European Journal of Ophthalmology / Vol. 11 no. 4, 2001 / pp. 333-337

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

H. KIRATLI[1], M. İRKEÇ[1], K. ÖZGÜL[2], A. ÖĞÜŞ[2]

[1] Department of Ophthalmology, School of Medicine
[2] Department of Biology, Faculty of Science, Hacettepe University, Ankara - Turkey

PURPOSE. *A large Turkish family with 52 members, 26 of whom had Groenouw type 1 corneal granular dystrophy was evaluated by genetic linkage studies and mutation analyses. Phenotype-genotype correlations were also assessed.*
METHODS. *DNA from peripheral blood lymphocytes of 22 family members was used in establishing linkage to chromosome 5q31. Single-strand conformation polymorphism analysis was done to detect mutations in exons 4 and 12 of the human transforming growth factor ß-induced gene located on chromosome 5q31. Automated sequencing was performed on exon 12 of an affected patient.*
RESULTS. *Patients yonger than 15 years of age had typical linear, granular opacities whereas adults had coarser, deeper granular stromal deposits. These changes were not associated with recurrent erosions or significant visual disabilities. The family was linked to chromosome 5q31 and a DNA shift was observed on exon 12 of affected patients. CGG to TGG transition producing R555W mutation was found.*
CONCLUSIONS. *Segregation of Arg555Trp has been described as causing Groenouw type I corneal dystrophy of variable severity in patients of various ethnic backgrounds. In this large Turkish pedigree, the Arg555Trp mutation was associated with a mild phenotype that became clinically evident at five years of age but which remained asymptomatic in terms of corneal erosions. (Eur J Ophthalmol 2001; 11: 333-7)*

KEY WORDS. *Cornea, Granular dystrophy, Chromosome 5q31, BIGH3 gene, R555W mutation*

Accepted: December 13, 2000

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 ß-induced gene (*BIGH3*) on chromosome 5q31 cause the phenotypic expression of five distinct autosomal dominant corneal dystrophies including CDGGI, Reis-Bücklers' (CDRB), granular-lattice (CDA), lattice type I (CDLI) and lattice type IIIA (CDLIIIA) (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.

 1120-6721/333-05$02.50/0

PATIENTS AND METHODS

The familu 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 ocula 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 *BIGH3* gene were amplified using exon 4F-4R and *BIGH*1621 F-1721R primers respectively. The amplification con-

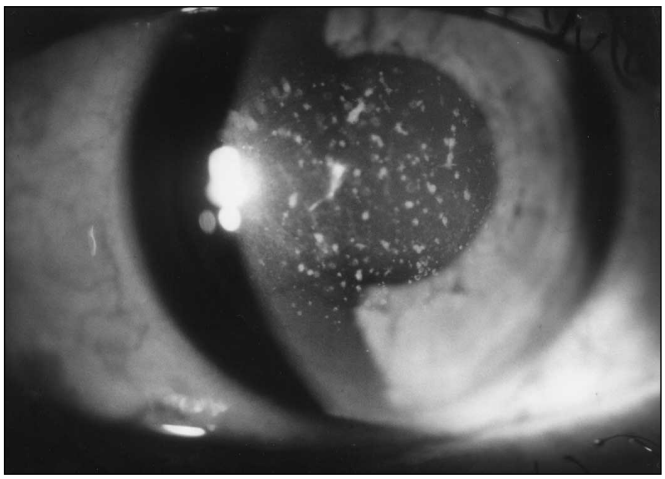

Fig. 1 - *Slit-lamp photograph of the left cornea of the proband, showing well-defined, coarse stromal opacities.*

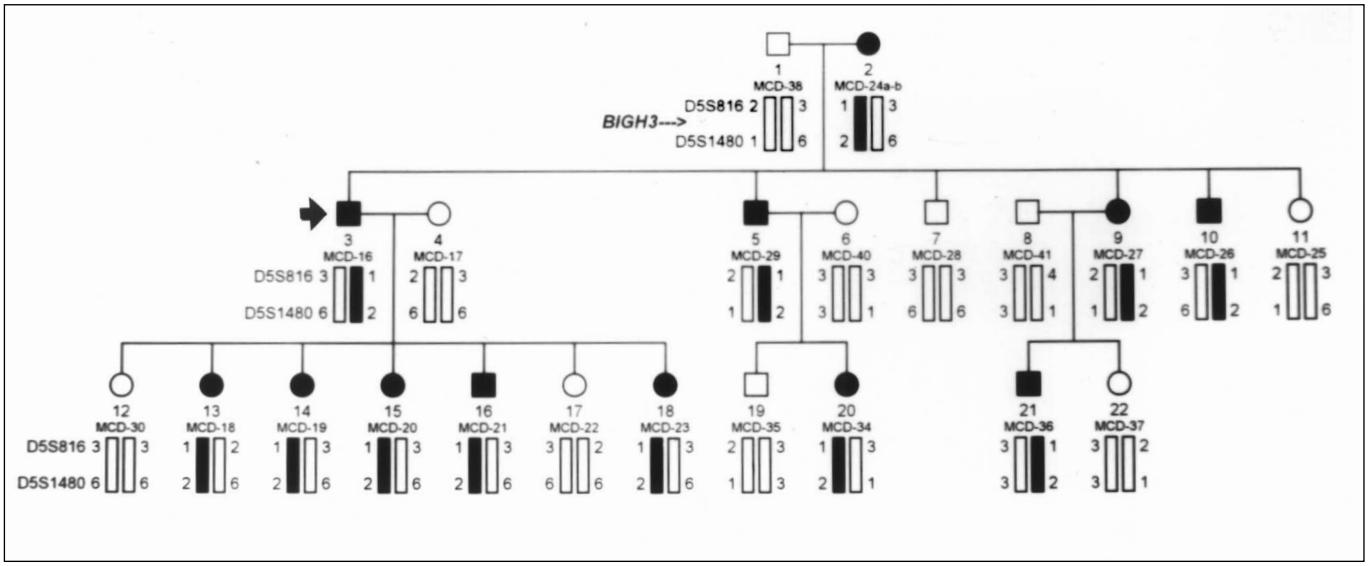

Fig. 2 - *The pedigree of the family with corneal granular dystrophy; solid symbols indicate affected individuals and open symbols unaffected members. The proband is marked by an arrow.*

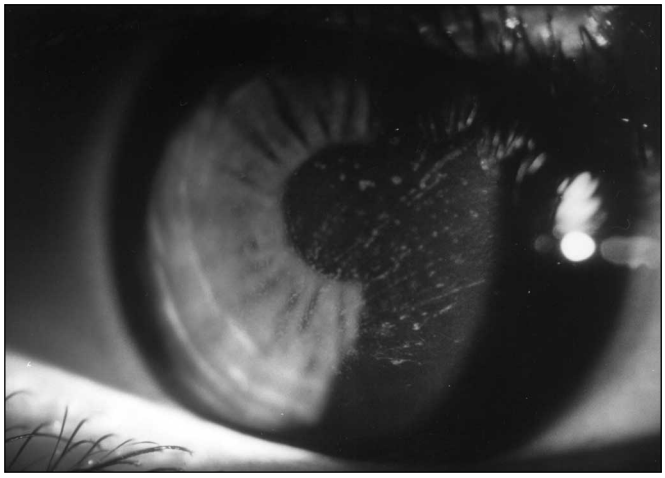

Fig. 3 - *Slit-lamp photograph of the left cornea of the 11-year-old daughter shows the rather linear arrangement of small, more superficial stromal deposits.*

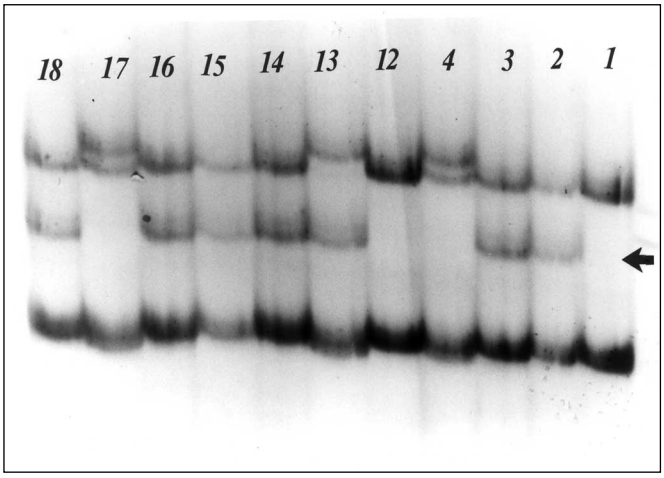

Fig. 4 - *Single-strand conformation polymorphism analysis shows a DNA shift (arrow) segregating with affected individuals in exon 12 of the* BIGH3 *gene. Upper numerals are the patient numbers in the pedigree.*

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.

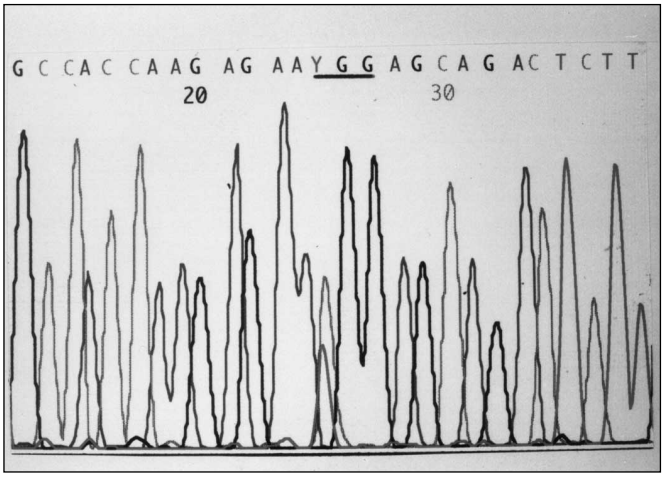

Fig. 5 - *Direct sequencing of exon 12 of the* BIGH3 *gene of an affected individual (patient no. 16) shows the C → T mutation in the position of nucleotide 1710.*

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 (comprenhensive 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 presentaion 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.

ACKNOWLEDGEMENTS

Supported in part by Tubitak, Turkish Scientific Council, project no. SBAG-AYD-185 and BAYG-BDP.

Reprint requests to:
Hayyam Kiratli, MD
Haccettepe Hastanesi Göz ABD
Sıhhiye TR-06100 Ankara, Turkey
hayyamk@ttnet.net.tr

REFERENCES

1. Møller HU. Granular corneal dystrophy Groenouw type I. Clinical and genetic aspects. Acta Ophthalmol 1991; 69: (suppl): S1-40.
2. Møller HU. Inter-familial variability and intra-familial similarities of granular corneal dystrophy Groenouw type I with respect to biomicroscopical appearance and symptomatology. Acta Ophthalmol 1989; 67: 669-77.
3. Munier FL, Korvatska E, Djemai A, et al. Kerato-epithelin mutations in four 5q31-linked corneal dystrophies. Nat Genet 1997; 15: 247-51.
4. Fujiki K, Hotta Y, Nakayasu K, et al. A new L527R mutation of the beta IGH3 gene in patients with lattice corneal dystrophy with deep stromal opacities. Hum Genet 1998; 103: 286-9.
5. Korvatska E, Munier FL, Chaubert P, et al. On the role of kerato-epithelin in the pathogenesis of 5q31-linked

corneal dystrophies. Invest Ophthalmol Vis Sci 1999; 40: 2213-9.
6. Møller HU. Granual corneal dystrophy Groenouw type I. Clinical aspects and treatment. Acta Ophthalmol 1990; 68: 384-9.
7. Rodriguez MM, Gaster RN, Pratt MV. Unusual superficial confluent form of granular corneal dystrophy. Ophthalmology 1983; 90: 1507-11.
8. Møller HU, Ridgeway AEA. Granular corneal dystrophy Groenouw type 1. A report of a probable homozygous patient. Acta Ophthalmol 1990; 68: 97-101.
9. Korvatska E, Munier FL, Djemai A, et al. Mutation hot spots in 5q31-linked corneal dystrophies. Am J Hum Genet 1998; 62: 320-4.
10. Mashima Y, Konishi M, Nakamura Y, et al. Severe form of juvenile corneal stromal dystrophy with homozygous R124H mutation in the kerato-epithelin gene in five Japanese patients. Br J Ophthalmol 1998; 82: 1280-4.
11. Okada M, Yamamoto S, Watanabe H, et al. Granular corneal dystrophy with homozygous mutations in the kerato-epithelin gene. Am J Ophthalmol 1998; 126: 169-76.
12. Mahima Y, Nakamura Y, Noda K, et al. A novel mutation at codon 124 (R124L) in the *BIGH3* gene is associated with a superficial variant of granular corneal dystrophy. Arch Ophthalmol 1999; 117: 90-3.
13. Konishi M, Mashima Y, Yamada M, Kudoh J, Shimuzu N. The classic form of granular corneal dystrophy associated with R555W mutation in the *BIGH3* gene is rare in Japanese patients. Am J Ophthalmol 1998; 126: 450-2.