Recurrent Granular Dystrophy of the Cornea: An Unusual Case

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Purpose: To describe a case of severe corneal granular dystrophy with clinicopathologic and molecular genetic findings.

Methods: The DNAs of a 53-year-old male patient suffering from corneal granular dystrophy and nonaffected family members was analyzed by molecular genetic methods. Clinical features, and histopathologic and immunohistochemical findings from the penetrating keratoplasty specimen, are described.

Results: Histopathologic and molecular genetic findings confirmed the diagnosis. A new genetic polymorphism is described. Histopathologic evidence supports the assumption of the epithelial origin of the described dystrophy.

Conclusions: A severe course of corneal granular dystrophy can be present in the absence of evidence of a homozygous mutational status, or a novel mutation. Molecular genetic analysis revealed a new polymorphism in this patient. The histopathologic findings support the assumption of an epithelial origin of the granular corneal deposits. Phototherapeutic keratectomy and penetrating keratoplasty may improve vision, but cannot prevent recurrence of the disease.

Key Words: corneal granular dystrophy, recurrence after corneal transplantation and phototherapeutic keratectomy, mutation of gene BIGH 3

(*Cornea* 2006;25:614–617)

Corneal granular dystrophy, also called Groenouw's dystrophy type 1, represents the most common hereditary corneal dystrophy with an autosomal dominant trait. It is a bilateral corneal disorder characterized by the deposition of small, discrete, sharply demarcated, grayish-white opacities in the anterior central stroma, which appear in the first or second decade of life. The intervening stroma remains clear, and vision is usually not affected early in the course. As the condition advances, individual lesions increase in size and number and may coalesce. They extend into the deeper and more peripheral stroma. However, the peripheral cornea usually remains free of deposits. Visual impairment is rarely severe until after the fifth decade and usually occurs secondary to intervening stromal opacification. Traditionally, granular, lattice, and macular dystrophy have been regarded as the 3 classic stromal dystrophies.[1] However, clinical evidence,[2,3] the results of histopathologic and electron microscopic examinations,[4–7] immunohistochemical analysis of the deposits,[8,9] and, in particular, current methods of molecular genetics[10–12] have resulted in new insights into the pathogenesis of corneal dystrophies, which thus require a new classification. The BIGH 3 gene and its product keratoepithelin are primarily expressed in the normal corneal epithelium.[13] Mutations of the BIGH 3 gene have been detected in anterior membrane dystrophies (Reis-Bücklers, Thiel-Behnke) and in the more common "classic" stromal dystrophies (granular dystrophies types 1 and 2, lattice dystrophies types 1 and 3A).[10–12] This supports the assumption that these entities may be regarded as epithelial in origin. We present the case of a patient with unusually severe corneal granular dystrophy, and discuss the results of the molecular genetic analysis.

MATERIALS AND METHODS

This case report describes the clinical, histopathologic, and molecular genetic aspects of an unusually severe case of granular corneal dystrophy. The sections of the penetrating keratoplasty specimen were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Congo red, and masson trichrome. Genomic DNAs were extracted by standard protocols from peripheral blood leukocyte samples collected from the patient and 3 healthy family members (maternal grandaunt, brother, and son) after informed consent was obtained. Exons 4 and 12 of the BIGH 3 gene were amplified from genomic DNA of each participant by polymerase chain reaction (PCR), using the following primers: ex 4f 5′-CCC CAG AGG CCA TCC CTC CT-3′, ex 4r 5′-CCG GGC AGA CGG AGG TCA TC-3, ex 12f 5′-GTT GAC AGG TGA CAT TTT CT-3′, ex 12r 5′-TAT CAA AAA GGA TCA CTA CT-3′. Each PCR product was purified and sequenced on both strands using the big dye terminator cycle sequencing kit. The products were resolved on genetic analyzer 310. Nucleotide sequences were compared with the nucleotide sequence of BIGH 3 human complementary DNA reported by Skonier et al.[14]

RESULTS

Clinical Findings and Family History

A 53-year-old white male was referred to our clinic to undergo repeated keratoplasty for granular corneal dystrophy. Corneal opacities were first observed when the patient was 5 years old, and bilateral reduced vision occurred 1 year later. He further reported the development of increased glare during his teens. Lamellar keratoplasty was performed in the right
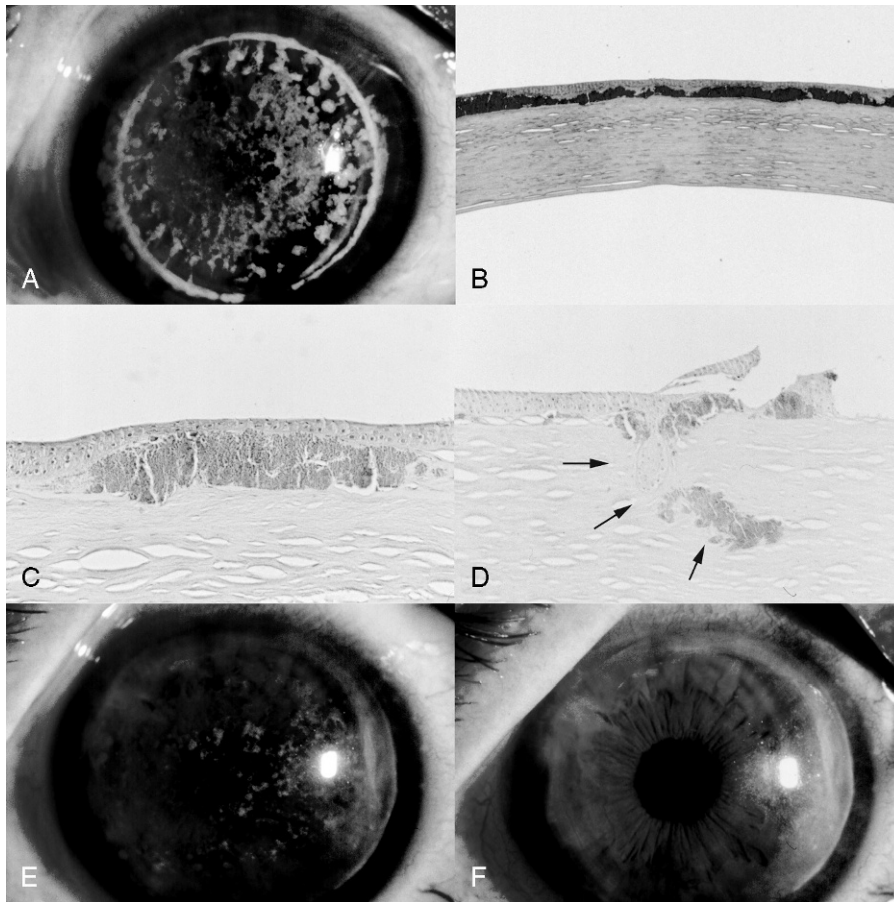
FIGURE 1. A, Corneal transplant of the right eye with multiple opacities in the superficial stroma, most severe in the central part of the transplant, at the junction to the recipient cornea and at the site of previous corneal sutures. B, Histopathologic section of the corneal transplant of the right eye with granular deposits in the subepithelial zone (masson trichrome, original magnification ×100). C, Same specimen showing the granular deposits in relation to a fibrous pannus, and at the level of the focally destroyed Bowman's layer (hematoxylin and eosin, original magnification ×200). D, Deep stromal deposits in this specimen occurring in the course of previous suture canals accompanied by epithelial invasion (arrows) (hematoxylin and eosin, original magnification ×200). E, Corneal transplant of the left eye with multiple opacities in the superficial stroma, most pronounced in the central part. F, Cornea of the left eye after phototherapeutic keratectomy.

eye at the age of 22 years; this was followed by penetrating keratoplasty due to recurrent corneal opacities at age 38. Corneal opacities of the left eye were treated with penetrating keratoplasty when the patient was 23 years old. After each intervention, the corneal transplant remained clear for only about 5 years before recurrence occurred. The patient reported that his deceased mother and maternal grandmother had very likely also suffered from the same corneal disease, but without notable visual impairment even in later life. Detailed ophthalmologic data on these women were not available. We examined the patient's maternal grandaunt, brother, and 32-year-old son, all of whom exhibited no corneal disease.

Best corrected visual acuity was 20/200 in the right eye and 20/100 in the left eye. Slit-lamp examination disclosed multiple opacities in the superficial stroma of both corneal transplants, typical of granular corneal dystrophy. Opacities were most severe in the central part of the transplants. In the right eye, there were also prominent opacities at the junction to the recipient cornea and at the site of previous single corneal sutures (Figs. 1A, E). The peripheral recipient cornea of both eyes was transparent. The other ocular structures were normal.

The patient underwent penetrating keratoplasty of the right eye. The surgical specimen was fixed in 4% formalin. Best corrected visual acuity in the treated eye was 20/40 at 24 months after surgery. However, superficial opacities in the transplant recurred after 34 months. The superficial granular

opacities in the corneal button of the left eye were removed by phototherapeutic keratectomy at an ablation depth of 0.04 mm (Fig. 1F). Best-corrected visual acuity in this eye was 20/30 1 month after surgery. However, superficial opacities in the transplant causing impairment of vision recurred after 22 months, and a second phototherapeutic keratectomy was performed.

Histopathologic and Immunohistochemical Findings

Light Microscopy

The corneal epithelium seemed unremarkable with the exception of some focal thinnings. The entire corneal transplant showed granular deposits primarily in the subepithelial zone (Fig. 1B), which were associated with a fibrous pannus (Fig. 1C). The deposits were further observed both at the level of the focally destroyed Bowman's layer and, although sparsely, in the anterior stroma (Fig. 1C). Deep stromal deposits were found exclusively at the junction of the host and donor cornea, and in the course of former suture canals in the presence of concurrent epithelial invasion (Fig. 1D). The granules seemed to be strictly extracellular. They were eosinophilic in the sections stained with hematoxylin and eosin (Figs. 1C, D) and seemed bright red in those stained with masson trichrome (Fig. 1B). Sections stained with PAS and

Congo red were negative, and the granules lacked birefringence on polarization.

Immunohistochemistry

The expression of cytokeratins 8, 13, 17, 18, and 19 and of vimentin was analyzed. In the presence of adequate positive controls and the demonstration of typical staining properties of the corneal tissue, all granular deposits were negative for those intermediate filament proteins.

Molecular Genetic Analysis

Sequence analysis of PCR products from exon 12 of the patient's BIGH 3 gene revealed a heterozygous single base pair transversion from C to T of the second nucleotide position of codon 555 (CGG/TGG). This causes the replacement of arginine by tryptophan at codon 555 of kerato-epithelin (R555W). The described mutation is characteristic of granular corneal dystrophy type 1 (granular Groenouw's type 1 corneal dystrophy). Furthermore, the analysis of exon 12 revealed a heterozygous single base pair transversion from G to A of the first nucleotide position of codon 549 (GCC/ACC). This causes the replacement of alanine by threonine at codon 549 of kerato-epithelin (A549T). Exon 4 showed no mutation.

Sequence analysis of PCR products from exon 4 to 12 of the BIGH 3 gene of 2 healthy family members (maternal grandaunt, brother) showed no alteration. Exon 12 of the son, however, did show the same heterozygous single base pair transversion at codon 549 as described for the patient (GCC/ACC). The 32-year-old son showed no corneal pathology (or: alterations). This base pair transversion can be, therefore, accorded to a polymorphism. A polymorphism is a natural variation in DNA sequence that has no adverse effects on the individual and occurs at a relatively high frequency in the general population.

DISCUSSION

Granular corneal dystrophy is known to be one of the 5q31-linked corneal dystrophies (CD), a clinically heterogeneous group of disorders caused by allelic mutations of the BIGH 3 (TGFBI) gene, correlating to specific phenotypes.[10] Mutation hot spots have been reported at positions R124 in exon 4 and R555 in exon 12. Thus, granular CD (Groenouw's type 1) is correlated to the mutation R555W, Thiel-Behnke CD to R555Q, Avellino CD to R124H, lattice type 1 CD to R124C,[10] Reis-Bücklers CD to R124L,[11] and lattice type 3A to P501T.[12] Clinical visual impairment results from progressive loss of corneal transparency secondary to the corneal deposition of aberrantly processed kerato-epithelin mutants.[8]

New mutations of the BIGH 3 gene are increasingly described in patients with atypical corneal dystrophies.[15,16] They also occur primarily in exons 4 and 12. Molecular genetic analysis of the BIGH 3 gene is, therefore, increasingly recommended in cases of atypical corneal dystrophies, as even a slight variation in the phenotype may indicate the presence of a new genotype.[15]

Severe cases of granular corneal dystrophy similar to that observed in our patient have been related to a homozygote mutational status by other authors,[6] or have been described as atypical, or special, forms of granular dystrophy.[17,18]

In view of the unusually severe expression of granular dystrophy in our patient, a molecular genetic analysis was initiated and, surprisingly, revealed the described mutation R555W of typical granular corneal dystrophy. A second, initially suspected disease-causing base pair transversion was subsequently also found in the patient's healthy son, thus providing evidence of the presence of a polymorphism. On the basis of our findings, the described highly severe form of granular dystrophy may be attributable to the classic BIGH 3 mutation and does not necessarily confirm the existence of a novel or a homozygous mutational status. However, the combination of the described polymorphism and the R555W mutation might explain the atypical course of granular dystrophy in this particular patient. For other diseases, a functional polymorphism in combination with a disease-causing mutation is reported to have phenotypical consequences.[19,20] To our knowledge, the described polymorphism in the BIGH 3 gene (GCC/ACC, codon 549, exon 12) has not previously been reported in the literature.

The present case is consistent with granular corneal dystrophy type 1 based on slit-lamp[1,21] and histologic findings[21] and on the family history of an autosomal dominant mode of inheritance with complete penetrance.[10] However, the development of severe corneal opacities at an early age is unusual. Visual impairment due to granular dystrophy is generally known to occur at an advanced age, and keratoplasty is required in only a small number of patients, mostly after the fourth decade.[1,2,22] Furthermore, the intrafamilial variation of the phenotype in the present case seems unusual,[23] with a view to the fact that there was less visual disturbance in the antecedents.

An (at least partial) epithelial origin has been described for granular corneal dystrophy. Similar to early findings in this dystrophy,[21] recurrent deposits in the corneal transplant, for example, are found almost without exception in the epithelium or, as in our patient, in the subepithelial zone.[2–5] Comparable to our findings, deep stromal deposits have been identified in the course of former suture canals,[6] or at the junction of the host and donor cornea.[2,6] These may be due to facilitated stromal migration of the epithelial deposits to these sites. There was, however, no evidence in our patient of the presence of cytokeratin 18 or vimentin in the granular deposits in support of the argument in favor of an epithelial origin brought forward by other authors.[9]

CONCLUSIONS

An unusually severe case of granular corneal dystrophy is described. The diagnosis of granular corneal dystrophy was confirmed by molecular genetic analysis. Our case illustrates that an unusually severe course of the disease, necessitating keratoplasty with the patient in his early 20s, may also occur in the presence of the classic BIGH 3 mutation. A severe form of granular corneal dystrophy is thus not always due to a homozygous mutational status. Furthermore, molecular genetic analysis revealed a new polymorphism. It is not excluded that the combination of this polymorphism and the R555W

mutation offers an explanation for a more severe course. The histopathologic findings in the corneal transplant of this patient support the assumption of an epithelial origin of the granular corneal deposits.

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