A Novel Mutation at Codon 124 (R124L) in the BIGH3 Gene Is Associated With a Superficial Variant of Granular Corneal Dystrophy

Yukihiko Mashima, MD; Yu Nakamura, MD; Kohsuke Noda, MD; Minako Konishi, MD; Masakazu Yamada, MD; Jun Kudoh, PhD; Nobuyoshi Shimizu, PhD

Objective: To identify the mutation in a human transforming growth factor β–induced gene (BIGH3) in a Japanese family with a severe form of granular corneal dystrophy of early onset associated with recurrent corneal erosions.

Patients: The tentative clinical diagnosis in this family was Reis-Bücklers corneal dystrophy; 4 persons affected with this disorder have been identified in 4 generations, and 3 of the 4 were examined. The proband underwent keratoplasties in our hospital (Keio University Hospital, Tokyo, Japan).

Methods: The BIGH3 gene was examined for a mutation by the polymerase chain reaction and direct sequencing. Corneal buttons of the proband were stained and examined by electron microscopy.

Results: Three affected persons were shown to have a heterozygous G→T transversion at the second nucleotide position of codon 124 (Arg→Leu) of the BIGH3 gene. In the proband, corneal deposits between the epithelium and the Bowman layer stained red with Masson trichrome stain. Electron microscopy revealed numerous electron-dense, rod-shaped bodies next to the epithelial basement membrane but no curly fibers suggestive of Thiel-Behnke dystrophy.

Conclusion: A novel R124L mutation of the BIGH3 gene was associated in this family with a superficial variant of granular corneal dystrophy.

Clinical Relevance: This mutation causes a severe form of superficial granular corneal dystrophy by producing abnormal keratoepithelin between the epithelium and the Bowman layer and thus clinical similarities to Reis-Bücklers corneal dystrophy.

Arch Ophthalmol. 1999;117:90-93

Four disorders of the corneal stroma that are linked to an autosomal dominant inheritance result from mutations in the human transforming growth factor β–induced gene (BIGH3), of which the product is keratoepithelin. These disorders are granular corneal dystrophy (GCD), associated with R555W; lattice corneal dystrophy type 1, associated with R124C; Avellino corneal dystrophy, associated with R124H; and Reis-Bücklers corneal dystrophy (RBCD), associated with R555Q mutation. Each of these 4 mutations affects the CpG dinucleotide of an arginine codon. Two types of the R124 mutation in the BIGH3 gene are associated with 2 different clinical phenotypes of corneal dystrophies, Avellino corneal dystrophy or lattice corneal dystrophy type 1, both of which are associated with amyloid deposition. Thus, keratoepithelin associated with the R124 mutation may form amyloidogenic intermediates that precipitate in the cornea.

The clinical appearance of GCD varies from the presence of a few granules to the formation of diffuse opacities. According to these published reports, GCD can be classified into at least 3 types based on the overall appearance of the cornea: Avellino corneal dystrophy, the classical form of GCD, and the superficial variant of GCD. Each type has in common the characteristics of staining bright red histologically with Masson trichrome stain and showing electron-dense rodlike bodies on electron microscopy.

We observed 7 members of a Japanese family with a severe form of GCD of early onset that was associated with recurrent corneal erosions. The clinical features of this disorder resembled those of the superficial type of GCD or RBCD, corneal dystrophy of the Bowman layer. In our patients, the disorder was associated with a novel mutation of codon 124 (R124L) in the BIGH3 gene, a third mutation in codon 124. Our data further highlight R124 as a “hot spot” within the BIGH3 gene.
PATIENTS AND METHODS

PATIENTS

Figure 1 shows the pedigree of the Japanese family with corneal dystrophy studied. The severely affected proband was referred to Keio University Hospital, Tokyo, Japan, for surgical treatment. Four affected persons have been identified in 4 generations. Seventy normal Japanese subjects (20 men and 50 women) served as controls for a mutation of codon 124.

METHODS

Formalin-fixed, paraffin-embedded sections of corneal specimens obtained at the surgery from the proband were stained with hematoxylin-eosin, Masson trichrome, or Congo red. Amyloid deposits were examined by birefringence and dichroism under cross-polarized light after staining with Congo red. The remainder of the tissue was fixed in glutaraldehyde, dehydrated in increasing concentrations of ethanol, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy.

After the 7 members of the family had given informed consent, DNA was extracted from their peripheral blood leukocytes by standard methods. Exons 4 to 16 of the BIGH3 gene were amplified from genomic DNA of each subject by the polymerase chain reaction with primers described previously, and the amplification products were sequenced on both strands. Sequencing of these amplified products was performed with an automatic fluorometric DNA sequencer (model ABI R5400, Applied Biosystems, Foster City, Calif) and a cycle sequencing kit (PRISM DyeDeoxy Terminator, Applied Biosystems) according to the manufacturer’s recommendations.

gene. We report the clinical and histological features of this novel mutation.

REPORT OF CASES

The proband, a 52-year-old woman, was referred to Keio University Hospital in April 1987 for severe corneal opacities that seriously impaired vision bilaterally. Her visual acuity was 20/200 OD and counting fingers OS. She had painful recurrent corneal erosions during her teens, and during her 20s, her vision progressively decreased. A lamellar keratoplasty was performed in the right eye at age 15 years and in the left eye at age 16 years. He later underwent superficial keratectomy in each eye. Diffuse subepithelial opacities occurred in both grafts due to disease recurrence (Figure 2, B). As a child, he had had painful recurrent corneal erosions. Recently, however, erosive episodes were rare.

The proband’s 5-year-old grandson had had painful recurrent corneal erosions since age 2 years. Slit-lamp examination of the child revealed geographic, fine, grayish opacities localized in the most superficial part of the corneal stroma of both eyes (Figure 2, C). Visual acuity was 20/25 OD and 20/30 OS. The initial clinical diagnosis in this family was RBCD.

HISTOLOGICAL STUDIES

Histological evaluation of the specimens of cornea exhibiting recurrent disease and obtained in 1997, 10 years after a previous keratoplasty, showed granular deposits that stained red with Masson trichrome stain. These were arranged in a confluent, lamellar pattern between epithelium and the Bowman layer (Figure 2, D). In some areas, the Bowman layer was completely replaced by deposits. These deposits also showed moderate staining with Congo red but equivocally exhibited birefringence and dichroism under polarized light. Electron microscopy revealed multiple electron-dense, rod-shaped bodies next to the epithelial basement membrane that were characteristic of GCD (Figure 3). No curly fibers were observed, however.

MOLECULAR GENETIC ANALYSIS

We detected a heterozygous R124L (G418T) mutation in exon 4 in all 3 affected members studied (Figure 4). This base change was not shown by the members of this family who were not affected or by 80 normal Japanese control subjects.
The clinical appearance of GCD varies from the presence of a few granules to the formation of diffuse opacities.\textsuperscript{4-7} Rarely reported has been an unusual superficial variant of GCD that shows fine corneal opacities that are confluently distributed and that appear in the most anterior central stroma in the first 5 years of life.\textsuperscript{8-10} This type of GCD is rapidly progressive and is frequently associated with recurrent corneal erosions in the first decade of life. It can be confused with RBCD because of the frequency of the recurrent erosions.\textsuperscript{8,10-14} Reis-Bücklers corneal dystrophy, an autosomal dominant inherited corneal dystrophy of the Bowman layer, is considered by some authors\textsuperscript{5,6,11} to be a superficial variant of GCD or that there seem to be at least allelic forms of the same biochemical defect.\textsuperscript{12} It is characterized by recurrent corneal erosions that appear in childhood, accompanied by an early loss of vision.\textsuperscript{5,7}

In this family, the histological findings of band-shaped, granular, Masson-positive subepithelial deposits and rod-shaped bodies on electron microscopy were compatible with a diagnosis of the superficial variant of GCD or “true” RBCD.\textsuperscript{11}

Molecular genetic analysis of the \textit{BIGH3} gene in the family we investigated did not show the R555Q mutation that has been identified in white persons with RBCD;\textsuperscript{1} instead, a novel R124L mutation was found. We questioned whether the family we described may have a superficial variant of GCD or a variant of RBCD. Because of the confusion in the literature regarding the diagnosis of RBCD, Küchle et al\textsuperscript{13} reviewed 2 types of corneal dystrophy that involved the Bowman layer and the anterior stroma—true RBCD and Thiel-Behnke dystrophy (honeycomb-shaped dystrophy). They concluded that these were 2 distinct corneal diseases, with most of the cases reported as RBCD actually being Thiel-Behnke dystrophy; most authors had incorrectly accepted peculiar curly filaments as the ultrastructural hallmark of RBCD.\textsuperscript{3} Because the clinical and histological features have not been reported in whites with RBCD and the R555Q mutation,\textsuperscript{1} we do not know whether a person with the R555Q mutation may have true RBCD or Thiel-Behnke dystrophy.

Haddad et al\textsuperscript{8} reported the cases of 2 patients with an unusual superficial variant of GCD. Both had had numerous episodes of eye irritation and a progressive decrease in visual acuity beginning at an early age. The initial histopathologic diagnosis was RBCD; however, electron microscopic observations of electron-dense, rod-shaped structures established a diagnosis of GCD. The clinical and histological features of our patients resembled those reported by Haddad et al. However, no familial history was obtained in those 2 patients. Küchle et al\textsuperscript{13} reviewed pub-
lished cases of corneal dystrophies of the Bowman layer and the anterior stroma and classified as true RBCD in both of the patients reported by Haddad et al.

CONCLUSIONS

This family with the R124L mutation has a superficial variant of GCD or true RBCD. The term “superficial variant of GCD” used in this family is based on histopathological features. Granular material that is more anterior in the cornea will give rise to erosions. Thus, affected persons in this family have more clinical similarities to true RBCD than they do to GCD. Although some authors have claimed that these 2 disorders are the same, the performance of molecular genetic analysis in other affected persons with these disorders is required to verify this suggestion. This method can now be used to definitively diagnose the various types of corneal dystrophies that previously presented an insurmountable challenge.

Accepted for publication September 16, 1998.

Toshiaki Yamamura, MD, Anamizu General Hospital, Ishikawa Prefecture, Japan, provided blood specimens for DNA analysis and clinical data.

Reprints: Yukihiko Mashima, MD, Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan (e-mail: mashima@mc.med.keio.ac.jp).

REFERENCES