• Basic Research •

Two mutations in the transforming growth factor beta-induced gene associated with familial Lattice corneal dystrophy

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Abstract

• AIM: To report a phenotypic variant pedigree of lattice corneal dystrophy (LCD) associated with two mutations, R124C and A546D, in the transforming growth factor beta-induced gene (TGFBI).

• METHODS: A detailed ocular examination was taken for all participants of a LCD family. Peripheral blood leukocytes from each participant were extracted to obtain the DNA. Polymerase chain reaction (PCR) of all seventeen exons of TGFBI gene was performed. The products were sequenced and analyzed. Histological examination was carried out after a penetrating keratoplasty from the right eye of proband.

• RESULTS: Genetic analysis showed that the proband and all 6 affected individuals harbored both a heterozygous CGC to TGC mutation at codon 124 and a heterozygous GCC to GAC mutation at codon 546 of TGFBI. None of the 100 control subjects and unaffected family members was positive for these two mutations. Ocular examination displayed multiple refractile lattice-like opacities in anterior stroma of the central cornea and small granular deposits in the peripheral cornea. The deposits were stained positively with Congo red indicating be amyloid in nature and situated mainly in the anterior and middle stroma.

• CONCLUSION: We observed a novel LCD family which carried two pathogenic mutations (R124C and A546D) in the TGFBI gene. The phenotypic features were apparently different from those associated with corresponding single mutations. The result reveals that although the definite mutation is the most important genetic cause of the disease, some different modifier alleles may influence the phenotype.

• **KEYWORDS:** corneal dystrophy; mutation; phenotype; transforming growth factor beta-induced gene
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INTRODUCTION

TGFBI (OMIM 601692, formerly called BIGH3) gene was first identified by Skonier *et al*[1] as a transforming growth factor beta-induced gene in a human lung adenocarcinoma cell line. In 1997, Munier *et al*[2] identified TGFBI on chromosome 5q31 and discovered 4 different mutations that associated with 4 inherited corneal dystrophies: R555W resulting in the granular dystrophy (GCD), R124H resulting in the Avellino dystrophy (ACD), R124C resulting in the lattice dystrophy type I (LCD-I), R555Q resulting in the Reis-Bücklers dystrophy (RBCD).

Subsequently, several additional mutations of TGFBI throughout the world were found to be responsible for diverse corneal dystrophies and a phenotype-genotype correlation had been established[3-4].

LCD is one of common stromal dystrophy which manifests typically as linear, radially oriented, branching opacities in the anterior stroma[5]. The opacities have been found to correspond with accumulations of amyloid. At least four different types of LCDs are recognized based on clinical features and the histologically natures. But it is difficult to distinguish these subtypes from each other in the absence of genetic analysis. Mutations in the TGFBI gene have been found in patients with LCD type I and IIIA. Mutations in the GSN gene have been found in cases of LCD type II[6-7]. The autosomal recessive form of the disease, LCD type III, has been mapped to chromosome 1p31[8]. To date, several distinct single mutations of TGFBI which including R124C, A546D, P501T, L527R, A620P, L518P and H626R have been associated with LCD[3,9-13]. It seems that these single nucleotide changes are important disease-producing mutations.

Several researchs have observed patients who harbored two different mutations in TGFBI with variant phenotype[14-15].

Table 1 Clinical findings and genotype in family members

Individual case	Gender	Age (a)	Status	Mutations in TGFBI	Age of onset (a)	Symptoms of onset	Visual acuity	
							OD	OS
Ⅰ 2	F	90	Affected	R124C A546D	19-20	FBS; Ph	10 cm/CF	10 cm/CF
Ⅱ 2	M	56	Unaffected	None	-	-	0.8	1.0
Ⅱ 3	F	63	Affected	R124C A546D	15	FBS; Ph; VA↓	20 cm/CF	20 cm/CF
Ⅱ 4	F	61	Affected	R124C A546D	17	FBS; Ph; VA↓	0.01	20 cm/CF
Ⅲ 2	M	28	Unaffected	None	-	-	1.0	1.0
Ⅲ 3	M	41	Affected	R124C A546D	18	FBS; Ph	0.4	0.4
Ⅲ 4	M	39	Unaffected	None	-	-	1.0	1.0
Ⅲ 5	M	33	Affected	R124C A546D	21	FBS; Ph	0.6	0.6
Ⅲ 6	F	39	Unaffected	None	-	-	1.0	1.0
Ⅳ 3	F	16	Affected	R124C A546D	16	FBS	0.8	0.8

OD: Right eye; OS: Left eye; FBS: Foreign body sensation; VA↓: Visual acuity decreased; Ph: Photophobia. -: Information is not provided.

Nevertheless, the reason of the phenotype in patients with such a double mutations differs from that with single mutations is still unclear. In this study, we report the first cases of a LCD family in Chinese associated with both R124C and A546D mutations of TGFBI gene.

SUBJECTS AND METHODS

Subjects The research strictly followed the Declaration of Helsinki and was adhered to the Review Board of Heilongjiang. A four-generation LCD pedigree was collected in the north of China in March 2014 (Figure 1). All 17 participants (6 affected and 11 unaffected) provided informed written consents. The ages of family members ranged from 5 to 90y. In addition, 100 unrelated, unaffected, healthy volunteers from China were collected as normal controls. The ages of normal controls ranged from 28 to 58y.

Clinical Evaluations Extensive ophthalmologic examinations were performed to determine the status of the corneas of each individual (affected or unaffected). The corneal phenotypes of affected members were documented by slit-lamp photography. The clinical history that including the age of onset, innitial presenting signs, clinical symptoms, and the treatment procedures were obtained in detail.

Molecular Analysis Peripheral blood was obtained in a total of 17 family members and one hundred volunteers. DNA was extracted with a QIAamp DNA blood Mini Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of all 17 exons of TGFBI gene were performed using the appropriate primers and conditions previously reported by Munier *et al*[2] or Afshari *et al*[16]. Each PCR product was sequenced with ABI BigDye Terminator Cycle Sequencing kit v3.1, (ABI Applied Biosystems, Foster City, CA, USA) for both strands. Then the results of sequencing were compared with the cDNA of TGFBI in GenBank (NM-000358) in order to detect any nucleotide changes. All Chinese controls were amplified and scanned the exon 4 and exon 12 of TGFBI gene.

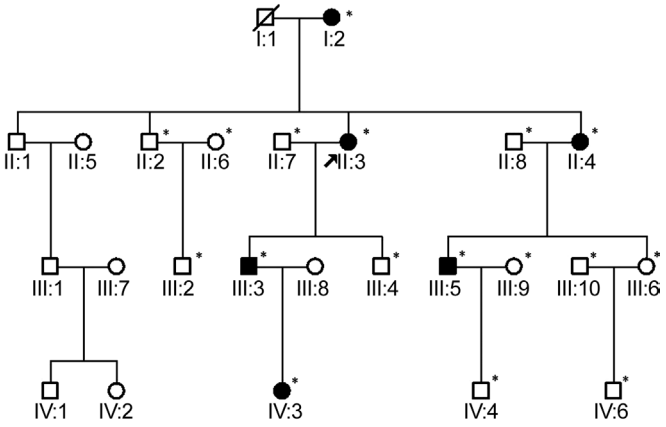

Figure 1 The pedigree of LCD family The proband was indicated by the arrow. Affected members in the family were filled with black and individuals who underwent research were indicated by the asterisks.

Histologic Evaluations One corneal button from the right eye of the proband was obtained after penetrating keratoplasty. Corneal section was stained by hematoxylin-eosin (HE), Congo red, periodic acid Schiff (PAS) and the Masson's trichrome for histologic examination.

RESULTS

Clinical Manifestations Clinical findings of the LCD pedigree were summarized (Table 1). Age of onset was 15 to 21y in the six affected family members. The initial symptoms and main complains included visual acuity decreased, foreign body

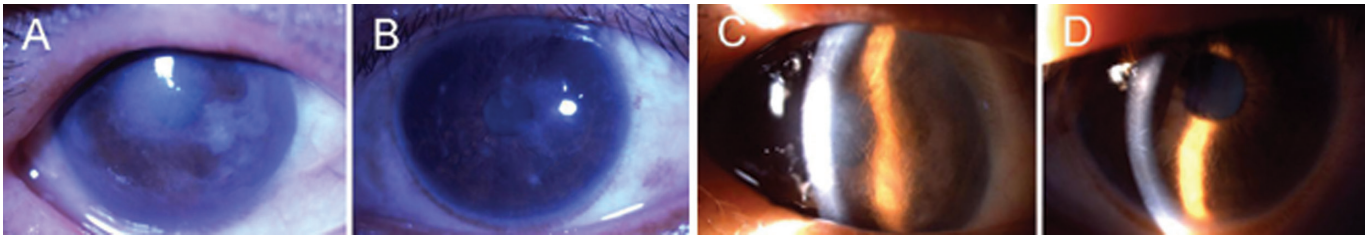

Figure 2 Slit-lamp photography of patients with LCD that associate with both R124C and A546D A: Photography of the proband showed gray and white confluent opacities involved almost whole cornea of her right eye; B: Clinical penotype of Ⅳ 3 (granddaughter of the proband) revealed several slight opacities in superficial stroma of the right corneal; C, D: Multiple refractile lattice-like opacities were finded in anterior stroma of the central cornea and small granular deposits could be seen in the peripheral cornea of Ⅲ 3 and Ⅲ 5 in their right eyes.

sensation and photophobia. Corneal erosion appeared in Ⅲ 3 and Ⅲ 5 in their third decade of life. The progress of corneal opacities in all affected members was commonly bilateral symmetry.

The proband of the family was a sixty-three years old Chinese woman who came to hospital for corneal transplantation. She complained a long-term bilateral decrease of visual acuity. Slit-lamp examination revealed gray and white confluent opacities involved almost entire cornea (Figure 2A). Severe corneal edema presented and the typical lattice opacities could not be observed. Her visual acuity decreased to 20 cm/CF in both eyes. Ultimately, penetrating keratoplasty was performed on her both eyes respectively.

Ⅲ 3 and Ⅲ 5 had their initial symptoms at 18 and 21y and both of them complained with foreign body sensation and photophobia. Slit-lamp examination observed that multiple refractile lattice-like opacities in anterior stroma of the central cornea and small granular deposits could be seen in the peripheral cornea (Figure 2C, 2D). The visual acuity of both eyes decreased to 0.4 in Ⅲ 3 and 0.6 in Ⅲ 5 respectively.

Ⅳ 3 was the granddaughter of proband (Figure 2B). No severe symptom or visual defect was complained until participating this research. Clinical penotype showed that several slight opacities in superficial stroma of her right cornea.

Molecular Genetic Analysis Direct sequencing was taken for all exons of TGFBI gene from the proband, and we identified two heterozygous mutations (Figure 3A, 3C). The exon 4 exhibits a C > T heterozygous substitution at nucleotide position 417 and causes a arginine to cystine acid variation at protein level (R124C). The exon 12 exhibits a C > A heterozygous substitution at the 1637 position and results in a alanine to aspartic acid variation at protein level (A546D). Additionaly, 5 affected (I2, II4, III3, III5 and Ⅳ 3) individuals who carrying the same two variations in heterozygous state were identified. None of 11 unaffected (II2, II6, II7, II8, III2, III4, III6, III9, III10, Ⅳ 4 and Ⅳ 6) participants and the one hundred normal controls was positive for these two mutations (Figure 3B, 3D).

Histologic Examination The proband underwent a penetrating keratoplasty for her right eye and the corneal button was excised for further histologic examination. It characterized by presence of variably sized, irregularly shaped deposits within the cornea. The deposits were stained positively with Congo red indicating be amyloid in nature and situated mainly in the anterior and middle stroma (Figure 4).

DISCUSSION

The authors presented the first report of a LCD pedigree which associated with two missense mutations in TGFBI gene, R124C in exon 4 and A546D in exon 12. These two heterozygous mucleotide changes not only cosegregated with keratopathy in the family, but also were predicted to change two amino acids of TGFBI-induced protein. It seems that both of these changes were important pathogenic mutations. As reported[17], the typical LCD caused by R124C was recognized by the characteristic net of linear opacities in the anterior stroma. The patients often had signs and symptoms at the first decade of their lives and the recurrent epithelial erosion was common. However, the atypical LCD caused by A546D presented polymorphic, chipped ice-appearing corneal opacities or combined with filamentous opacities in the peripheral cornea[18-19]. The amyloid deposits were larger and situated predominantly in the mid and posterior corneal stroma. They often had a later onset and rarely complain of corneal erosions. In the current studied family, the age at onset for affected individuals was between 15 and 21y, with a mean of 17.75y. In contrast to the typical LCD, mild corneal erosion only appeared in Ⅲ 3 and Ⅲ 5. Multiple refractile lattice-like opacities were presented in their anterior stroma of central cornea and small granular deposits were observed in peripheral cornea. Histologic result showed that the amyloid deposits situated mainly in the anterior and middle stroma compared with the atypical LCD. These clinical features of studied patients were apparently different from those associated with corresponding single mutations. At this point of view, the phenotype of our pedigree was seemed to be a summation of two types. But we think that this atypical presentation was not a simply superposition, but a result of the interaction between two pathogenic gene mutations.

To date several cases of double mutations associated with TGFBI gene have been described but the clinical phenotype of affected patients differs markedly from each other. Ha *et al*[20] observed a heterozygous P501T mutation of TGFBI gene

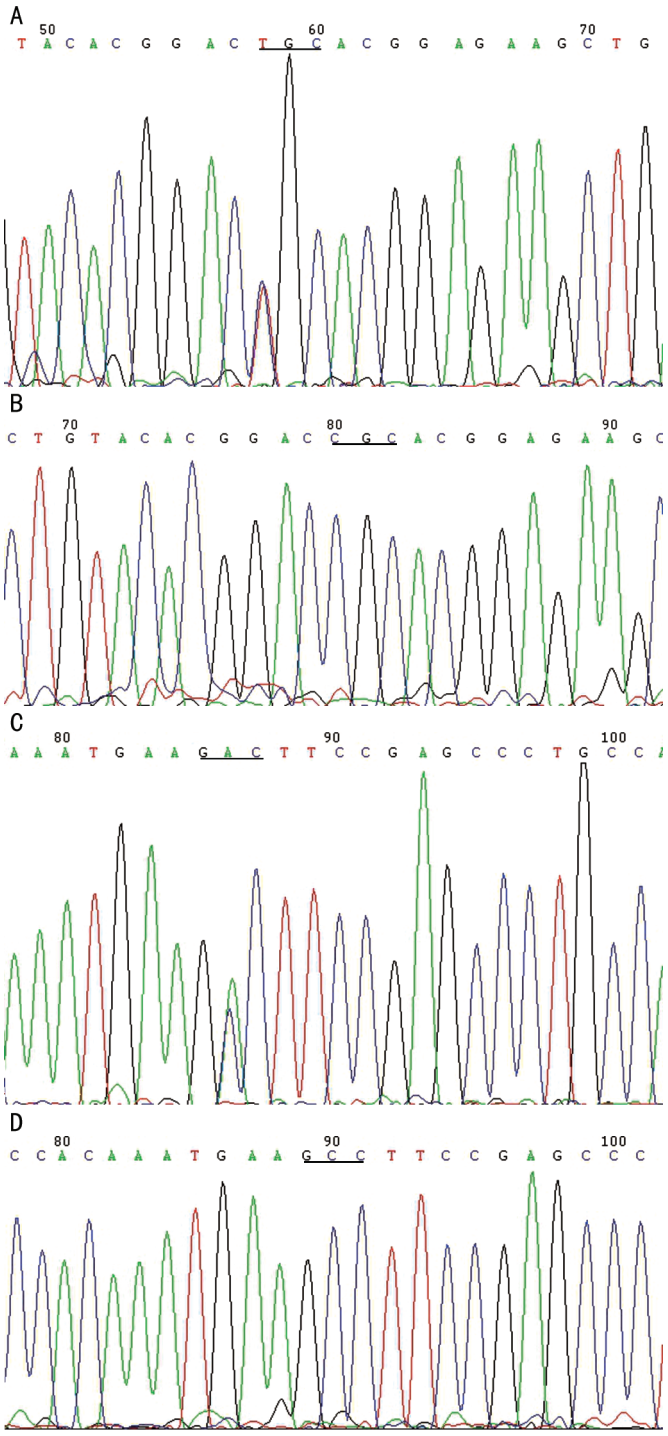

Figure 3 Sequence analysis of exon 4 and exon 12 of TGFBI gene in the family A: The exon 4 of proband exhibits a C > T heterozygous substitution at nucleotide position 417 and results in a arginine to cystine acid variation (R124C); B: A normal sequence of exon 4 of TGFBI encodes arginine in the codon 124; C: The exon 12 of proband exhibits a C > A heterozygous substitution at the 1637 position and results in a alanine to aspartic acid variation (A546D); D: A normal sequence of exon 12 is shown, which encodes alanine in codon 546.

and a homozygous Q118X mutation of MISI gene in same patient. The MISI gene was identified as responsible for the typical gelatinous drop like corneal dystrophy (GDLD) and usually in the autosomal recessive inheritance. It was reported that P501T mutation of the TGFBI gene could cause LCD IIIA[10] . Ha *et al*[20] finded that the patient with P501T and

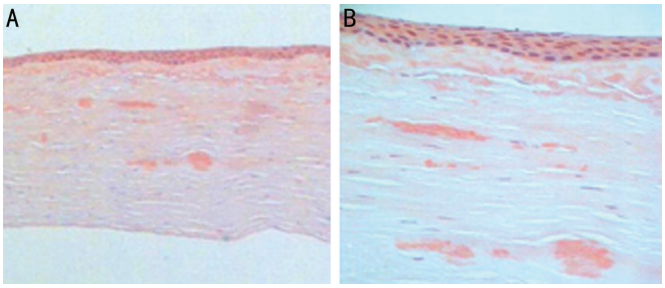

Figure 4 The histopathological results of excised corneal stained with Congo red of the proband Light microscopy observed irregularly shaped and variably sized deposits in the anterior and middle stroma of the corneal tissue. A:10×; B:40×.

Q118X resembled GDLD penotype but not LCD. Dighiero *et al*[21] reported a French family affected with GCD and founded two heterozygous mutations in the TGFBI gene-R124L and ΔT125-ΔE126. These two mutations caused a variant of GCD that was intermediate in severity between the classical and superficial variant forms. Klintworth *et al*[14] and Aldave *et al*[22] presented a phenotypic variant of LCD associated with the A546D and P551Q missense changes in exon 12 of the TGFBI gene. The affected members had not only lattice-like corneal stromal deposits but also discrete, short, irregularly shaped opacities. Klintworth *et al*[14] believed the P551Q allele could play a modifier role on the phenotype that associated with the A546D mutation. Yamada *et al*[15] reported two cases with a double mutations in the TGFBI gene (R124H and N544S). The phenotype of the patients seemed to be a summation of the LCD and ACD. To sum up, these double mutations more or less produced phenotypic variations. It indicated that the interaction between these pathogenic mutations was exist and these alleles were modified by each other.

The modifier genes could influence the phenotypic variability in some monogenic disorders had been proven[23]. And many factors such as environment, age, and sex may play an important role on expressivity and penetrance of the disease[24]. However, the genetic interactions between mutated alleles and the modifier alleles or the effect of environmental factors are unclear. If those genetic interactions and modifier alleles can be confirmed, it may lead to a possible way to inhibit or delay the occurrence of genetic diseases.

In conclusion, our study observed a novel LCD pedigree which carried two pathogenic mutations (R124C and A546D) in the TGFBI gene. Its phenotypic features were obviously different from that reported LCD types. The result confirms that although the definite mutation is the most important genetic cause of disease, some different modifier alleles may influence the phenotype to some extent. Uncovering the interactions between them may lead to a possible way to inhibit the occurrence of the corneal dystrophy.

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Conflicts of Interest: Cao WP, None; **Yuan HG**, None; **Liu P**, None; **Li X**, None; **Hu Q**, None.

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