



Mutation Analysis of the *TGFBI* Gene in Consecutive Korean Patients With Corneal Dystrophies

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Background: Mutations in the transforming growth factor β -induced gene (*TGFBI*) are major causes of genetic corneal dystrophies (CDs), which can be grouped into *TGFBI* CDs. Although a few studies have reported the clinical and genetic features of Korean patients with *TGFBI* CD, no data are available regarding the frequency and spectrum of *TGFBI* mutations in a consecutive series of Korean patients with clinically diagnosed CDs.

Methods: Patients with any type of CD, who underwent both ophthalmologic examination and *TGFBI* gene analysis by Sanger sequencing at a tertiary care hospital in Seoul, Korea from 2006 to 2013, were enrolled in this study.

Results: Among a total of 89 patients, 77 (86.5%) were diagnosed as having clinical *TGFBI* CD. Seventy-three out of 74 patients (98.6%) with granular CD type 2 (GCD2), had the p.R124H mutation. Of particular note, one patient with rapidly progressive CD had the p.R124H mutation as well as a novel nonsense variant with unknown clinical significance (p.A179*). In three patients with lattice CD type 1 (LCD1), one known mutation (p.R124C) and two novel variants (p.L569Q and p.T621P) in the *TGFBI* gene were identified.

Conclusions: This study provides epidemiological insight into CDs in a Korean population and reaffirms that GCD2 is the most common *TGFBI* CD phenotype and that p.R124H is the only mutation identified in patients with GCD2. In addition, we broaden the spectrum of *TGFBI* mutations by identifying two novel missense variants in patients with LCD1.

Key Words: Granular corneal dystrophy type 2, Korean, Lattice corneal dystrophy type 1, Mutation, *TGFBI*

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INTRODUCTION

Corneal dystrophies (CDs) comprise a group of hereditary and non-inflammatory corneal diseases with progressive accumulation of deposits in different layers of the cornea, resulting in a loss of corneal transparency and visual impairment [1]. The genes that are currently known to be responsible for CD include *TGFBI*, *GSN*, *K12*, *K3*, *MIS1*, *CHST6*, *COL8A2*, and *SLC4A11* [2]. Among these, mutations in the transforming growth factor

beta-induced gene (*TGFBI*; OMIM 601692) have been associated with several CDs. These CDs include granular CD type 1 (GCD1 or classic CD); granular CD type 2 (GCD2 or Avellino CD); granular CD type 3 (GCD3 or Reis-Bücklers CD); and lattice CD type 1 or *TGFBI* type (LCD1). According to the International Committee for Classification of Corneal Dystrophies (IC3D) classification, these CDs belong to category 1, meaning that they are well-defined CDs for which the gene has been mapped and identified and specific mutations are known [3].

An apparent genotype-phenotype correlation has emerged from *TGFB1* molecular studies. Several recurrent mutations have been shown to be associated with four specific phenotypes: p.R124H in GCD2, p.R555W in GCD1, and p.R124C in LCD1 [4]. Moreover, a number of cases with phenotypic variation for the same mutation, including atypical or variant phenotypes [5-8], have been reported, and novel mutations have also been described. Cumulatively, these data suggest phenotypic and allelic heterogeneity with respect to the *TGFB1* gene.

A few studies reported the clinical and genetic features of Korean patients with CDs associated with *TGFB1* mutations; however, these studies selected only patients with genetically confirmed mutations [9-12]. Owing to the phenotypic and allelic heterogeneity in the *TGFB1* gene, we analyzed the *TGFB1* sequences in Korean patients with multiple types of clinically diagnosed CD. The purpose of this study was thus to investigate the frequency of *TGFB1* mutations in patients with multiple types of clinically diagnosed CD and to examine genotype-phenotype correlations and the spectrum of *TGFB1* mutations.

METHODS

We retrospectively enrolled a consecutive series of unrelated patients who had been clinically diagnosed as having CD and underwent *TGFB1* gene analysis by Sanger sequencing. Patients were enrolled at a tertiary care hospital in Seoul, Korea, from 2006 to 2013. Slit-lamp examination was performed by professional ophthalmologists for all patients, and environmental and systemic factors were excluded. In addition, none of the patients

showed any response to steroid treatment. The clinical phenotype of CD was categorized according to the IC3D classification into *TGFB1* CDs and other CDs [3]. This study was approved by the Institutional Review Board of the Samsung Medical Center.

Genomic DNA was extracted from peripheral blood leukocytes by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Each *TGFB1* exon was amplified by PCR, and the resultant amplicons were sequenced on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For all patients, *TGFB1* exon 4 (which can harbor the p.R124H mutation) was analyzed. If negative, other exons (1, 5, 6, 11, 12, 13, and 14) that are also known to harbor frequently detected mutations were analyzed. The remaining exons were also sequenced in patients without any mutations in these exons using primers designed by the authors (available on request). *TGFB1* NM_000358.2 and *TGFB1* NP_000349.1 were used as cDNA and protein reference sequences, respectively.

For variants of unknown significance (VUS) identified in the *TGFB1* gene, *in silico* analysis was performed with Polymorphism Phenotyping v2 (PolyPhen-2; <http://genetics.bwh.harvard.edu/pph2>) and Sorting Intolerant From Tolerant (SIFT; <http://sift.jcvi.org>) to predict pathogenicity. In addition, the loci with VUS were queried in dbSNP142 (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), 1000 Genome Project data (<http://www.1000genomes.org/>), the National Heart Lung Brain Institute Exome Sequencing Project (ESP) database (<http://evs.gs.washington.edu/EVS/>), and an in-house collection of 150 exomes to determine the VUS allele frequencies.

Table 1. Clinical phenotype and spectrum of mutations in the *TGFB1* gene for 89 patients with corneal dystrophies

Clinical phenotype		TGFB1 mutation		
TGFB1 corneal dystrophies	Granular corneal dystrophies type 2 (Avellino corneal dystrophies)	N = 74	p.R124H p.R124H (;) p.A179* Not detected	N = 72 N = 1 N = 1
	Lattice corneal dystrophies, TGFB1 type	N = 3	p.R124C p.L569Q p.T621P	N = 1 N = 1 N = 1
	Other corneal dystrophies			
	Macular corneal dystrophies	N = 3	Not detected*	N = 3
	Fuchs endothelial corneal dystrophies	N = 3	Not detected†	N = 2
	Fleck corneal dystrophies	N = 2	Not detected	N = 2
	Posterior polymorphous corneal dystrophies	N = 1	Not detected	N = 1
	Epithelial basement membrane dystrophy	N = 1	Not detected	N = 1
	Lattice corneal dystrophies, gelsolin type	N = 1	Not detected	N = 1
	Other‡	N = 1	Not detected	N = 1

*Two patients were analyzed for only exon 4; †One patient was analyzed for only exon 4; ‡Clinical phenotype is not in accordance with a specific category.

RESULTS

A total of 89 patients with CD were included in this study. The median patient age at diagnosis was 48 yr (range, 16-74 yr), and 38.2% of the patients were male. The proportion of patients with *TGFBI* CD was 86.5% (77/89). Seventy-four patients (83.1%, 74/89) were diagnosed as having as having GCD2 and three (3.3%, 3/89) were diagnosed as having as having LCD1. No patients with GCD1 or GCD3 were identified in this study (Table 1).

Among the 74 patients clinically diagnosed as having GCD2, 73 (82.0%, 73/89) had the p.R124H mutation, while one patient had no mutations in the *TGFBI* gene (Fig. 1). One patient with the p.R124H mutation had an additional nonsense variant, p.A179* (c.535C>T) that is predicted to produce a truncated *TGFBI* protein (Fig. 2A). The patient's father was also diagnosed as having GCD2 and had the p.R124H mutation but not the p.A179* variant, while her mother did not have an eye problem but refused *TGFBI* gene testing.

Of the three patients with LCD1, one patient had the p.R124C mutation, while two other patients had novel variants, p.L569Q (c.1706T>A) in exon 13 and p.T621P (c.1861A>C) in exon 14 (Fig. 2B and C). These novel variants were predicted to be pathogenic by *in silico* analyses and were not identified in dbSNP142, 1000 Genome Project data, the ESP database of more than 13,000 control alleles, or an in-house collection of 150

exomes. A *TGFBI* mutation was not detected in any of the 12 patients with other types of CD (13.4%, 12/89).

DISCUSSION

This study reaffirms that GCD2 is the most common type of CD in the *TGFBI* CDs as well as in the multiple types of CD. This high proportion of GCD2 is consistent with the fact that, worldwide, exacerbation of GCD2 after refractive corneal surgery is frequently reported [13]. Thus, our results highlight the importance of early diagnosis of GCD2 through genetic screening in Korea.

The patient with GCD2 who lacked any mutations in the *TGFBI* gene was a young 26-yr-old woman who showed a few discrete granular deposits in the anterior stroma with unilateral manifestation (Fig. 3A). Although fine, discrete round opacities in the anterior stroma are indicative of early GCD2 symptoms, unilateral manifestation is somewhat atypical [14]. Because the patient refused to provide information regarding her family history, we could not determine whether this condition was inherited or not.

A previous study on 42 Chinese patients with particular CD subtypes did not detect *TGFBI* mutations in three patients with clinically diagnosed GCD2; interestingly, whole gene sequencing revealed that GCD2 was caused by non-*TGFBI* mutations in these patients [15]. Since *TGFBI* CD appears to represent a dis-

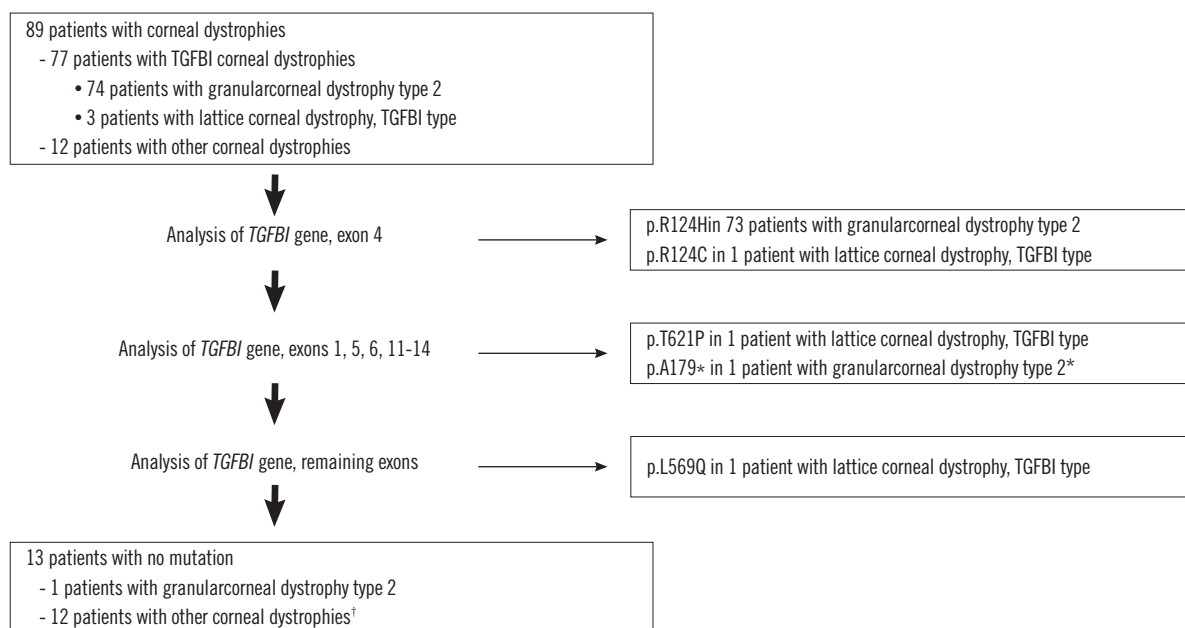


Fig. 1. Flow chart of the *TGFBI* mutation analysis protocol and the results of the 89 patients included in the present study.

The p.A179 mutation was additionally found in one patient with the p.R124H mutation; †Three patients underwent sequence analysis of only exon 4.

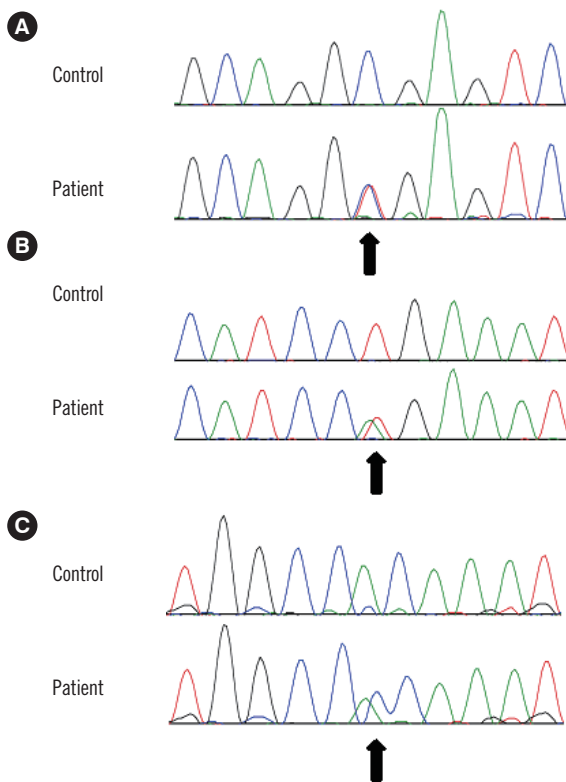


Fig. 2. Sequence electropherograms of novel *TGFB1* variants. (A) The c.535C>T (p.A179*) variant identified in a patient with granular corneal dystrophy type 2 (arrow). (B) The c.170G>A (p.L569Q) variant identified in a patient with lattice corneal dystrophy, *TGFB1* type (arrow). (C) The c.1861A>C (p.T621P) variant identified in a patient with lattice corneal dystrophy, *TGFB1* type (arrow).

ease spectrum, rather than discrete phenotypes, with increasingly atypical or variant phenotypes, genetic diagnosis sometimes challenges the apparent clinical diagnosis when few *TGFB1* mutations are found in the atypical group [16].

Both a novel p.A179* variant and the p.R124H mutation were identified in a 35-yr-old woman who experienced sudden deterioration of visual acuity in both eyes. In addition, both corneas exhibited progressed granular opacities with a dense confluent pattern that covered most of the corneal surface (Fig. 3B). We confirmed that the p.R124H mutation was inherited from her father, who also had GCD2. However, we could not test whether the patient's mother had the p.A179* variant or not. Assuming the p.A179* variant did not occur *de novo*, the patient was likely compound heterozygous for the two variants. The clinical significance of p.A179* is not clear because this variant might be inherited from the patient's mother who had no apparent eye problems. Previously, a Japanese patient with compound heterozygous variants (p.R124H and p.G470*) has been reported and

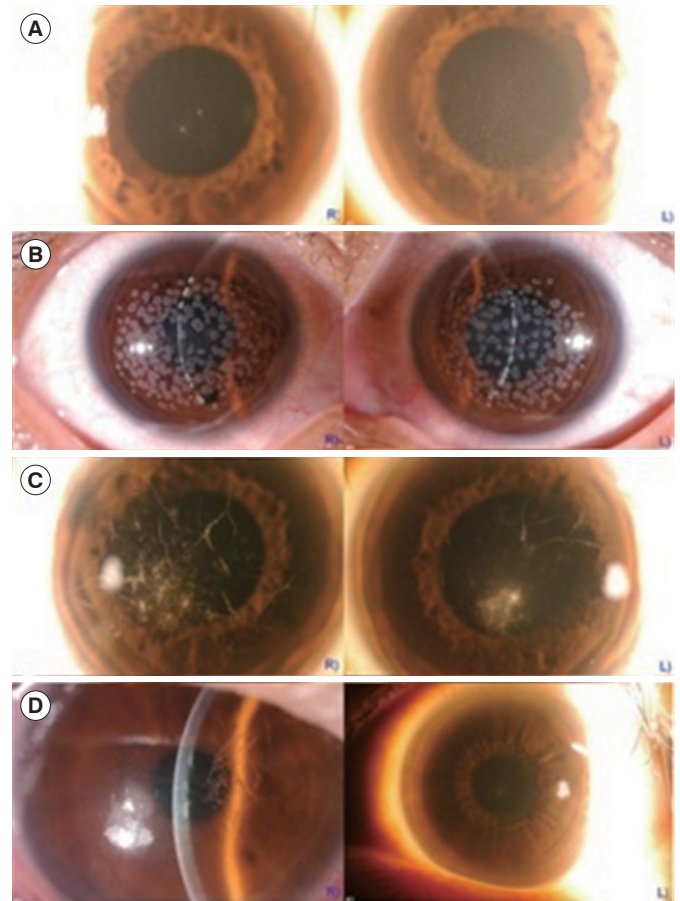


Fig. 3. Slit-lamp photographs. (A) Patient with GCD2 without any *TGFB1* mutations and a few discrete granular deposits in the anterior stroma with unilateral manifestation. (B) Patient with GCD2 carrying a novel p.A179* variant, as well as the p.R124H mutation, with progressed granular opacities in a dense confluent pattern covering most of the corneal surface. (C) Patient with LCD1, carrying the p.L569Q variant and large lattice lines. (D) Patient with LCD1, carrying the p.T621P variant with recurrent corneal erosions with typical lattice lines.

the pathogenicity of the nonsense variant (p.G470*) was unclear because the patient's daughter with the p.G470* variant was unaffected [17].

Two novel variants, p.L569Q and p.T621P, were detected in each patient affected by LCD1. These two patients showed a phenotype of larger lattice lines and recurrent corneal erosions with typical lattice lines, retrospectively (Fig. 3C and D). Lattice CD variants (types IIIA, I/IIIA, IV, and polymorphic amyloidosis), which belong to LCD1, are caused by more than two dozen distinct heterozygous mutations and are known to exhibit mutational and phenotypic variability [7]. In particular, lattice CD, type IIIA may exhibit larger lattice lines, although the lattice pattern depends on age; moreover, corneal erosions are a typical sign of

lattice CD, types IIIA and I/IIIA [3]. Nearly all of these mutations are located in the fourth FAS1 domain of *TGFB1*; two novel variants in these two patients were also located in the fourth FAS1 domain of *TGFB1* [3].

Other non-*TGFB1* CD phenotypes were not uncommon. None of the 12 patients with these types of CD had a mutation in the *TGFB1* gene. However, among these patients, two with macular CD and one with Fuchs endothelial CD only underwent sequence analysis of exon 4. To exclude *TGFB1* mutations, analysis of all exons is needed for these two patients.

This is the first study to investigate the clinical phenotypes and spectrum of mutations in the *TGFB1* gene in consecutive Korean patients with CD. Previous reports studying *TGFB1* focused only on patients with *TGFB1* mutations confirmed by genetic testing. The population of the present study is thus a wider representation of the range of clinical and genetic characteristics of patients with CD. GCD2 was the most common CD phenotype observed, and the p.R124H mutation was the only causal mutation of GCD2 while two novel missense variants in lattice CD and one novel nonsense variant in GCD2 were also identified. Moreover, sequence analysis of the entire *TGFB1* gene in patients with clinical phenotypes suggestive of homozygous mutations is needed to rule out the possibility of compound heterozygosity.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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