The phenotype of Arg555Trp mutation in a large Turkish family with corneal granular dystrophy

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INTRODUCTION

Corneal granular dystrophy (CDGGI) is an autosomal, dominantly inherited, progressive, bilateral symmetric condition in which nonamyloid deposits accumulate in the superficial stroma of the central cornea, becoming larger and deeper with age (1). At least two clinical phenotypes with differing severity have been recognized in different families (1, 2). Missense mutations on human transforming growth factor β-induced gene (BIGH3) on chromosome 5q31 cause the phenotypic expression of five distinct autosomal dominant corneal dystrophies including CDGGI, Reis-Bücklers¹ (CDRB), granular-lattice (CDA), lattice type I (CDLI) and lattice type IIa (CDLIIA) (3, 4). The BIGH3 gene encodes kerato-epithelin, a protein whose exact function is yet to be clarified. The R555W mutation causes misfolding of the protein which in turn results in accumulation of deposits in Bowman’s layer and the anterior central stroma (5).

In this study, genetic linkage and mutation analyses were done in a large Turkish family which presented with classic CDGGI, making evaluation of the disease possible at various stages during its clinical course. This is the first large scale mutation result from the Turkish population concerning CDGGI.
PATIENTS AND METHODS

The family came from Hakkari province, in southeast Turkey. The proband, a 38-year-old man, first presented with the complaint of recent-onset blurry vision and received the diagnosis of typical CDGGI (Fig. 1). The rest of the family was examined by two of the authors at a regional hospital in the province where all the members of the family live. The ocular examination included assessment of best-corrected visual acuity, anterior segment biomicroscopy and ophthalmoscopy.

The pedigree consisted of a total of 52 members (26 affected, 18 normal and 8 spouses) in three generations. The diagnosis of CDGGI was based on the autosomal dominant inheritance pattern and discrete central corneal stromal deposits (6). The largest branch of the family who presented for examination without anyone missing was selected for genetic analysis (Fig. 2). A total of 22 blood samples were obtained from all relevant family members and DNA was extracted for molecular evaluation.

Linkage analysis was done to see if the family was also linked to chromosome 5q31. The DNA markers D5S816 and D5S1480 from the region were used to detect linkage to chromosome 5q31. DNA samples were amplified using these site-specific primers. Amplification was carried out by a Hybaid Omn-E thermocycler. The polymerase chain reaction (PCR) products were then run on 7% denaturing polyacrylamide gel and visualized by silver staining. For amplification initial denaturation at 94 °C for 3 minutes was followed by 32 cycles of PCR amplification at 94 °C for 30 seconds, 55-60 °C for 30 seconds, 72 °C for 30 seconds, with a final extension step at 72 °C for 10 minutes.

For mutation screening, exons 4 and 12 of the \textit{BIGH3} gene were amplified using exon 4F-4R and BIGH1621F-1721R primers respectively. The amplification con-
ditions were as in the method described previously (3). The single-strand conformation polymorphism (SSCP) technique and PCR methods were used to detect mutations. For SSCP analysis, 5 µl of PCR product was mixed with 5 µl denaturing formamide solution (95% formamide, 20 mM EDTA), denatured for 5 minutes at 94 °C then placed immediately on ice. The samples were run on 10% polyacrylamide gels (acrylamide:bisacrylamide ratio 19:1) in the cold room at 40 watts for 4 hours. Gels were silver stained and manually photographed. DNA sequencing was only used for PCR products of exon 12 using one affected member of the pedigree (patient no. 16). Automated sequencing was done commercially using an ABI Prism 310 Genetic Analyser by Iontek Co., Bursa, Turkey.

RESULTS

The patients ages ranged from 3 to 58 years. The youngest affected patient was a five-year-old girl. The proband had best-corrected visual acuity of 20/40 in each eye. In all other patients in whom a reliable assessment could be made, visual acuity was not worse than 20/30. The typical corneal findings in members of the family younger than 15 years were small white granules located superficially and clustered in lines (Fig. 3). In older patients, the refractile deposits were coarser, more numerous and located deeper in the stroma without the formation of lines (Fig. 1). Except for the proband, the patients were asymptomatic and none had recurrent erosions. There has been no instance where penetrating keratoplasty was indicated and no histopathologic study has been done on the corneas of this large family.

The respective order of the BIGH3 gene and the DNA markers are D5S816-BIGH3-D5S1480 within a 7cM interval (comprehensive genetic maps in The Center For Medical Genetics, Marshfield, US: http://www.marshmed.org/genetics). Haplotype analysis showed a total of 17 informative meiosis (11 phases known) perfectly segregated with the
markers selected from the region.

Once we had proved that the family was linked to chromosome 5q31 we did SSCP analysis of exons 4 and 12 of the **BIGH3** gene in all screened family members. We detected a DNA shift in SSCP, segregating with affected family members in exon 12 (Fig. 4). As SSCP demonstrated segregation of the mutant allele in all family members tested, only one affected individual (patient no. 9) was then sequenced. This study showed a C → T transition in position 1710 (CGG to TGG), producing a R555W mutation (Fig. 5).

**DISCUSSION**

The clinical presentation of CDGG1 shows wide variability. There are probably at least two major clinical phenotypes of CDGG1, with several subtypes. One important feature is that there appears to be a constant expressivity within a given family (2). An early-onset form involves superficial stromal changes, severely affected visual acuity, recurrent erosions and the need for keratoplasty as early as five years of age. (1, 7) A late-onset milder form is usually associated with fewer stromal granules and opacities, less visual disturbance and erosions that rarely require keratoplasty (1, 6). In the rare event of homozygous patients, the disease may become manifest at infancy and have a more severe clinical course (8).

Recent advances in molecular genetics have made it possible to pinpoint the missense mutations at codon 124 (arginine→histidine) and codon 555 (arginine→tryptophan) in 5q31-linked corneal dystrophies (9). These findings also explain the phenotypic variations and severity of the disease. While patients with heterozygous R124H mutations usually do not have visual symptoms in their second and third decades of life, homozygous individuals often need keratoplasty at the age of 16 and corneal deposits recur within a year (10). Okada et al (11) described three patients with homozygous Arg555Trp mutation, who had a severe form of CDGG1, with large placoid opacities and early recurrence after surgery. A new mutation at codon 124 (R124L) has been linked to a severe type of superficial CDGG1 (12). It is noteworthy that these mutations may show geographic variations: reports from Japan indicate that the R555W mutation is rare compared to R124H, while the opposite seems to be true in Europe (3-5, 9, 10, 12, 13).

The large family described in this study had the R555W mutation. From the geographic standpoint, this population is located between Europe and the Far East, at the crossroads of historic trade routes. All the affected members displayed a mild form of the disease with no significant visual disabilities or recurrent erosion problems. Younger patients had both linear and granular opacities whereas with increasing age larger granules predominated. Our findings in this family with CDGG1 support the growing evidence that the heterozygous mutation Arg555Trp may be responsible for a milder form of the disease in a multitude of distant populations or ethnic groups.

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