

BIGH3 mutation in a Bangladeshi family with a variable phenotype of LCDI

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LABORATORY STUDY

Abstract

Aims To report a Bangladeshi family displaying intrafamilial phenotypic heterogeneity of lattice corneal dystrophy type I (LCDI) and to identify the causative mutation.

Methods Molecular genetic analysis was performed on DNA extracted from all members of the family. Exons of *BIGH3* gene were amplified by polymerase chain reaction. Gene mutation and polymorphisms were identified by heteroduplex and sequence analyses. Segregation of the mutation in the family was confirmed by restriction digestion of amplified gene fragments.

Results A heterozygous C→T transition at the first nucleotide position of codon 124 of the *BIGH3* gene was detected in the three affected members and not in the unaffected members of the family.

Conclusions This is the first report of *BIGH3* gene mutation in a Bangladeshi family with phenotypic heterogeneity. This study confirms that *BIGH3* gene screening should be undertaken for proper classification of corneal dystrophy, especially in the absence of histopathological examination.

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Introduction

Corneal dystrophies are a genetically and clinically heterogeneous group of disorders that occurs most frequently bilaterally and are manifested around the time of puberty, progressing slowly throughout life.¹ Most corneal dystrophies are inherited as an autosomal dominant trait.²

Several phenotypic variants of corneal dystrophies with an autosomal dominant inheritance were reported to result from specific mutation(s) in *BIGH3* (or transforming growth factor, β -induced [*TGFB1*]) gene on chromosome 5q31 (Table 1). Lattice corneal dystrophy type I (LCDI, OMIM 122200),^{3–6} LCD type IIIA (LCDIIIA),^{7–11} intermediate type I/IIIA (LCDI/IIIA),^{9–12} deep stromal LCD (LCDIV),^{13–15} granular corneal dystrophy (GCD, OMIM 121900),^{3,4,15} Avellino corneal dystrophy (ACD, OMIM 121900),^{3,4,16} Thiel Behnke corneal dystrophy or corneal dystrophy of Bowman layer type 2 (CDB2, OMIM 602082)^{3,4,17,20}, and Reis-Bücklers' corneal dystrophy (RBCD, OMIM 121900) all result from mutations in *BIGH3*.^{17–20} These conditions are defined by their clinical manifestations and the histological characteristics of corneal deposits.²¹

LCDI (OMIM 122200) has an autosomal dominant mode of inheritance with variable expressivity. The disease presents towards the end of the first or second decade of life and slowly progresses to cause discomfort and visual impairment, usually before the sixth decade. It is characterised by thin greyish, linear branching deposits of amyloid material that progressively accumulate in the subepithelial and stromal layers of the cornea.²² The specific cause of the amyloid deposits is unclear, however, they may be secondary to collagen degeneration or produced by abnormal keratocytes.

Histopathologically, the white dots and lattice lines consist of amyloid in the corneal stroma, which is confirmed histochemically by the pink to orange staining of the deposits by Congo red.²

It has been reported that both R124C and L518P mutations were responsible for LCDI in both Caucasian and Japanese families.^{3–6}

In this study, a Bangladeshi family has been studied both clinically and genetically. Our

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Table 1 Reported *BIGH3* mutations in different phenotypes of corneal dystrophy

Phenotypes	Mutations	References
LCD type I	R124C	3–5
	L518P	6
LCD type IIIA	P501T	7,8
	N622H	9
	A546T	10
	V626S	11
LCD type I/IIIA	H626R	9,12
	NVP629-630ins	12
	H626P	11
	G623D	11
	T538R	11
LCD type IV	L527R	13,14
	V124S	15
GCD	R555W	3,4
	R124S	15
ACD	R134H	3,4,16
CDB2	R555Q	3,4,17,20
RBCD	R124L	17,18,20
	ΔF540	19,18

results revealed a heterozygous C to T transition at the first nucleotide position of codon 124 in the *BIGH3* gene in all affected members. Clinically, the phenotype of LCDI in the proband and his brother was not typical. However, the finding of only single mutation in the three affected members of the family, including the mother with her typical LCDI phenotype, further indicates that screening of *BIGH3* gene is a mandatory step to arrive at an unambiguous diagnosis of corneal dystrophy.

Materials and methods

Report of cases

The cases were examined by MFE and LAF.

- *Case II-2 (the proband)*: A 10-year-old boy who was referred to Moorfields Eye Hospital complaining of intermittent ocular irritation and mild visual impairment. The episodes of ocular irritation began at the age of 5 years. The patient's best corrected visual acuity was 6/18 in both eyes. His intraocular pressure (IOP) was 12 mmHg in the right eye and 11 mmHg in the left eye. He had no history of keratoplasty. Slit-lamp biomicroscopic examination revealed bilateral confluent dense geographic subepithelial corneal opacities resembling that of RBCD, but with intervening very fine lattice lines (Figure 1a).
- *Case II-3 (the proband's brother)*: An 8-year-old boy affected by the same phenotypic presentation as the proband and having no history of eye surgery (Figure 1b).

- *Case I-1 (the proband's mother)*: A 42-year-old female had also been examined. She had a past history of photophobia, lacrimation, and chronic ocular irritation since childhood. Her visual acuity was 6/24 in both eyes and her IOP was 14 mmHg in the right eye and 12 mmHg in the left eye. She had no history of keratoplasty. Slit-lamp examination showed dense subepithelial opacities with fine lattice lines in the superficial and midstromal layers of both corneas (Figure 1c). A full medical history was obtained and no systemic manifestations of amyloidosis such as cranial and peripheral neuropathies, skin changes such as lichen amyloidosis, cutis laxa, blepharochalasis, protruding lips, and mask facies were detected.
- *Case I-2 (the proband's father)*: Unaffected as determined by the slit-lamp biomicroscopic examination.
- *Case II-1 (the proband's brother)*: Unaffected as determined by the slit-lamp biomicroscopy.

Methods

The study had the approval of Moorfields Eye hospital Ethics Committee and conformed to the tenets of the Declaration of Helsinki. An informed consent from all participants was obtained and blood samples were collected from the parents, the two affected, and the one unaffected offspring.

Genomic DNA was extracted from peripheral leucocytes by standard methods and was amplified using the polymerase chain reaction (PCR). The intronic primers used are those listed in (Table 2) spanning exon/ intron boundaries. Amplification was performed in a DNA thermal cycler using the following conditions: 3 min at 94°C followed by 35 cycles of 94°C for 1 min, 60–64°C for 1 min, and 72°C for 1 min, with a final extension step at 72°C for 5 min.

DNA from the participants was amplified using the intronic primers (4–16) of the *BIGH3* gene and analysed on a heteroduplex gel. The amplified DNA for the exons that showed positive results with heteroduplex analysis was purified using a Qiaquick PCR purification kit (Qiagen) and sequenced using an automatic fluorescent DNA sequencer (ABI Prism 373A, Perkin Elmer, Foster City, CA, USA) following the manufacturer's instructions. Nucleotide sequences for the coding regions were compared with the nucleotide sequence of the published *BIGH3* human cDNA.²³ Following identification of the C→T mutation by sequencing, a cosegregation study was performed using *AvaII* restriction enzyme (Promega). The recognition site for *AvaII* is 5'...G▼G(A/T)CC...3' and the C→T change abolishes the recognition site for the mutated allele. The

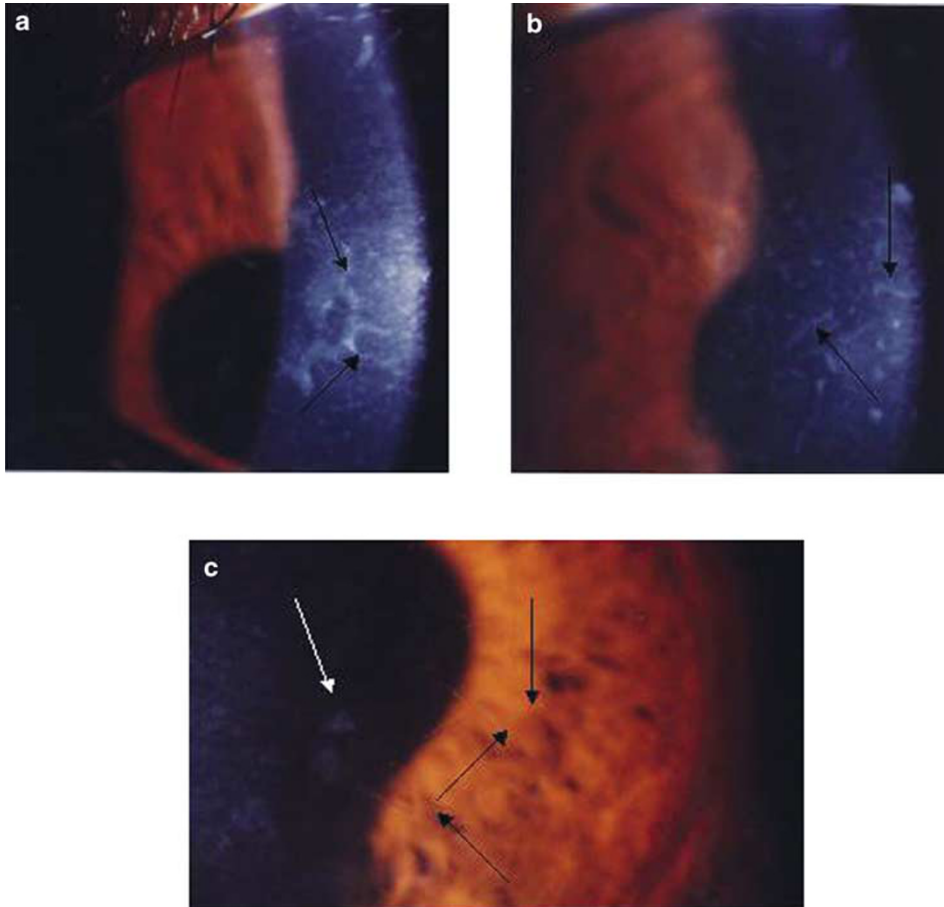


Figure 1 Slit lamp biomicroscopic findings: (a) the right eye of the proband (II-2) showing geographic subepithelial deposits (atypical LCDI), (b) the right eye of the proband's brother (II-3) showing diffuse superficial corneal haze with superimposed amorphous deposits (atypical LCDI) (c) the left eye of the proband's mother (I-1) showing the lattice lines typical of LCDI.

novel sequence alteration found in intron 7 was tested in 100 control chromosomes using *DdeI* restriction enzyme (Promega) with recognition sequence 5'...C▼TNAG...3'. The T→C change abolishes the recognition site of the enzyme.

Results

Mutation screening of *BIGH3* gene by heteroduplex analysis revealed positive results for exons 4, 7, 11, and 12 in all affected members with the exception of exon 7, which was positive for the mother only. No changes were detected in other exons. Direct sequencing analysis of the proband (II-2) and his mother (I-1) for exon 4 revealed a heterozygous base pair transition at nucleotide position 417 (C→T), which converts an arginine at codon 124 into cysteine as shown in Figure 2. This mutation has previously been reported in LCDI.³⁻⁶ The unaffected members of the family did not have the mutation as determined by a cosegregation study of the restriction

digest analysis in which the mutation abolishes the recognition site of *AvaII*. The three affected members showed one undigested product of the expected size (310 bp) for the mutant allele plus two restriction digest products (190 bp and 110 bp) from the normal allele. Unaffected members depicted two restriction digest fragments (190 bp and 110 bp) as shown in Figure 3. Sequence analysis of the same exon revealed a polymorphism in intron 4 (IVS4-16C→T) that was previously reported.^{4,12}

Sequence analysis of the other three PCR products that were aberrantly migrating as identified by heteroduplex analysis showed polymorphisms that did not change the amino-acid sequence of the protein. The polymorphisms were identified in intron 7 (IVS7+46T→C), exon 11 at nucleotide 1416 with C→T change and in exon 12 at nucleotide 1620 with T→C change.

Two of these polymorphisms have previously been identified,^{4,12} however, the polymorphism in intron 7 is

Table 2 Primers designed for amplification of *BIGH3* gene

Exon No.	Length	Tm	Product size	MgCl ₂	Gc%	Sequence
Exon 4 forward	20	63.50	310 bp	1.5 mM	50.00	tcctcctctgtcttctgc
Exon 4 reverse	20	63.90			50.00	agactcccatcatcatgcc
Exon 5 forward	20	65.10	330 bp	1.5 mM	50.00	tgttcccagagttgcaagg
Exon 5 reverse	21	65.10			47.62	ccacacatcggacagaaatg
Exon 6 forward	18	64.30	310 bp	1.5 mM	61.11	ggggcttgggactatgc
Exon 6 reverse	21	64.30			57.14	gcagaagagttcctgctagc
Exon 7 forward	20	64.10	315 bp	2 mM	55.00	accagtgaagctgtgtgctc
Exon 7 reverse	20	63.40			50.00	tggcagtggtatgttcatc
Exon 8 forward	20	59.50	313 bp	1.5 mM	60.00	ggacctgacttgacctgag
Exon 8 reverse	20	58.50			50.00	cacaaaggatggcagaagag
Exon 9 forward	20	61.36	320 bp	1.5 mM	55.00	cctgctgatgtgtcatgc
Exon 9 reverse	20	56.69			55.00	gggtgctgtaaatcggagag
Exon 10 forward	26	61.50	310 bp	1.5 mM	34.62	ttatctctcatcactcttcattg
Exon 10 reverse	26	61.00			34.62	ttttactaataacaagtcccacag
Exon 11 forward	22	64.30	330 bp	1.5 mM	50.00	ggataatgacctgctacatgc
Exon 11 reverse	20	62.00			50.00	tcccaaggtagaagaaagc
Exon 12 forward	20	63.50	330 bp	1.5 mM	45.00	tcaatcctgatgtgccaac
Exon 12 reverse	21	62.90			42.80	aaaatacctctcagcgtggtg
Exon 13 forward	20	64.10	290 bp	1.5 mM	55.00	catctgggggtgagatag
Exon 13 reverse	18	64.90			66.67	ctctgggccctcttgac
Exon 14 forward	26	61.70	317 bp	2 mM	34.62	tttagaacagttttctctctccac
Exon 14 reverse	24	63.00			33.33	tccatctcaaaaacaaagaacaag
Exon 15 forward	20	63.00	250 bp	1.5 mM	60.00	gctggagaggctcctctatg
Exon 15 reverse	21	64.20			52.38	ccctcagtcacgggtgttatg
Exon 16 forward	18	66.60	250 bp	1.5 mM	61.11	agcagatggcaggcttgg
Exon 16 reverse	19	65.90			57.89	tgagtgggggtgcaatgg

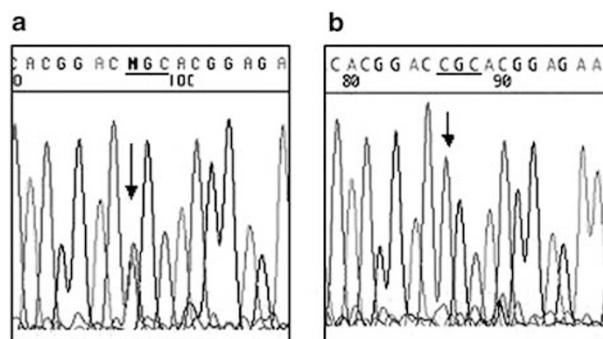


Figure 2 Direct sequencing of exon 4 of the *BIGH3* gene. (a) the proband (II-2) showing heterozygous single base pair transition (CGC → TGC, R124C). (b) the unaffected father (I-2) showing the wild-type allele.

novel. Although the polymorphism in intron 7 was detected in the mother affected by the typical picture of LCDI and was not detected in the proband and his brother, it did not cosegregate with the disease and was detected in a control population of the same ethnic origin at a frequency of 92 out of 100 chromosomes.

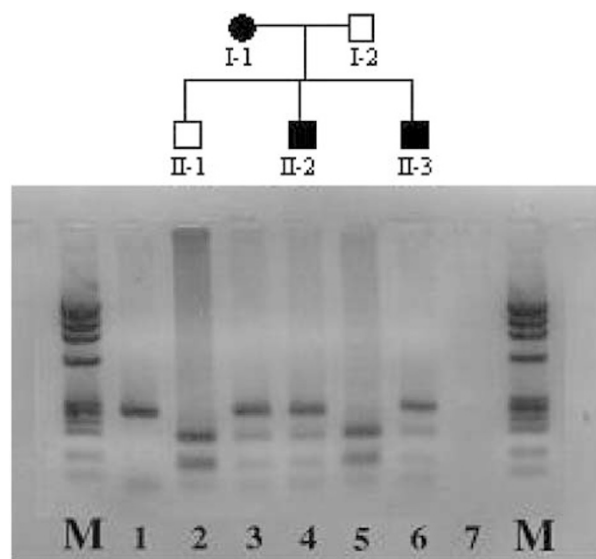


Figure 3 The results of cosegregation study by restriction analysis. M = ϕ x174-*Hae*III marker; lane 1: undigested 310 bp PCR product; lanes 2 and 5: *Ava*II digested product from unaffected members (II-1) and (I-2) respectively, showing the digested wild-type alleles (190 bp and 110 bp); lanes 3, 4, and 6: *Ava*II digested product from affected members (I-1), (II-2), (II-3), respectively, carrying the R124C mutation showing the undigested mutated allele (310 bp); lane 7 contains no sample.

Discussion

In this study, we report a family from Bangladesh with two different phenotypes, the two affected offspring have a phenotype that resemble that of RBCD, whereas the mother has a typical LCDI phenotype.

All affected members of the family have an R124C mutation in the *BIGH3* gene. The same mutation was found in Caucasian³ and Japanese patients with LCDI.²⁴ This suggests that this mutation occurs independently in several ethnic groups.⁵

Molecular genetic analysis of the *BIGH3* gene in the present family did not reveal the R555Q mutation previously found in Caucasians with CDB2³ or the R124L mutation found in Japanese individuals with RBCD.¹⁷ Also, it did not reveal the L518P previously reported with LCDI.⁶ Instead, the same R124C mutation previously associated with LCDI was found in both the mother (LCDI) and the two affected offspring (atypical LCDI).

It has been postulated that RBCD may be a variant of LCD, with a mild to nonexistent deep stromal pathology.²⁵ Our findings based on clinical and genetic studies of the family support this theory. It is possible that the mother's phenotype originally resembled that of her offspring and that the development of lattice lines in her cornea is secondary to chronic irritation produced by numerous episodes of superficial erosions.

Another possible explanation for the observed variant phenotype is the effect of a modifier locus in which other genes affect the phenotypic expression of the mutated *BIGH3* gene. There are well-documented examples of intrafamilial variable expressivity for deafness in humans, which have been attributed to modifier gene.²⁶

The fact that the affected members in this family had not undergone keratoplasty made the categorisation of corneal dystrophy difficult. However, the genetic analysis declared that all affected members in this family belong to one type of corneal dystrophy.

Thus, we conclude that the possibility of RBCD being a variant of LCDI is reinforced by the study of this family and that *BIGH3* gene screening should be mandatory for proper classification of corneal dystrophies, especially in the absence of histopathological examination. Also, the detection of R124C mutation in a Bangladeshi family for the first time adds to the previous reports that *BIGH3* mutations occur independently in different ethnic origins.

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