Original Article

Genetic and Phenotypic Investigation of a Chinese Pedigree with Lattice Corneal Dystrophy IIIB Subtype

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Abstract

Purpose: To investigate phenotypes and disease-causing mutation in the transforming growth factor b-induced gene (TGFBI) in a Southern Chinese pedigree with lattice corneal dystrophy (LCD) IIIB with complicated cataract.

Methods: A Southern Chinese pedigree with lattice corneal dystrophy IIIB with complicated cataract was recruited. Comprehensive ophthalmic investigations were performed before and after cataract surgery of phacoemulsification and intraocular lens implantation in the proband's both eyes. Peripheral blood was collected from the proband, and genomic DNA was extracted. All exons of the TGFBI gene were sequenced to screen possible mutations.

Results: A bilateral LCD IIIB subtype was observed in the proband. Optical coherence tomography further revealed superreflective changes in the subepithelial and stroma layers of the cornea, with reduced central corneal thickness. Notably, bilateral cataract was found in the proband. Direct sequencing detected a recurrent heterozygous missense c.1877A>G mutation in exon 14 of the TGFBI gene, resulting in substitution of histidine with arginine (p.H626R).

Conclusion: The current study was the first report of the TGFBI p.H626R mutation in Southern Chinese, suggesting that it could be a mutation hotspot across populations. Moreover, the mutation was associated with LCD IIIB subtype with complicated cataract, which had not been reported before, pointing to clinical heterogeneity of the mutation. (***Eye Science 2013; 28:144-147***)

Keywords: lattice corneal dystrophy; TGFBI; mutation; cataract

Introduction

Lattice corneal dystrophy (LCD) is a progressive hereditary disease characterized by the bilateral progressive visual impairment due to amyloid deposition in the cornea[1]. Nevertheless, disease symptoms can be variable among different LCD cases, suggesting the existence of clinical heterogeneity in LCD. According to clinical manifestations, LCD is classified into six subtypes, including LCD I[1,2], LCD II[3,4], LCD III[5,6], LCD IIIA[7-10], LCD IIIB[1], and LCD IV[11,12].

At present, various disease-causing mutations have been reported in LCD, indicating genetic heterogeneity in the disease. Most of these mutations are found in the transforming growth factor b-induced gene (TGFBI, also known as BIGH3, OMIM 601692) at 5q31, which encodes a 68 kDa protein involved in cell-collagen interactions. Different TGFBI mutations have been associated with different subtypes of LCD. For example, p.L527R is found to be associated with LCD type IV[13]. Phenotype-genotype correlation analysis could provide important insights into the etiology of LCD.

In the current study, a Southern Chinese pedigree with LCD IIIB subtype and complicated cataract was recruited, which exhibited different phenotypes from existing reports in literature. We investigated these clinical phenotypes and the underlying TGFBI mutation in the proband from the pedigree.

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Materials and methods

Subject recruitment and clinical examination

This study was approved by the Ethics Committee of Joint Shantou International Eye Center and was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from each participating subject after explanation of the nature of the study.

A Southern Chinese pedigree with LCD IIIB subtype and complicated cataract was recruited in Joint Shantou International Eye Center, Shantou University & the Chinese University of Hong Kong, Shantou, China. The proband (II–6) received combined phacoemulsification and intraocular lens implantation in both eyes. Ophthalmic examinations including best correct visual acuity and slit lamp photographs were performed before and after the sugary. In addition, time domain optical coherence tomography (OCT) was performed using a Visante™ OCT Model 1000 (Carl Zeiss Meditec, Inc., Dublin, CA) to detect corneal changes. Peripheral blood was collected from the proband, and genomic DNA was extracted by using the QIAmp Blood kit(Qiagen, Hilden, Germany).

Mutation screen

Seventeen pairs of primers(Table 1) for amplicons targeting all exons and their adjacent splicing junctions in TGFBI (NCBI human genome build 37.2, NC_000005.9 for gDNA, NM_000358.2 for mRNA, and NP_000349.1 for protein), were designed using Primer 3 (http://frodo.wi.mit.edu/primer3/), as pre viously described[14,15]. Polymerase chain reaction (PCR)amplification was performed using the GeneAmp PCR System 9700 (ABI, USA) in a 25–μl mixture containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Sangon, Shanghai, China), 1 U Taq DNA polymerase (Invitrogen, Carlsbad, USA), 0.2 μM primers, and 20 ng of genomic DNA. Bidirectional sequencing of PCR products was performed using the BigDye Terminator Cycle Sequencing v3.1 kit (ABI, Foster City, USA) and the 3130xl Genetic Analyzer (ABI, USA). Sequencing data were analyzed using novoSNP software. Mutation naming followed the nomenclature recommended by the Human Genomic Variation Society (http://www.hgvs.org/mutnomen/).

Results

Clinical phenotypes

The 41 year-old male proband(II-6)presented with blurred vision in both eyes for 1 year. He had positive family history of LCD IIIB subtype (Figure 1). Slit lamp photographs showed gray-white thick lattice lines extending across the cornea. OCT showed corneal erosion in both eye, with hyperreflective changes located at subepithelial and stromal layers of the cornea. The cornea was not smooth, and was thinner than normal, with a central corneal thickness only about 480 nm (Figure 2). Complicated cataract was observed in both eyes, which had not been found in any other existing reports for LCD IIIB subtype. After cataract sugary, the visual acuity of the right eye improved from 0.02 to 0.04, and that of the left eye improved from 0.01 to 0.1.

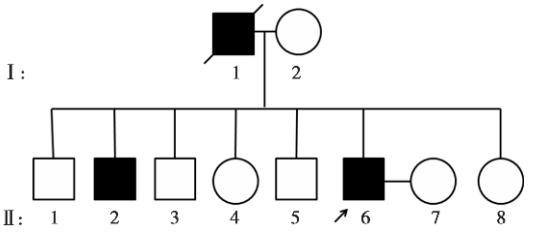

Figure 1 A Southern Chinese pedigree with LCD IIIB subtype and complicated cataract. Filled squares and circles denote affected males and females, respectively. Normal individuals are shown as empty symbols.The proband is indicated by an arrow.

Genetic analysis

Direct sequencing in the proband revealed a heterozygous missense mutation of single-base A→G transition (p.1877A>G) in exon 14 of the TGFBI gene, resulting in the substitution of histidine (CAT) with arginine (CGT) at codon 626 (Figure 4). No other TGFBI mutation was found in the proband.

Discussion

In the current study, we investigated clinical phenotypes and the mutation in the TGFBI gene in a proband from a Southern Chinese pedigree with LCD IIIB subtype combined with complicated cataract. The current study, for the first time, reported the p.H626R mutation in a Chinese family with

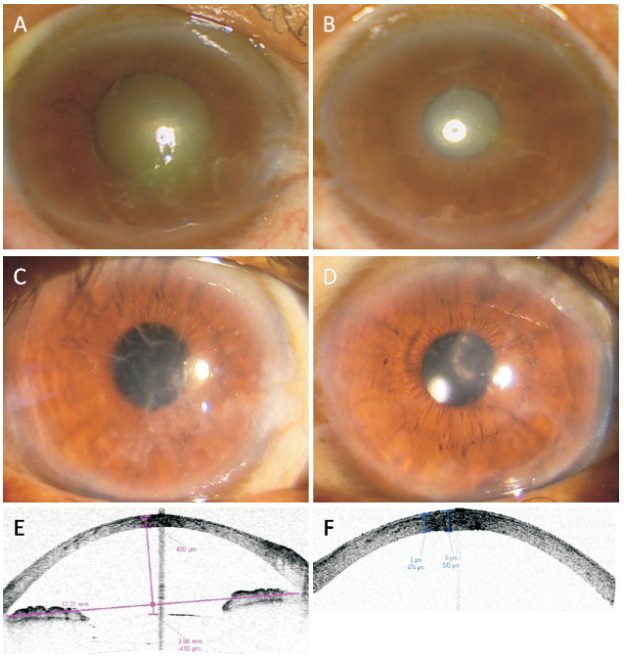

Figure 2 Photographs and optical coherence tomography of the proband′s both eyes. Photographs were taken before cataract surgery (A: OD, B: OS), B and C: after cataract surgery (C: OD, D: OS). Examinations showed gray-white thick lattice lines extending across the cornea, and the complicated cataract. The optical coherence tomography (OCT) of both eyes showed hyperreflective changes located at subepithelial and stromal layers of the cornea. The cornea was not smooth and was thinner than normal (central corneal thickness is only 480 nm) (E: OD; F: OS)

LCD IIIB subtype and complicated cataract, pointing to clinical heterogeneity for the mutation.

The LCD IIIB subtype is characterized by bilateral progressive visual impairment and has an intermediate age of disease onset between LCD I and LCD IIIA. The LCD IIIB subtype was first described in England[16], and then was named by Chau[1]. In 2010, Yang et al found this mutation in Northern Chinese, with clinical features as an intermediate subtype between LCD I and IIIA[17]. Our study showed that the proband had an intermediate age of disease onset at around 40 years, with a p.H626R mutation in TGFBI, suggesting this could be a mutation hotspot across different populations for LCD IIIB.

Complicated cataract, caused by changes in ocular structures by other diseases, can result in visual impairment[18,19]. Complicated cataract can be observed in cornea disease[20]. In the current study, complicated cataract caused the patient to be imbalanced in both

Table 1 Primer sequences used for PCR and sequencing of TGFBI exons and adjacent regions

Exon	Primer	5′ to 3′	Annealing temp (°C)	Product size(bp)
1	TGFBI-1F	CCGCTCGCAGCTTACTTAAC	58	362
	TGFBI-1R	AGCGCTCCATGCTGCAAGGT		
2	TGFBI-2F	GGGCTGATGGGAATCTAGG	55	460
	TGFBI-2R	TGGCCAGCTTCCTAAAAATG		
3	TGFBI-3F	TCCTAAGAGGAGAGTGTTTCACC	55	383
	TGFBI-3R	GCTGTTTCCAGCTTCCATTC		
4	TGFBI-4F	GTCTTTGGGCTTTCCCACAT	55	478
	TGFBI-4R	GCATTAGACGCAACCTGGTA		
5	TGFBI-5F	AAGACTTGGGTGCTTCCTGA	55	478
	TGFBI-5R	CAAAATGTGGGTTCCACAAG		
6	TGFBI-6F	CTCTTTGTGCCTGCTTCTCC	55	478
	TGFBI-6R	GGTCAAGCTGAATGTCTGGTC		
7	TGFBI-7F	GGCTTCTGTTTTCGTCTTGG	55	427
	TGFBI-7R	TGCTCACCTCTCAGGGCTTC		
8	TGFBI-8F	AGAAGGCGAGGAGGATCTG	55	484
	TGFBI-8R	GGTCCAGGCAGGGAAGTC		
9	TGFBI-9F	TGTTCCCCTGATGACACAAG	55	499
	TGFBI-9R	CAAAGGCATTCTCTGCACTG		
10	TGFBI-10F	CCCTCTTAGGGCAAAACTCC	55	540
	TGFBI-10R	TCCTTCTGCAGGATCTCATT		
11	TGFBI-11F	ACATCATTTTATCCCAGCCTTA	55	483
	TGFBI-11R	ACCCTTCTTCCCAATGTAAGC		
12	TGFBI-12F	AGCCTGGAATCACTCCCTCT	55	499
	TGFBI-12R	AGGGTTGCTAGTCCCTGGTT		
13	TGFBI-13F	TGCTTTGTGTCCTCTGACCA	55	500
	TGFBI-13R	TGATTTCCCTGAAGACCCTCT		
14	TGFBI-14F	CTGTTCAGTAAACACTTGCT	55	350
	TGFBI-14R	CCCAATTCACTCTGCAATCA		
15	TGFBI-15F	ACAGCATCTCACCTCAGTGT	55	360
	TGFBI-15R	AACCTAGCAGGCATCTTACC		
16	TGFBI-16F	GCTTGCACAACTTATGTCTG	58	250
	TGFBI-16R	CAGGTCTGCAATGACTTC		
17	TGFBI-17F	CCTGGTCCTTGAGATTCTGA	55	490
	TGFBI-17R	GAGGCTGGATTGCTTGATTC		

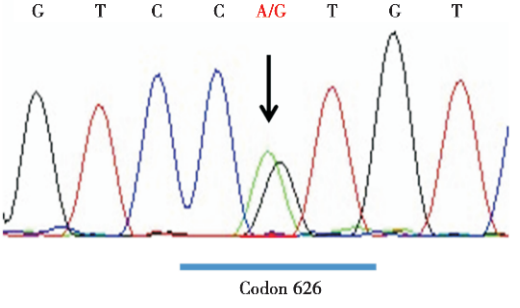

Figure 3 Direct sequencing of a p.H626R missense mutation in exon 14 of the TGFBI gene in the proband. The chromatogram shows a heterozygous, single-base A→G transition, resulting in the substitution of histidine (CAT) with arginine (CGT) at codon 626.

eyes. After cataract surgery, the visual acuity of left eye improved significantly. However, no existing literature has yet reported complicated cataract in a LCD IIIB subtype, as described in the current study for the first time.

OCT can provide information about the range of corneal opacities and measures corneal thickness in corneal dystrophy. In the current study, time-domain OCT was able to detect hyperreflective changes located at subepithelial and stromal layers of the cornea, and thinning of corneal epithelium in LCD IIIB subtype. The manifestations were comparable to LCD variants, but differed from the LCD I subtype reported by Nowińska in Polish[21].

In the current study, we found a TGFBI p.H626R mutation in a Southern Chinese family affected by a LCD IIB subtype with complicated cataract. Our findings therefore provide new insight into the genotype-phenotype correlation and the roles played by TGFBI in the etiology of LCD.

Disclosure statement

There is no conflict of interest to declare.

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