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SHORT COMMUNICATION

First genetic analysis of lattice corneal dystrophy type I in a family from Bulgaria

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PURPOSE. *To report a new family belonging to a previously non-investigated geographic area with a rare form of lattice corneal dystrophy (LCD).*
METHODS. *Detailed ophthalmologic analysis was carried out on a Bulgarian woman, enrolled for perforating keratoplasty. In order to obtain a final diagnosis both histology and genetic analysis were performed.*
RESULTS. *Upon transplantation, histologic analysis of the dystrophic cornea revealed the typical staining pattern and amyloid deposits of lattice corneal dystrophies. Genetic analysis of the subject and her daughter confirmed the presence of an autosomal dominant R124C mutation within exon 4 of the BIGH3 gene, encoding for keratoepithelin, while showing no abnormalities in her son.*
CONCLUSIONS. *The identification of this mutation allows the unambiguous classification of this corneal dystrophy as LCD type I. A first case of LCD I in a family from Eastern Europe could help to better clarify the molecular epidemiology of the disease. (Eur J Ophthalmol 2005; 15: 804-8)*

KEY WORDS. *Amyloid deposits, Genetic analysis, Keratoepithelin, Lattice corneal dystrophies, Perforating keratoplasty.*

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INTRODUCTION

Lattice corneal dystrophies (LCD) are a class of inherited stromal amyloidoses characterized by pathognomonic, branching "pipestem" lattice figures in the cornea, leading to progressive opacification and severe visual handicap. Based on phenotypic and clinical analysis, a large number of LCD subtypes have been identified so far in many different geographic areas (1). Recently, at least some LCDs have been considered a result of mutations in the ?ig-h3 gene, located on chromosome V and encoding for the adhesion molecule keratoepithelin (2). In fact, it has been shown that granular dystrophy Groenouw type I (CDGG1), Reis-Bucklers (CDRB), lattice type I (CDL1), and Avellino (ACD) are four 5q31-linked human autosomal dominant corneal dystrophies (2).

The BIGH3 gene could be typically induced by transforming growth factor-β (TGF-β) in a human adenocarcinoma cell line derived from lungs and preferentially expressed in the corneal epithelium as an extracellular protein (3, 4), and, accordingly, the most current designation for this gene is TGFBI (transforming growth factor-β-

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induced). The BIGH3 protein consists of 683 amino acids and has a carboxyl terminal Arg-Gly-Asp (RGD) motif. Moreover, it contains four tandemly repeated homologous domains of circa 140 aminoacids that display a remarkable sequence similarity to regions belonging to the Drosophila melanogaster cell adhesion molecule known as fasciclin 1 (5, 7).

These four FAS1 domains correspond to amino acids 100–238 (domain 1), 239–374 (domain 2), 375–501 (domain 3), and 502–635 (domain 4).

Inherited mutations of the BIGH3 gene that have been associated with lattice corneal dystrophies were identified in several countries (8-11). Among others, for CDL1, a C-to-T mutation at nucleotide position 417 in exon 4, leading to a R124C mutation within FAS1 domain 1, was frequently found in several families throughout the world (12-14).

The apparent universality of this association with the R124C mutation makes it very likely that such codon change is alone sufficient for generating this phenotype and that this protein site is critical for the accumulation of amyloid deposits (15).

Herein we report the presence of R124C mutations in two Bulgarian women (mother and daughter) with lattice corneal dystrophy.

To our knowledge, this is the first report about the presence of this specific mutation in an Eastern European family.

MATERIALS AND METHODS

Clinical and histologic analysis

In the Operative Unit of Ophthalmology at Latina Hospital “S. Maria Goretti” (Italy), a complete ophthalmologic examination, including anamnesis, was performed on a 40-year-old woman with bilateral corneal disease, and on her two children (a 16-year-old boy and a 14-year-old girl).

The patients underwent visus examination (Snellen charts), slit lamp biomicroscopy, corneal topography, applanation tonometry, fundus examination, and anterior segment photography.

Tissue sections of the corneal specimen were examined by light microscopy after being stained with hematoxylin-eosin and Congo red following standard protocols. Informed and written consent on experimental investigation were obtained from the patients.

Genetic analysis

Genomic DNAs of the three subjects under analysis were extracted from blood samples containing EDTA, using a commercially available kit (High Pure PCR template preparation kit, Roche Diagnostic GmbH, Germany). The TGFBI gene was analyzed by direct genomic DNA sequencing of exon 4 and exon 12. As reference we used the locus AY149344 sequence which was already available at the Gene Bank.

The two couples of primers used for the amplification and subsequently for sequence analysis were those already described by others (2). The PCR products were of circa 240 bp for exon 4 and circa 320 bp for exon 12. Sequence analysis was performed by means of BigDye terminator technique 3.1 vs (Applied Biosystems) on purified templates, following standard protocols.

Dyed templates were purified with Centrisep columns (Applied Biosystems) and then loaded on the 3100 Avant Genetic Analyzer (Applied Biosystems). Sequence analysis and alignments were performed using SeqScape software version 2 (Applied Biosystems).

RESULTS

The aim of this study was to determine the histologic and genetic basis of a corneal dystrophy affecting a Bulgarian woman enrolled for perforating keratoplasty (PK) in Italy. The patient underwent a complete ophthalmologic examination. Best-uncorrected visual acuity (BUVA) in her right eye (RE) was 12/20, while best-corrected visual acuity (BCVA) could not be achieved with by correction. BUVA in left eye (LE) was 1/20, while BCVA in LE was 2/20 with +2 sphere = +3.50/180. Slit-lamp examination showed both in RE and in LE signs of stromal corneal opacities in the periphery, coupled with central anterior opacities more pronounced in her LE (Fig.1, A, B). Some areas of the corneal tissues clearly presented with amyloid deposits. Corneal topography showed no significant alterations in both eyes. Fundus examination by means of indirect ophthalmoscopy showed no abnormalities in both eyes. She then had PK in her LE in April 2004, leading to a BUVA of 2/20 and a BCVA of 8/20 with +5/95 TABO after 1 month. Clinical follow-ups are still taking place.

During her interview, she remarked that her sister had the same pathology, as well as some of her relatives living in Bulgaria: the genealogic tree of her family is reported in

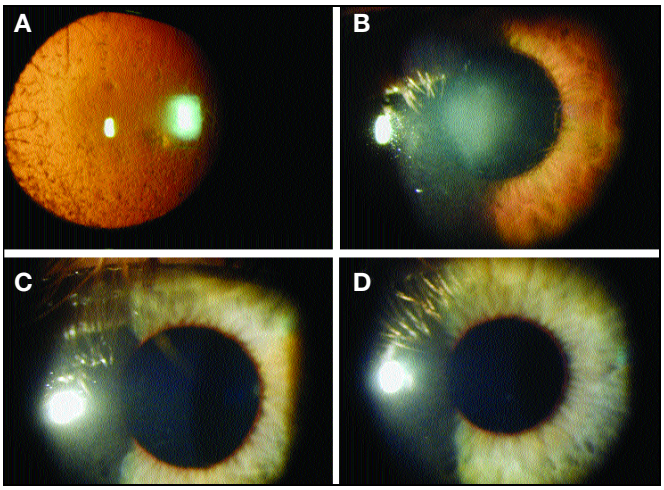

Fig. 1 - (A) *Mother's right eye as seen through retroillumination technique, showing typical pipestem branches.* **(B)** *Mother's right eye as seen on slit lamp examination, showing also central whitish opacities.* **(C)** *Daughter's right eye showing initial features of corneal dystrophy (arrow).* **(D)** *Daughter's left eye showing initial features of corneal dystrophy (arrow).*

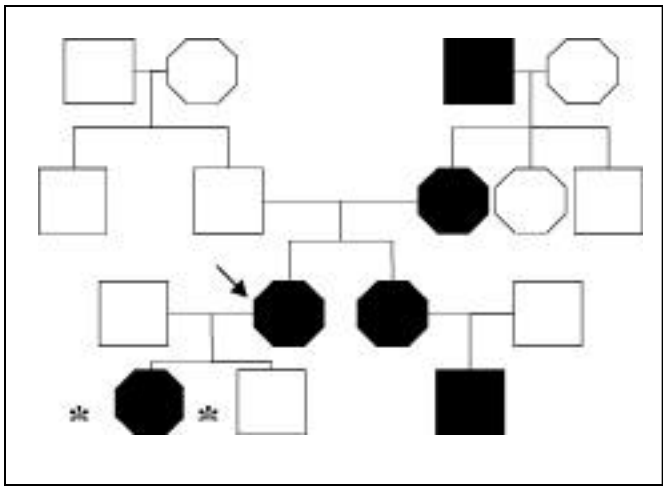

Fig. 2 - *The family hereditary pattern. The individuals affected by corneal blindness are reported in filled circles (females) or squares (males). Arrow indicates the mother and asterisks her children analyzed.*

Figure 2. It was important to verify whether her children (a 16-year-old boy and a 14-year-old girl) also had corneal blindness, and therefore they also were examined at our unit.

The bilateral corneal lesions appeared in the mother as bright irregular shaped opacities within the peripheral corneal stroma by confocal microscopy, showing whitish and more severe central opacities in LE>RE, responsible for visual impairment. Clinical symptoms and cornea features were indeed considered representative of the CDL1 disease and these findings, accompanied by severe visual impairment, suggested PK. In addition, initial corneal signs could be seen in both eyes of the patient's daughter (Fig.1, C, D), while they were not present in her brother. This led us to submit her daughter to future and close follow-ups during the years, in order to determine any change in her objective signs. In July 2004 her daughter had 20/20 BUVA in both eyes and no other abnormalities could be evidenced (Fig. 1, C, D).

Upon explantation, the histologic features of the diseased cornea were analyzed (Fig. 3). The corneal tissue was similar to that reported in an earlier study (17) and characterized by the presence of eosinophilic, variably

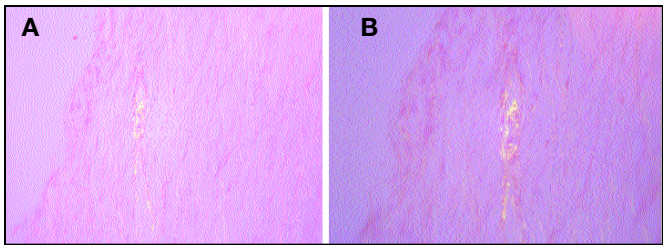

Fig. 3 - *Histologic analysis on cross-sections (circa 5 mm) of the cloudy cornea.* **(A)** *Hematoxylin-eosin staining: amyloid deposits within the subepithelial and corneal stroma are evident (100x).* **(B)** *The amyloid substance in lattice corneal dystrophy is birefringent when stained with Congo red (250x).*

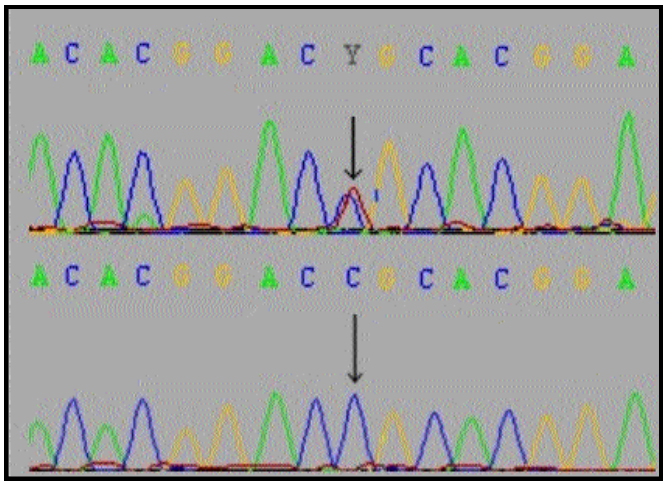

Fig. 4 - *Graphical output of genomic DNA sequencing. The patient ➤ and her daughter both display the same heterozygous pattern (C/T = Y) at the level of nucleotide 19260 (arrow) (upper panel) while her son does not show any mutation in the same position (lower panel).*

sized, irregularly shaped, roundish deposits of amyloid within the corneal stroma (Fig. 3A). The deposits were situated mostly in the superficial central cornea.

These accumulations, when stained with Congo red, exhibited apple-green dichroism under polarized light (Fig. 3B). Descemet's membrane and the corneal endothelium were unremarkable in the initial grafts (data not shown).

In order to obtain a final molecular picture, we carried out genetic analysis. It is worth noting that the lack of annotated CDL1 cases in eastern Europe made such analysis even more interesting. As previously stated, during anamnesis, the patient mentioned that in her family there were several cases of such visual impairment and we determined that her daughter started to display the same corneal anomalies reported in her mother. Therefore, the genomic DNAs were purified from blood samples collected from her two children, and the two BIGH3 exons (e.g., 4 and 12) known for displaying mutations were analyzed. The analysis of exon 4 of the two females showed the presence of heterozygous C/T in the base 19260 (Fig. 4) corresponding to R124C amino acidic substitution in the FAS1 domain1, while the son did not carry this mutation.

DISCUSSION

In recent years, a subset of hereditary corneal dystrophies has been related to mutations in the TGFBI (BIGH3) gene. At least 15 different mutations in TGFBI, accompanied by amyloid deposition in the cornea, have been identified in families with different clinical variants of lattice corneal dystrophy (LCD): R124C, R124H, L518P, P501T, L527R, A546T, L569R, A622H, H620R, H626R, L527R, A546T, A546D, H620R, 9-bp insertion at nt 1885-1886, and missense at nt 1887. Therefore, due to such genetic heterogeneity, in order to assess a final diagnosis it is strictly necessary to identify which specific mutations are carried by the patient (1-5).

All hereditary corneal disorders caused by mutations in TGFBI are associated with an anomalous extracellular deposition of proteins within the cornea. LCD type I is characterized by prominent delicate linear opacities that tend to be mainly in the superficial corneal stroma, with epithelial erosions. Because TGFBI is normally transcribed in the corneal epithelium, where it is preferentially expressed on the corneal external surface, the corneal epithelium might be the major source of the amyloid that accumulates in LCD type I (18). The protein composition of the amyloid deposits that occurs in individuals carrying mutations within the TGFBI gene has been reported to include apolipoprotein J (apoJ) and apolipoprotein E (apoE), which contribute to amyloid formation also in patients with Alzheimer disease (16).

Recently, a homology model of the human FAS1 domain 4 showed that the R124C mutations, as well as other common mutations found in position 555, are likely to affect protein-protein interactions leading to amyloid or granular deposits depending on the particular mutation (7). On the other hand, exon 12 did not contain any mutation or polymorphism in all the samples tested. These findings were in full agreement with the previous diagnostic and histologic analysis, finally confirming the corneal lesions as a corneal lattice dystrophy type 1 (CLD1). The clinical features of the daughter as well as the handout of the genetic analysis strongly suggest the necessity of monitoring the evolution of clinical symptoms, which will very likely lead to a surgical treatment.

In conclusion, this is the first case report describing the presence of R124C mutation in Bulgaria. As outlined by other authors (19), there is an evident scarcity of molecular data on eye pathologies in eastern European countries. Our new findings may prompt further molecular and genetic screening of other subjects, displaying similar corneal diseases, from the same geographic area, and can be of help for the design of blindness prevention global protocols (20).

The authors have no proprietary interest in any aspect of the article.

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