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Role of BIGH3 R124H mutation in the diagnosis of Avellino corneal dystrophy

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PURPOSE. *To report the presence of the R124H mutation in two Spanish families with Avellino corneal dystrophy (ACD).*
METHODS. *Two families with subjects who presented biomicroscopic features of ACD were included in this study. They have no relatives of Italian origin. Genomic DNA of the patients was isolated from peripheral blood. DNA was amplified using primer pairs corresponding to exons 4 and 12.*
RESULTS. *In Family 1, Patients I-1, II-1, and II-3 presented granular deposits in the anterior stroma. In Family 2, Patients I-1 and II-1 presented similar deposits in anterior stroma; Patient I-2 presented biomicroscopic findings similar to granular corneal dystrophy (GCD) and isolated fine lattice deposits. Patient II-2 presented isolated central granular deposits and remarkable lattice deposits in the form of Christmas tree. An identical point mutation in the BIGH3 gene (TGFBI) was observed in all affected members of the two families. The mutation consisted of a substitution of arginine by histidine at amino acid residue 124. It was reflected in the sequence analysis by the presence of a G to A transition at nucleotide 418. The mutation was not found in unaffected family members.*
CONCLUSIONS. *The detection of the R124H BIGH3 mutation confirmed the diagnosis of ACD in the reported families. This is the first study that shows the presence of such mutation in Spain. The authors found the same mutation reported in other countries. In earlier stages, BIGH3 mutation analysis may help to distinguish ACD from GCD, particularly in young patients. (Eur J Ophthalmol 2008; 18: 345-50)*

KEY WORDS. *Corneal dystrophy, Granular corneal dystrophy, Avellino corneal dystrophy, ACD, R124H, BIGH3 gene*

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INTRODUCTION

Corneal dystrophies are hereditary conditions which are mostly slowly progressive throughout life. Granular corneal dystrophy (GCD) (Groenouw type I) is a bilateral disorder characterized by the deposition of small, discrete, sharply demarcated, grayish white opacities in the anterior stroma. The opacities can vary in shape and usually are grouped into three morphologic types: drop-shaped, crumb-shaped, and ring-shaped. Initially, the stroma between the opacities remain clear (1). GCD is the commonest of the corneal dystrophies, and usually results in visual disability in the fourth or fifth decades. It is characterized by the presence of small, discrete grayish white punctuate or elongated, sharply defined opacities. Lattice corneal dystrophy (LCD) is an inherited, primary, localized corneal amyloidosis. Clinical features of this dystrophy include discrete ovoid or round subepithelial opacities, anterior stromal white dots, and small refractile filamentary lines. With time, the patients may develop a diffuse central anterior stromal haze. With further progression, the lesions can appear as small nodules, dots,

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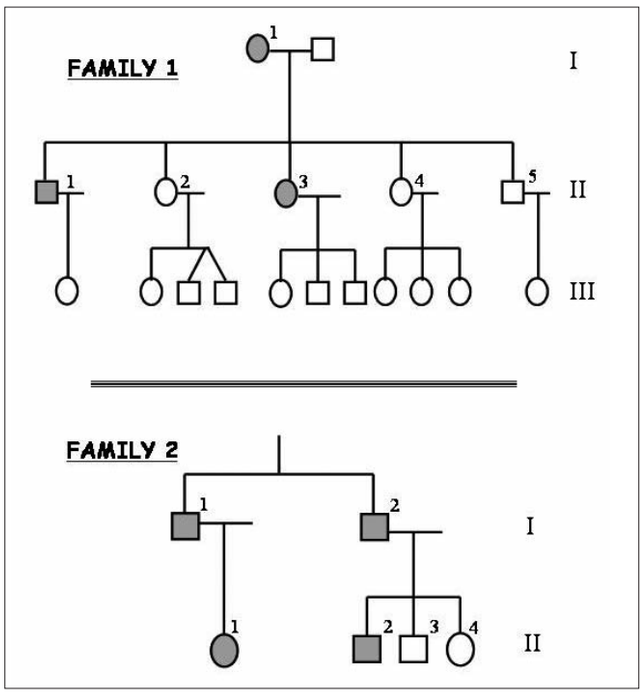

Fig. 1 - *Pedigree of Family 1 showing index case (I-1) and five offspring: II-1, affected 56-year-old man; II-2, a 54-year-old woman; II-3, affected 51-year-old woman; II-4, a 49-year-old woman; II-5, a 42-year-old man. Affected cases present the R124H mutations of BIGH3 gene. Pedigree of Family 2 showing four affected cases (I-1, an 81-year-old man; I-2, a 79-year-old man; II-1, a 50-year-old woman; II-2, a 48-year-old man). II-3, a 46-year-old man, and II-4, a 42-year-old woman, were unaffected.*

threadlike spicules, or thicker, radially oriented branching lines (1). Avellino corneal dystrophy (ACD) (OMIM 121900) is a variant of corneal dystrophy, which is characterized by concurrence of the features of granular and lattice dystrophies (2, 3). These patients have evidence of well circumscribed central opacities similar to those seen in granular dystrophy. However, on histologic examination they present lattice-like deposits in addition to the granular lesions (1).

The clinical manifestations that define ACD are 1) anterior, stromal discrete gray white granular deposits; 2) mid to posterior stromal lattice lesions; and 3) anterior stromal haze (3). The earliest clinical evidence of this disorder is discrete sharply demarcated granular deposits in the subepithelial and anterior stromal layers of the cornea. Lattice lesions develop after the granular deposits appear. Because the pedigrees reported by Holland et al (3) and three families reported by Folberg et al (2) all traced their family origin to the Italian province of Avellino, Holland et al (3) suggested the name of Avellino corneal dystrophy for this entity. No patients were seen with lattice lesions without granular opacities (1-3). The last sign to emerge is the corneal haze (3).

The inheritance of the dystrophy is autosomal dominant, which histologically is characterized by a mixed type of granular and amyloid deposits in anterior stroma and amyloid lattice deposits in deep stroma (4). In other words, ACD show pathologic overlapping features between GCD and LCD. Further studies demonstrated that GCD, LCD, and ACD were caused by mutations within the same gene on chromosome 5 (5, 6). It was then seen that mutations in the *BIGH3* gene caused the stromal dystrophies linked to chromosome 5q (7-10). In ACD, the corneal phenotype is induced by a G to A transition at the second nucleotide position of codon 124. This mutation changes an arginine residue to a histidine (R124H) (10). Since Munier et al (7) identified the *BIGH3* gene as a corneal dystrophy causing gene, there have been numerous reports confirming the same mutation in patients from different nationalities. However, the R124H mutation has never been described in patients with ACD from Spain. ACD and GCD may have similar biomicroscopic features, particularly in young individuals. The clinical diagnosis for ACD is usually more evident with advancing age. Until the identification of the responsible genes, there was no way to test the accuracy of the clinicopathologic diagnosis.

In this article, we describe two unrelated Spanish families with clinical features of ACD, and we report the presence of the R124H mutation for the first time in Spain. We also emphasize the importance of genetic testing in the diagnosis of ACD, particularly at early stages of the disease, when differential diagnosis with GCD is challenging.

MATERIALS AND METHODS

Clinical study

Two families with subjects who presented biomicroscopic features of ACD were included in this study. In Family 1, Patient I-1 was referred to our service for cataract operation and possible keratoplasty. In Family 2, Patient II-2 was referred for corneal study. The diagnoses of stromal corneal dystrophy and possible ACD were made after the biomicroscopic study. Subsequently, other family members were recruited and subjected to clinical and genetic

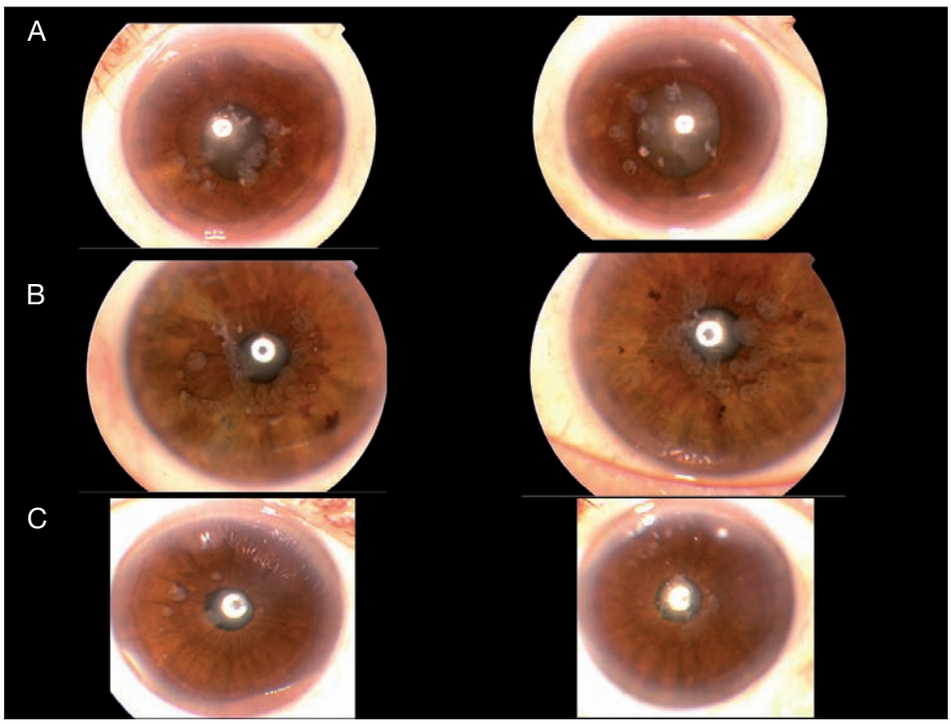

Fig. 2 - *Phenotypical features of affected members in Family 1.* **(A)** *Case I-1, Granular deposits and discrete reticular deposits resembling a granular corneal dystrophy.* **(B)** *Case II-1, Several confluents granular corneal deposits in the anterior stroma.* **(C)** *Case II-3, Isolated and confluents granular deposits in anterior stroma.*

evaluation. Figure 1 shows the pedigree of Family 1 and Family 2. Informed consent from all individuals was obtained for clinical and molecular analysis.

The two families were unrelated. All members of each family were born in Spain, and both families' origin has been traced to Spain. They have no relatives of Italian origin.

Molecular analysis

Genomic DNA of the patients was isolated from peripheral blood by organic extraction (DNeasy®, Qiagen GmbH, Hilden, Germany). DNA was amplified using primer pairs corresponding to exons 4 and 12 (10) of the *BIGH3* gene (OMIM 601692). PCR amplification was performed in 20 µL reaction volumes that contained 100 ng of DNA, 75 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 0,2 µM of each primer, 0.2 mM of each dNTP, and 1 unit of Taq DNA polymerase (BIOTOOLS, B&M Labs, S.A., Spain). Both exons 4 and 12 were amplified with the following PCR conditions: an initial 5 min denaturation at 94 ºC followed by 35 cycles of 1 min at 94 ºC; 1 min at 60 ºC; 1 min at 72 ºC; and a final extension of 10 min at 72 ºC. For DNA sequencing, PCR products were first purified using the MinElute PCR Purification Kit (Qiagen GmbH) and were bidirectionally sequenced using the original primer pair and Applied Biosystem Cycle Sequencing kit (Applied Biosystem Inc., Santa Clara, CA). Samples were analyzed on the ABI Prism 3100-Avant instrument, using standard run parameters. The separation matrix used was POP-6 using 1X TBE with EDTA running buffer (Applied Biosystem Inc.).

RESULTS

Clinical study

Family 1. Figure 2 shows the corneal deposits in the three members who were phenotypically affected. In this family, Patients I-1, II-1, and II-3 presented granular deposits in the anterior stroma. Lattice deposits were noticeable in deep stroma more remarkable in the first son. The other family members did not present evidence of corneal dystrophy. Patient I-1 was operated for cataracts with visual improvement, and keratoplasty was not required.

Family 2. Patients I-1 and II-1 presented discrete granular stromal opacities in anterior stroma. The stroma between deposits remained clear. The vision was not affected. Patient I-2 presented biomicroscopic findings similar to the

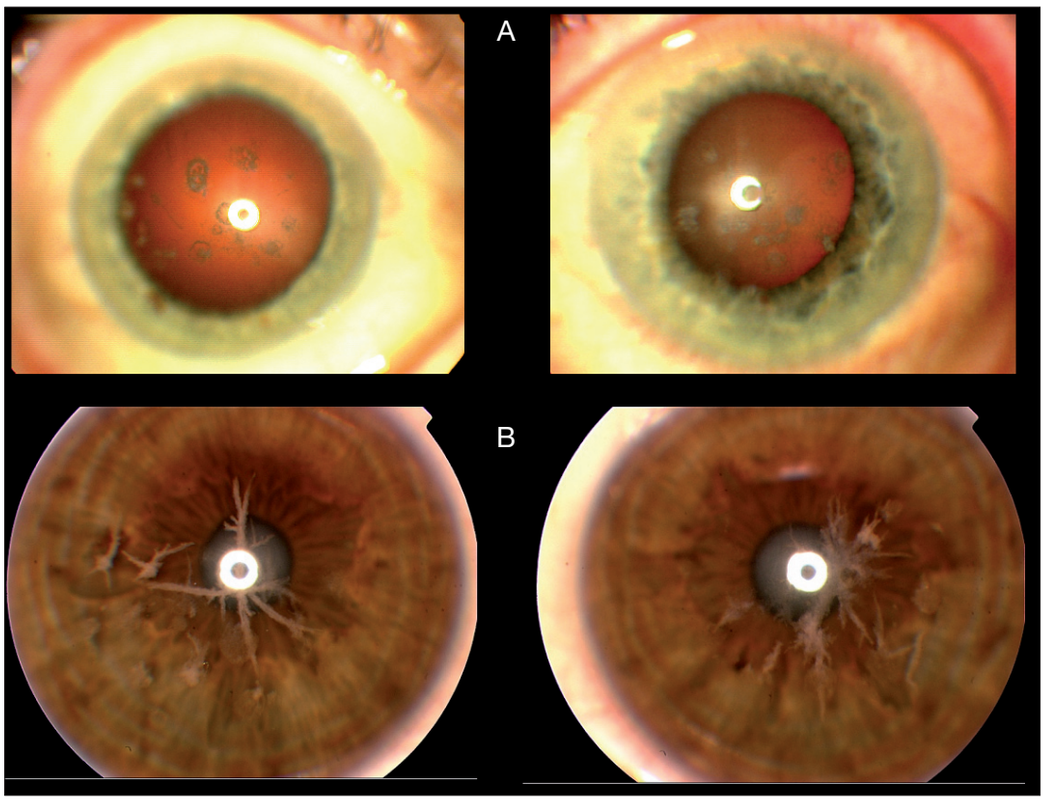

Fig. 3 - *Phenotypical features of affected members in Family 2.* **(A)** *Case I-2, Granular corneal deposits similar to the GCD and isolated fine lattice deposits.* **(B)** *Case II-2, Isolated corneal granular deposits and lattice deposits in form of Christmas tree.*

GCD, and isolated fine lattice deposits were also observable. Patient II-2 presented isolated central granular deposits and remarkable lattice deposits in the form of Christmas tree. Best-corrected visual acuity was 20/30 in both eyes. Keratoplasty was not required. The other family members did not present corneal abnormalities. Figure 3 shows the clinical features of the affected members in this family.

Molecular study

An identical point mutation in the *BIGH3* gene (TGFBI) was observed in all affected members of the two families (Family 1: I-1, II-1, and II-3; Family 2: I-1, I-2, II-1, and II-2). The mutation consisted of a substitution of arginine by histidine at amino acid residue 124. It was reflected in the sequence analysis by the presence of a G to A transition at nucleotide 418 and it always was present in heterozygous form. The mutation was not found in unaffected family members (Family 1: II-2, II-4, and II-5; Family 2: II-3 and II-4) (Fig. 4). Family 1 generation III was not subjected to analysis. All members of these two families (clinically affected and clinically unaffected) were also checked for mutations in exon 12 of *BIGH3* gene, and none of them exhibited any mutation.

DISCUSSION

The clinical features of the two families reported here are similar to those described in previous series of ACD. It is interesting to notice that lattice deposits may be localized as seen in Family 1. A lack of relation between the intensity of these lattice deposits in members of the same family was observed in Family 2.

Lattice lesions in ACD develop after the granular deposits appear and may be discrete as in some patients included in the present study. At early age, only granular deposits may be evident (3). In other words, lattice lesions develop gradually, starting later in life, and increasing with age. Thus, the correct diagnosis of ACD is not always easy in young individuals. Under such circumstances, the distinction between GCD and ACD may be difficult if the family is not of Italian origin. Genetic analysis is an interesting

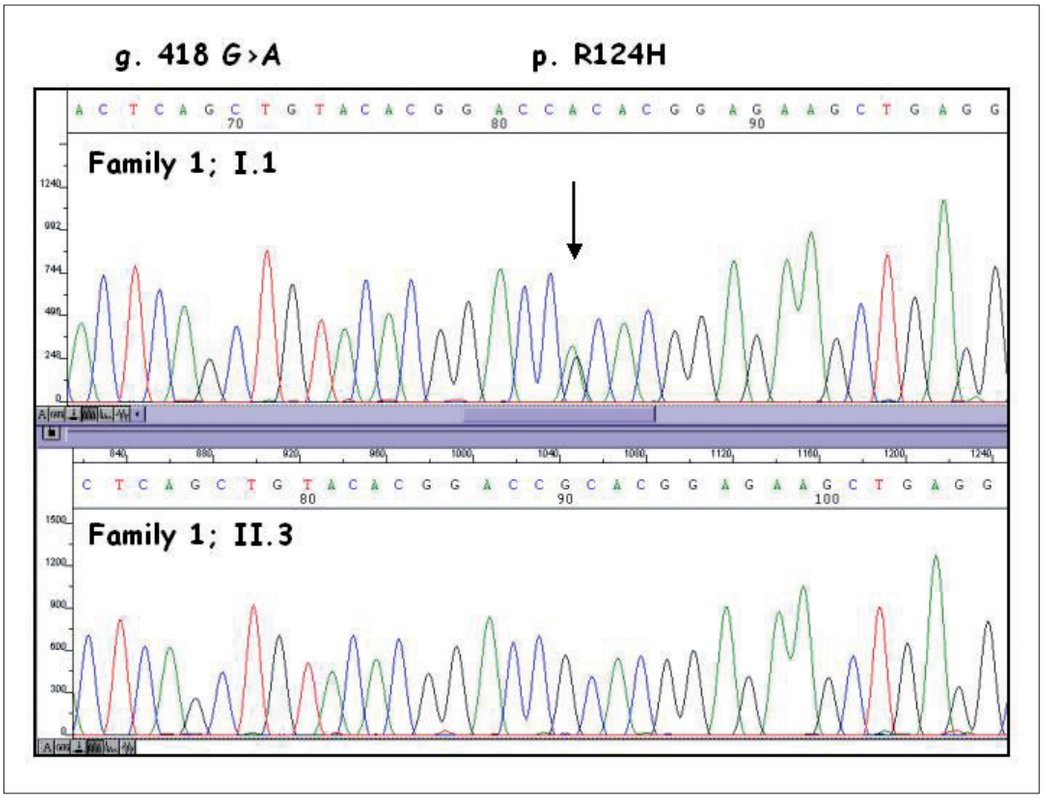

Fig. 4 - *DNA sequence phenograph showing Family 1 cases I-1 (index) and II-2 (unaffected). G to A transition at nucleotide 418 converts arginine to histidine at aminoacid residue 124.*

tool for confirming the diagnosis in young patients.

The *BIGH3* gene encodes for a 683 amino acid protein, also known as TGFBI protein or keratoepithelin. Mutations in the *BIGH3* gene have been found to be responsible for a group of hereditary cornea-specific deposition diseases: the group of 5q31-linked corneal dystrophies. BIGH3 is expressed in many tissues, including the cornea, skin, and matrix of many connective tissues. TGFBI protein is involved in the cell adhesion process, and is upregulated by transforming growth factor-beta. Mutations in BIGH3 were identified in six distinct autosomal dominant dystrophies (10): R555W with granular corneal dystrophy type I (Groenouw type I) (7), R124H with ACD (7), R124L with granular type III (Reis-Bucklers) (11), R555Q in honeycomb (Thiel-Behnke) (7), R124C with Lattice type I (Biber-Haab) (7), and P501T in lattice type IIIa corneal dystrophy (12). Heterozygous R124H mutations are characteristic of ACD. However, homozygous mutations have also been described, and they occur in patients with the most severe phenotypes.

The two unrelated families included in this study resulted heterozygous for the R124H mutation. They exhibit the same mutation. It has been observed that the clinical features are more moderated in the third generation. Although the third generation was not screened in these two families, the corneal appearance was more severe in the second generation in comparison to the first. This observation may indicate that a second modifier is involved in the accumulation of the deposits in the cornea as has been proposed (13).

R124H mutation has been found in the Italian phenotype families of ACD (8-10). However, the same mutation has been detected in families from France, Austria, and Germany (8). Subsequent studies have demonstrated high frequency of this mutation in countries from Asia. Many Japanese patients with ACD exhibit the R124H mutation (14). Up to 91% of patients initially diagnosed with GCD in Japan presented the R124H mutation (15), and they correspond to ACD patients, since the R124H mutation is exclusively found in this clinical setting. Our study has demonstrated for the first time that the same mutation may be present in patients with ACD from Spain. R124H mutations may occur independently in several ethnic groups and these mutations do not reflect a putative

founder effect (8). The BIGH3 protein polymerizes to form a fibrillar structure and strongly interacts with type I collagen, laminin, and fibronectin. The mutations reported in the 5q31-linked CD do not significantly affect these properties and it has been suggested that mutant forms of BIGH3 may require other cornea specific factors to form the abnormal accumulations in the 5q31 linked dystrophies (16). The phenotype in our second family may confirm these observations.

There is a different form of lattice corneal dystrophy, the so-called Finnish form of amyloidosis (Meretoja), that is due to a specific mutation in the gelsolin gene (9, 17). This form of corneal dystrophy can be found outside Finland, and the clinical diagnosis also can be confirmed by mutation analysis of the gelsolin gene (9, 18).

In conclusion, detection of the R124H BIGH3 mutation confirmed the diagnosis of ACD in the two Spanish families reported here. This is the first study that shows the presence of such mutation in Spain. We postulate that mutation analysis of the *BIGH3* gene may be required to establish a correct diagnosis of ACD, particularly in young patients. In earlier stages, BIGH3 mutation analysis may help to distinguish ACD from GCD, which is characterized by R555W mutation in the same gene (10). Probably, in the future, the correct classification of CD will rely more on the results of BIGH3 mutation analysis rather than on the clinical manifestations of the disease.

Proprietary interest: None.

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