

# An Analysis of *BIGH3* Mutations in Patients with Corneal Dystrophies in the Kyushu District of Japan

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**Purpose:** To assess the involvement of *BIGH3* in corneal dystrophies (CD) with an autosomal dominant trait, in patients referred to a hospital in the Kyushu district of Japan.

**Methods:** Forty-five CD patients from 44 families were studied. Genomic DNA was extracted from peripheral blood, and exons 4 and 12 of the *BIGH3* gene were amplified by polymerase chain reaction followed by direct sequencing.

**Results:** In exon 4, an R124H mutation associated with Avellino corneal dystrophy (ACD) was found in 39/44 families (86.4%) and an R124C mutation associated with lattice corneal dystrophy type 1 (LCD1) was detected in 2/44 families (4.5%). In exon 12, an R555W mutation associated with granular corneal dystrophy (GCD) was detected in 4/44 families (9.1%).

**Conclusions:** Codons R124 and R555 of the *BIGH3* gene represent mutational hotspots in the genomes of Japanese patients with autosomal-dominant CD. **Jpn J Ophthalmol** 2002;46:469–471 © 2002 Japanese Ophthalmological Society

**Key Words:** Avellino corneal dystrophy, *BIGH3* gene, granular corneal dystrophy, lattice corneal dystrophy.

## Introduction

The 5q31-linked corneal dystrophies (CD) are autosomal-dominant disorders, characterized by age-dependent progressive accumulation of protein deposits in the cornea and resulting in visual impairment. In 1997, granular corneal dystrophy (GCD), lattice corneal dystrophy type 1 (LCD1), Avellino corneal dystrophy (ACD), and Reis-Bucklers corneal dystrophy were found to result from mutations in the human transforming growth factor (*BIGH3*) gene.<sup>1</sup>

Mutations in the *BIGH3* gene have been reported in patients at institutes both in the eastern and the western regions of Japan.<sup>2</sup> Mutations in R124H, which is normally associated with ACD, were the

most common in the Japanese CD patients, followed by R124C mutations, which are associated with LCD1 in both eastern and western Japan. In contrast, the P501T mutation was found only in western and the L527R mutation only in eastern Japan.<sup>2,3</sup> No molecular genetic analysis has yet been reported from the Kyushu district in western Japan. Because there may be a specific regional distribution of patients with particular mutations in *BIGH3*, molecular studies of *BIGH3* mutations in CD cases are needed. In this study in the Kyushu district, we analyzed *BIGH3* gene mutations in patients suffering from two of the most common and distinct autosomal-dominant eye diseases, in order to evaluate the incidence and clinical characteristics of GCD and LCD1.

## Materials and Methods

### Patients

This study was approved by the Ethics Committee of the Ohshima Hospital of Ophthalmology. Begin-

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ning in June 1998, CD outpatients at Ohshima Hospital of Ophthalmology (Fukuoka, Japan) were invited to participate in a molecular genetic study. Forty-five CD patients from 44 Japanese families were studied. All patients were residents of the Kyushu district in Japan. This group included 12 men and 33 women, ranging in age from 16–86 years (median age =  $62 \pm 16.1$  years). All patients were re-examined by the same ophthalmologist (Y.K.). The corneas of each subject were photographed several times with a slit-lamp camera. All patients gave their informed consent prior to inclusion in the study.

### Molecular Analysis

DNA was extracted from the blood of the 45 CD patients using standard protocols in order to screen for genetic mutations. Blood samples from a group of 24 unrelated, healthy individuals were analyzed as controls. Genomic DNAs of exon 4 and exon 12 of the *BIGH3* gene were amplified using appropriate forward and reverse primers (exon 4: 5'-CCCCA GAGGCCATCCCTCCT-3' and 5'-TGAGGCCT CAGCTTCACCG-3'; exon 12: 5'-GGACTGACG GAGACCCTCAA-3' and 5'-GCATCTCCCAA GAGTCTGCT-3'). Polymerase chain reaction (PCR) conditions were as follows: for exon 4, 10 minutes at 94°C, followed by 35 cycles of 30 seconds at 72°C, 94°C for 30 seconds, and 60°C for 30 seconds, with a final extension step at 72°C for 5 minutes; for exon 12, 10 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 60°C for 30 seconds, 72°C for 30 seconds, with a final extension step at 72°C for 5 minutes. The PCR products were purified and sequenced using the Big Dye Terminator sequencing kit (Perkin-Elmer Applied Biosystems, Branchburg, NJ, USA). The products were resolved on an ABI Prism 377 sequencer (Perkin-Elmer).

### Results

Forty-five patients with a history of CD have been investigated for mutations in the *BIGH3* gene and clinically assessed and/or treated in our hospital since 1998. Because population analysis revealed two mutational hotspots in the *BIGH3* gene, we initially screened exons 4 and 12 of *BIGH3* for mutations.

Table 1 shows the three different mutations detected among the 45 patients from 44 families. In exon 4, an ACD-associated missense mutation (G418A) that changes the arginine in codon 124 to histidine (R124H) was found in 39/44 families (86.4%). Another LCD1-associated missense alteration (C417T) that changes arginine to cysteine

**Table 1.** *BIGH3* Gene Mutations in 45 Corneal Dystrophy Patients

Mutations		No. of Cases	No. of Families	Disease*
R124H	Heterozygous	39	38	ACD
R124C	Heterozygous	2	2	LCD
R555W	Heterozygous	4	4	GCD
Total		45	44	

\*ACD: Avellino corneal dystrophy, LCD: Lattice corneal dystrophy, GCD: Granular corneal dystrophy.

(R124C) was detected in 2/44 families (4.5%). In exon 12, a GCD Groenouw type-I-associated missense alteration (C1710T) was detected in 4/44 families (9.1%). All the patients possessed heterozygous mutations. None of the sequence alterations described above were detected in any of the 24 healthy unrelated individuals without eye diseases used as controls. Slit-lamp examination of corneas showed that each patient's genotype correlated with a distinct phenotype. The corneas of patients with ACD had variable appearance (data not shown).

### Discussion

We screened 45 Japanese outpatients who presented at our clinic with autosomal, dominantly inherited corneal dystrophy for *BIGH3* mutations. Consistent with the previous report that ACD associated with R124H mutation is the most common form of corneal stromal dystrophy in Japan,<sup>2</sup> we found that the CD patient group exhibited a high incidence (86.4%) of the R124H mutation. In contrast, we encountered a relatively high percentage of patients (9.1%) with the R555W mutation, although it has been reported that the classic form of GCD associated with the R555W mutation is rare.<sup>4</sup> Because the percentage of patients with R555W mutation is higher in the western (Kansai district: 6/91 or 6.6%) than in the eastern (Kanto district: 1/73 or 1.4% and 4/88 or 4.5%) part of Japan, and our hospital is located in western Japan, it is likely that GCD patients predominate in western Japan. The possibility exists that the number of patients with the classic form of CD is higher in Japan than previously recognized. We encountered only 2 patients (4.5%) with LCD1 (associated with R124C mutation), a much lower frequency than seen in other districts (13/73 or 17.8% and 13/88 or 14.8% in the Kanto district; 10/91 or 11.0% in the Kansai district). We are continuing to recruit patients in order to establish a more precise figure for LCD1 frequency.

Although we screened only two mutational hotspots in *BIGH3*, in exons 4 and 12, we completed

molecular diagnoses in all 45 patients, suggesting that this type of analysis gives sufficient information for an initial screening. Other mutations in the *BIGH3* gene have been reported, including the R555Q mutation in Reis-Bucklers CD,<sup>1</sup> the L527R mutation in an LCD with deep stromal opacities,<sup>3</sup> and the H626R and N622H mutations in late-onset forms of LCD.<sup>5</sup> While a larger scale study is required to confirm the incidence of the *BIGH3* mutation in Japan, these mutations appear to be rare and/or region-specific.

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