BIGH3 Mutation Spectrum in Corneal Dystrophies

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PURPOSE. To investigate the molecular pathology underlying BIGH3-related corneal dystrophies (CDs) and to further delineate genotype–phenotype specificity.

METHODS. Sixty-one index patients with CDs were subjected to phenotypic and genotypic characterization. The corneal phenotypes of all patients were assessed by biomicroscopy and documented by slit lamp photography. The BIGH3 gene was amplified ex by exon from constitutional DNA to perform single-strand conformation polymorphism (SSCP) analysis, followed by direct bidirectional sequencing of abnormal formers.

RESULTS. The phenotypes of CDs were classified as lattice CD in 30 patients, Groenouw type I in 12 (CDGGI), Avellino in 7 (CDA), Reis-Bückler in 8 (CDRB), and Thiel-Behnke in 4 (CDTB). Fifty occurrences of 16 distinct mutations were identified, including 8 novel mutations responsible for lattice type IIa in three patients (CDLIIA), intermediate type I/IIIA (CDLI/IIIA) in four patients, and atypical CDL with deep deposits in one patient (CDL-deep).

CONCLUSIONS. Disease-causing mutations were identified in 80% of the patients (50/61). All mutations localize in two regions of kerato-epithelin: the amino acid R124 and BIGH3 fasc domain 4. This study also confirms the mutation hot spot at positions R124 and R555 with nearly 50% of the mutations targeting these two amino acids (24/50). In addition the corneal phenotypes induced by changes at R124 and R555 are amino acid specific: R124C in CDLI, R555W and R124S in CDGGI, R124H in CDA, R124L in CRRB, and R555Q in CDTB. In CDLIIA, CDLI/IIIA, and CDL-deep the genotype–phenotype correlation is domain specific, with all changes occurring at the boundary or within the fasc4 domain. (Invest Ophthal Vis Sci. 2002; 43:949–954)

The 5q31-linked CDs represent a clinically heterogeneous group of disorders caused by allelic mutations of the BIGH3 (TGFBI) gene, markedly correlating to specific phenotypes. These conditions are inherited as autosomal dominant traits with complete penetrance and incomplete dominance.1,2 In a previous report,3 we showed that the occurrence of four distinct heterozygous recurrent mutations was responsible for four specific phenotypes of CD: R555W in granular Groenouw type I CD (CDGGI), R124C in lattice type I CD (CDLI), R124H in Avellino CD (CDA), and R555Q in Thiel-Behnke CD (TBBCD) initially described as Reis–Bückler (CDRB; honeycomb type). Soon thereafter, the list of BIGH3-related phenotypes was further extended to the superficial granular CDRB (geographic type)4 and to lattice type IIIa CD (CDLIIIa)5 in association with R124L and P501T, respectively. In addition to CDRB, two other superficial variants of granular dystrophy were described as the result of homozygosity for R555W in the diffuse placoid form6 and R124H in the juvenile confluent form.7 Clinical visual impairment results from progressive loss of corneal transparency secondary to the corneal deposition of aberrantly processed kerato-epithelin mutants.8

Histologically, these eight clinical entities can be classified into four categories, based on the type of deposits: hyalin in CDGGI and in the three superficial variants of granular dystrophy; amyloid in CDLI and CDLIIA; hyalin and amyloid in CDA; and fibrocellular in TBBCD. Genetically, all these mutations, except P501T in exon 11, target the two arginine residues at positions 124 and 555 in exons 4 and 12, respectively. This relatively simple picture gained in complexity when four additional mutations were reported in atypical and/or asymmetrical late-onset forms of CDL with deep stromal deposits (CDL-deep) and intermediate type I/IIIA (CDLIIA).7,9

The purpose of this study was to further characterize the pathologic molecular characteristics underlying 5q31-linked CDs by reporting novel disease-causing mutations and to clarify the nature of genotype-phenotype correlations.

PATIENTS AND METHODS

The present study was approved by the ethics committee of the University of Lausanne School of Medicine and adhered to the tenets of the Declaration of Helsinki. The corneal phenotype of all index patients was assessed by slit lamp examination and/or review of biomicroscopic photographs by an investigator blinded to the genetic status. The diagnosis was further documented by histopathology, when possible. Genomic DNA of the patients was isolated from blood peripheral leukocytes by organic extraction (Nucleon; Amersham, Amersham, UK). Constitutional DNAs were then subjected to exon-by-exon single-strand conformation polymorphism analysis (SSCP), followed by direct bidirectional sequencing of abnormal formers, as previously described.9 Later in the study and because of the great number of

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Single-nucleotide polymorphisms that were seen in our patients, direct sequencing of exons 4 and 12 was performed, followed, when no mutation was observed, by direct sequencing of all the other exons in preference to the SSCP approach. Amplification from genomic DNA was performed with the primers and conditions described in Table 1. For SSCP analysis, aliquots of labeled amplified DNA were added to formamide loading buffer, denatured at 90 °C for 5 minutes, and then loaded on a 1× mutation detection enhancement (MDE) gel (FMC). Electrophoresis (300–600 V) was performed overnight at 4 °C and 20 °C. DNA bands were revealed by autoradiograph. Direct sequencing of double-stranded PCR products was performed with specific 5' fluoresceinated primers, using one of two sequencers (ALF; Pharmacia, Uppsala, Sweden, or model 310; PE-Applied Biosystems, Foster City, CA), according to the manufacturers' protocols.

RESULTS

Patients

The population under study consisted of 61 consecutive unrelated index patients who were from various ethnic backgrounds and had CDs that included 30 CDL, 12 CDGGI, 7 CDA, 4 CDTB, and 4 CDRB. Patients previously reported by our grounds and had CDs that included 30 CDL, 12 CDGGI, 7 CDA, 4 CDTB, and 4 CDRB. Patients previously reported by our.

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Table 1. Primers and PCR Conditions

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence of Primers</th>
<th>PCR Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'-CCGCTGAGACTACTTAAC-3'</td>
<td>Q-sol., 58°C</td>
</tr>
<tr>
<td>2</td>
<td>5'-GGCAAGTGTAGTGTGACT-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>3</td>
<td>5'-CCGGGGACGAAGGCTAC-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>4</td>
<td>5'-CTTGGGCTGGTTACAGATAC-3'</td>
<td>2% FA, 58°C</td>
</tr>
<tr>
<td>5</td>
<td>5'-CCGGGCAGACGGAGGTCATC-3'</td>
<td>2% FA, 55°C</td>
</tr>
<tr>
<td>6</td>
<td>5'-TTTATATTGACCAAAGGG-3'</td>
<td>4% FA, 55°C</td>
</tr>
<tr>
<td>7</td>
<td>5'-AGGTGCGAGGTAGTGTGAC-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>8</td>
<td>5'-CCGGGGACGAAGGCTAC-3'</td>
<td>4% FA, 55°C</td>
</tr>
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<td>9</td>
<td>5'-CCGGGGACGAAGGCTAC-3'</td>
<td>4% FA, 55°C</td>
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<tr>
<td>10</td>
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<td>55°C</td>
</tr>
<tr>
<td>11</td>
<td>5'-AGGGCTGCTGGTTACAGATAC-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>12</td>
<td>5'-CTTGGGCTGGTTACAGATAC-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>13</td>
<td>5'-AGGGCTGCTGGTTACAGATAC-3'</td>
<td>55°C</td>
</tr>
</tbody>
</table>

FA, formaldehyde; Q-sol, Q solution from Qiagen, Chatsworth, CA; temp, temperature (centigrade) of annealing.

like, or very peculiar fractal-like deposit. The Avellino, Reis-Bückler, and Thiel-Behnke groups were clinically homogeneous in their specific types (Fig. 1).

Mutation Screening

Mutation analysis revealed 50 occurrences of 16 distinct disease-causing mutations (Table 2). Eight previously reported mutations were detected in 39 families: 11 with CDLI (R124C), 4 with CDLI/IIIA (H626K, S540), 12 with CDG1 (R555W, R124S), 6 with CDA (R124H), 2 with CDRB (R124L), and 4 with CDL (R124S). In addition, eight novel mutations were identified in 11 families: 5 CDLIIIA, 3 CDL-deep, and 5 CDL/IIIA (Fig. 2). None of these mutations were present in the 200 control chromosomes.

Phenotype

CDLIIIA. The CDLIIIA group consisted of one Italian patient with sporadic disease and two South American patients with a family history compatible with autosomal dominant transmission. Direct sequencing of exons 14 revealed two different transversion mutations at nucleotide position 1913—T to A and T to G—both predicting an N622K change in KE (Fig. 2). The third mutation associated with CDLIIIA was the deletion of a G at nucleotide position 1926, resulting in a frameshift encoding an abnormal C-terminal protein and a premature truncation at amino acid 669, resulting in the following alignments:

Wild-type: VITNQLPPANRPQERGDELDADSLFEKFQAASFS-RASQVRLAPVYKQLRKH

Mutation (V627S) SSPMFCSLQPTDLKKEGMNQLTTLRLSN-7

KOHQPFPILRGLCD

These three patients had a similar history of late-onset progressive loss of vision and recurrent corneal erosions in the fourth and fifth decades of life. Slit lamp examination documented the presence of large,ropy lattice lines in the anterior stroma (Fig. 1). Successful surgery was performed in both patients with the N622K mutations (T1913G and T1913A) consisting of lamellar and perforating keratoplasty, respectively. Histologic examination using Congo red staining showed large amyloid deposits in the anterior stroma and notably beneath the Bowman layer. Excimer-mediated therapeutic photoablation was performed in the patient with delG1926.

Intermediate Type CDL/I/IIIA. Another four novel mutations occurred in four presumed unrelated patients from France, (n = 1), Switzerland (n = 2), and Italy (n = 1). All patients had a positive family history of CD. The phenotype was atypical and the age of onset delayed to between the third and fifth decades of life. In two patients from one family from the United States, we observed a C-to-G transversion at position 1660, which generated a T538R mutation. The patients had experienced corneal erosions as teenagers. Histopathology obtained after perforating keratoplasty in a 17-year-old patient confirmed the presence of amyloid deposits predominating subepithelially.

Two index patients were found to have a G-to-A transition at base 1915, resulting in a G626D mutation. The first symptoms were red, painful eyes and photophobia at approximately the third and fourth decades of life, sometimes complicated by corneal erosions. Biomicroscopic examination showed discrete subepithelial and very thin linear deposits in the anterior stroma, leaving the middle and posterior third of the stroma free of opacifications (Fig. 1). One index patient had an A-to-C transversion at cDNA position 1924, resulting in an H626P mutation. The corneal phenotype was characterized by a dense haze associated with lattice lines. Histology from the first perforating keratoplasty was not available, but analysis of a subsequent corneal button
showed multiple fusiform amyloid deposits in the corneal stroma.

The last novel mutation identified consisted of a T-to-G transversion in position 1600, causing an L518R substitution at the protein level in a patient from Italy. This mutation is associated with a severe phenotype characterized by subepithelial and stromal amyloid deposits, as was documented by light microscopy after perforating keratoplasty performed in the patient at age 47.

Finally, the ΔF540 mutation was observed in a patient originating from Arbus, Sardinia, who initially had a diagnosis of CDRB. Closer examination of the cornea revealed lattice I/IIIA CD composed of tiny branching subepithelial deposits, leaving the stroma entirely free of lesion. Taking into consid-

**Table 2. Mutation Analysis in 61 Families**

<table>
<thead>
<tr>
<th>Corneal Phenotype</th>
<th>Total Number of Families</th>
<th>Exon</th>
<th>Codon</th>
<th>Nucleotide</th>
<th>Number of Families with Mutation</th>
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</thead>
<tbody>
<tr>
<td>CDLI</td>
<td>11</td>
<td>4</td>
<td>R124C</td>
<td>417C &gt; T</td>
<td>11</td>
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<tr>
<td>CDLIIIA</td>
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<td>14</td>
<td>N622K</td>
<td>1913T &gt; G</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>N622K</td>
<td>1913T &gt; A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>V626x</td>
<td>ΔG1926</td>
<td>1</td>
</tr>
<tr>
<td>CDLI/IIIA</td>
<td>13</td>
<td>14</td>
<td>G625D</td>
<td>1915G &gt; A</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>H626R</td>
<td>1924A &gt; G</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>H626P</td>
<td>1924A &gt; C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>T538R</td>
<td>1660C &gt; G</td>
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<td>ΔF540</td>
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<td>11</td>
<td>L518R</td>
<td>1600T &gt; C</td>
<td>1</td>
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<tr>
<td>CDL-deep</td>
<td>3</td>
<td>14</td>
<td>V651D</td>
<td>1939A &gt; T</td>
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<tr>
<td>CDGGI</td>
<td>12</td>
<td>14</td>
<td>R124H</td>
<td>417C &gt; A</td>
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<tr>
<td></td>
<td></td>
<td>12</td>
<td>R555W</td>
<td>1710C &gt; T</td>
<td>11</td>
</tr>
<tr>
<td>CDA</td>
<td>7</td>
<td>4</td>
<td>R124H</td>
<td>418G &gt; A</td>
<td>6</td>
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<tr>
<td>CDRB</td>
<td>8</td>
<td>4</td>
<td>R124L</td>
<td>418G &gt; T</td>
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<tr>
<td>CDTB</td>
<td>4</td>
<td>12</td>
<td>R555Q</td>
<td>1712G &gt; A</td>
<td>4</td>
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</table>
onset with unilateral disease, at least, at initial examination. The anterior third of the stroma is mostly intact, as is the corneal epithelium. Penetrating keratoplasty is usually necessary at approximately 50 years of age.

**DISCUSSION**

We reviewed the phenotype and searched for mutations in the *BIGH3* gene in 61 families affected with different forms of CD. Using a combined approach based on SSCP and direct sequencing of both strands of all exons, we identified mutations in more than 80% of the families. Mutation hot spots have been reported at positions R124 and R555. Our analysis confirms these previous results: Almost half (24/50) of the mutations identified by us are located at these two amino acids. A worldwide study would probably increase this number. Indeed, we can assume that we have been contacted by medical centers only when the classic mutations have not been observed. Our analysis also indicates a strong correlation between specific mutations at these positions and the phenotype observed in several CD subtypes. These families are divided into two groups; group A in which the phenotype-genotype correlation is amino acid specific (R124 and R555) and group B in which a continuum of lattice phenotypes is associated with domain-specific mutations (fasc4).

CDLI, CDTB, CDGGI, CDA, and CDRB belong in group A. Among the 42 families with these phenotypes, the disease-causing mutations were identified in 35 (85%) of them. All 11 families with the classic forms of CDLI exhibited the R124C classic mutation. Similar results were observed for CDTB in which the classic R555Q mutation was identified in all four families examined. So far, we have associated CDGGI with only two very specific mutations, R555W and R124S, and only the C124H and the R124L mutations were observed in CDA and CDRB, respectively, although in several families with these two phenotypes, no mutation could be identified. A review of the literature showed that only one other mutation representing most probably a “private” event, was associated with CD from group A: a complex mutation (R124L and ΔT125-E126 on the same chromosome) in a form of CDGGI. No other mutation has so far been reported in association with CDs in group A.

The CDs in group B—CDLIIIA, CDLIIA/IIIA, and CDL-deep—are more heterogeneous, and molecular analysis detected mutations in 79% (15/19) of the families. Ten different mutations, of which eight are novel, were identified in 19 families with CDLIIIA, CDLII/IIIA, or CDL-deep. In addition, the Sardinian ΔF540 mutation, previously associated with CDRB, was clinically reassessed and ultimately reclassified as CDLIIIA (late onset during the third decade of life and presence of tiny linear subepithelial deposits). A review of the literature indicates that group B is also associated with the largest variety of mutations: 17 of the 24 mutations reported so far in *BIGH3* are associated with a large spectrum of atypical and/or asymmetrical lattice phenotypes. The amyloid nature of the deposits was documented histologically in three of four of those with lattice-type CD, of which two had unusual posterior amyloid deposits. Gelsolin mutations responsible for lattice CD type II (D187N and D187Y) were excluded by sequencing in all four patients. The patient with CDA had a phenotype indistinguishable from its *BIGH3*-related counterpart. The six patients with CDRB were poorly characterized clinically, and none had the diagnosis confirmed histologically. It is possible that mutations

**FIGURE 2.** DNA sequence fluorogram showing the eight novel *BIGH3* mutations.

- L518R (T1600G)
- N622K (T1913A)
- T538R (C1661G)
- G623D (G1915A)
- H626P (A1924C)
- V627S (del. G1926)
- V631D (T1939A)
- F540 mutation, previously associated with CDRB, was clinically reassessed and ultimately reclassified as CDLIIIA (late onset during the third decade of life and presence of tiny linear subepithelial deposits). A review of the literature indicates that group B is also associated with the largest variety of mutations: 17 of the 24 mutations reported so far in *BIGH3* are associated with a large spectrum of atypical and/or asymmetrical lattice phenotypes. The amyloid nature of the deposits was documented histologically in three of four of those with lattice-type CD, of which two had unusual posterior amyloid deposits. Gelsolin mutations responsible for lattice CD type II (D187N and D187Y) were excluded by sequencing in all four patients. The patient with CDA had a phenotype indistinguishable from its *BIGH3*-related counterpart. The six patients with CDRB were poorly characterized clinically, and none had the diagnosis confirmed histologically. It is possible that mutations
in introns or in the promoter could be responsible for several of them. It is also possible, that cases have been misdiagnosed and actually represent phenocopies or even do not represent cases of ADCD5. We are analyzing the available corneal samples from these cases to investigate by immunohistology whether the deposits are made of KE.

From Figure 3 showing the position of the 24 mutations of BIGH3 reported so far, two important regions are discernible: the amino acid R124 and BIGH3 fasc domain 4. The importance of R124 has already been stressed, whereas there has not yet been a systematic worldwide study undertaken to analyze the implication of mutations at that position, but from discussion with colleagues conducting investigations in this field, we can estimate that mutations at R124 are present in more than half of all the patients with ADCD5. R124 represents therefore a key position for the generation of intracorneal amyloid deposits. Computer analysis of the structure at that position shows a high hydrophilic region and suggests that substitution of R124 by a cysteine would induce a \( /H9252 \) turn in the protein. Additional studies on the structure of KE will help in understanding the role of this region in the making of amyloid.

Computer analysis of BIGH3 located four domains with homology to the Fasciclin I gene. All the pasc domain 4 is located between residues 502 and 632. All the BIGH3 mutations described so far, not related to R124, are located exactly at the boundary or in this domain. Fasciclin I is involved in cell differentiation in Drosophila melanogaster and is expressed in the growing nerve cones, where it is involved in guidance of cone growth. Because BIGH3 is only poorly expressed in the brain, a specific role in nerve growth is unlikely. However, mouse developmental studies have identified BIGH3 expression in the mesenchyme of several organs, and BIGH3 could therefore be important for spatial modeling.

Our mutation analysis indicates that not all four domains are equally important in mediating KE’s normal action and that a yet to be determined specific action is performed by domain 4 — either directly or by interaction with an unknown protein. It would be interesting to identify the protein partners of BIGH3.

References

8. Stewart H, Black GC, Donnai D, et al. A mutation within exon 14 of the TGFBI (BIGH3) gene on chromosome 5q31 causes an


