

# An Arg124Cys mutation in transforming growth factor $\beta$ -induced gene associated with lattice corneal dystrophy type I in a Chinese pedigree

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**Purpose:** To identify a clinical and genetic form of a large Chinese family with an autosomal-dominant lattice corneal dystrophy type I (LCD I). **Methods:** The patients' eyes were examined on the basis of slit-lamp microscopy, and other clinical records were also collected. Genomic DNA was extracted from peripheral leukocytes of the affected patients and their unaffected family members. Each previous reported mutation of the transforming growth factor  $\beta$ -induced gene (TGFB1) gene was amplified by touch-down polymerase chain reaction and directly sequenced to verify the disease-causing mutation. **Results:** Typical clinical features of LCD I were found by slit-lamp photography in these affected Chinese pedigrees. A heterozygous single base-pair transition from C to T (c.418 C > T), leading to amino acid substitution Arg124Cys (R124C) in the encoded TGFB1 protein, was detected in all of the eighteen affected patients. The same mutation was not found in unaffected family members. **Conclusion:** The R124C mutation hot spot, which was relatively rare in China, was responsible for LCD I in the large family. Molecular genetic analysis of TGFB1 gene can offer an accurate diagnosis of patients with lattice corneal dystrophies in the clinical treatment.

**Key words:** LCD I, mutation, R124C, TGFB1

More than 30 types of inherited corneal dystrophy have so far been categorized based on disease features including physical appearance, clinical features and age of onset in their clinical features.<sup>[1,2]</sup> These have proved to be limited in effectiveness because the same phenotypes of disease maybe with different genotypes or the same mutation site expressing various types diseases.<sup>[3]</sup> With the development of biomedicine, molecular genetic studies can further confirm which mutations in gene contribute to the disease generation. These contain 11 chromosomes (chr: 1, 2, 5, 9, 10, 12, 13, 16, 17, 20, and X) and several genes (the transforming growth factor beta-induced gene: *TGFB1*, the carbohydrate sulfotransferase 6 gene: *CHST6*, the gelsolin gene: *GSN*, the keratin 3 gene: *KRT3*, the keratin 12 gene: *KRT12*, and the surface marker 1 gene: *M1S1*).<sup>[1,4]</sup>

*TGFB1* (BIG-H3) which was first isolated by Skonier *et al.* is identified as a transforming growth factor- $\beta$ 1 inducible gene in a human lung adenocarcinoma cell line.<sup>[5]</sup> It is located on the long

arm of chromosome V (5q31), and encoded protein played an important role in the skin and cornea tissues.<sup>[6]</sup> A large number of missense mutations (R124C,<sup>[7]</sup> L518P,<sup>[8]</sup> P501T,<sup>[9]</sup> L527R,<sup>[10]</sup> N544S,<sup>[11]</sup> A546T,<sup>[12]</sup> and N622K (T1913G or T1913A)<sup>[13]</sup> in this gene were the main cause of lattice corneal dystrophy (LCD). LCD is classified into LCD I (OMIM 122200),<sup>[14]</sup> LCD II (OMIM 105120),<sup>[15]</sup> LCD III (OMIM 204870),<sup>[16]</sup> and LCD IIIA.<sup>[17]</sup> LCD I is characterized by the early onset of fine lattice lines associated with a faint haze in the midstroma. It is characterized by thin lattice lines in the midstroma, leading to progressive opacification, grayish nodular deposits, and other visual defect.

Here, we report six related Chinese families, who are both Han People, with the classical mutation of R124C in *TGFB1*. Affected patients in these families show typical phenotypes of LCD I. It is indicated that Chinese LCD I patients with R124C mutation have similar clinical features within the same family with the R124C mutation described before.

## Methods

All of participants are informed, written consents according to the Declaration of Helsinki. The research was carried out by the National Laboratory of Medical Molecular Biology, Department of Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), (Beijing, China). Six

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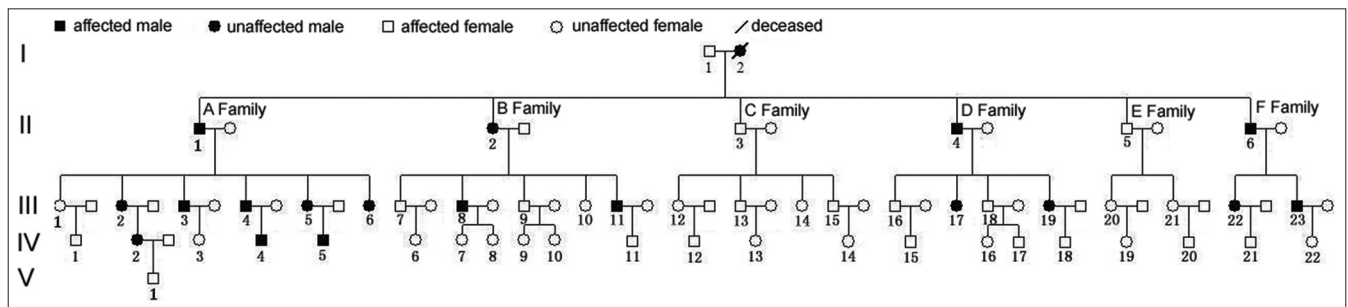
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**Table 1: Primers for TGFB1 gene**

Gene/Exon	Primer sequence (5'-3')	Temperature (°C)	Produce size (bp)
TGFB1/4	R124_F: GAGTCGTTGGATCCACCACC	62.7	406
TGFB1/4	R124_R: CATGTTCTCAGCCCTCGTGA	62.4	406
TGFB1/11	Pro501/Val505/Arg514_F: GAGGTACGGGACCCTGTTC	62.3	500
TGFB1/11	Pro501/Val505/Arg514_R: GTGCTCTTGGCTCACTTGGAG	62.4	500
TGFB1/12	R555_F: TGTGTGCATTCCAGTGGC	60.3	411
TGFB1/12	R555_R: TTCCTTTAGTCCC GCCCA	61.5	411
TGFB1/12	Ile522/Thr538/Ala546_F: GAGAGAGCTGGAGCCTGGAA	62.1	401
TGFB1/12	Ile522/Thr538/Ala546_R: CCAACTGTTTGCTGCTGGG	62.8	401
TGFB1/13	His572_F: ACTCCTTCTCAGCAGCCAGG	62	460
TGFB1/13	His572_R: GGGAAATTTAGCCAGCCTTTG	62.1	460
TGFB1/14	R626_F: TGGGCGACAAGATTGAAA	58.7	465
TGFB1/14	R626_R: CCGCAAAGACAATGTGT	58.4	465



**Figure 1:** The family hereditary patterns of six Chinese pedigrees. The pedigrees show autosomal dominant transmission of the disease. The black symbols represent affected subjects

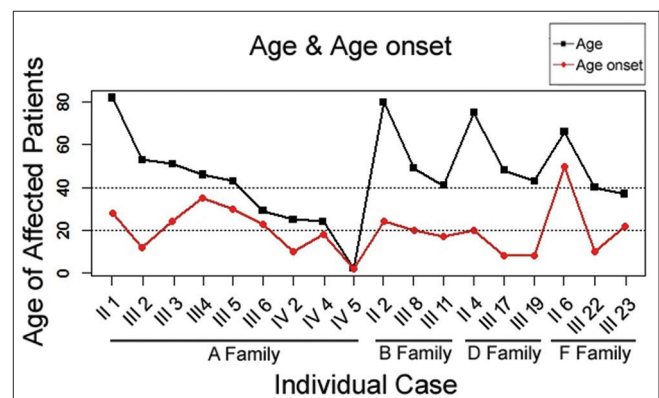
related Chinese pedigrees of LCD I were obtained for our study. Fifty-four family members from these Chinese families were recruited from The Department of Ophthalmology Chinese PLA General Hospital in our study. The time approval from the ethics committee is obtained in March, 2014.

### Clinical examinations

Complete ophthalmologic examinations were performed on the patients of these six Chinese families. The patients underwent vision examination, slit-lamp biomicroscopy, and cornea fluorescein staining.

### Genetic analysis

Genomic DNA was isolated from the whole blood of participants by the QIAamp DNA Blood Mini kit according to the manufacturer's protocol. *TGFB1* was analyzed by direct genomic DNA sequencing of mutation. The forward and reverse specific primers were designed from TianYi HuiYuan Company. Touch-down polymerase chain reaction (PCR) was performed in patients. A four-temperature touch-down PCR program was as follows: 5 min at 95°C followed by 40 cycles of 95°C for 1 min, 61°C for 1 min (with temperature decreasing from 61 to 58°C by 10 per cycle), and 72°C for 1 min with a final extension step at 72°C for 10 min. Each PCR fragment was purified by The Vigorous Biotechnology of Gel-Spin DNA Extraction Kit, and then plus strands were subsequently analyzed by direct sequencing from TianYi HuiYuan Company. Sequence results were compared with the control sequence of unaffected family members.



**Figure 2:** Scatterplot of 18 affected patients' age and age onset. Black line represents the age of 18 affected patients; the red line represents the onset age of the 18 affected patients

## Results

### Clinical features

The transmission patterns of these six Chinese pedigrees (19 affected, 35 unaffected) are consistent with autosomal dominant inheritance [Fig. 1]. General clinical data of patients from the six families are shown in Table 1. The average age of onset was 21 years old [Fig. 2], and gender was irrelevant to the disease [Fig. 3]. For example III2, the proband of A family, showed typical clinical features of LCD I. He was

12 years old when he had bilateral recurrent corneal erosions and progressive visual defect. The slit-lamp examination of both the eyes revealed thin-branching refractive lines in the anterior corneal stroma. The small lattice-shaped opacity is the characteristic feature of LCD I, which can be observed by

direct illumination and retroillumination [Fig. 4]. Such typical clinical features of some patients illustrated the diagnosis of LCD I in these six families.

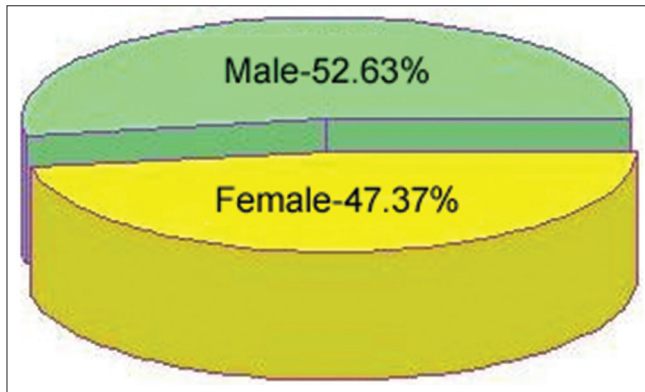
**Genetic analysis**

The *TGFBI* gene (bp: 135,364,584–135,399,507) is known to be related to LCD I. Each previous reported mutation of the *TGFBI* gene was amplified by touch-down PCR [Table 2] and directly sequenced to verify the disease-causing mutation [Fig. 5].

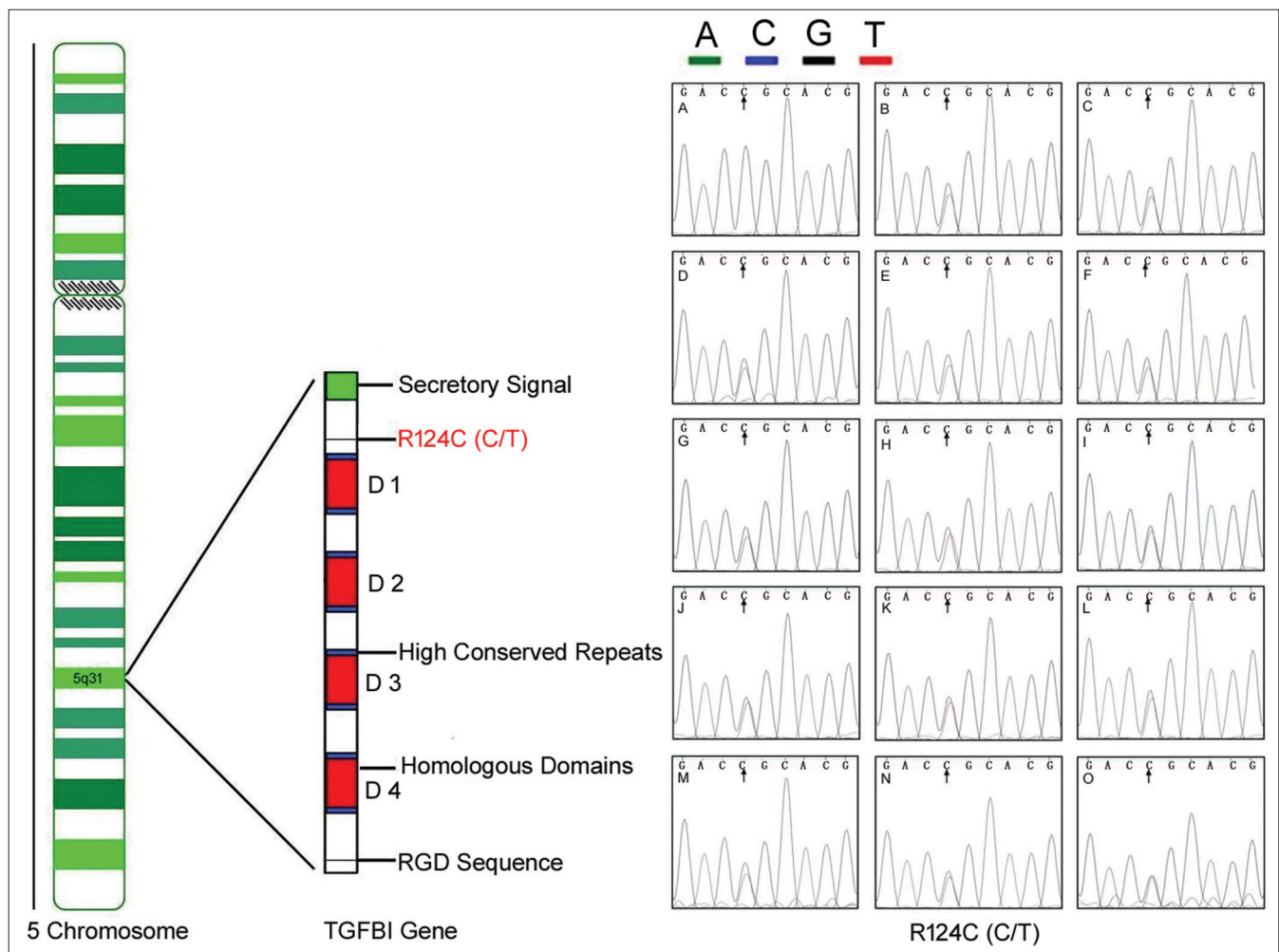
**Discussion**

The LCD can be categorized according to the specific layer of cornea mainly affected: epithelial, subepithelial, stromal, and endothelial dystrophies.<sup>[18-21]</sup> About 30 different mutations in *TGFBI* have been identified in families with variants of LCD. Some reports from different ethnic groups have described an R124C mutation of the *TGFBI* gene as the most common cause of LCD I;<sup>[22-25]</sup> however, the classic LCD I is relatively rare in Chinese people.

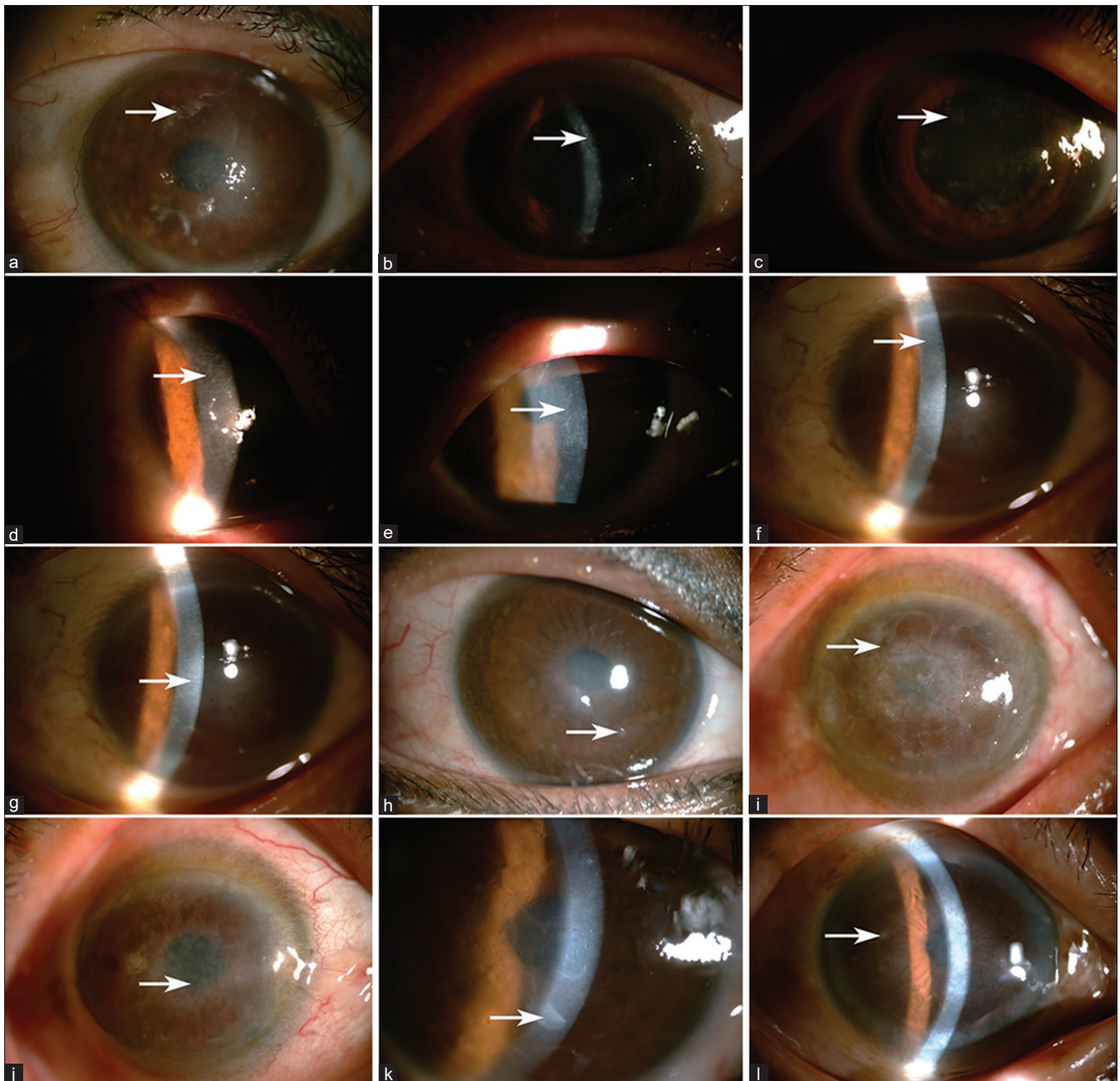
In our study, genetic analysis of *TGFBI* showed that all affected members of the family have a heterozygous mutation



**Figure 3:** The affected male and female have quite proportion that male accounted for 52.63% and female accounted for 47.37%



**Figure 4:** Direct sequencing of polymerase chain reaction products corresponding exon 5 of *TGFBI* in the region encompassing codon 124. A. The arrow indicates that the sequence of codon 124 in unaffected family members showed one blue peak. B-O. The arrow indicates the affected family members showed blue and red peaks resulting in a single heterozygous base-pair transition leading to an amino acid substitution, Arg124Cys. No equivalent mutation was detected in other unaffected family members



**Figure 5:** Corneal photographs of lattice corneal dystrophy type I patients. Eyes were displaying nodulolinear amyloid deposits (arrow). The deposits are mainly located in anterior stroma (arrowhead)

of R124C, which belongs to a classification of LCD I. However, whether other SNPs may influence the progression of disease have to further research.

Currently, genetic diagnosis plays an important role in establishing the classification of corneal dystrophy. Although excimer laser surgery is the most effective treatments for corneal dystrophy in clinical, molecular genetic analysis could establish more reliable diagnostic criteria and clinical diagnosis. The technique of gene examination could also make prenatal and postnatal gene diagnosis possible in our clinical practice. It is necessary to identify which specific mutations are carried by the patient to assess a final diagnosis. Once the R124C mutation is detected in an individual from these families at birth, it can be

expected that he or she will develop corneal lattice dystrophy, which may require keratoplasty in the future.

### Conclusion

The R124C mutation hot spot which was relatively rare in China was responsible for lattice corneal dystrophy type I in the large family. Molecular genetic analysis of TGFBI gene can offer an accurate diagnosis of patients with lattice corneal dystrophies in the clinical treatment.

### Disclosure statement

This submission has not been published anywhere previously and that it is not simultaneously being considered for any other publication.

**Table 2: Clinical data for the affected individuals in the family with the R124C mutation**

Individual case	Gender	Age	Age onset	Affected eye
A-II1	Male	82	28	Right
A-III2	Female	53	12	Left/Right
A-III3	Male	51	24	Left/Right
A-III4	Male	46	35	Right
A-III5	Female	43	30	Left
A-III6	Female	29	23	Left
A-IV2	Female	25	10	Right
A-IV4	Male	24	18	Left
A-IV5	Male	2	2	Left
B-II2	Female	80	24	Right
B-III8	Male	49	20	Left/Right
B-III11	Male	41	17	Left
D-II4	Male	75	20	Left
D-III17	Female	48	8	Right
D-III18	Female	43	8	Right
F-II6	Male	66	50	Left
F-III22	Female	40	10	Right
F-III23	Male	37	22	Left

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### Conflicts of interest

There are no conflicts of interest.

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