

# Chinese family with atypical granular corneal dystrophy type I caused by the typical R555W mutation in *TGFBI*

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## Abstract

• **AIM:** To investigate the clinical features and genetic defects in four generations of a Chinese family affected with atypical granular corneal dystrophy type I (GCD type I).

• **METHODS:** Family history and clinical data were recorded. Genomic DNA samples were obtained from peripheral blood leukocytes of all participated. Exons of the transforming growth factor- $\beta$ -induced (*TGFBI*) gene were directly sequenced after being amplified by polymerase chain reaction (PCR), and multi-point linkage analysis using microsatellite makers flanking the gene was applied to identify the disease-causing mutation.

• **RESULTS:** Clinical features were quite variable in patients, some patients only had opacities in the epithelium, and others revealed multiple bilateral circular, discrete, crumb-like opacities mainly in the epithelium, with several in different depths of corneal stroma, and the performance was different bilaterally, even in the same patient. Directly nucleotide sequencing revealed a heterozygous p.R555W mutation in the coding sequence of the *TGFBI* gene in all affected individuals of the family, but was not found in all unaffected. The maximum logarithm of odds (LOD) score obtained by multi-point analysis was detected at marker locus D5S393 (LOD = 2.740;  $\alpha=1.000$ ).

• **CONCLUSION:** Our case presented with clinical features and the pathogenic mutations in *TGFBI* gene, the phenotype of the pedigree was quite different from typical GCD type I, so we suggested that this phenotype

was a variant of GCD type I. These findings expand the knowledge about GCD type I, and demonstrate that molecular genetic analysis is important to make an accurate diagnosis of patients with variable corneal dystrophies in clinic.

• **KEYWORDS:** atypical; granular corneal dystrophy; *TGFBI*; gene mutation

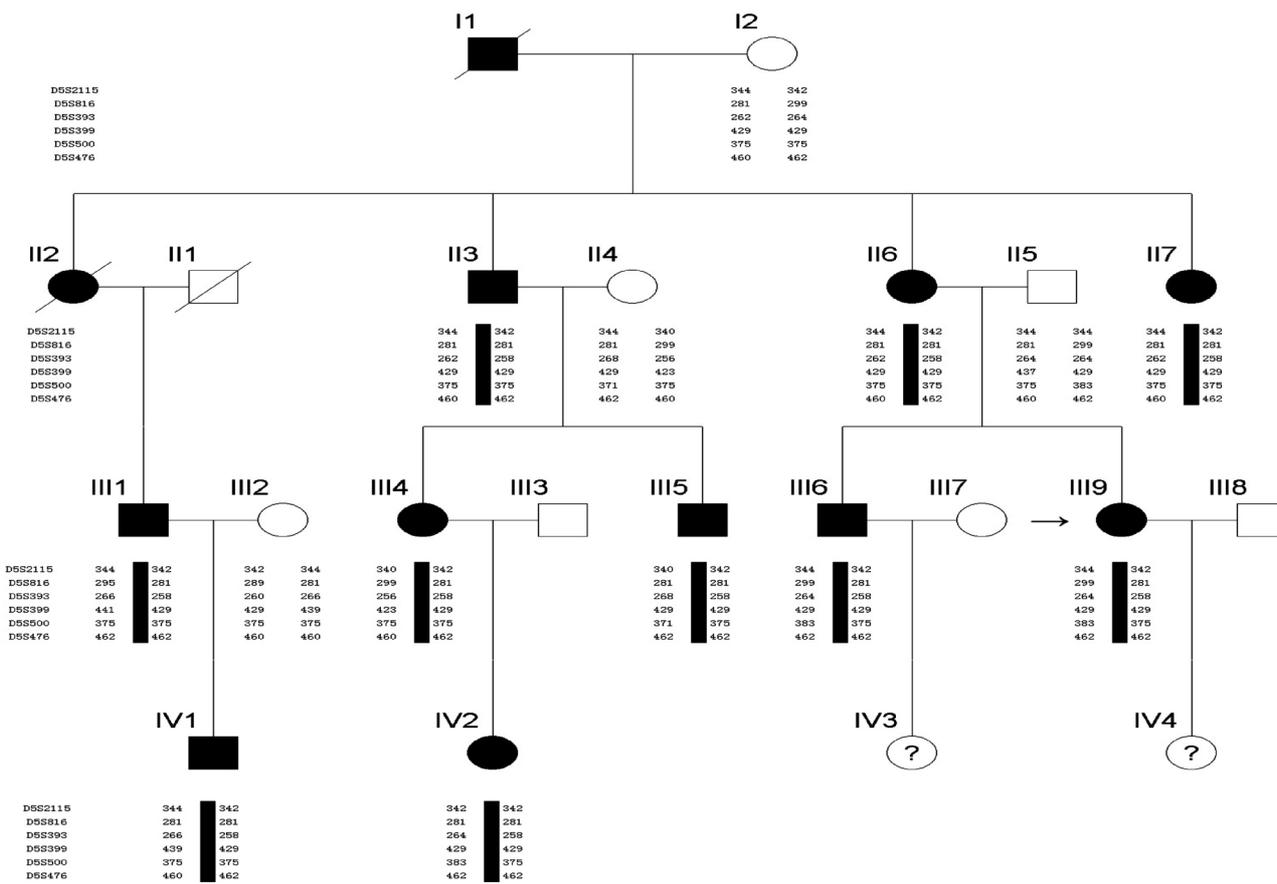
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## INTRODUCTION

Corneal dystrophies (CDs) are bilateral, clinically heterogeneous, and genetically determined diseases, characterized by deposition of nonnative protein or other material in different layers of the cornea which leading to the loss of corneal transparency and vision acuity<sup>[1]</sup>. In clinic, the corneal dystrophies are classified based on the results of slit-lamp examination, the morphology and depth of the corneal deposits. The majority of the corneal dystrophies are transmitted as autosomal dominant traits with a high degree of penetrance<sup>[2]</sup>. Since the transforming growth factor beta-induced (*TGFBI*) gene was identified as causative gene for stromal corneal dystrophies by Munier *et al*<sup>[3]</sup> in 1997, many types of corneal dystrophies have been described to associated with the *TGFBI* gene mutations including granular corneal dystrophy (GCD), lattice corneal dystrophy of types I, IIIA, I/IIIA, IIIB, and IV (LCD I, LCDIIIA, LCDI/IIIA, LCDIIIB, LCDIV), Reis-Bucklers' dystrophy (RBCD), and Thiel-Behnke corneal dystrophy (TBCD)<sup>[4]</sup>.

Granular corneal dystrophy type I (GCD I; MIM 121900) is one of the most common phenotypes of CDs, characterized by small, discrete, sharply demarcated grayish white opacities in the anterior central stroma resembling bread crumbs or snowflakes<sup>[5]</sup>. It is transmitted as autosomal dominant traits and caused by mutations in the *TGFBI* gene. To date, more than 50 distinct disease-causing mutations have been described in *TGFBI* that are associated with CDs<sup>[6]</sup>. As special corneal dystrophy can caused by particular mutations in *TGFBI*, but the correlation a genotype-phenotype is not always certain<sup>[7-10]</sup>. In the clinic, it is difficult to make an accurate diagnosis and classification for patients with variable and atypical phenotypes.



**Figure 1 Pedigree and haplotype of a Chinese family** The black symbols indicate individuals with a diagnosis of corneal dystrophy performed by genetic analysis. The arrow indicates the proband. The haplotype markers are shown at the left of each generation. The black bars depict the disease associated haplotype. Haplotype analysis identified the causative gene as being between D5S2115 and D5S476 on chromosome 5.

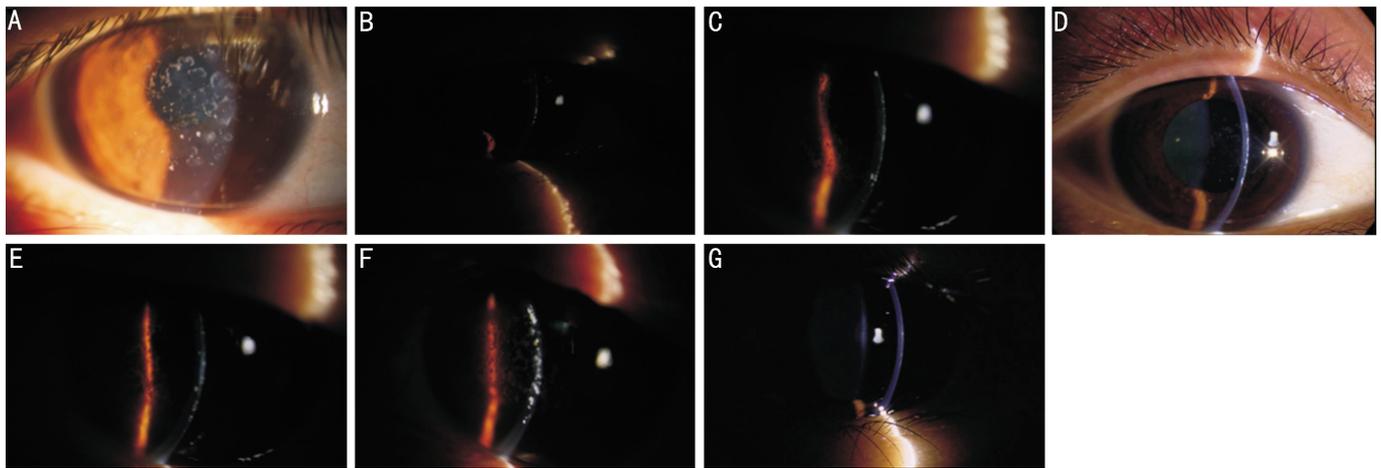
Thus far, the R555W mutation in *TGFBI* has been described to have apparent genotype-phenotype correlation with Granular corneal dystrophy type I [11-13]. In this study, we present a four-generation Chinese consanguineous family with an unusual GCD type I caused by the *TGFBI* R555W mutation. The particular opacities mainly affect the layer of epithelium, which is different from the typical of GCD type I previously reported for this same mutation. We propose that this unusual phenotype may be a variant of GCD type I. Our findings expand the genotypic-phenotypic spectrum of GCD type I, and demonstrate the importance of gene diagnosis in CDs.

**SUBJECTS AND METHODS**

**Subjects** We studied a four-generation Chinese consanguineous family with atypical phenotype of CDs. Ten patients and five unaffected relatives were recruited in our study (Figure 1). Informed consents were obtained from all participants according to the Declaration of Helsinki. All the patients participating in this study were diagnosed in the Eye Center of the Second Affiliated Hospital of Medical College, Zhejiang University. Complete ophthalmologic examinations were performed on all participants, including visual acuity, slit-lamp, and fundus examination.

**Genomic DNA preparation** Blood specimens (5mL) were collected in Ethylene Diamine Tetraacetic Acid (EDTA). We extracted genomic DNA in the peripheral blood leukocytes from all participants using a Simgen Blood DNA mini kit (Simgen, Hangzhou, China).

**Mutation screening** The 17 exons and flanking intronic sequences of *TGFBI* were amplified by polymerase chain reaction (PCR), using previously reported primers [11]. PCR was performed in a volume of 25μL in a DNA Thermal Cycler (Perkin Elmer, Norwalk, CT, USA). PCR cycling conditions were as follows: 5min of an initial denaturation step at 95°C, 10 cycles of denaturation at 95°C for 25s, initial annealing at 60°C for 25s, and extension at 72°C for 40s; the annealing temperature was reduced by 1°C per cycle until 50°C. This was followed by 30 cycles with denaturation at 95°C for 30s, annealing at 55°C for 25s, and extension at 72°C for 40s with a final extension step at 72°C for 10min. PCR products were isolated by electrophoresis on 1% agarose gels and sequenced using the BigDye Terminator Cycle sequencing kit V 3.1 (ABI Applied Biosystems; Sangon Co., China) on an ABI PRISM 3730 Sequence Analyzer (ABI), according to the manufacturer's instructions.



**Figure 2** Slit-lamp photographs of patients A: The proband's cornea (Figure 1, III9) showed multiple round grayish white opacities in the central stroma; B: The left eye of the proband's cornea showed opacities only in the epithelium; C: The right eye of the proband's cornea showed opacities in the epithelium and anterior stroma; D: The cornea in patient of III4 (Figure 1) showed opacities in the epithelium and anterior stroma; E: The cornea in patient of II7 (Figure 1) showed opacities in the epithelium, anterior stroma and middle stroma; F: The cornea in patient of II3 (Figure 1) showed opacities in the epithelium and total stroma; G: The left eye of patient of IV2 (Figure 1) showed opacities in the epithelium.

**Genotyping** We performed a partial genome scan in the vicinity of the *TGFBI* locus, and chose the 6 fluorescent short tandem repeat polymorphic markers for this locus (Figure 1). A 'touchdown' PCR was performed in a 20 $\mu$ L reaction volume containing 10ng of genomic DNA, 0.3mmol/L of each dNTP, 0.1 $\mu$ mol/L each of forward and reverse primers, 1U HotStarTaq polymerase (Qiagen, Hilden, Germany), 3.0mmol/L MgCl<sub>2</sub>, and 1 $\times$ HotStarTaq buffer. After an initial denaturation period of 15min at 95 $^{\circ}$ C, 11 cycles were performed at 94 $^{\circ}$ C for 20s, 65-56.5 $^{\circ}$ C for 40s (with a 0.5 $^{\circ}$ C decrease each step), then 68 $^{\circ}$ C for 2min; followed by 94 $^{\circ}$ C for 20s, 56 $^{\circ}$ C for 40s, 68 $^{\circ}$ C for 2min, for 24 cycles; a final extension at 60 $^{\circ}$ C for 1h was performed. The PCR products were appropriately pooled and an aliquot was loaded onto a 5% standard denaturing polyacrylamide gel and run in an Applied Biosystems 3130xl Genetic Analyzer. The size of each allele was determined on the basis of an internal size standard (GeneScanTM-500 Liz Size Standard, Applied Biosystems, USA) in each lane, and results were analyzed using the Applied Biosystems GeneMapper 4.0.

**Linkage analysis and haplotyping** Multi-point linkage analysis was calculated using Merlin. A gene frequency of 0.0001 with penetrance of 80%, 90%, 99% and 100% was assumed. Microsatellite markers, allele frequencies, and recombination distances between the marker loci were based on the Marshfield database and the UCSC database. Family and haplotype data were processed using Cyrillic software (version 2.1; Cyrillic, Oxfordshire, UK).

## RESULTS

**Clinical Findings** The transmission patterns of the four-generation Chinese family were consistent with autosomal dominant inheritance (Figure 1). Slit-lamp

**Table 1** Clinical evaluation of affected individuals

Patient's number (age, a)	Depth of corneal lesion
II3 (59a)	OU: Epithelium and total stroma
II6 (54a), II7 (49a)	OU: Epithelium, anterior and middle stroma
III1 (37a), III9 (23a)	OD: Epithelium, anterior stroma OS: Epithelium
III4 (31a), III5 (28a), III6 (27a)	OU: Epithelium, anterior stroma
IV1 (8a), IV2 (5a)	OU: Epithelium

OU: Oculus uterque; OD: Oculus dexter; OS: Oculus sinister.

examination of patients presented with round, discrete and crumb-shaped opacities mainly in the central region, which was almost the same with GCD type I, but of the lesion depths were distinctly different, the corneal opacities mainly involving the layer of epithelium, but only a few in different depth of the corneal stroma. Furthermore, the lesion depths were found to be different between the affected individuals (Table 1), even in the same patient the lesion depths was different bilaterally.

The patient III1 and III9 (Figure 1) only had opacities in the epithelium in the left eye, but in their right eye stroma was also affected (Figure 2A, B, C). In patient II3; III4 and II7 lesion mainly affected corneal stroma bilateral (Figure 2D, E, F). The corneal opacities only affected the epithelium of bilateral in the patient IV1 and IV2 (Figure 2G). Most of patients had experience of painful and visual defect after the age of ten, and their visual acuity decreased gradually with occasional photophobia, they would have to experience of eye painful and photophobia when their immunity decrease, and it will last for three days to ten days. Other ocular or systemic abnormalities was not found.

**Mutation Analysis** After direct sequencing all exons of *TGFBI*, a heterozygous C to T transition at nucleotide

position 1663 (c.1663C>T) in all affects were detected (Figure 3). This nucleotide substitution resulted in the amino acid change from Arginine to Tryptophan at amino acid position 555 (p.R555W). This mutation was not seen in the unaffected family members and 100 normal controls.

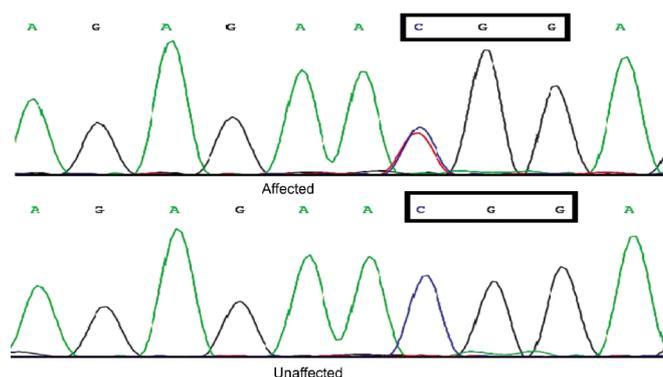
**Linkage and haplotype analysis** Genescan and linkage analysis were carried out using 6 microsatellite makers flanking the *TGFBI* gene, and positive results were obtained, including the maximum logarithm of odds (LOD) score 2.740 at D5S393 ( $\alpha=1.000$ ; Table 2). Haplotype analysis showed that affected individuals in the family shared a common haplotype, with a region flanked by the markers D5S2115 and D5S416 at 5q containing *TGFBI*.

### DISCUSSION

Clinically, the GCD is one phenotype of stromal corneal dystrophies. The typical GCD type I is characterized by multiple, discrete, sharply demarcated grayish white opacities resembling bread crumbs or snowflakes in the anterior central stroma, leading to painful bilateral, progressive opacification and visual defect. The age of onset usually within the second decade of life, as the condition advance, the opacities of the corneal increase in size and number and may coalesce, extending into the deeper and more peripheral stroma<sup>[12]</sup>.

Thus far, the majority of mutations in the *TGFBI* gene have been demonstrated associated with CDs, among them R124 and R555 have indentified as the two mutational hot spots in various populations<sup>[13-17]</sup>. In this study, we described an unusual phenotype of GCD type I associated with the p. R555W mutation in *TGFBI* gene. The patients we investigated presented with various clinical features, even in the same patient, the performance was different bilaterally. Slit-lamp examination of eye showed round, discrete and crumb-shaped opacities mainly in the layer of epithelium, but only a few in different depths of the corneal stroma. The proband (III9, Figure 1) here was a 23-year-old woman who initially noted reduced of visual acuity and recurrent corneal erosion after 15-year-old, and showed opacities mainly in the epithelium and a few in the anterior stroma in the left eye, and only affected the epithelium of the right eye. The first clinical impression was a phenotype of anterior CDs mainly affecting the corneal epithelium, but the patient (II3, Figure 1) had opacities in the epithelium and total strom, which was equivalent to the typical GCD type I. It is difficult to classify this phenotype from these clinical manifestations, so we sequenced the PCR product of gene *TGFBI* and found a typical mutation site (C.1663C>T) causing amino acid change from arginine to tryptophan (R555W).

The R555W mutation in *TGFBI* gene was first indentified associated with GCD type I by Munier *et al*<sup>[3]</sup>. Since then, this mutation has been demonstrated to have a clear



**Figure 3 Mutation analysis of the *TGFBI* gene** The sequence chromatogram (forward strand) showed a heterozygous C>T transition at codon 555 in the affected individuals. Codon 555 was in the box.

**Table 2 Multi-point LOD scores for linkage analysis**

Marker	0.800	0.900	0.990	1.000
D5S2115	2.700	2.700	2.701	2.701
D5S816	2.727	2.728	2.728	2.728
D5S393	2.739	2.739	2.740	2.740
D5S399	2.738	2.738	2.739	2.739
D5S500	2.715	2.716	2.716	2.716
D5S476	2.714	2.715	2.715	2.715

genotype-phenotype association with GCD type I<sup>[2,4,18]</sup>. In this present study, by directly genetic analysis, we found R555W mutation in *TGFBI* gene, which suggested they belong to a classification of GCD type I. However, except the patient II3 (Figure 1) all patients had a gray-white crumb-like opacity mainly in the layer of epithelium and only a few in the anterior to mid stroma of the cornea, and the youngest patient (IV2, Figure 1) was only 5-years old, which is different from previously reported for this mutation, even in the same patient, the performance is different bilaterally, for example, the proband and the patient of III9 (Figure 1) had opacity only in the epithelium of their left eye, but in the right eye the stroma was also affected. Moreover, there was no age-dependent phenotype was noted in this Chinese pedigree. Thus, we propose that this unusual disorder as atypical GCD type I.

The *TGFBI* gene, originally named beta-ig-h3 (*BIGH3*), was discovered by Skonier *et al*<sup>[19]</sup>, and being transcribed almost exclusively in the corneal epithelium and stromal keratocytes<sup>[20]</sup>. The protein  $\beta$ ig-h3 (*TGFBI*p), which is encoded by *TGFBI* gene, is a highly conserved protein that contains a COOH-terminal Arg-Gly-Asp (RGD) motif, NH-terminal Cys-rich EMI domain and four consecutive fasciilin 1 (FAS1) domains. The p.R555W mutation is located in the fourth FAS1 domain of *TGFBI*p, which is predicted to alter solubility or stability of protein rather than structure<sup>[21]</sup>, and they may be directly affect protein-protein interactions rather than misfolding in the endoplasmic

reticulum [22]. Moreover, in 2003, Morand *et al* [23] reported that in human corneal epithelial cells the mutation p.R555W in *TGFBI*p triggered apoptosis, and suggested that mutated *TGFBI*p affect the activation of the  $\alpha3\beta1$  integrin-related pathway is part of the pathophysiological process that leads to the *TGFBI*-related CDs.

Sometime it is difficult to make a precise diagnosis rely solely on the clinical features, especially for patients with variable and atypical phenotypes. In this condition gene diagnosis becomes necessary. In this study, we presented clinical and molecular information supporting this unusual GCD type I caused by the p.R555W mutation in the *TGFBI* gene. This finding is expected to give a valuable insights into the GCD type I and also demonstrated that genotype plays an important role in clinic.

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