reverse:	TGCACCATGATGTTCTTATC	
17	Forward:	AGTGAAGTTTCACAAACCAC	58
	Reverse:	CCACATTTGGGATAGGTC	

factor-beta-induced protein (TGFBIp) [11]. In this study, we conducted a clinical evaluation and molecular genetic analysis of TGFBI gene in a Chinese family of ACD with atypical phenotypes.

MATERIALS AND METHODS

Patients A three-generation Chinese family from Sichuan Province, China, with ACD was recruited to this study (Figure 1). This family includes five ACD patients and six unaffected relatives. This study was approved by a local institutional medical ethics committee, and informed consent conforming to the tenets of the Declaration of Helsinki was obtained from each of participants.

Methods

Clinical examination Snellen best-corrected visual acuity test, slit-lamp biomicroscopy, intraocular pressure measurement, and fundus examination were conducted by an experienced ophthalmologist for all subjects. Detailed clinical history such as the age of onset, initial signs and symptoms, progression of disease, and ocular therapeutic procedures was documented.

Molecular genetic analysis Peripheral blood was collected from all individuals involved in this study. Genomic DNA was extracted from leukocytes using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to standard protocol. DNA integrity was evaluated by 1% agarose gel electrophoresis. Exons of the TGFBI gene were amplified from genomic DNA of each participant by polymerase chain reaction (PCR). Briefly, PCR was performed using 30μL reaction mixtures, each containing 30-40 ng genomic DNA, 1.0 pmol of each of the forward and reverse primers (listed in Table 1), and 15μL 2×Taq Master Mix (SinoBio Biltech Co. Ltd, Shanghai, China). Cycling conditions included an initial denaturation at 94℃ for 5 minute, followed by 35 cycles of denaturation at 94℃ for 30 seconds, annealing at 52.6-64.3℃ for 30 seconds, extension at 72℃ for 30 seconds, and a final extension at

Table 2 Clinical feature in family members with the TGFB1 R124H mutation

Individual case	Gender	Age	Corneal Status	TGFBI genotype	Visual acuity at presentation	
					OD	OS
Ⅰ1	M	81	Affected	Wt/R124H	0.02	0.1
Ⅰ2	F	74	-	-	1.0	0.6/1.0
Ⅱ1	M	53	Affected	Wt/R124H	1.2	0.02
Ⅱ2	F	42	-	-	0.4/0.9	0.5/0.8
Ⅱ3	M	46	-	-	1.2	0.1/1.0
Ⅱ4	F	50	-	-	1.2	1.2
Ⅱ5	M	43	-	-	1.2	1.2
Ⅱ6	F	44	Affected	Wt/R124H	0.8/0.8	0.8/1.0
Ⅲ1	M	18	Unaffected	Wt/R124H	0.8/1.0	0.8/1.0
Ⅲ2	F	18	-		0.15/1.0	0.15/1.0
Ⅲ3	F	14	Unaffected	Wt/R124H	1.2	1.5

72℃ for 5 minutes. The amplified products were purified with a cycle-pure kit (OMEGA, Bio-Tek, USA) and sequenced on a ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA). Nucleotide sequences were compared with the wild type *TGFBI* sequence (GenBank NG_012646.1).

RESULTS

Clinical Findings Clinical features of the family members are summarized in Table 2. The proband(patient Ⅱ:6, Figure 1) , a 44-year-old female, was asymptomatic but presented with bilateral scattered grayish small dot, annular and snowflake-like opacities in the subepithelial area and Bowman's layer in the central cornea, grayish linear opacity in the stroma anteriorly in her left eye with peripheral cornea unaffected. Her physical examination was otherwise normal.. Her best corrected visual acuity was 0.8 OD and 1.0 OS (Figure 2A).

The second patient (Patient Ⅰ:1,Figure 1) was proband's father (81-year-old), who was asymptomatic. Corneal examination revealed a few grayish spot-like confluent opacities in the anterior stroma and one or two granular deposits in the middle stroma of the central cornea (Figure 2B). The best corrected visual acuity was 0.02 OD and 0.1 OS, likely due to age-related macular degeneration.

Patient Ⅱ:1 (Figure 1) was proband's 53-year-old brother. Slit lamp examination showed a few spot-like opacities in his left cornea (Figure 2C), and nearly no opacities were found in his right cornea (Figure 2D). His best corrected visual acuity was 1.2 OD and 0.02 OS, due to high myopia in his left eye.

In addition, 2 younger family members (III: 1, 18-year-old; III: 3, 14-year-old, Figure 1) who were the descendants of the affected father or mother, presented with no changes in the corneas. Their visual acuity was 1.0 OD/1.0 OS and 1.2 OD/1.5 OS respectively, and they carried the same variation in the heterozygous state.

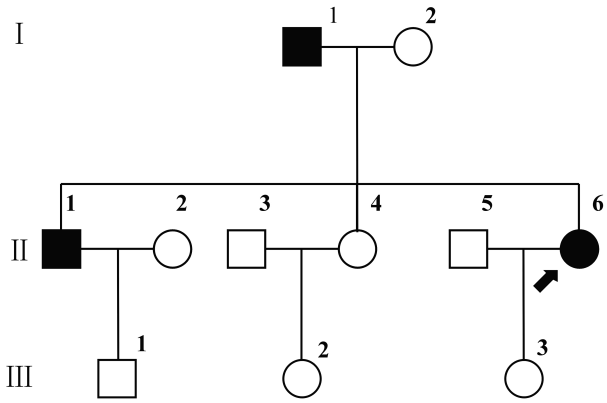

Figure 1 The pedigree of the family with Avellino corneal dystrophy (ACD). The arrow indicates the proband. The filled circle or square indicates the affected individual

TGFBI gene analysis Seventeen exons of TGFBI were analyzed by direct sequencing for the affected and unaffected members of this pedigree. A heterozygous mutation T>A in exon 4 which caused an amino acid substitution from Arginine (CGC) to Histidine (CAC) at codon 124 (R124H) (Figure 3), was identified in all affected members and their children, who were unaffected by the time the study was done, as shown in Figure 1 and Table 1).

DISCUSSION

Granular-lattice dystrophy, also known as Avellino corneal dystrophy, consists of both granular and lattice changes in the same cornea. The disorder appears to progress with age. To date, most cases studied with molecular genetic techniques for ACD have had the R124H mutation in TGFBI, but the phenotype of affected individuals varied markedly in severity from family to family [12-16]. Typically, granules develop at a younger age and lattice occurs later in life. Granules become visible and reach their mature quantity early, and remain nearly stationary in size. Lattice changes occur gradually, increase proportionally with age. Affected offsprings appear to have fewer lattice changes than their parents [9]. In our study, we found three affected

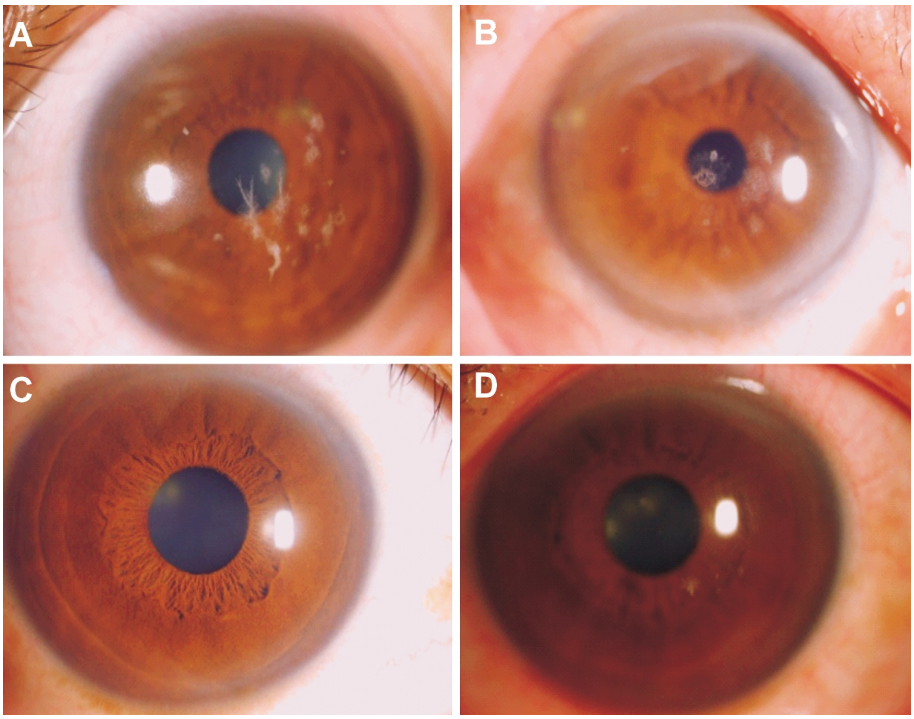

Figure 2 Slit-lamp photomicrographs A: The proband Ⅱ:6; B: The father of the proband Ⅰ:1; C, D: The brother of the proband Ⅱ:1

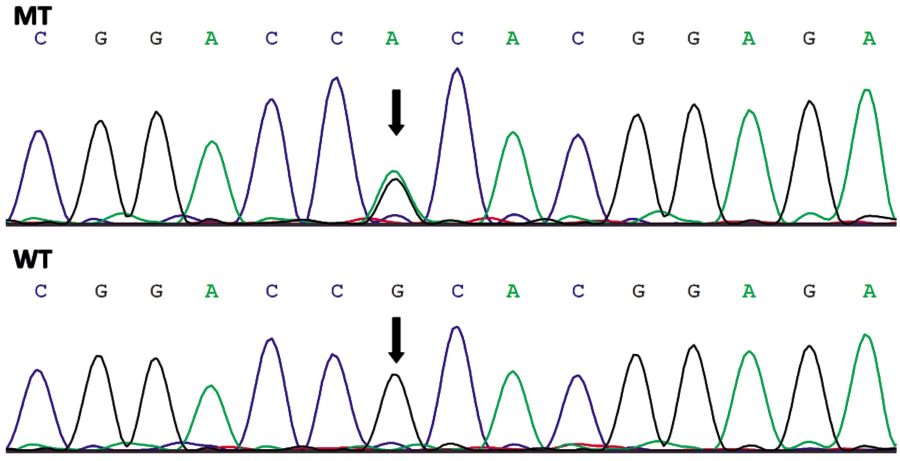

Figure 3 Sequence analysis showed heterozygous mutation consisting of a G>A transversion in exon 4(red arrow)

family members and two unaffected family members carried the same heterozygous TGFBI R124H mutation. The father (Ⅰ:1) exhibited granular changes only, while his daughter, the proband (Ⅱ:6) appeared to have both granular and lattice changes. His son (Ⅱ:1) who carried the same variation in heterozygous state only had a few spot-like opacities in the left cornea of his eye. Phenotypically, the pedigree we documented here exhibited atypical features of ACD.

In the pedigree, particularly, the patient (Ⅱ:1, Figure 2C, D), a 53-year-old male, had only a few spot-like opacities in his left eye, but presented no detectable changes in the other eye by the time the study was done. Therefore, the phenotypes were mild and presented differently in his eyes. Moreover, we also found two individuals (III: 1, III: 3) who carried the same heterozygous R124H mutation presented no changes to their corneal transparency and visual acuity at the age of 18 and 14 respectively. Therefore we reported the intrafamilial clinical variability observed in this pedigree with TGFBI dystrophy which 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. On the other hand, it is proposed that there might be some modifier genes which could influence the phenotype of the disease gene, which means TGFBI might have some modifier genes that could ameliorate the effect of the mutated allele [17]. The modifier genes could play an important role in phenotypic

variability in monogenic disorders [17]. However, the genetic interactions between modifier genes and the causative genes in corneal dystrophies still remain to be elucidated, although the causative genes have been identified. Thus, the molecule mechanism and the pathogenesis of the diseases are worthy of further studies. Revealing the mechanism and identifying the modifier gene is critical to understand the pathogenesis of the disease.

Today, excimer laser surgery is becoming one of the most effective treatments for corneal dystrophy and ametropic patients. However Avellino dystrophy was considered as a contraindication for laser-assisted in-situ keratomileusis (LASIK) surgery because of the increased deposition of granular material and a decrease in best-corrected visual acuity. Recurrence of ACD as well as granular and lattice dystrophies has been documented after treatment by excimer laser phototherapeutic keratectomy (PTK) and Photo Refractive Keratectomy (PRK) [18, 19], and worsening of ACD after laser in situ keratomileusis (LASIK) has also been reported [20-25]. Studies show that keratoepithelin is secreted by keratocytes after trauma. The accumulation of deposits at the LASIK flap interface in the laser ablation zone is thought to be the result of keratocyte stimulation from the microkeratome and laser ablation or the formation of a potential space at the flap-stromal interface as a result of the surgery [26]. For our pedigree with atypical phenotype and low penetrance, without molecular genetic analysis, it would be difficult to have an accurate genetic counseling and preoperative diagnosis.

In conclusion, our study elucidated the correlation between genotype and phenotype of ACD. Molecular genetic analysis was used as an effective means for future clinical diagnosis. It also enhanced our understanding of corneal dystrophy and showed the limitations of the current phenotypic method of corneal dystrophy diagnosis.

Acknowledgements: We thank the family for their cooperation and continued interest.

REFERENCES

1 Weiss JS, Møller HU, Lisch W, Kinoshita S, Aldave AJ, Belin MW, Kivelä T, Busin M, Munier FL, Seitz B, Sutphin J, Bredrup C, Mannis MJ, Rapuano CJ, Van Rij G, Kim EK, Klintworth GK. The IC3D classification of the corneal dystrophies. *Cornea* 2008;27 Suppl 2:S1-83

2 Klintworth GK. Corneal dystrophies. *Orphanet J Rare Dis* 2009; 4:7

3 Yu J, Zhou LH, Lv L. Penetrating keratoplasty in the treatment of corneal dystrophy. *Journal of Clinical Ophthalmology* 2003;11:102

4 Munier FL, Korvatska E, Djemaï A, Le Paslier D, Zografos L, Pescia G, Schorderet DF. Kerato-epithelin mutations in four 5q31-linked corneal dystrophies. *Nat Genet* 1997;15:247-251

5 Klintworth GK. Advances in the molecular genetics of corneal dystropies. *Am J Ophthalmol* 1999;128:747-754

6 Afshari NA, Mullally JE, Afshari MA, Steinert RF, Adamis AP, Azar DT, Talamo JH, Dohlman CH, Dryja TP. Survey of patients with granular, lattice, Avellino,and Reis-Bucklers corneal dystrophies for mutations in the BIGH3 and gelsolin genes. *Arch Ophthalmol* 2001;119:16-22

7 Poulaki V, Colby K. Genetics of anterior and stromal corneal dystrophies. *Semin Ophthalmol* 2008; 23(1):9-17

8 Wenping Cao, Hongyan Ge, Xiaobo Cui, Lu Zhang, Jing Bai, Songbin Fu, Ping Liu. Reduced penetrance in familial Avellino corneal dystrophy associated with TGFBI mutations. *Molecular Vision* 2009;15:70-75

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; 111:1546-1552

10 Mashima Y, Yamamoto S, Inoue Y, Yamada M, Konishi M, Watanabe H, Maeda N, Shimomura Y, Kinoshita S. Association of autosomal dominantly inherited corneal dystrophies with BIGH3 gene mutations in Japan. *Am J Ophthalmol* 2000;130:516-517

11 Kannabiran C, Klintworth GK. TGFBI gene mutations in corneal dystrophies. *Hum Mutat* 2006;27(7):615-625

12 Eifrig DE Jr, Afshari NA, Klintworth GK. The clinical spectrum of granular corneal dystrophy caused by the R124H Mutation in the TGFBI Gene. *Invest Ophthalmol Vis Sci* 2004;45: E-Abstract 1516

13 Mashima Y, Imamura Y, Konishi M, Nagasawa A, Yamada M, Oguchi Y, Kudoh J, Shimizu N. Homogeneity of kerato-epithelin codon 124 mutations in Japanese patients with either of two types of corneal stromal dystrophy. *Am J Hum Genet* 1997;61(6):1448-1450

14 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;18:424-429

15 Zhang T, Yan N, Yu W, Liu G, Wu X, Lian J, Liu X. Molecular genetics of Chinese families with TGFBI corneal dystrophies. *Mol Vis* 2011 Feb 4;17: 380-387

16 Watanabe H, Hashida Y, Tsujikawa K, Tsujikawa M, Maeda N, Inoue Y, Yamamoto S, Tano Y. Two patterns of opacity in corneal dystrophy caused by the homozygous BIG-H3 R124H mutation. *Am J Ophthalmol* 2001;132:211-216

17 Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher S, Katsanis N. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature* 2006;439:326-330

18 Dogru M, Katakami C, Nishida T, Yamanaka A. Alteration of the ocular surface with recurrence of granular/Avellino corneal dystrophy after phototherapeutic keratectomy: report of five cases and literature review. *Ophthalmology* 2001;108: 810-817

19 Dinh R, Rapuano CJ, Cohen EJ, Laibson PR. Recurrence of corneal dystrophy after excimer laser phototherapeutic keratectomy. *Ophthalmology* 1999;106: 1490-1497

20 Jun RM, Tchah H, Kim TI, Stulting RD, Jung SE, Seo KY, Lee DH, Kim EK. Avellino corneal dystrophy after LASIK. *Ophthalmology* 2004;111:463-468

21 Lee WB, Himmel KS, Hamilton SM, Zhao XC, Yee RW, Kang SJ, Grossniklaus HE. Excimer laser exacerbation of Avellino corneal dystrophy. *J Cataract Refract Surg* 2007;33:133-138

22 Roh MI, Grossniklaus HE, Chung SH, Kang SJ, Kim WC, Kim EK. Avellino corneal dystrophy exacerbated after LASIK: scanning electron microscopic findings. *Cornea* 2006;25:306-311

23 Aldave AJ, Sonmez B, Forstot SL, Rayner SA, Yellore VS, Glasgow BJ. A clinical and histopathologic examination of accelerated TGFBIp deposition after LASIK in combined granular-lattice corneal dystrophy. *Am J Ophthalmol* 2007; 143:416-419

24 Chiu EK, Lin AY, Folberg R, Saidel M. Avellino dystrophy in a patient after laser-assisted *in situ* keratomileusis surgery manifesting as granular dystrophy. *Arch Ophthalmol* 2007;125:703-705

25 Banning CS, Kim WC, Randleman JB, Kim EK, Stulting RD. Exacerbation of Avellino corneal dystrophy after LASIK in North America. *Cornea* 2006;25: 482-484

26 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;21:223-226