

A Novel Mutation of the *TGFBI* Gene Found in a Vietnamese Family with Atypical Granular Corneal Dystrophy

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Background: Mutation of the human transforming growth factor beta-induced (*TGFBI*) gene causes granular corneal dystrophy (GCD) in various ethnic groups. In this report, we identify the genetic defect on the *TGFBI* gene in a Vietnamese family with atypical GCD.

Cases: The patient and her relatives were examined clinically. Genomic DNA was extracted from blood leukocytes. Fifty normal Vietnamese were used as controls. Analysis of the *TGFBI* gene was performed using polymerase chain reaction and direct sequencing.

Observations: The 42-year-old proband clinically showed multiple white dot-like opacities scattered in the anterior and mid-stroma of the central cornea. Unlike GCD, these deposits were smaller, localized deeper and less severe. DNA analysis revealed a nucleotide transversion at codon 123 (GAC → CAC), causing Asp → His substitution (D123H). This mutation was also detected in 3 out of 5 unaffected family members, but was absent in the 50 normal controls.

Conclusions: The novel D123H mutation of the *TGFBI* gene was not co-segregated with GCD in the family studied, and did not exist in the control population. It probably was a disease-causing mutation, thus expected to cause a novel variant of GCD in the proband. The detection of the D123H mutation in three unaffected family members indicates that it has low penetrance for GCD. *Jpn J Ophthalmol* 2003;47:246–248 © 2003 Japanese Ophthalmological Society

Key Words: Granular corneal dystrophy, human transforming growth factor beta-induced gene, low penetrance, novel mutation, Vietnamese.

Introduction

Granular corneal dystrophy (GCD) Groenouw type I is an autosomal dominant inherited corneal disorder that is characterized by discrete white granular opacities in the centrosuperficial region of the cornea, appearing in the first or second decade of life.¹ With age, these opacities increase in number, extent, and depth. Visual impairment is due to progressive corneal opacification as a result of recurrent erosions. Avellino corneal dystrophy (ACD) has been described as a combined dystrophy that shows both granular and lattice deposition in the same cornea. The human transforming growth factor β -induced (*TGFBI*)

gene was identified by Skonier et al.² Subsequently, five autosomal dominant corneal dystrophies: GCD Groenouw type I, lattice type I, lattice type IIIA, ACD, and Reis-Bücklers have all been reported to be caused by specific point mutations in the *TGFBI* gene. The R555W and R124H mutations of the *TGFBI* were reported to cause GCD and ACD, respectively, first in white patients by Munier et al,³ then in Japanese.⁴ Recently, a novel 6-bp deletion, involving codons 125 and 126 associated with R124L, was reported to cause a variant of GCD in a French patient.⁵

Herein, we examined a Vietnamese family with an atypical form of GCD and found a novel mutation in the *TGFBI* gene.

Materials and Methods

A Vietnamese family, including a female patient affected with atypical GCD and five family members were

Received: September 5, 2002

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examined clinically. No particular ocular history of the proband, including trauma and infection, was recorded. After obtaining informed consent, peripheral blood samples were collected from the proband and the five family members. Fifty healthy individuals of Vietnamese origin served as controls. Leukocytes were pelleted, and transferred to Japan for molecular analysis. Genomic DNA was extracted from the leukocytes by standard procedures. Exons 4 and 12 of the *TGFBI* gene were amplified by polymerase chain reaction (PCR) each using a pair of primers.³ PCR products were purified using the High Pure PCR Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), then subjected to direct sequencing. 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 the *TGFBI* (BIGH3) human cDNA published by Skonier et al.²

This study was performed in accordance with the tenets of the World Medical Association of Helsinki regarding research involving human subjects. It was a joint research project of the National Institute of Ophthalmology, Hanoi, Vietnam, and the Department of Ophthalmology, Juntendo University, Tokyo, Japan, approved by the Ministry of Health of Vietnam (Agreement No 10887 YT/QT) and the Ethics Committee of Juntendo University (Permit No. 109).

Results

Slit-lamp examination of the 42-year-old proband revealed multiple small white dot-like opacities in the central region of the corneas scattered in the anterior and mid-stroma (Figure 1A), unlike typical GCD seen in Vietnam (Figure 1B). No lattice lines or corneal erosions were observed.

In the proband, sequencing of the *TGFBI* gene within exon 12 showed no change at codon 555. Sequencing of exon 4 revealed a nucleotide transversion at codon 123 (GAC → CAC), causing Asp → His substitution (D123H) (Figure 2A). This mutation was also detected in three out of five unaffected family members: the proband's mother, her elder son, and one of three brothers (Figure 2B). This genetic defect was absent in the 50 normal control subjects, as well as from 53 Vietnamese individuals with other types of corneal dystrophy (data not shown).

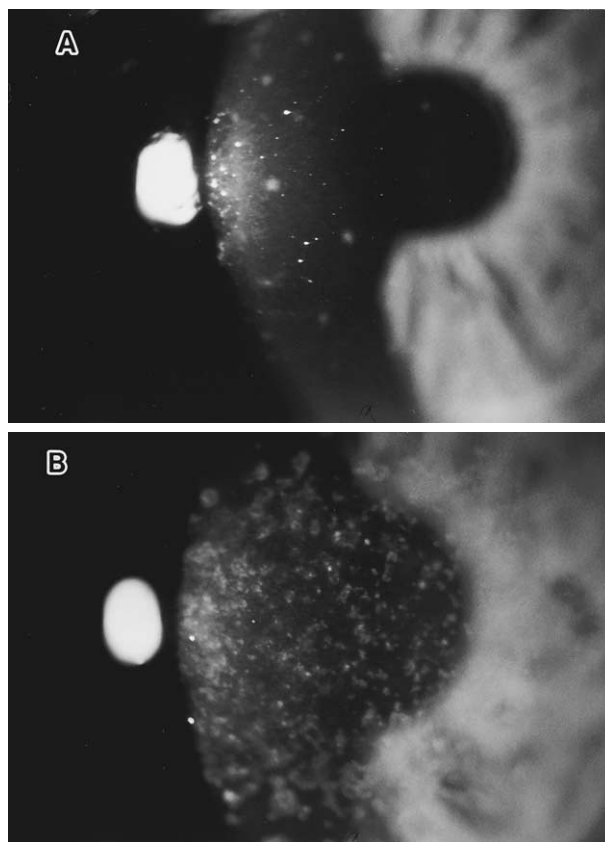


Figure 1. (A) Slit-lamp photo of the cornea of the proband showing multiple white dot-like deposits scattered in the anterior and mid-corneal stroma. (B) Typical granular corneal dystrophy seen in Vietnam (caused by R555W mutation of the *TGFBI* gene). Magnification: $\times 16$.

Discussion

The novel Asp123His (D123H) mutation was identified in the patient with atypical GCD and in three of five unaffected family members, and thus was not cosegregated with the phenotype in the family studied. The D123H mutation may be one of the rare polymorphisms; however, the fact that it was absent in the control population adds weight to our interpretation that this is a true disease-causing mutation. Therefore, D123H mutation was thought to cause the GCD in the proband. As clinical findings of this case showed, the corneal lesions were characterized by multiple white dot-like deposits, scattered in the anterior and mid-stroma. These deposits were smaller in size, localized deeper in the stroma, and less severe than those seen in GCD Groenouw type I or ACD. At the age of 42, the proband showed a mild corneal manifestation, without corneal erosion. Here, the detected genetic defect also differed from those previously reported for typical GCD and ACD. The clinical and genetic

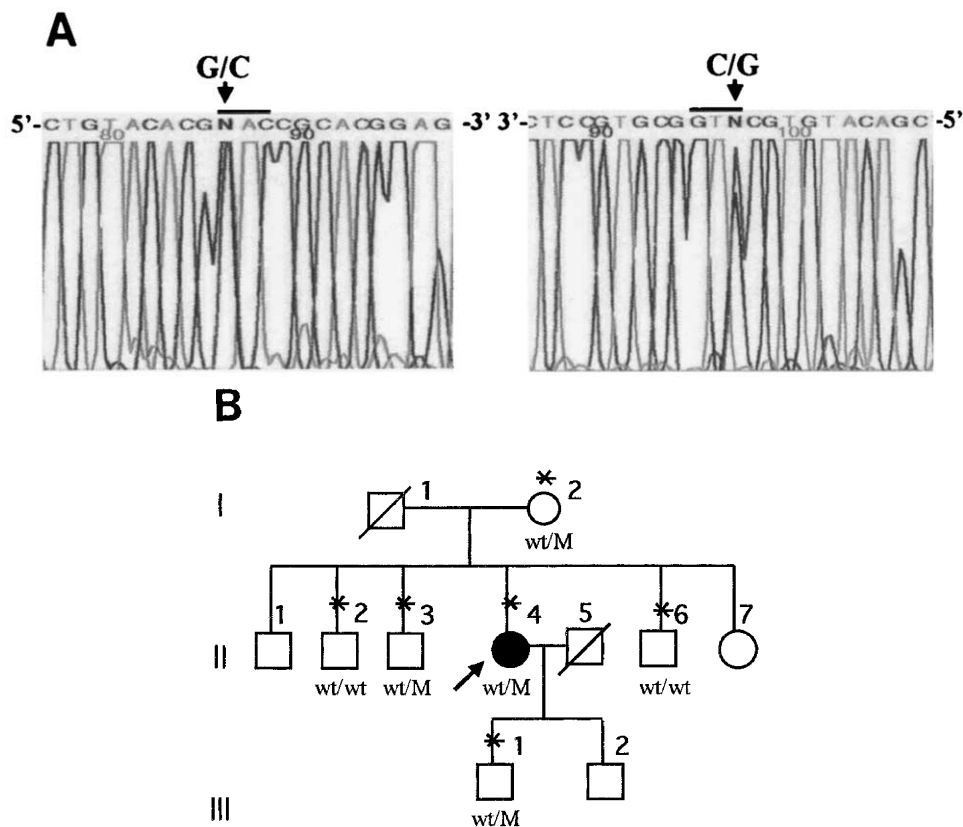


Figure 2. (A) Sequence of the *TGFBI* gene around codon 123 within exon 4 in the proband: A nucleotide transversion of a single nucleotide of codon 123 (GAC → CAC), causing Asp → His substitution (D123H). (B) Pedigree of proband. Open symbols: unaffected, solid black: affected. Asterisks indicate the members who were examined clinically and genetically. Arrow indicates the proband. wt/M: individuals (I-2, II-3, II-4, and III-1) having heterozygous D123H mutation on the *TGFBI* gene, wt/wt: individuals (II-2 and II-6) with wild-type codon 123 on both chromosomes.

features seemed to show that the proband had a novel phenotypic variation of GCD caused by the D123H mutation of the *TGFBI* gene. Because this family was from the vicinity of Hanoi, Vietnam, we named this rare variant as the Hanoi type of GCD.

However, D123H mutation was detected not only in the patient, but also in three of five unaffected relatives, who, at present, show no manifestation of GCD, even the proband's 81-year-old mother. This indicates that D123H mutation has low penetrance for GCD, as previously reported regarding the P501T mutation of the *TGFBI* gene for lattice corneal dystrophy type IIIA in a Japanese family.⁶

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