

SCIENTIFIC CORRESPONDENCE

H626R and R124C mutations of the TGFBI (BIGH3) gene caused lattice corneal dystrophy in Vietnamese people

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Background/aims: Mutations of the human transforming growth factor β induced gene (*TGFBI*) were reported to cause lattice corneal dystrophy (LCD) in various nationalities. This study analysed the *TGFBI* gene in Vietnamese people with LCD.

Methods: 13 unrelated families, including 34 patients and 21 unaffected members were examined. 50 normal Vietnamese people served as controls. Blood samples were collected. Genomic DNA was extracted from leucocytes. Analysis of *TGFBI* gene was performed using the polymerase chain reaction and direct sequencing. Corneal buttons were studied histopathologically.

Results: Two clinically distinguishable forms of LCD were revealed: one was typical of LCDI; the other was characterised by the late onset, thick lattice lines, and asymmetry between two eyes. Sequencing of the *TGFBI* gene revealed R124C mutation in three families and H626R mutation in 10 families. Congo red staining of the H626R-LCD cornea showed amyloid deposits in the subepithelial and stromal layers.

Conclusions: R124C and H626R mutations of *TGFBI* gene caused LCD in Vietnamese people. R124C, a common cause of LCDI in many nationalities, was relatively rare, whereas H626R reported in several white people but not yet in Asians was most common (>75%) in Vietnamese people. Since the phenotype caused by H626R represents a new variant intermediate between LCDI and LCDIIIA, we proposed to consider it as LCD type IIIB.

Lattice corneal dystrophy (LCD), a type of stromal amyloidosis, is one of the most common inherited corneal diseases. LCD type I, a classic form (MIM 122200) is an autosomal dominant disorder of childhood onset, characterised by thin greyish, linear, branching deposits of amyloid material in subepithelial and stromal layers of the cornea. Recurrent corneal erosion is characteristic, and keratoplasty is frequently required.^{1,2} LCD type II, Meretoja syndrome (MIM 105120), is accompanied by systemic amyloidosis involving the cornea, skin, kidney, and other tissues, and is characterised by thicker, but fewer and more peripheral, lattice lines.³ LCD type III (OMIM 204870) is characterised by late onset (70–90), thick lattice lines extending from limbus to limbus. This type has a presumably autosomal recessive mode of inheritance and affects Japanese individuals.⁴ LCD type IIIA, also of late onset, in contrast has an autosomal dominant inheritance, and is characterised by thick “ropy” lattice lines extending across the cornea frequently associated with superficial corneal erosions.⁵

The human transforming growth factor β induced gene, *TGFBI* (BIG-H3) was identified by Skonier *et al.*⁶ In 1997, Munier *et al* identified mutations in the *TGFBI* for four distinct autosomal dominant corneal dystrophies: R555W with granular corneal dystrophy type I, R124H with Avellino, R555Q with

Reis-Bucklers, and R124C with LCDI in white people.⁷ After reports also described the R124C mutation of the *TGFBI* gene as a common cause of LCDI in various nationalities, including Japanese, American, Canadian, French, and Korean.^{8–11} In addition, a variety of novel mutations in *TGFBI* gene were also found to cause atypical, non-classic forms of LCD.^{12–16} Recently, P501T mutation of *TGFBI* gene was identified as a cause of LCDIIIA in Japanese families.¹⁷

In this study, we report the clinical and genetic findings in 13 unrelated Vietnamese families with LCD.

PATIENTS AND METHODS

The study was performed in accordance with the tenet of the World Medical Association of Helsinki regarding research involving human subjects. This was a joint research between the National Institute of Ophthalmology (NIO), Hanoi, Vietnam, and the Department of Ophthalmology, Juntendo University, Tokyo, Japan, approved by Ministry of Health of Vietnam (agreement No 10887 YT/QT) and the ethics committee of Juntendo University (No 109). Thirteen unrelated families of Vietnamese origin, including 34 patients and 21 unaffected members from eight different northern and central provinces of Vietnam were examined. The patients, 15 males and 19 females were aged 28–72 (average 50.5) years old. All of them had been followed at the Department of Corneal and External Diseases, NIO. Clinical diagnosis of LCD was based on slit lamp finding of lattice deposits in the cornea bilaterally. The pedigree of each family was also recorded. After informed consent was obtained from all participating subjects, blood samples were taken from peripheral blood vessels. Fifty normal Vietnamese subjects were used as controls. Leucocytes were pelleted and transferred to Juntendo University. Each genomic DNA was extracted by a standard procedure.¹⁸ At first, exons 4, 11, 12 of the *TGFBI*, as “hot spots,” were amplified by the polymerase chain reaction (PCR) using each pair of primers.⁷ Exon 14 was amplified as described by Stewart *et al.*¹⁵ PCR products were purified using the High Pure PCR Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), then subjected to direct sequencing.^{19,20} The terminator reaction for sequencing was performed using the DNA Sequencing Kit, Dye Terminator Cycle Sequencing, Ready Reaction (Perkin Elmer Applied Biosystems, Foster City, CA, USA). Sequencing was carried out in an automated DNA Sequencer Model 373A (Applied Biosystems) in both sense and antisense strands. Nucleotide sequences for coding regions were compared with the nucleotide and deduced amino acid sequence of *TGFBI* (BIGH3) human cDNA published by Skonier *et al.*⁶

Two corneal buttons obtained at keratoplasty were fixed in 10% buffered formaldehyde solution for routine paraffin wax embedding and light microscopy. Cross sections of each button were prepared and stained with Congo red.

RESULTS

Clinical examination revealed two distinguishable forms of LCD. In one group of patients, LCD type I was recognised; it

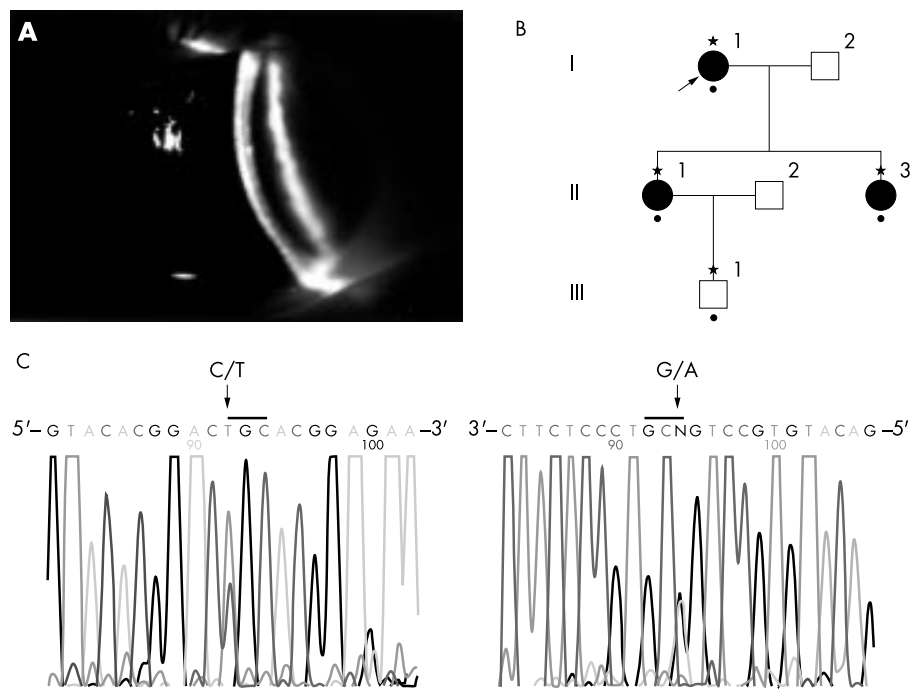


Figure 1 LCD type I in Vietnamese family. (A) Corneal photograph shows numerous thin refractile lattice lines in subepithelial and stromal layers of the central cornea. (B) Pedigree of family L with typical LCDI. Open symbols indicate the unaffected individuals; solid symbols indicate the affected members. Asterisks indicate the members who were examined clinically and genetically. Arrows indicate the proband. (C) Sequence around codon 124 within exon 4 in this group of patients revealed a nucleotide transition at codon 124 (CGC→TGC), causing an Arg→Cys substitution (R124C).

was characterised by numerous thin linear branching deposits in the subepithelial and stromal layers (Fig 1A). The onset of symptoms was during childhood and visual impairment became obvious in the second decade of life, associated with frequent recurrent corneal erosions. Of the few families having typical LCDI, a pedigree was shown in Figure 1B. Sequencing of *TGFBI* gene within exon 4 in these families revealed a nucleotide transition (CGC→TGC) of codon 124, causing an Arg→Cys substitution (R124C) (Fig 1C).

In the majority of families, we encountered clinical features clearly different from the typical LCDI described above. This group of patients showed fewer but thicker and deeper lattice lines, extending more to the periphery than those of LCDI (Fig 2A and B). The clinical characteristics also did not fit with LCD type IIIA, as the lattice deposits were somewhat thinner than those of LCDIIIA. As patients were followed, progress was most commonly asymmetric between two eyes, especially in the 38 year old patient who showed the lattice deposits in the right eye initiated 3 years earlier, but not yet in the left eye (data not shown). At the time of this study, all affected individuals with this atypical form of LCD were older than 33 years most of them were over 40 years. Corneal erosion was not uncommonly observed in patients aged about 50 years or older (Fig 2B). Of these families, a pedigree was displayed in Figure 2C, showing numerous members affected with the atypical LCD in two generations. Sequencing within exon 14 of *TGFBI* gene in these families revealed a nucleotide change at codon 626 (CAT→CGT), replacing histidine with arginine (H626R) (Fig 2D). Two corneal buttons obtained from patients having H626R mutation were stained with Congo red, confirming the amyloid nature of corneal deposits. The amyloid deposits were found in the mid and

anterior stroma, and in a linear, band-like pattern above and below Bowman's layer (Fig 2E).

Results of the analysis of *TGFBI* gene in 13 Vietnamese families with LCD are summarised on the Table 1. Both R124C and H626R mutations were not detected in the unaffected family members and also excluded from 50 control subjects (data not shown).

DISCUSSION

Clinical examination of patients in 13 families revealed two forms of LCD that differed from each other by the onset, and characteristics of lattice deposits. In the group of patients with typical LCD type I, sequencing of *TGFBI* gene within exon 4 revealed an R124C mutation. The other group of patients with the atypical LCD were shown to have another, H626R mutation within exon 14 in *TGFBI* gene. These genetic defects were not detected in healthy family members or in normal controls. This indicates that R124C and H626R mutations were co-segregated with the disease phenotype, thus causing LCD in Vietnamese people.

Many reports from different ethnic groups have described an R124C mutation of the *TGFBI* gene as the most common cause of LCDI⁸⁻¹¹; however, as the sequencing data showed, only three out of 13 families had the R124C, indicating that classic LCD type I is relatively rare in Vietnamese people. However, the H626R mutation, rarely reported before, was found at high frequency (>75%) in the majority of families (Table 1), indicating that the LCD phenotype caused by H626R mutation of *TGFBI* gene is most common in Vietnamese.

The H626R mutation of *TGFBI* gene was previously reported to cause LCD in several white families^{15 16 21} but not in Asians.

Table 1 Mutations of the *TGFBI* gene found in Vietnamese people with LCD

LCD	Mutations	No of families	No of patients
Type I	R124C	3	5
Intermediate between type I and IIIA*	H626R	10	29
Total		13	34

*Proposed to be classified as LCD IIIB.

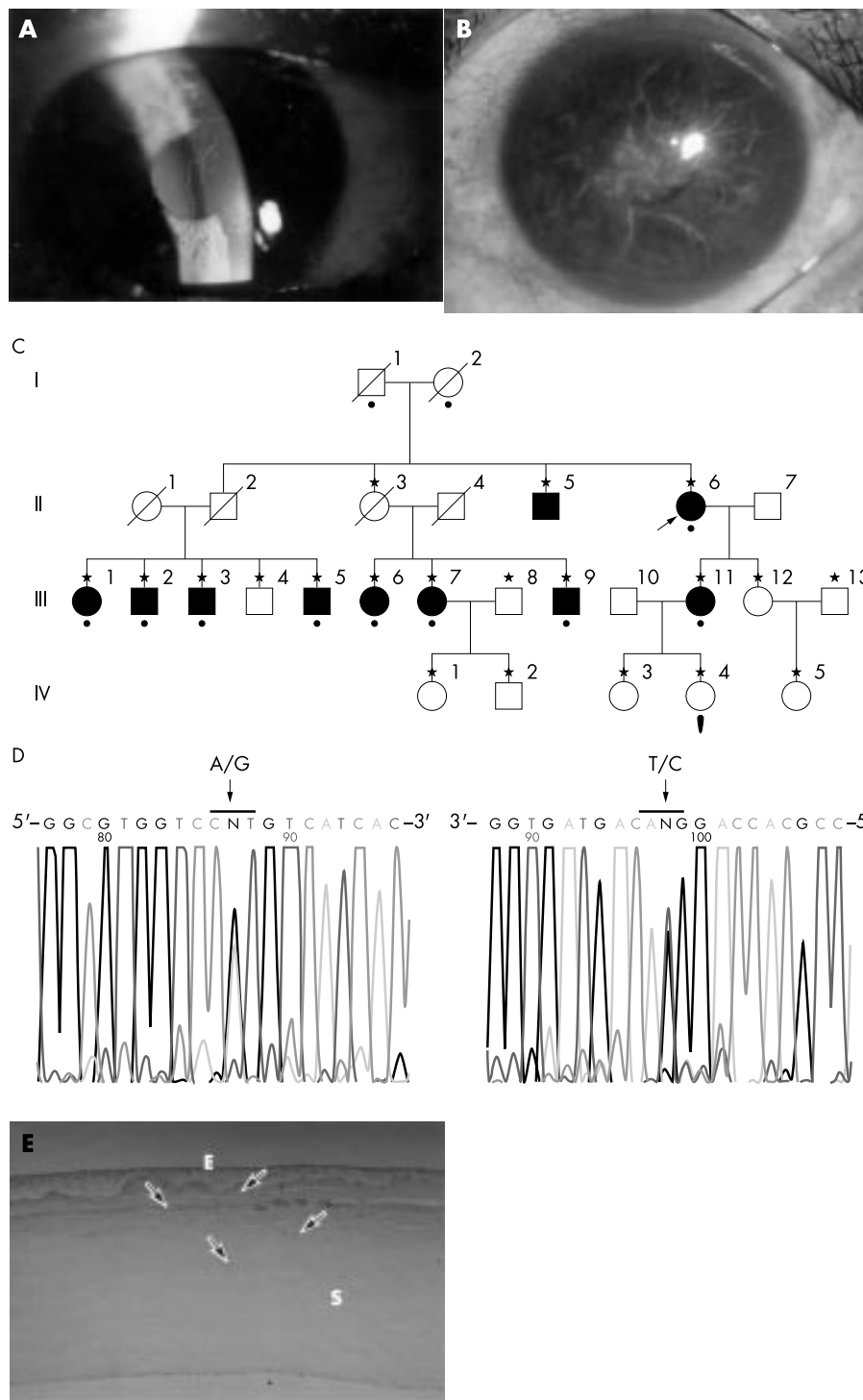


Figure 2 (A) Corneal photographs of a 42 year old patient with an atypical form of LCD shows few thick and deep lattice lines. (B) Corneal photographs of a 49 year old patient shows thick lattice lines extending more to the periphery. A superficial scar was seen as a result of recurrent corneal erosions. (C) Pedigree of family N affected with the atypical LCD. Open symbols indicate the unaffected individuals; solid symbols indicate the affected members. Asterisks indicate the members who were examined clinically. Dots beneath the symbols indicate the member examined genetically. Arrow indicate the proband. (D) Sequence around codon 626 within exon 14 in the patients with atypical LCD shows a nucleotide change at codon 626 (CAT→CGT), replacing a histidine with an arginine (H626R). (E) Histopathological appearance of the corneal buttons obtained from patients having H626R mutation on *TGFBI* gene. The corneal deposits were stained with Congo red in both mid and anterior stroma, and in a linear, band-like pattern above and below Bowman's layer. (E) Epithelium; (S) stroma. Arrows indicate amyloid deposits.

Our patients with the H626R mutation showed clinical features that clearly differed from the classic LCD type I—later onset, fewer but thicker lattice lines extending more to the periphery, and significant asymmetry between two eyes. This clinical variant also did not fit with LCD IIIA; firstly, the onset of H626R-LCD was usually in the third or fourth decades of life, thus earlier than LCD IIIA; secondly, the lattice deposits were thinner than those of LCD IIIA. In fact, the H626R-LCD encountered in our study represents an intermediate form between LCDI and LCD IIIA, whose clinical appearance corresponded to previously described cases.^{15 16 21} In our experience, the H626R-LCD is clinically easy to distinguish from LCD type I and can also be differentiated from LCD IIIA. Recently,

Schmitt-Bernard *et al* studied the clinical, histopathological, and ultrastructural characteristics of LCDI, H626R-LCD, and LCD IIIA and pointed out that these were three different clinically and histopathologically distinguishable forms of LCD. The LCD caused by the H626R mutation on the *TGFBI* gene constituted a separate group of LCD as intermediate type.²² Our data add weight to the H626R-LCD phenotype as a new type of LCD. Since H626-LCD has clinical characteristics similar to LCD IIIA and in the category of LCD already classified as types I, II, III, IIIA, and IV²³ we propose to consider this new variant as LCD type IIIB. Thus, in a group of LCD III with late onset and thick lattice deposits, LCD III is an autosomal recessive with no known mutation; LCD IIIA is autosomal

dominant caused by the P501T of the *TGFBI* gene and mostly affects Japanese; and LCDIIB is also autosomal dominant with intermediate late onset, caused by the H626R mutation on the *TGFBI* gene, and mostly affects Vietnamese people. As H626R mutation was found at relatively high frequency (>75%), codon 626 within exon 14 is also considered a "hot spot" in the *TGFBI* gene for Vietnamese patients with LCD.

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