Am. J. Hum. Genet. 62:320–324, 1998

Mutation Hot Spots in 5q31-Linked Corneal Dystrophies

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Summary

Mutations in the *BIGH3* gene on chromosome 5q31 cause four distinct autosomal dominant diseases of the human cornea: granular (Groenouw type I), Reis-Bücklers, lattice type I, and Avellino corneal dystrophies. All four diseases are characterized by both progressive accumulation of corneal deposits and eventual loss of vision. We have identified a specific recurrent missense mutation for each type of dystrophy, in 10 independently ascertained families. Genotype analysis with microsatellite markers surrounding the *BIGH3* locus was performed in these 10 families and in 5 families reported previously. The affected haplotype could be determined in 10 of the 15 families and was different in each family. These data indicate that R555W, R124C, and R124H mutations occurred independently in several ethnic groups and that these mutations do not reflect a putative founder effect. Furthermore, this study confirms the specific importance of the R124 and R555 amino acids in the pathogenesis of autosomal dominant corneal dystrophies linked to 5q.

Introduction

Granular (Groenouw type I [CDGG1]; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]), lattice type I (CDL1; OMIM 122200 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?122200]), Avellino (ACD) and Reis-Bücklers (CDRB; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]) corneal dystrophies are bilaterally symmetrical disorders that are inherited in an autosomal dominant pattern and are characterized by progressive accumulation of corneal deposits that begin to appear during the 1st or 2d decade of life. With time, these opacities cause serious visual handicaps, often requiring phototherapeutic keratectomy or corneal transplantation (Ridgway and Møller 1992; Rogers et al. 1993). Histopathologically, CDGG1 displays an accumulation of discrete granular opacities, starting in the anterior third of the cornea. The clinical and histological characteristics of CDGG1 and CDRB overlap considerably. However, CDRB can be distinguished by an earlier onset, painful recurrent erosions, and more superficially localized corneal opacities (Küchle et al. 1995). In the case of CDL1, also classified as single-organ amyloidosis, the branching linear amyloid deposits are localized in the central part of the cornea (Klintworth 1967). ACD, a mixed type, presents both CDGG1- and CDL1-like clinical features (Folberg et al. 1988).

We recently reported four missense mutations in the *BIGH3* gene, encoding the kerato-epithelin (KE) protein. Each form of 5q31-linked autosomal dominant corneal dystrophy was associated with a different mutation: CDL1 with R124C, ACD with R124H, CDGG1 with R555W, and CDRB with R555Q (Munier et al. 1997). To better define the genotype-phenotype correlations and to determine whether mutations in *BIGH3* are solely responsible for 5q31-linked autosomal dominant corneal dystrophies, we screened 10 additional families affected with CDGG1, CDL1, or ACD, and we observed that the mutations reported previously occurred in all 10 families. Haplotype analysis revealed that these mutations have arisen independently several times and that they do not originate from a common founder. Therefore, CpG dinucleotides at amino acids R124 and R555 represent hot spots for mutations in the 5q31-linked corneal dystrophies.

Patients, Material, and Methods

Patients

Blood samples were obtained after informed consent from individuals diagnosed with 5q31-linked corneal

Received August 18, 1997; accepted for publication December 2, 1997; electronically published February 6, 1998.

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0002-9297/98/6202-0016$02.00

Table 1

Haplotype Analysis among Families with Corneal Dystrophy

Marker	Haplotype in Family				
	A. CDGG1				
	G-1[a]	G-2	G-3	G-4	G-5
R555W	+	+	+	+	+
D5S393	8	10	4	6	(3 7)
D5S399	3	7	1	8	(9 1)
D5S500	6	9	6	10	(1 6)
D5S396	3	2	2	2	(5 2)
	B. CDL1				
	L-1[a]	L-2[a]	L-3	L-4	L-5	L-6
R124C	+	+	+	+	+	+
D5S393	4	4	4	(4 3)	(4 4)	3
D5S399	9	4	5	(9 3)	(5 12)	3
D5S500	8	6	4	(8 4)	(6 8)	4
D5S396	2	3	4	(2 2)	(3 5)	3
	C. ACD			
	A-1[a,b]	A-2[a,b]	A-3	A-4
R124H	+	+	+	+
D5S393	5	(5 3)	(4 8)	4
D5S399	9	(9 4)	(4 4)	4
D5S500	8	(8 4)	(6 9)	6
D5S396	4	(2 2)	(2 2)	5

Note.—Affected haplotype is given; where not determined, both alleles are given in parentheses.
[a] Mutations from these families were reported previously.
[b] No histology available.

dystrophies. These individuals were members of 10 different families. All diagnoses were confirmed histologically after corneal transplantation in at least one affected family member, with the exception of two patients with ACD (see table 1). One of the non–histologically documented cases of ACD was in a patient from Avellino, Italy, and the other was in a patient from France. More than 100 Caucasians participated as control individuals for mutation search. DNA extraction from peripheral blood was performed according to standard protocols.

Haplotyping

Haplotypes were constructed by use of a minimum-recombination strategy in 15 families (including 5 families reported elsewhere [Munier et al. 1997]). Microsatellite analysis was performed with fluorescent-labeled primers by use of a standard semiautomated method on an automated laser fluorescent (ALF) DNA sequencer (Pharmacia Biotech), as described elsewhere (Korvatska et al. 1996).

Screening of Mutations

All exons of the *BIGH3* gene were analyzed by means of SSCP, as described elsewhere (Munier et al. 1997). To screen the intron portions flanking exon 12, we used two new primers for this region: BIGexon12-2F (5′-CATTCCAGTGGCCTGGACTCTACTATC-3′) and BIGexon12-2R (5′-GGGGCCCTGAGGGATCACTACTT-3′).

Sequencing

PCR amplicons showing abnormal migrating patterns on SSCP were subcloned and sequenced with the AutoRead sequencing kit (Pharmacia Biotech), on both strands, by use of vector and internal primers. Sequencing products were resolved on an ALF DNA sequencer. To control for PCR-induced errors, we sequenced at least 10 independent clones in the region of each mutation/polymorphism.

Genomic DNA amplification of exons 4 and 12 was performed by use of the corresponding forward and reverse primers. PCR products were reamplified with a fluorescent nested primer (exon 4: 5′-GAGGCCATCCCTCCTTCTGT-3′; exon 12: 5′-CCAGTGGCCTGGACTCTACTATCCTCA-3′) and were sequenced directly with the Thermo Sequenase fluorescent-labeled primer cycle-sequencing kit (Amersham Life Science). Samples were resolved on an ALF DNA sequencer. The mutations were deposited in GenBank (accession numbers AF035626, AF035627, AF035628, and AF035629).

Table 2

Mutation Analysis in 16 Families with 5q31-Linked Corneal Dystrophies

Mutation	Corneal Dystrophy Type	No. of Families with Mutation/Total No. of Families
R124:		
R124C	CDL1	6/6
R124H	ACD	4/4
R555:		
R555W	CDGG1	5/5
R555Q	CDRB	1/1

Results

Mutation Screening

DNA samples from 10 probands of various ethnic origins (American, French, Italian, Vietnamese, German, Austrian, and Norwegian) affected with CDGG1 (4 families), CDL1 (4 families), or ACD (2 families) were screened for mutations within the complete coding region of *BIGH3* (table 1). Initially, we searched, by direct sequencing of exons 4 and 12, for the four previously described mutations: R124C, R124H, R555Q, and R555W. We then analyzed *BIGH3* exons, including intron boundaries, by means of SSCP, as described elsewhere (Munier et al. 1997).

Table 1 summarizes the data for 15 families, including the 5 families reported elsewhere (Munier et al. 1997). We observed the presence of the R555W mutation in all five families with CDGG1, the R124H mutation in all four families with ACD, and the R124C mutation in all six families with CDL1. When pooled, these data indicate that recurrent mutations at CpG dinucleotides of R124 and R555 are responsible for the 5q31-linked corneal dystrophies in all 16 families, including the CDRB family reported elsewhere (table 2; Munier et al. 1997).

Haplotype Analysis

To test the hypothesis of a common ancestor versus multiple independent occurrences of an identical mutation in each 5q31-linked corneal dystrophy, we determined the affected haplotype by genotyping the available family members by use of four microsatellite markers within 1 cM of the disease locus (Korvatska et al. 1996). For five families, we received DNA from only one affected member; therefore, we were unable to determine the affected haplotype. According to our data (table 1), families with recurring mutations could be related in only four cases: L-1 and L-4 (possible common haplotype over the 1-cM region analyzed); G-3 and G-5 (possible shared fragment D5S399-D5S500-D5S396); and A-1 and A-2, as well as A-3 and A-4 (possible shared fragment D5S393-D5S399-D5S500). Not surprisingly, cases in which the haplotypes could be identical were in families whose affected haplotypes have not been determined.

Identification of Variants or Polymorphisms

Probands from families with 5q31-linked corneal dystrophies, as well as patients with other forms of corneal dystrophy, were screened for mutations in the *BIGH3* exons, by means of SSCP analysis. Electrophoretic-mobility shifts were detected in some probands and/or in control individuals, for exons 4, 7, 8, 11, and 12 and for introns 4 and 12. PCR products demonstrating bands that, relative to wild type, were aberrantly migrating, were sequenced. We identified a total of eight different nucleotide changes. These alterations were classified as polymorphisms because they do not change the amino acid sequence of the protein, they do not cosegregate with the disease, and all but two of them are present in normal control individuals (table 3). The P135P and A284A variants were observed in only one individual each.

Table 3

Variants or Polymorphisms in *BIGH3*

Location	Variant or Polymorphism		Confirmation Method	No. of Chromosomes Tested	Frequency in Control Population (%)
	Nucleotide	Codon			
Exon 4	294A/T	P98P	Sequencing	24	100
Exon 4	405C/T	P135P	*Apa*I digestion	200	0
Intron 4	IVS4−16C→T	. . .	Sequencing	100	10
Exon 7	852C/A	A284A	*Stu*I digestion	200	0
Exon 8	981A/G	V327V	Sequencing	50	18
Exon 11	1416C/T	L472L	Sequencing	50	18
Exon 12	1620T/C	F540F	Sequencing	50	25
Intron 12	IVS12+23A→G	. . .	Sequencing	50	66

Discussion

We recently established that CDGG1, CDL1, ACD, and CDRB—four clinically distinct 5q31-linked autosomal dominant corneal dystrophies—are caused by allelic mutations of *BIGH3*. Four missense mutations were observed at two amino acid positions. Occurrence of identical mutations in two families with CDL1 and in two families with ACD could be explained by an unknown—and possibly very remote—relationship, by the presence of two hot spots, or by chance. It is therefore important to establish whether 5q31-linked corneal dystrophies are due to numerous different alterations in *BIGH3* or to preferential mutations. To gain insight into the molecular basis of these corneal dystrophies, in the present study, we searched for mutations in 10 additional families. We established the affected haplotypes in 6 of the 10 additional families and in 4 of the 5 families reported elsewhere.

All five CDGG1 families shared the R555W mutation. In four families, the affected haplotypes could be established, and all four were different (table 1). Although the affected haplotype for G-5 cannot be individualized, since we obtained DNA from only one affected family member, it appears to be different. The four ACD R124H-positive families, from Italy, France, Austria, and Germany, showed at least two different genetic origins. Haplotype analysis in six CDL1 R124C-positive families from three different ethnic groups (three Swiss, one French, and two American) showed that recurrent mutations have occurred in at least five families.

In our collection of 16 families, including 6 reported elsewhere, there have been perfect correlations between CDGG1 and R555W, between ACD and R124H, and between CDL1 and R124C. We have analyzed only one CDRB family (mutation R555Q); therefore, no comment on the prevalence of the R555Q mutation can be made. However, it is evident that independent mutations have occurred on numerous occasions at both the R124 and R555 codons and that these mutations clearly are not due to a founder effect.

Observing independent mutations at two amino acid positions, as opposed to establishing a founder effect, has important implications for the molecular diagnosis and for the understanding of the pathophysiology of the 5q31-linked corneal dystrophies. It is interesting to note that both amyloid-related phenotypes (CDL1 and ACD) involve R124 mutations and that both nonamyloid-related phenotypes (CDGG1 and CDRB) involve R555 mutations. How R124- and R555-mutated KE results in the accumulation of corneal deposits is not yet clear; analysis of additional families with 5q31-linked corneal dystrophies will be necessary to establish the true prevalence of the R124 and R555 mutations and to determine whether mutations at other amino acids also lead to amyloid or nonamyloid deposits.

Two hypotheses might explain the pathogenesis of the deposits. On one hand, the phenotype could be due to an aberrant function resulting from a specific alteration of KE by the R124 and R555 mutations. R555 is located in a predicted coiled-coil domain, and its mutation could lead to a modification of this domain. This, in turn, could impair binding to stromal proteins such as collagen VI, for example (Hirano et al 1996). R124C and R124H abolish a putative phosphorylation site; thus, they could have important consequences on the structure of KE. On the other hand, degradation of the mutated KE could be disturbed by these mutations, and either an amyloidogenic part of the protein (in the case of CDL1 and ACD, since this has been reported for many amyloidoses) or the complete protein (in the case of CDGG1 and CDRB) could aggregate to form the corneal deposits.

In summary, we have presented data indicating that 5q31-linked corneal dystrophies are due to recurrent independent mutations at two specific codons of *BIGH3* and that, so far, the phenotype is perfectly correlated with these mutations: R124C (in the case of CDL1), R124H (in the case of ACD), and R555W (in the case of CDGG1).

Acknowledgments

These investigations were supported by Swiss National Science Foundation research grants 32-042195.94 and 32-43619.95. We thank Dr. Phil Shaw for discussion, and we thank F. Ahmad, A.-C. Magnin, and M. Fiaux for technical assistance.

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