AN AUTOSOMAL DOMINANT GRANULAR CORNEAL DYSTROPHY FAMILY ASSOCIATED WITH R555W MUTATION IN THE BIGH3 GENE

Yu-Chih Hou, Fung-Rong Hu, and Muh-Shy Chen

Abstract: Autosomal dominant granular corneal dystrophy is a stromal corneal dystrophy characterized by discrete granular opacities that cause recurrent corneal erosion and blurred vision. Four different corneal dystrophies, including granular dystrophy, are caused by mutations of the BIGH3 gene. We report a case of autosomal dominant granular corneal dystrophy in a 45-year-old woman with bilateral blurred vision and recurrent eye pain since adolescence. Numerous diffuse granular opacities were found in the superficial stroma of the cornea. Her 3 sons had a similar history and clinical presentation. Autosomal dominant granular corneal dystrophy was diagnosed. Mutation analysis by single-strand conformation polymorphism and direct sequencing in 2 of the affected family members revealed R555W mutation in the BIGH3 gene. This independent R555W mutation has been previously found in different ethnic populations including Caucasians and Japanese with granular dystrophy of Groenouw type I. These findings indicate the importance of R555 amino acid in the pathogenesis of autosomal dominant granular corneal dystrophy.

Key words: Corneal dystrophies, hereditary; Exons; Mutation


Granular corneal dystrophy (Groenouw type I; GCD) was first described by Groenouw in 1890 as an autosomal dominant corneal disorder with characteristic sugar granule-like opacities located centrally in the cornea transmitted through 4 generations.1 GCD usually becomes apparent in the first or second decade of life. Corneal opacity consists of grayish white granules with discrete borders in the center of the subepithelial layer and anterior stroma. The intervening stroma remains clear and vision is usually not affected early in the course. These granular opacities may slowly become larger and coalesce later on, but the peripheral cornea usually remains uninvolved. The granular deposits may involve Bowman’s layer and result in irregular surface and recurrent corneal erosions. Most patients with GCD usually require no treatment or penetrating keratoplasty. Although recurrent corneal erosion may occur, this tends to be self-limited.

In a large 7-generation Danish pedigree, Eiberg et al found by linkage analysis that the GCD gene is located on 5q between interleukin-9 at 5q22-q32 and D5S119 at 5q31.3-q33.3.2 Two other phenotypically seemingly distinct forms of corneal dystrophy were mapped to the same region: lattice corneal dystrophy type I and the so-called Avellino form, in which both lattice corneal dystrophy type I and granular dystrophy coexist in the same individuals.3 Four autosomal corneal dystrophies have been mapped to 5q31: granular dystrophy of Groenouw type I (CDGG1)2; Reis-Bücklers corneal dystrophy (CDRB)4; lattice corneal dystrophy type I (CDL1)5; and Avellino corneal dystrophy (ACD).5 Clinically, all 4 show progressive opacification of the cornea leading to severe visual handicap.

The BIGH3 gene is a transforming growth factor (TGF)-β–induced gene that was first isolated from human adenocarcinoma cells treated with TGF-β.6 This gene contains 17 exons corresponding to 683 amino acids which are highly conserved between species.7 The BIGH3 gene contains 4 homologous internal domains, which can be folded into a bivalent structure to bind a surface protein on 2 different cells and participate in the transmission of intercellular signals.8 In 1997, Munier et al generated a yeast artificial chromosome (YAC) contig of the linked area.
and, following cDNA selection, recovered the \textit{BIGH3} gene that encodes keratoepithelin.\textsuperscript{7} Four different missense mutations of the \textit{BIGH3} gene were detected in the CpG dinucleotide of 2 arginine codons, 124 and 555. Arg124His, Arg124Cys, Arg555Trp, and Arg555Gln mutations were reported to cause ACD, CDL1, CDGG1, and CDRB, respectively.\textsuperscript{7} Severe, early-onset, superficial forms of CDGG1 have also been described as a homozygous manifestation in the offspring of a consanguineous marriage in which both affected parents suffered from a mild form of the disease.\textsuperscript{8} All previously reported granular corneal dystrophy families were caused by the mutations in the \textit{BIGH3} gene.\textsuperscript{7,8}

\section*{Case Reports}

A 45-year-old female had blurred vision and repeated attacks of intermittent ocular irritation on both eyes since adolescence. Her best-corrected visual acuity at the initial examination was 20/40 in both eyes. Slit-lamp examination revealed bilateral breadcrumb-like stromal opacities. Clinical diagnosis of granular corneal dystrophy was made. Tracing back her family history, the pedigree of this family showed an autosomal dominant inheritance pattern (Fig. 1). Examination of her second son (III-2), an 18-year-old man, revealed fine, discrete, grey-white dots or radial lines in the anterior central stroma and the clear intervening stroma in both eyes (Fig. 2). He had suffered from progressively decreased visual acuity in both eyes, accompanied by many episodes of ocular irritation since childhood. His best-corrected visual acuity was 20/30 in both eyes. These findings prompted us to survey whether the \textit{BIGH3} gene mutation was also responsible for this CDGG1 family in Taiwan.

For mutation analysis, we collected 20 mL of venous blood from the proband and her second son (II-8 and III-2) after obtaining informed consent. Genomic DNA was extracted from leukocytes by a standard procedure. We screened the mutations in 13 exons (exon 4 to 16) of the \textit{BIGH3} gene by single-strand conformation polymorphism (SSCP) analysis\textsuperscript{9} and sequenced both exon 4 and exon 12 containing codons 124 and 555, where the originally reported \textit{BIGH3} gene mutations are located. The oligonucleotide primer sets used for the \textit{BIGH3} gene were 5’-CCCGAGAGGCCATCCCTCCT-3’ and 5’-CCGGGCAGACGGAGGAGGTCATC-3’ to amplify exon 4; and 5’-GTTGACAGGTGACATTTTCT-3’ and 5’-TATCAAAAAGGATCACTACT-3’ to amplify exon 12. Other primer sets used for other exons were based on the reports of Munier et al.\textsuperscript{7} Samples of genomic DNA were amplified by the polymerase chain reaction (PCR), according to the following thermocycling protocol: 94°C for 5 minutes; 40 cycles of 94°C for 1 minute; 55°C for 1 minute; and 72°C for 1 minute with a final extension step at 72°C for 7 minutes.

Cold SSCP technique was used to assess 13 exons of the \textit{BIGH3} gene. For SSCP analysis, 10 µL of PCR samples and 10 µL of loading dye (95% formamide and 5% of 6 x loading dye) were mixed well and denatured at 95°C for 10 minutes. Twenty mL of solution in total was loaded in parallel on a 12% non-denaturing polyacrylamide tris-boric acid-EDTA (TBE) gel (39:1 acrylamide to bis-acrylamide cross-linking in 0.5 x TBE). The polyacrylamide mini-gel (8.0 cm x 8.0 cm x 1.5 mm) was run at 5 W at 4°C on the Novex XCELL Mini Cell (Novex). The gels were stained with 0.5 µg/ml ethidium bromide in 0.5 X TBE and photographed under UV light.

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Granular Dystrophy with BIGH3 Mutation

in exon 12 (Fig. 3) but not in exon 4 or any other exon (data not shown). Exon 12 was sequenced using a direct sequencing method. Amplified PCR DNA was purified using a PCR purification kit, and sequenced with an automatic fluorescent DNA sequencer (ABI Prism 373; Applied Biosystems; Foster City, CA, USA) and an ABI PRISM Cycle Sequencing kit (Applied Biosystems). Sequencing this abnormal conformer of exon 12 revealed a heterozygous C → T transition at nucleotide 1710 (CGG → TGG) in codon 555 that resulted in a substitution from Arg to Trp (R555W) [Fig. 4]. Exon 4 (another hot spot of mutation in the BIGH3 gene) was also sequenced and showed no mutation. These sequencing results corresponded to the SSCP results.

Discussion

We performed a molecular analysis in an autosomal dominant granular corneal dystrophy family. A heterozygous Arg555Trp mutation in the BIGH3 gene was discovered in this family. This mutation can be screened out by SSCP analysis in the BIGH3 gene and further confirmed by DNA sequencing analysis. The result is identical to the mutation previously reported by Munier et al to cause CDGG1.7 The diagnosis of hereditary corneal dystrophies is originally based on slit-lamp and histopathologic findings. However, the clinical appearance of GCD varies from the presence of a few granules to the formation of diffuse and dense opacities.10,11 Definition of criteria to classify subtypes on the basis of clinical signs and symptoms (age of onset, frequency of corneal erosion, impairment of visual acuity, and depth of granules in stroma) or histological features is problematic, and variation in terminology causes confusion in the literature. Other corneal dystrophies may also share some similar clinical presentations with GCD. For example, ACD has clinical and histologic features of granular dystrophy but also exhibits features of lattice corneal dystrophy.12,13 The granular opacities are more superficial and of earlier onset than lattice deposits. In the early stage, ACD may be diagnosed clinically as granular dystrophy and in some reports is described as a variant of granular dystrophy.12,13 CDB1 (Reis-Bücklers type), an autosomal dominant corneal dystrophy of Bowman’s layer, is also considered by some authors to be a superficial variant of GCD.14 Molecular genetic analysis of the BIGH3 gene in ACD and CDB1 has shown that they share similar clinical features to GCD, and are caused by the R124H and the R124L mutations, respectively.15,16

Stewart et al reported 3 families with granular corneal dystrophy that presented with different clinical features.17 GCD in these 3 families was shown to be caused 3 separate mutations in BIGH3 gene: 418G → A, a mutation initially described as causing
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References