Clinical and Genetic Profile of Avellino Corneal Dystrophy in 2 Families From North India

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Objective: To report Avellino corneal dystrophy and underlying R124H mutation in 2 families of Indian origin.

Methods: Peripheral blood was collected in EDTA for genomic DNA isolation from leukocytes of all affected and unaffected individuals. Amplification of transforming growth factor β-induced gene (TGFBI) using polymerase chain reaction followed by direct sequencing was carried out to determine the mutations underlying the disorder. A detailed clinical evaluation was undertaken to establish a genotype-phenotype correlation.

Results: R124H mutation resulting from a missense heterozygous substitution of G to A at nucleotide 418 of TGFBI was detected in all affected members of the 2 families. The affected individuals were clinically diagnosed as having granular corneal dystrophy. Histopathological examination was not done because no surgical intervention was undertaken.

Conclusions: To our knowledge, this is the first report of Avellino corneal dystrophy from India clinically diagnosed as granular corneal dystrophy, emphasizing that TGFBI screening is essential for the accurate diagnosis and classification of corneal dystrophies.

Clinical Relevance: Molecular genetics is a useful tool for accurate diagnosis and classification of corneal dystrophies. All autosomal dominant stromal dystrophies should be screened for underlying mutations in TGFBI because the clinical and phenotypic appearance is variable.

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VELLINO CORNEAL DYSTROPHY is characterized by the presence of gray-white discrete granular deposits in the subepithelial and anterior stromal corneal layers with or without discernible lattice lines in the stroma. These lattice lines develop in the second and third decade or even later and differ from the ones present in typical lattice corneal dystrophy in being larger, denser, whiter, and more spiculated. In the most advanced form of the disorder, stromal haze emerges. Histologically, these deposits stain with Masson trichrome and Congo red and are seen as discrete hyaline and fusiform deposits of amyloid in the corneal stroma.

Explicit mutations in TGFBI are responsible for specific types of 5q31-linked corneal dystrophies, such as Groenouw type I (R555W), Avellino (R124H), Reis-Bücklers (R124L), Thiel-Behnké (R553Q), and lattice type I (R124C). The R124H mutation known to be associated with Avellino corneal dystrophy (ACD) (OMIM 121900) was initially described in families originating from the Italian province near Naples. Subsequently, with widespread availability of molecular diagnostic techniques, it has also been reported from Germany, Ireland, Europe, Japan, France, South Korea, the United Kingdom, and Iran.

Despite specific TGFBI mutations being associated with each of these corneal dystrophies, atypical and variable phenotypes along with extensive intrafamilial and interfamilial variations are seen and genotype-phenotype correlation is not always possible.

We studied 5 affected individuals from 2 unrelated, nonconsanguineous Indian families who presented clinically with granular corneal dystrophy and hereby report their clinical features with genetic analysis.

METHODS

The study had the approval of the institute research ethics committee and conformed to the tenets of the Declaration of Helsinki. Informed consent from all participants was obtained for slitlamp examination, in vivo white-light con-
focal microscopy (Confoscan 4; Nidek Technologies, Padova, Italy), and molecular genetic studies. Detailed family history was taken and the available family members were examined. All members of both families were born in India and have their origin in the North Indian state of Haryana. They have no relatives of Italian origin, making them the first Indian families, to our knowledge, to be reported to have ACD. The pedigree charts of the 2 families are shown in Figure 1.

Peripheral blood samples (5 mL) were collected in EDTA and genomic DNA was extracted from all the samples. The DNA was then subjected to polymerase chain reaction (PCR) amplification using a set of primer pairs, as described previously. The constituents and the conditions used for PCR reaction are described herein. The 25-µL reaction mixture contained genomic DNA (200 ng), primers (0.5pM each), magnesium chloride (1.5mM), deoxyribonucleotide triphosphate (0.2mM), 1× PCR buffer (containing 10mM TRIS–hydrochloric acid, pH 8.3; 50mM potassium chloride; and 0.1% gelatin), and Taq polymerase (0.5 U; Roche, Basel, Switzerland). The PCR machine was programmed with the following amplification conditions of 3 minutes of denaturation at 94°C followed by 35 cycles at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute, and final extension was given at 72°C for 5 minutes.

Amplified products were sequenced directly with BigDye Terminator Mix version 3.1 (Applied Biosystems, Foster City, California), according to the manufacturer’s instructions, and then were analyzed on an ABI-3100 Genetic Analyzer (Applied Biosystems). Nucleotide sequences for the coding regions were compared with the nucleotide sequence of the published TGFBI human complementary DNA (GenBank NM_000358).

RESULTS

FAMILY A

Case II-1 (proband) was first examined at the Cornea and External Eye Disease Clinic at the age of 51 years when he presented with visual disturbance. The patient’s best-corrected visual acuity on initial examination was 6/9 OU. Slitlamp biomicroscopy showed discrete disc and ribbonlike opacities in the anterior stroma of the right eye (Figure 2A and B) while the left eye (Figure 2E and F) was found to have predominantly ribbonlike discrete opacities in the anterior stroma. The condition was diagnosed as granular corneal dystrophy.

Case III-2 (proband’s daughter) was referred to the Cornea and External Eye Disease Clinic at the age of 19 years when she complained of redness and watering. The patient’s unaided visual acuity was 6/6 OU. Slitlamp biomicroscopy revealed discrete ring- and crumb-shaped opacities distributed in a radiating pattern in the anterior stroma of both eyes (Figure 3), suggestive of granular corneal dystrophy. In addition, she also had a few discrete, non-branching, linear, gray-white opacities in the anterior stroma (Figure 3B and D). Examination of the unaffected family members revealed a disease-free cornea.

FAMILY B

The second family was also clinically diagnosed as having granular corneal dystrophy and consisted of a 53-year-old...
proband (case II-1), her 32-year-old daughter (case III-1), and a 28-year-old son (case III-2). Slitlamp examination of the proband (case II-1) showed discrete rings, crumbs, and a few raylike, linear, gray-white opacities in the anterior axial corneal stroma of both eyes, with more prominent clinical features in the right eye (Figure 4). The daughter (case III-1) also had asymmetric involvement, the right eye (Figure 5A and B) being more prominently affected, with similar features as the mother but more pronounced. The son (case III-3) had a unilateral presentation with a single discrete opacity in the right eye (Figure 6A).

Direct sequencing analysis of exon 4 of TGFBI in the 2 affected members from family A and the 3 affected members of family B revealed a single heterozygous missense substitution at nucleotide position 418 (G to A) converting arginine at codon 124 to histidine (Figure 7). This mutation results in a change in the structure of the protein coded by TGFBI, resulting in hyaline and amyloid deposition in the corneal stroma, and is known to be associated with cases of ACD. Similar change at this position was not seen in the unaffected members of both the families.

**COMMENT**

The families with ACD initially reported in the literature were found to trace their ancestry to a large region of Campania in Italy comprising cities like Naples, Avellino, Lioni, and Stio. Many cases of ACD have subsequently been reported from other countries. This study describes for the first time, to our knowledge, ACD from India in 2 families with
R124H mutation, thus emphasizing the geographic diversity of the disease.

Avellino corneal dystrophy is an autosomal dominant disorder, sharing its features with both granular and lattice corneal dystrophy, and results from a specific mutation (R124H) in TGFBI. In our study of the 5 affected individuals, 3 individuals (family A case II-1 and family B cases II-1 and III-1) showed the presence of a few fine, gray-white linear deposits in the superficial stroma. These deposits were different from those classically seen in lattice corneal dystrophy as these were not refractile, translucent, or branching in pattern. Two affected individuals in their sixth decade (family A case I-1 and family B case II-1) harbored the heterozygous R124H mutation but did not show any lattice lesions that are classically described with progression of age in ACD. Confocal microscopy of 3 affected individuals (family A case II-1 and family B cases II-1 and III-1) revealed highly reflective granular lesions in the superficial stroma of the central cornea with no other images corresponding to latticelike lesions.

Histopathologically, corneal dystrophies are classified based on the nature of the deposits seen in the cornea. Granular corneal dystrophy and its subtypes are marked by the presence of hyaline deposits while lattice corneal dystrophy, along with its variants, is characterized by the presence of amyloid deposits. Avellino corneal dystrophy is unique as it shows the presence of both hyaline and amyloid deposits. However, histopathological examination does not obviate the need for genetic analysis because it may be inconclusive and may not be available if surgical intervention is not undertaken. Classification of corneal dystrophies based only on clinical and histopathological characteristics is becoming increasingly difficult with the identification of new genotypic variants with different phenotypic features. This is evident from 1 such study that reports a new combined granular lattice dystrophy with atypical presentation that could not be classified based only on clinical and histopathological evidence. Molecular genetic analysis revealed a new causative mutation M619K in TGFBI in the family, without which the dystrophy would have been classified as a phenotypic variant of ACD and the new mutation would have been missed. It has also been reported that the heterozygous R124H mutation of TGFBI resulted in an atypical form of granular corneal dystrophy that was characterized histopathologically by the absence of amyloid deposits, thereby indicating that for the correct diagnosis of such dystrophies, histopathological and genetic evaluations are both essential. In our study, histopathological examination was not done because none of the affected individuals required any surgical intervention.

Our results confirm the importance of TGFBI screening as a mandatory step for the accurate diagnosis and classification of corneal dystrophies that would facilitate timely intervention and management, particularly in the younger age group.

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REFERENCES


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