

RESEARCH NOTE

A clinical and molecular-genetic analysis of Chinese patients with lattice corneal dystrophy and novel Thr538Pro mutation in the *TGFBI* (*BIGH3*) gene

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Lattice corneal dystrophy (LCD), characterized by the accumulation of amyloid within the cornea, is one of the most common inherited corneal diseases. Mutations in the human transforming growth factor beta-induced gene (*TGFBI*), formerly designated *BIGH3*, have been shown to be associated with different forms of LCD, including LCD type I (LCDI) and type IIIA (LCDIIIA) (Munier *et al.* 2002). Here we report the clinical and molecular-genetic analysis of LCD in Chinese patients. Clinical evaluation showed presence of corneal defects with variations in both age of onset and severity of amyloid deposits in these patients. Sequence analysis identified a novel A-to-C transversion at position 1612 (A1612C) in the *TGFBI* gene in a pedigree with LCDI. The A1612C mutation is expected to result in a Thr538Pro (T538P) substitution in *TGFBI* protein. Our study showed that P501T mutation with its disequilibrium-linked polymorphism IVS10-3T→C, previously identified only in Japanese LCDIIIA patients (Tsujikawa *et al.* 2002), was also present in Chinese LCDIIIA patients.

Patients and methods

Subjects and ophthalmological examinations

As part of a genetic screening programme for vision impairment, two members from one family (figure 1a) and three sporadic cases with LCD were diagnosed at the Eye Clinic, First Affiliated Hospital of Zhejiang University School of Medicine. All cases were reexamined and ascertained by the same ophthalmologists (Drs Yangshun Gu and Lili Chen), and blood samples were collected. The corneas of some

subjects were photographed with a slit-lamp camera. Informed consent was obtained from all subjects prior to their participation in the study, in accordance with the regulations of the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Zhejiang University.

Molecular-genetic analysis

DNA samples were extracted from peripheral blood of the five patients using the salting-out method described by Miller (Miller *et al.* 1988). A group of 53 unrelated, healthy individuals were analysed as control. Based on the data of a patient population analysis showing that there were four mutational hotspot exons (exons 4, 11, 12 and 14) in the *TGFBI* gene (Munier *et al.* 2002), we screened these exons using the method of polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP). The primers used were identical to those described in previous reports (Munier *et al.* 1997; Korvatska *et al.* 1998). Thermal cycling was performed on a GeneAmp PCR System 9700 with appropriate conditions. PCR products were denatured and electrophoresis was performed in 8% nondenaturing polyacrylamide gels with 6% glycerol or 5% sucrose in 1×TBE buffer at room temperature. The gels were silver-stained. The PCR products with a mobility shift in gels were purified (Qiaquick gel extraction kit, Qiagen) and sequenced on both strands by using Mega BACE 1000 sequencer (Amersham Pharmacia Biotech).

Results

Clinical manifestations

Clinical information of family A (figure 1b) and three sporadic cases with LCD is summarized in table 1.

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Molecular-genetic analysis

In family A, we found a shifted band on PCR-SSCP analysis in the *TGFBI* gene exon 12 of the proband (III-1). Sequencing assay identified a novel mutation, ACA→CCA, at codon 538 (figure 1c). The proband's mother (II-2) showed the same mutation (figure 1c). This mutation, confirmed by sequencing both the sense and antisense strands, was never present in 106 chromosomes of 53 unrelated control subjects.

Cases B and C were shown by PCR-SSCP and sequencing analysis to carry a common C417T/Arg124Cys mutation in exon 4 of *TGFBI* gene. A Pro501Thr substitution, a previously reported common mutation in Japanese LCD patients, was identified in exon 11 of *TGFBI* gene in case D (data not shown).

We also identified four different single nucleotide polymorphisms (SNPs) in *TGFBI* gene. The SNPs 1620T/C (F540F) and 1416C/T (L472L), located in the coding regions of the gene (figure 1d), are present in 16% and 13% of our control subjects and of lower frequency than the reported 25% and 18% in an earlier study (Korvatska *et al.* 1998). The other two SNPs, IVS10-3T→C and IVS12+23A→G, residing in introns 10 and 12, have frequencies of 3% and 6% respectively. None of them cosegregates with the disease.

Discussion

In this study, we conducted a clinical and molecular-genetic analysis in Chinese patients with LCD. Multiple forms of LCD have been described (Munier *et al.* 2002), including

LCDI and LCDIIIA. Our subjects fall into two of them based on their clinical features: LCDI (cases A, B and C)—autosomal dominant, with early onset, characterized by thin grayish, linear, branching deposits of amyloid material in subepithelial and stromal layers of the cornea; and LCDIIIA (case D)—autosomal dominant, with late onset, characterized by thick lattice lines extending across the cornea frequently associated with superficial corneal erosions. While cases A, B and C are categorized as LCDI, they varied in age of onset and clinical manifestations. The patients of family A and case C developed the symptoms much later than case B and patients of previous reports. This suggests that not only do different mutations in the *TGFBI* gene differently alter its function, but also genetic modifiers and/or environmental factors might affect clinical consequence of this disease.

We report here a novel mutation A1612C in the *TGFBI* gene in a Chinese family with LCDI. The mutation, which is a first-base alteration of codon 538 and results in threonine to proline substitution, is different from the previously reported mutation C1613G, which causes threonine to arginine substitution and LCDI/IIIA clinically (Munier *et al.* 2002). Two different pathogenic mutations occur at the same codon, indicating that threonine 538 might be important to the biological function of the protein.

Two sporadic cases with LCDI have been associated with the R124C mutation in our study. R124C alteration abolishes a putative phosphorylation site, which might change the tertiary structure of kerato-epithelin and lead to amyloid conversion (Munier *et al.* 1997). R124C mutation has been

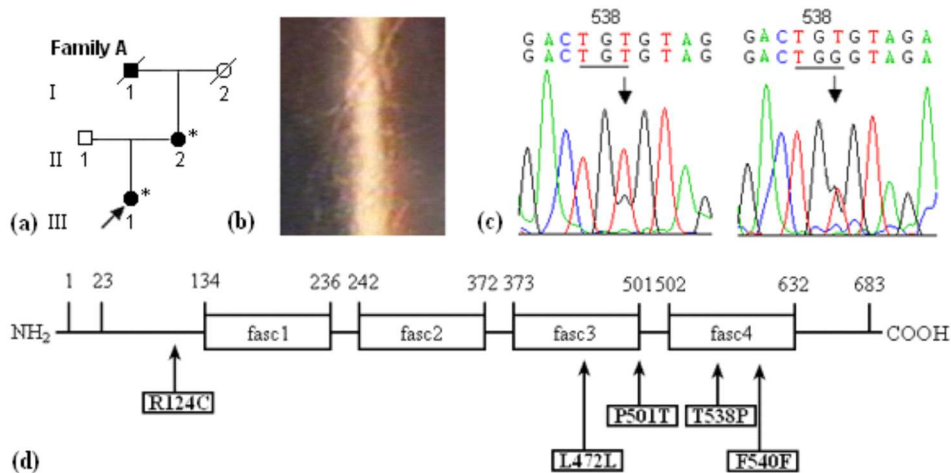


Figure 1. Identification of a new mutation in a family with LCDI. (a) Pedigree of family A with LCDI. Solid symbols indicate the affected members, and open symbols indicate the unaffected members. The arrow indicates the proband. Asterisks indicate the members who were examined. A diagonal line through the symbol indicates deceased members. (b) Slit-lamp photograph of the left eye of proband A-III-1 showing lattice-like lines in the central cornea. (c) Left: nucleotide sequence of *TGFBI* gene exon 12 of the proband A-III-1 (antisense strand). Right: nucleotide sequence of *TGFBI* gene exon 12 of proband's mother A-II-2 (antisense strand). Top lane: normal sequence; bottom lane: mutated sequence. Arrows indicate an A-to-C transversion at the first nucleotide position in codon 538, which results in a Thr538Pro substitution. (d) Domains of the *TGFBI* protein and the positions of the mutations and the SNPs. fasc1 to fasc4: internal domains homologous to the *Drosophila melanogaster* fasciclin gene.

Table 1. Summary of clinical manifestations in the subjects.

Case no.	Age	Sex	Age of onset	Vision	Symptoms	Slit-lamp examination	Clinical diagnosis
A-III-1, proband	25	F	19	LE:20/100 RE: 20/70	Low vision and photophobia in both eyes for 5 years	Numerous fine, refractile lattice lines and dots in the anterior stroma of the central cornea in both eyes	LCDI
A-II-2, mother	55	F	35	LE:N/A RE:N/A	Low vision and ocular pain in both eyes for 25 years; underwent penetrating keratoplasty in right eye at age 47 years and in left eye at age 49 years	Grafts are transparent in both eyes	LCDI
B	26	F	5	LE:20/60 RE:20/50	Low vision, ocular pain and photophobia in both eyes for 20 years	Irregularity of the epithelial surface with fine branching lattice lines in the subepithelial and anterior stroma in both eyes	LCDI
C	22	F	19	LE:20/50 RE:20/60	Low vision and photophobia in both eyes for 3 years	Irregularity of the epithelial surface with fine branching lattice line in the subepithelial and anterior stroma in both eyes	LCDI
D	71	M	68	LE:20/80 RE:20/100	Gradual reduction in visual acuity in both eyes for 3 years	Thick, ropy branching lattice lines throughout the cornea in both eyes	LCDIIIA

found to be the cause of LCDI in a wide spectrum of ethnic groups (Munier *et al.* 1997). Among Japanese LCDI patients, 23% had the R124C mutation (Yoshida *et al.* 2002). Further study is required to determine whether this site is a mutation hotspot in Chinese LCD patients.

The Pro501Thr mutation, associated with LCDIIIA, was reported previously only in western Japan, with an incidence of about 6% (Yamamoto *et al.* 1998; Kawasaki *et al.* 1999) and reduced penetrance (Stewart *et al.* 1999; Ha *et al.* 2002). Tsujikawa speculated that the Pro501Thr may be a founder mutation rather than a hotspot since there was a disequilibrium-linked polymorphism, IVS10-3T→C, in all the Japanese patients with this mutation (Tsujikawa *et al.* 2002). Interestingly, our Chinese patient who carries the Pro501Thr mutation also carries IVS10-3T→C. Thus, this mutation might be a common ancestral mutation shared by the two ethnic groups.

The *TGFBI* gene codes for the protein BIGH3, which is highly conserved between species. This protein contains

an N-terminal secretory signal peptide, four 140-amino-acid repeats (fasc1-4, figure 1d) with internal homology, and an arg-gly-asg (RGD) motif at the C terminus. More than 25 mutations in the *TGFBI* gene have been identified in hundreds of affected individuals of different ethnic groups (Endo *et al.* 1999; Mashima *et al.* 2000; Kim *et al.* 2001; Chau *et al.* 2003). Overexpression of mutated *TGFBI* gene in human corneal epithelial cells induces apoptosis (Morand *et al.* 2003), indicating apoptosis might be a key element in the pathophysiology of TGFBI-related corneal dystrophies. However, the molecular mechanism underlying the disease remains elusive and should be further studied.

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