AUTOSOMAL DOMINANT GRANULAR CORNEAL
DYSTROPHY CAUSED BY A TGFBI GENE MUTATION IN A
MEXICAN FAMILY

DISTROFIA CORNEAL GRANULAR AUTOSÓMICA DOMINANTE
CAUSADA POR MUTACIÓN DEL GEN TGFBI EN UNA FAMILIA
MEXICANA

ZENTENO JC1, SANTACRUZ-VALDÉS C2, RAMÍREZ-MIRANDA A1

ABSTRACT

Objective: To describe the clinical data and the results of molecular analyses of the TGFBI gene in a patient with classic granular stromal corneal dystrophy (type I).

Methods: A female patient aged 60-years complaining of a long-standing decrease of visual acuity bilaterally associated with photophobia and foreign body sensation, underwent a complete ophthalmologic examination. Molecular analyses of DNA from the patient and from an affected brother included PCR amplification of exons 4, 11, 12, and 14 of the TGFBI gene and direct automated sequencing of the PCR products.

Results: The affected patient showed a pattern of corneal stromal lesions that was compatible with a diagnosis of classic granular dystrophy. No involvement of other corneal layers was evident. Molecular analysis disclosed a point mutation in exon 14 of the TGFBI gene which consisted of an adenine to guanine change at nucleotide position 1924, predicting a substitution of arginine instead of histidine at residue 626 of the TGFBI protein (H626R). An identical mutation was detected in DNA from her affected brother.

RESUMEN

Objetivo: Las distrofias corneales son un grupo de alteraciones hereditarias en las que una acumulación progresiva de material amiloide, hialino o mixto en las distintas capas corneales produce disminución de la transparencia corneal. Se describen las características clínicas y los estudios moleculares del gen TGFBI en una paciente Mexicana con una distrofia corneal estromal de tipo granular.

Métodos: Examen oftalmológico completo, caracterización fenotípica de la distrofia corneal, y análisis del gen TGFBI por reacción en cadena de la polimerasa (PCR) y por secuenciación nucleotídica, en DNA de la propósita y de un hermano afectado.

Resultados: Las lesiones corneales observadas en la paciente fueron compatibles con el diagnóstico de distrofia corneal estromal de tipo granular (clásica). No se observaron lesiones en las otras capas corneales. El análisis del gen TGFBI en DNA de la paciente y de un hermano afectado reveló una mutación puntual, de adenina a guanina, en el exón 14 de TGF-BI que origina un cambio de histidina a arginina en el aminoácido 626 (H626R) de la proteína TGFBI.

Conclusiones: Éste es el primer caso en el que se demuestra que una distrofia corneal granular es...
**INTRODUCTION**

Corneal dystrophy is a group of hereditary diseases characterized by loss of corneal transparency caused by progressive accumulation of abnormal deposits in corneal layers. These alterations are very heterogeneous at the clinical and genetic level, they begin in the first decades of life, mainly involve the central corneal area and are not associated to inflammatory processes (1). With the passage of time, said deposits lead to visual alterations and surgical procedures such as penetrating keratoplasty are frequently required to reestablish visual acuity. The current classification of corneal dystrophy is based on the corneal layer which is involved and on the biomicroscopic characteristics of the deposits, which can be amyloid, hyaline or a combination thereof (2). The majority of corneal dystrophies are inherited as dominant autosomic traits with variable clinical expressions and high degree of penetration (3). In recent years research on the molecular base of different types of corneal dystrophy has led to the identification of a group of stromal dystrophies which originate in mutation of the **TGFBI** gene (Transforming Growth Factor, Beta-Induced) (also known as **BIGH3**), located in the chromosomal region 5q31 (2,4-6). The mutations defined in this gene uniformly correlate with specific dystrophy types which selectively affect the corneal stroma. To date **TGFBI** mutations have been demonstrated in patients of several ethnic groups with four different dominant autosomic stromal corneal dystrophies, i.e., the granular corneal dystrophy type 1 (6-8), the granular corneal dystrophy type 2 or Avellino type (6,8,9), the granular corneal dystrophy type 3 or Reis-Bucklers type (8,10,11) and the lattice corneal dystrophy types 1, 3A, 1/3A, 3B and 4 (12-14). All corneal dystrophies caused by mutations in **TGFBI** are characterized by abnormal extracellular deposits of **TGFBI** proteins which mute in the corneal stroma (15).

The granular corneal dystrophy type 1, also known as «classical» granular corneal dystrophy, is characterized by multiple discrete opacities in the cornea similar to breadcrumbs. It is the most frequent type of stromal dystrophy (16). Generally, the alteration becomes evident in the first or second decade of life or in puberty with the appearance of white-grayish opacities which involve the superficial stromal layer of the cornea (17). In time, the lesions tend to get bigger, to aggregate and extend deeper and towards the periphery. Typically, a clear area is preserved around the corneal-scleral limbus and, in the fourth decade of life, central disc-shaped opacities may appear in the center. Affected subjects are able to maintain normal vision for a long time because visual acuity reduces very gradually (18).

A uniform phenotype-genotype correlation has been observed in the granular corneal dystrophy type 1 because the vast majority of subjects with this dystrophy have a mutation from cytosine (C) to thymine (T) on the bases 1710 of exon 12 of gene **TGFBI** which gives rise to a change from arginine to tryptophan in aminoacid 555 of the **TGFBI** protein (2,6).

**Key words:** Corneal dystrophy; stromal dystrophy; corneal genetic disease; **TGFBI** mutation.

**Conclusions:** This is the first time that a case of stromal granular dystrophy has been demonstrated to be caused by the H626R mutation, a molecular defect classically detected in the phenotypically distinct lattice corneal dystrophy. Our data indicate that the same molecular defects in the **TGFBI** gene lead to different phenotypes in stromal dystrophies, thus expanding the genotypic-phenotypic spectrum in this group of corneal diseases (Arch Soc Esp Oftalmol 2006; 81: 369-374).

**Palabras clave:** Distrofia corneal, distrofia estromal, enfermedad corneal hereditaria, mutación del gen **TGFBI**.
BI gene. This is the first description of a mutation in TGFBI in Mexican subjects with hereditary corneal dystrophy, and our results provide an expansion of the mutational range associated to stromal granular dystrophies.

**SUBJECTS, MATERIAL AND METHODS**

The leading case is a 60-year-old patient (BSA) who came to the consulting practice complaining of progressive reduction of visual acuity in both eyes since age 38. Ophthalmological exploration gave a visual acuity of 20/200 in both eyes, with deferred refraction due to the presence of important, mainly central, corneal opacities which involves the anterior and middle stroma, with clear spaces interspersed between them and respecting the corneal-scleral limbus (fig. 1). The epithelial and endothelial levels of the cornea exhibited normal characteristics. IOP was 14 mmHg in both eyes while eye movements did not exhibit alterations and no abnormalities were observed in eye fundus exploration. Associated ocular symptoms included photophobia, feeling of having a foreign body in the eye and perceiving halos surrounding lights. No associated facial dysmorphia or somatic malformations were found. The patient’s family history and genealogy analysis revealed that the father (deceased), a brother and a paternal aunt had been ophthalmologically assessed in the past and diagnosed with «corneal degeneration» and that the hereditary transmission of the disease in the family followed a dominant autosomic pattern (fig. 2). Even though the relatives involved were not directly assessed by our group, a DNA sample was obtained from one (a brother) for genetic study and to confirm the status of diseased.

Molecular studies of the TGFBI gene: after obtaining the approval of the patients and of the institutional Ethics committee, we extracted genomic DNA from leucocytes of a peripheral blood sample of the subject and a brother, by means of standard techniques. An amplification via chain reaction of polymerase (PCR) of exons 4, 11, 12 and 14 of the TGFBI was carried out, utilizing 4 pairs of specific oligonucleotids described previously (6). The PCR-amplified products were analyzed in 1.5% agarose gel from where the strips with the amplified products were sliced and purified utilizing the Qia-ex II kit (Qiagen, Charlesworth, USA).

**Fig. 1:** Phenotype of corneal dystrophy in the patient with direct lighting (A) and backlighting (B), showing thin granular opacities in the stroma which mainly involve the central area of the cornea and respect the corneal-scleral limbus.

**Fig. 2:** Simplified genealogy of the family affected by granular corneal dystrophy. A dominant autosomic transmission of the disease can be seen. The arrow signals the subject case. The squares and circles indicate men and women, respectively. The symbols in black indicate subjects with corneal dystrophy.
Automated nucleotide sequencing of the PCR purified products was carried out by means of ddNTPs terminals marked with fluorescence (Applied Biosystems, Foster City, USA). All the samples were analyzed in a Genetic Analyzer 310 (Applied Biosystems) DNA analyzer. The sequence variations were verified in both DNA chains. By way of control DNA, 60 alleles of the TGFBI gene of healthy Mexican subjects were analyzed (n=30). The nucleotide sequences were compared to the normal sequence of the TGFBI gene published in the Ensembl database (ENST00000305126).

RESULTS

After carrying out the sequencing of nucleotide bases corresponding to exons 4, 11, 12 and 14 of the TGFBI gene in the patient’s DNA, a mutation was identified in exon 14 in heterozygosis, which consisted in the change of an adenine to a guanine in nucleotide 1924. This mutation causes a substitution of the CAT normal codon, which encodes histidine aminoacid in position 626 of the protein by a CGT codon, which encodes an arginine (fig. 3). The DNA analysis of the patient’s affected brother revealed an identical change, thus confirming the familial character of the mutation and of the corneal dystrophy. This mutation was not detected in any of the 60 alleles of normal Mexican subjects.

DISCUSSION

Corneal dystrophy is a group of hereditary diseases characterized by abnormal deposits of amyloid, hyaline or mixed material in the cornea which cause significant reductions in the refractive index and loss of corneal transparency. In this study we performed a molecular analysis of the TGFBI gene in a Mexican family affected by granular stromal corneal dystrophy type 1 (classical). Previous studies in subjects of different ethnic origin have proved that this type of dystrophy is caused almost exclusively by the substitution of arginine to triptophane in aminoacid 555 of TGFBI.

In the patient analyzed in this study, the corneal phenotype is compatible with a type 1 or classical granular stromal corneal dystrophy. The diagnostic of granular stromal corneal dystrophy type 2 or Avellino, an entity which has some clinical simila-
correlation between genotype and phenotype in classical granular dystrophy, because in at least two non-related subjects affected by this type of dystrophy a mutation from C to A was found in TGFBI nucleotide 417, which leads to the substitution of arginine by serine in position 124 (Arg124Ser) (6,19).

Even though in most corneal dystrophy cases associated to TGFBI there is a strict correlation between genotype and phenotype, it is becoming evident that in some cases additional genetic and/or environmental factors are capable of modulating the phenotypic expression of identical mutations in this gene. The participation of these «modifying» genes has been suggested for other monogenic diseases (20,21).

The characterization of specific molecular defects which cause corneal dystrophy in sporadic or familial cases facilitates the early identification of other family members carrying the mutating gene and will develop the disease. This information is of great importance not only for familial genetic counseling but also in the design and application of future treatments aimed at delaying or inhibiting deposits of material which finally lead to the development of corneal dystrophy. The analysis of additional corneal dystrophy cases in different population groups will facilitate the spectrum of TGFBI mutations associated to this group of stromal dystrophies and define the corneal phenotypes which are the result of specific mutations.

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