

A Novel Nonsense Mutation with a Compound Heterozygous Mutation in TGFBI Gene in Lattice Corneal Dystrophy Type I

Tohru Sakimoto*, Hitoshi Kanno[†], Jun Shoji*,
Yoji Kashima*, Shigeki Nakagawa[‡], Shiro Miwa[§] and Mitsuru Sawa*

*Department of Ophthalmology,

Nihon University, School of Medicine, Tokyo, Japan;

[†]Department of Transfusion Medicine, Tokyo Women's Medical University,
Tokyo, Japan; [‡]Department of Biochemistry, Nihon University, School of Medicine,
Tokyo, Japan; [§]Okinaka Memorial Institute for Medical Research, Tokyo, Japan

Purpose: We examined transforming growth factor beta-induced (TGFBI) gene mutations in a family with lattice corneal dystrophy type I.

Methods: The proband was one of the offspring of a consanguineous marriage; 4 affected and 3 unaffected individuals of the family were investigated. Genomic DNA of each case was extracted and used for polymerase chain reaction (PCR). The exon 4, 11, and 12 of the TGFBI gene were directly sequenced. The mutations were confirmed by PCR restriction fragment length polymorphism analysis.

Results: There was no significant difference in phenotype between the proband and the other 2 patients, except for progression of the corneal opacity with age. R124C mutation was detected in all affected individuals. In addition, G470X, a novel nonsense mutation, was detected in the proband, resulting in the proband being a compound heterozygote with the TGFBI gene. Her unaffected daughter was found to be heterozygous for G470X.

Conclusion: It is most likely that the novel nonsense mutation is not pathogenic, and that the mutant keratoepithelin protein with R124C is responsible for the phenotype. **Jpn J Ophthalmol 2003;47:13–17** © 2003 Japanese Ophthalmological Society

Key Words: Corneal dystrophy, nonsense mutation, transforming growth factor beta-induced gene.

Introduction

Most hereditary corneal dystrophies are bilateral and progressive. Lattice corneal dystrophy (LCD) constitutes a heterogeneous group of corneal dystrophies, which are thought to be inherited in an autosomal dominant manner. LCD has been classified into four distinct subtypes, Type I, II, III, and III A.

In 1997, Munier et al reported that several corneal dystrophies were caused by specific mutations in the transforming growth factor beta-induced (TGFBI) gene, which encoded keratoepithelin protein.¹ These involve the LCD type I and IIIA, granular corneal dystrophy, Avellino corneal dystrophy, and Reis-Bückler corneal dystrophy.^{2,3} These autosomal dominant inherited corneal dystrophies are thought to be associated with a heterozygous or homozygous missense mutation in the TGFBI gene. In addition, insertion of three amino acids and deletion of two amino acids were also reported recently.^{4–6} We report a family with the LCD type I associated with compound heterozygous mutations including a novel nonsense mutation.

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Correspondence and reprint requests to: Tohru SAKIMOTO, MD, Department of Ophthalmology, Nihon University, School of Medicine, 30-1 Oyaguchi Kamimachi, Itabashi-ku, Tokyo 173-8610, Japan

Materials and Methods

Family Study

We investigated a family comprising 4 LCD type I patients and 3 unaffected individuals. No clinical signs of systemic amyloidosis were observed in any of the individuals. The affected family members were examined and were also being followed up at the Department of Ophthalmology, Nihon University School of Medicine, Itabashi Hospital, Tokyo. They were examined with a slit-lamp biomicroscope. The genetic investigation was approved by the Ethics Committee in Nihon University, School of Medicine. Informed consent was obtained from the examined individuals.

Deoxyribonucleic Acid Analysis

After obtaining informed consent for genetic investigations, we extracted genomic deoxyribonucleic acid (DNA) from leukocytes of peripheral blood samples (QIAamp DNA Blood Mini Kit; QIAGEN, Hilden, Germany). We examined exon 4, 11, and 12, where many mutations in the TGFBI gene have been reported. Exon 4, 11, and 12 of the TGFBI gene were amplified, using sense and antisense primers, which were reported by Munier et al (exon 4F/R,11F/R, 12F/R),¹ by polymerase chain reaction (PCR) in a total volume of 50 μ L. PCR products contained the following final components: 0.2 mM deoxyribonucleotide triphosphate, 10 \times PCR buffer (Perkin-Elmer, Branchville, NJ, USA), 0.5 μ M sense and antisense primers, 2.5 U *Taq* DNA polymerase (Ampli*Taq*; Perkin-Elmer), distilled water, and DNA. PCR products were purified with a commercial kit (QIAquick PCR Purification kit; QIAGEN), followed by direct sequencing. The samples were sequenced with an automatic fluorescent DNA sequencer (ABI Prism 377; Applied Biosystems, Foster City, CA, USA) and a BigDye terminator cycle sequencing FS ready reaction kit (Applied Biosystems), and both sense and

antisense strands were sequenced. The mutations were confirmed by PCR restriction fragment length polymorphism (PCR-RFLP) methods using primers and restriction endonucleases denoted in Table 1.

Results

Family Study

Figure 1 shows the pedigree of the studied family. Asterisks indicate affected individuals. They all live in Ibaragi Prefecture. The proband, case 1, is a 71-year-old woman who was one of six offspring of a consanguineous marriage. Because her parents and all of her sisters were already deceased, we were unable to perform genetic research of the ancestry. The proband had suffered from progressively poor visual acuity since childhood. Slit-lamp examination disclosed dense, severe subepithelial opacities without accompanying typical lattice lines, and also mild nuclear cataracts in both eyes (Figure 2). At the time of the first examination at our hospital, her best-corrected visual acuity was 0.01 for the right eye and 0.03 for the left eye.

Case 4, a 44-year-old woman, a daughter of the proband, underwent lamellar keratoplasty for the right eye in 1991 at our hospital. She was treated at the eye clinic close to her home after the surgery, but recurrent corneal opacities exacerbated. In 1999, slit-lamp examination disclosed dense, but not as severe as in the case of the proband, subepithelial honeycomb-like opacities in the left eye (Figure 3), as well as recurrence of opacities in the right corneal graft. Her best-corrected visual acuity was 0.3 for the right eye and 0.1 for the left.

Case 7, an 18-year-old girl, a granddaughter of the proband, had been diagnosed as having lattice corneal dystrophy at the age of 3 years at an eye clinic close to her home. In the first decade, her visual acuity was good, although she sometimes was suffering from eye pain due to recurrent corneal erosion. At the time of the first examination at our hospital, slit-

Table 1. Strategy for Identification of the TGFBI Mutation Spots in Polymerase Chain Reaction (PCR) Restriction Fragment Length Polymorphism Analysis

Mutation	Primers	PCR Products (bp)	Restriction Enzyme	Normal Allele	Mutant Allele
G470X (1408CAG→TAG)	G470XF*/11R	193	<i>FspI</i>	175 + 18	193
R124C (370CGC→TGC)	R124CF†/4R	136	<i>ApaI</i>	112 + 24	136

*G470XF:5'-TTCTCTGTCCCTCTTCTGCG-3'.

†R124CF:5'-CCACCACTCAGCTGTACAGGGCC-3'.

lamp examination disclosed dense, subepithelial honeycomb-like opacities in the center of the cornea of her right eye (Figure 4). Her best-corrected visual acuity was 0.2 for the right eye and 0.08 for the left.

Deoxyribonucleic Acid Analysis

The results of DNA analysis are shown in Figure 1. We examined the individuals indicated by the numbers in the figure. We found a novel nonsense mutation in the TGFBI gene, 1408CAG→TAG (Gly470Stop; G470X) in case 1. The proband was heterozygous for G470X. This mutation caused truncation of almost 32% of the TGFBI gene from its C-terminal. In addition, a missense mutation, 370CGC→TGC (Arg124Cys; R124C) was detected on the other allele. As a result, the proband was verified as a compound heterozygote with the TGFBI gene. The other affected offspring were heterozygous for R124C; however, G470X was not detected. On the other hand, heterozygous G470X was detected in case 2, the unaffected 48-year-old daughter of the proband.

In PCR-RFLP analysis for G470X, double bands (193 bp and 175 bp) were observed after the *Fsp* I digestion, confirming that the proband was heterozygous for G470X (Figure 5). We checked for G470X in 100 TGFBI alleles in Japanese individuals, but G470X was not detected in the normal population.

Discussion

In our study, all affected individuals had the R124C mutation as has been previously described.⁷ There was no significant difference in phenotype between the proband and the other 2 patients, except for progression of the corneal opacity with age. This mutation shows complete cosegregation of the mutations with the disease phenotype. As cysteine is expected to participate in disulfide bonding, R124C would influence the three-dimensional structure of the mutant protein.⁷ A recent investigation reported that a 22-amino-acid-mutated BIGH3 (TGFBI)₍₁₁₀₋₁₃₁₎ polypeptide including R124C formed amyloid fibrils in vitro, suggesting that amyloid fibril formation did

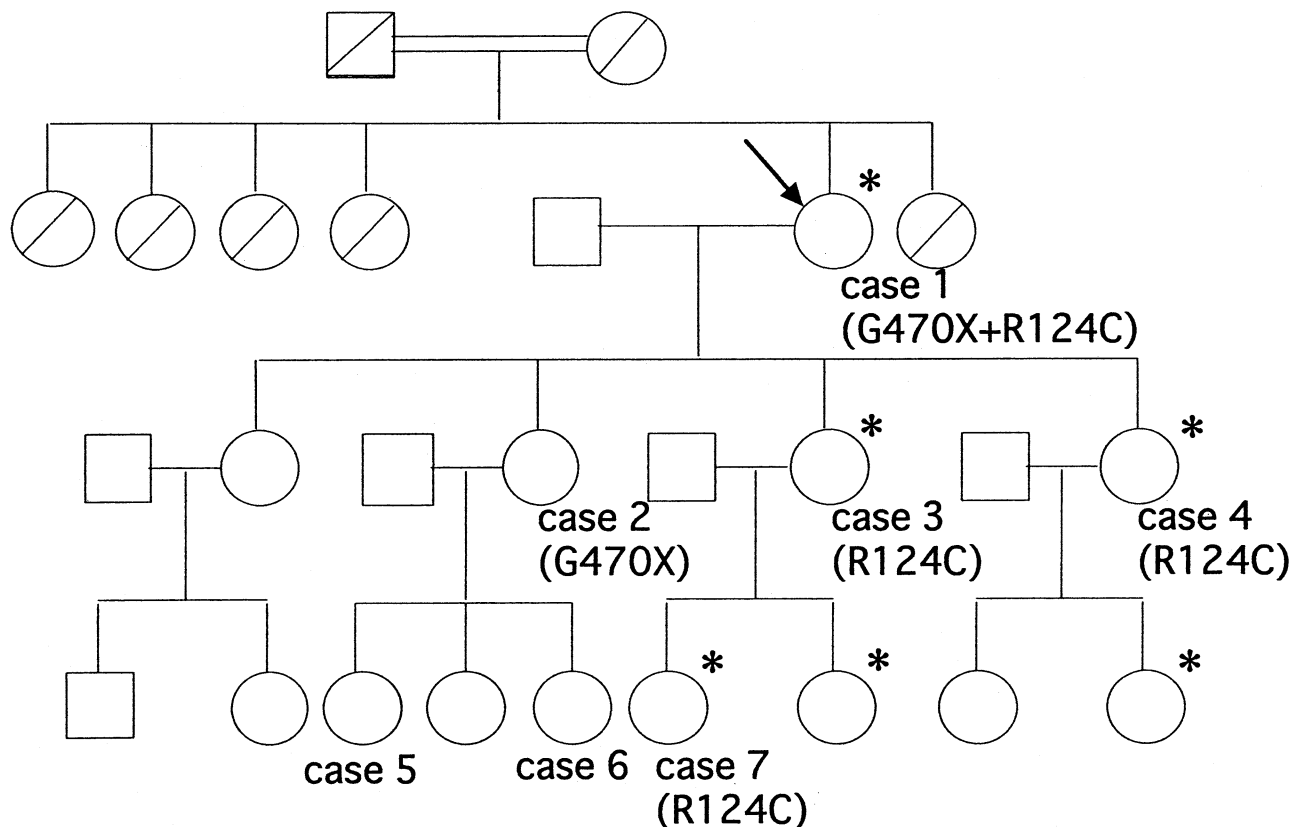


Figure 1. The pedigree of the family studied. Arrow indicates the proband, a 71-year-old woman. Asterisks indicate the affected individuals. The family members with mutations detected in this study are shown with case numbers. The slash in a symbol means family member is deceased.

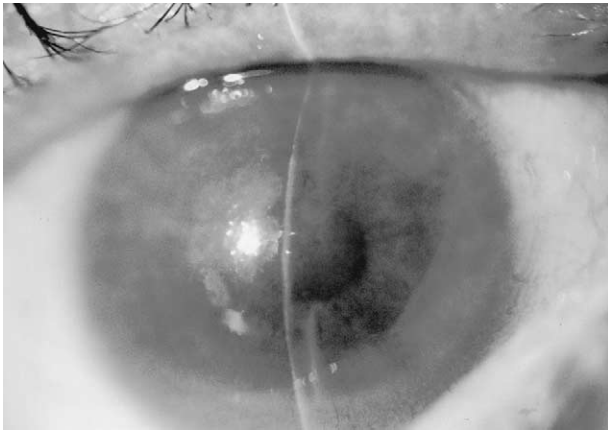


Figure 2. Slit-lamp photograph of the left eye of case 1, the proband. Dense and severe subepithelial opacities are present.

not require the presence of an entire molecule of the TGFBI protein.⁸ These studies indicated that mutant protein with R124C is responsible for the phenotype.

However, we detected a novel nonsense mutation in the proband. Almost one third of the TGFBI polypeptide will be lost by this mutation. In order to examine the pathophysiological significance of the nonsense mutation, we screened the family members.

All affected individuals had the R124C mutation, but only the proband was compound heterozygote for R124C and G470X. An unaffected daughter of the proband, being asymptomatic for corneal disorder, was heterozygous for G470X (Figure 1, case 2).

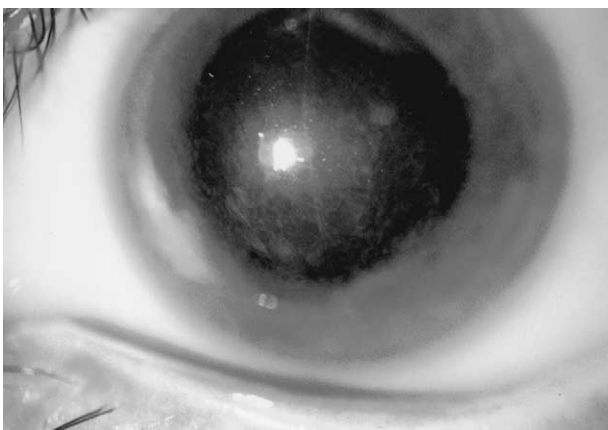


Figure 3. Slit-lamp photograph of the left eye of case 4, daughter of the proband. The opacities are dense but not severe in this case compared with those in the proband, case 1.

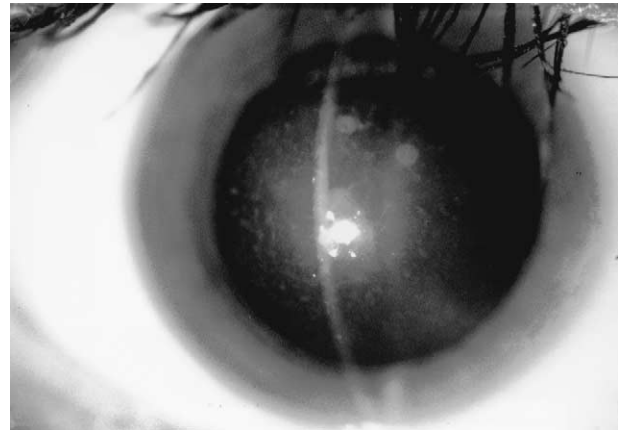


Figure 4. Slit-lamp photograph of the right eye of case 7, granddaughter of the proband. Subepithelial honeycomb-like opacities are limited to the center of the cornea.

These results suggest that G470X may not play a role in the disease phenotype.

Korvatska et al showed an unusual accumulation of keratoepithelin protein by a Western blotting study in an LCD type I patient's cornea.⁹ In their report, several keratoepithelin proteins with the N-terminal truncation have been shown to be present in the cornea. In addition, the R124C mutation caused an accumulation of the mutant proteins with the C-terminal truncation. Because the G470X was not cosegregated with the disease phenotype in the proband in our study, we speculated that the G470X protein had not accumulated in the cornea. However, because we investigated only the hot spots of the TGFBI

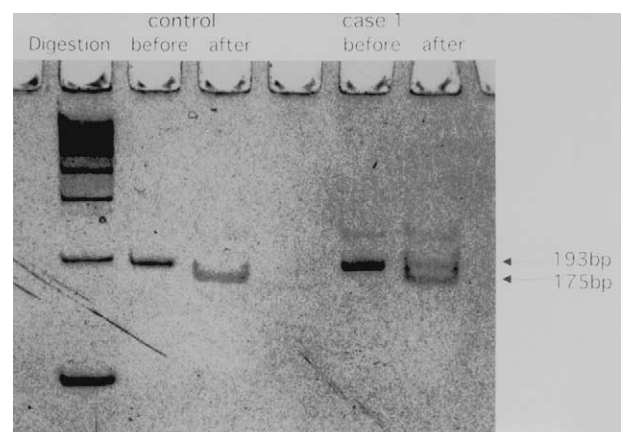


Figure 5. Polyacrylamide gel electrophoresis of polymerase chain reaction-restriction fragment length polymorphism for G470X, confirming proband was heterozygous for this mutation.

gene, exon 4, 11, and 12, further investigations would be required. In this investigation, we showed that the nonsense mutation in the TGFBI gene would not affect the phenotype, and that the mutant keratoepithelin protein with R124C was responsible for the corneal disorder of the proband.

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