Homozygous *CRYBB1* Deletion Mutation Underlies Autosomal Recessive Congenital Cataract

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Purpose. Some 30% of cases of congenital cataract are genetic in origin, usually transmitted as an autosomal dominant trait. The molecular defects underlying some of these autosomal dominant cases have been identified and were demonstrated to be mostly mutations in crystallin genes. The autosomal recessive form of the disease is less frequent. To date, only four genes and three loci have been associated with autosomal recessive congenital cataract. Two extended unrelated consanguineous inbred Bedouin families from southern Israel presenting with autosomal recessive congenital nuclear cataract were studied.

Methods. Assuming a founder effect, homozygosity testing was performed using polymorphic microsatellite markers adjacent to each of 32 candidate genes.

Results. A locus on chromosome 22 surrounding marker *D22S1167* demonstrated homozygosity only in affected individuals (lod score > 6.57 at $\theta = 0$ for *D22S1167*). Two crystallin genes (*CRYBB1* and *CRYBA4*) located within 0.1 cM on each side of this marker were sequenced. No mutations were found in *CRYBA4*. However, an identical homozygous delG168 mutation in exon 2 of *CRYBB1* was discovered in affected individuals of both families, generating a frameshift leading to a missense protein sequence at amino acid 57 and truncation at amino acid 107 of the 252-amino-acid CRYBB1 protein. Denaturing [d]HPLC analysis of 100 Bedouin individuals unrelated to the affected families demonstrated no *CRYBB1* mutations.

Conclusions. *CRYBB1* mutations have been shown to underlie autosomal dominant congenital cataract. The current study showed that a different mutation in the same gene causes an autosomal recessive form of the disease. (*Invest Ophthalmol Vis Sci.* 2007;48:2208–2213) DOI:10.1167/iovs.06-1019

In the developed world, the incidence of congenital cataract is 30 per 100,000 live births.[1] At least a third of such cases are familial.[2] Congenital cataracts may occur as an isolated anomaly, as part of generalized ocular developmental defects (nonsyndromic) or as a component of a multisystem syndrome. Although some congenital cataract loci have been determined in humans by genetic linkage analysis,[2–5] many genes that are involved have yet to be determined.

The transparency of the normal eye lens is dependent on its ability to express a high concentration of crystallin proteins. A higher concentration of such proteins corresponds to a higher refractivity of the medium. Crystallins are highly stable major constituents of the vertebrate eye lens and comprise approximately 90% of the water-soluble lens proteins.[6–8] They have a particular spatial arrangement critical to the transparency of the lens[9] and are hence good candidate genes for congenital cataract disease.[6] The three major classes of crystallins in the human lens—α-, β-, and γ-crystallin—are distinguishable by size, charge, and immunologic properties. The amino- and carboxyl terminal extensions of β-crystallins are presumed to be of importance in protein aggregation and orientation.[10]

Crystallin genes, which encode major structural proteins in the lens, are considered as obvious candidate genes of congenital cataracts owing to both their high levels of lenticular expression and their confirmed functions in maintaining lens transparency. Increasing evidence suggests the correlated relationship between mutations in the crystallin genes with the occurrence of congenital cataracts in humans.[11] Several reports indicate that mutations in mammalian crystallin genes are associated with congenital cataracts,[12] providing concurring evidence.

Autosomal dominant, highly penetrant mutations of β-crystallins appear to be the most common cause of congenital cataract disorders,[13–18] and, when present, typically occur in both eyes.[4] However, different mutations in the human *CRYAA* crystallin gene have been deduced to have different patterns of inheritance: a case of recessive inheritance in αA-crystallin (*CRYAA*) has been reported in a Persian Jewish family.[2] Other cases of autosomal recessive congenital cataract have been associated with homozygous mutations in *CRYBB3*,[19] as well as in several noncrystallin genes.[20,21]

In the present study, two large inbred Bedouin families from southern Israel were found to have an autosomal recessive form of congenital cataract. Assuming a homozygous mutation due to a founder effect, the locus wherein lies the expected mutation was sought.

Materials and Methods

Patients

Two unrelated Israeli Bedouin families were recruited and comprised 14 patients with congenital cataracts and 21 unaffected individuals. The patients were offspring of consanguineous marriages, suggesting that the disorder was due to a founder mutation in each family. The pedigrees are depicted in Figure 1. This study was approved by the Israeli National Genetics Helsinki Committee and the Soroka Medical Centre Institutional Review Board. Written informed consent was obtained from all subjects in accordance with the Declaration of Helsinki.

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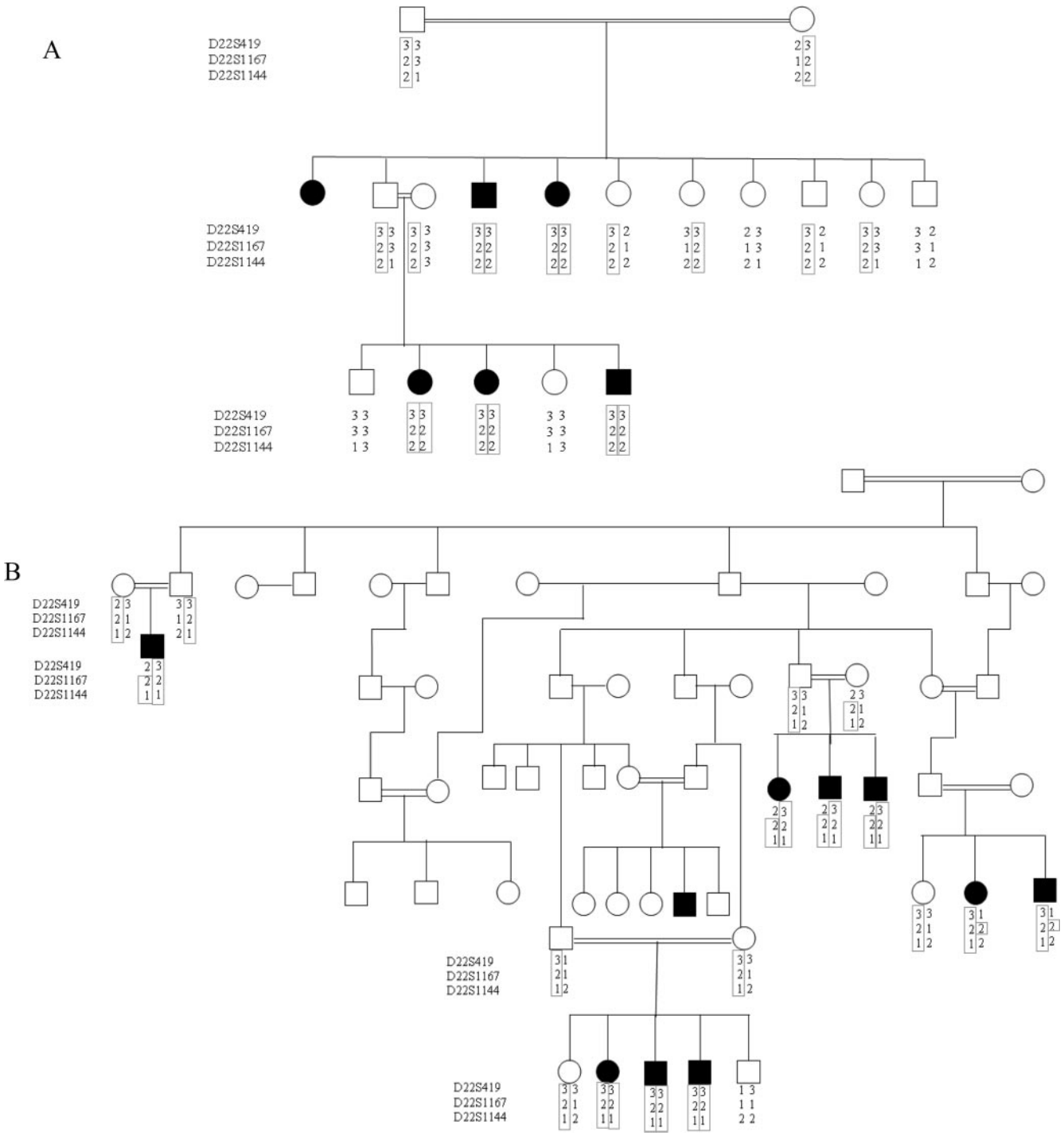

FIGURE 1. Pedigree of two large Israeli-Bedouin kindred with nuclear congenital cataract. Haplotype surrounding the *CRYBB1* gene is shown and the homozygosity region is boxed. Polymorphic marker *D22S1167* is immediately adjacent to *CRYBB1* and is surrounded by *D22S419* (~4 cM upstream) and *D22S1144* (~5 cM downstream). (**A**) Family 1, (**B**) family 2.

Clinical Details

The phenotype was determined via ophthalmic examination, which included the best corrected visual acuity (Snellen's), slit lamp biomicroscopy, and fundus examination. All affected individuals had bilateral confluent nuclear opacification.[22] All except two children underwent bilateral nonsimultaneous lens washout and posterior chamber intraocular lens implantation. Age at the procedure ranged between 19 and 51 months (average 31 months; median 29 months). In two children, the nuclear opacities were mild and surgery was deferred.

Linkage and Haplotype Analysis

Genomic DNA was extracted from whole blood (FlexiGene DNA AGF3000 Kit; Qiagen, Valencia, CA) per the kit instructions. Polymorphic microsatellites flanking 32 candidate genes (Table 1) that have been shown to be involved in lens or cataract development were selected by using the UCSC Human Genome Browser (University of California, Santa Cruz, CA) with Primer3 (http://fokker.wi.mit.edu/primer3/ developed by Steve Rozen and Helen Skaletsky, Whitehead Institute and Howard Hughes Medical Institute, Massachusetts Institute

TABLE 1. Thirty-two Candidate Genes Tested for Linkage to Inherited Congenital Cataract Disorder in Pedigrees 1 and 2

Gene Symbol	***GSTA4***	***ENO1***	***CTPP3***	***CRYBA2***	***CRYAB***	***HSF4***	***CZP3***	***CTAA2***
Marker	*D6S1623*	*D1S243*	*D20S874*	*D2S2359*	*D11S89S*	*D16S421*	*D13S1267*	*D17S938*
Marker	*D6S1956*	*D1S2404*	*D20S917*	*D2S433*	*D11S4192*	*D16S3086*	*D13S175*	*D17S926*
Gene Symbol	***GCNT2***	***CCA1***	***MIP***	***CAAR***	***LIM2***	***BFSP2***	***CRYBA1***	***CCSSO***
Marker	*D6S470*	*D17S802*	*D12S1644*	*D9S2153*	*D19S206*	*D3S1290*	*D17S1873*	*D15S1036*
Marker	*D6S1034*	*D17S2195*	*D12S1632*	*D9S257*	*D19S246*	*D3S1587*	*D17S841*	*D15S117*
Gene Symbol	***CRYL1***	***CRYZL1***	***GJA8***	***CRYGS***	***CRYGB***	***CRYGC***	***CRYGD***	***CRYBB2***
Marker	*D13S175*	*D21S1254*	*D1S442*	*D3S1262*	*D2S2208*	*D2S2208*	*D2S2208*	*D22S419*
Marker	*D13S250*		*D1S2612*	*D3S3570*	*D2S154*	*D2S154*	*D2S154*	*D22S1028*
Gene Symbol	***MAF***	***CRYGA***	***CRYBB1***	***CRYAA***	***CRYBA4***	***CCZS***	***FTL***	***CRYM***
Marker	*D16S3040*	*D2S2208*	*D22S419*	*D21S1890*	*D22S1167*	*D17S1872*	*D19S879*	*D16S3045*
Marker	*D16S750*	*D2S154*	*D22S1167*	*D21S1885*	*D22S310*	*D17S1157*	*D19S596*	*D16S3046*
Marker			*D22S1144*					

Flanking polymorphic markers for each gene were selected by using the UCSC Human Genome Browser and were tested for homozygosity.

of Technology, Cambridge, MA) used to design primers for PCR amplification. PCR amplification of flanking markers for each of these genes (primer sequences available on request) was performed according to standard procedures. All PCR reactions were as follows: denaturation at 95° for 1 minute, followed by 35 cycles of 95° for 30 seconds, 55° for 30 seconds, and 72° for 30 seconds, and a final extension at 72° for 7 minutes.

Products were separated by electrophoresis on a 6% polyacrylamide gel and visualized thereafter by silver staining.[23] Haplotypes were constructed manually and analyzed. Primers used for PCR amplification of polymorphic markers adjacent to the *CRYBB1* gene were: *D22S1167* (forward: 5′ACATGGCAAAACCCAGTCTC3′, reverse: 5′-GGGGCTTCAACAACATTCTTAAC-3′); *D22S419* (forward: 5′-GGCTCAGGGACTCTGGA-3′, reverse: 5′-GGCCAATCGGTAGGTCA-3′); and *D22S1144* (forward: 5′-GCTGAAATTGCCAAAGTTTA-3′, reverse: 5′-GAGCCTCTGGTCCTCTGT-3′). All PCR reactions were performed using PCR master mix (ABgene, Epsom, UK). Linkage was demonstrated by using Superlink,[24] assuming autosomal recessive heredity of the phenotype.

PCR Amplification of Genomic DNA for Sequencing

Primers designed to amplify exon 2 of the *CRYBB1* gene (GenBank accession number: gi: 21536279; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD): forward: 5′-acaggatgtggggctatgag-3′ and reverse: 5′-gtgcggaggagtaagaggtg-3′. The reaction was performed with PCR master mix (ABgene), and conditions were as follows: denaturation at 95° for 1 minute, followed by 35 cycles at 95° for 30 seconds, 58° for 30 seconds, and 72° for 30 seconds, and a final extension at 72° for 7 minutes.

DNA Sequencing

PCR products were gel purified (Qiagen), according to the manufacturer's instructions, and sequenced in both forward and reverse directions with the primers used in the respective PCR amplifications. Sequencing was performed with dye-termination chemistry (Prism BigDye terminator cycle sequencing kit; Applied Biosystems, Inc. [ABI], Foster City, CA, and PRISM 377 DNA sequencer; ABI). A second DNA sample from each patient was tested to confirm mutations identified during the first round of sequencing, with two different PCR products being sequenced for each.

Mutation Analysis

The *CRYBB1* gene which showed linkage to congenital cataracts was sequenced using primers designed by using the National Center for Biotechnology Information (NCBI) database and Primer3 software. PCR products were separated by electrophoresis on agarose gels and kit purified (Qiagen). Products were bidirectionally sequenced (Prism 377 DNA Sequencer; ABI) and analyzed by using Chromas software (Technelysium Pty Ltd., Tewantin QLD, Australia).

Comparison to published DNA sequences was thereafter performed. Further analysis of the deletion in exon 2 of the *CRYBB1* gene was performed with denaturing high-performance liquid chromatography (dHPLC), on PCR-amplified genomic DNA samples (WAVE system; Transgenomic, Elancourt, France), according to the manufacturer's protocol. Elution of PCR products from the column was effected by using an acetonitrile gradient in 0.1 M triethylamine acetate buffer (pH 7), at a constant flow rate of 0.9 mL · min^{-1}. The melting profile of the PCR product determined the temperature at which heteroduplex detection occurred and was calculated with the system software (WaveMaker software; Transgenomic). The DNA fragments were analyzed with a total run time of 7.8 minutes per sample. The linear acetonitrile gradient was adjusted to ensure that the relevant fragments were eluted between 4.5 and 5.5 minutes.

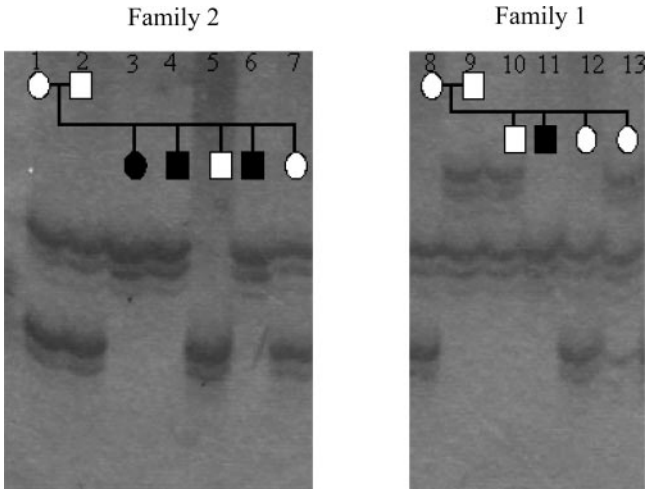

FIGURE 2. Linkage analysis at the *CRYBB1* locus. Individuals 1 to 7 belong to family 2, and individuals 8 to 11 belong to family 1 (Fig. 1). All patients (3, 4, 6, and 11 are shown) in both families exhibited a homozygous genotype for the polymorphic marker *D22S1167*. Their parents (individuals 1, 2, 8, and 9) were heterozygous carriers.

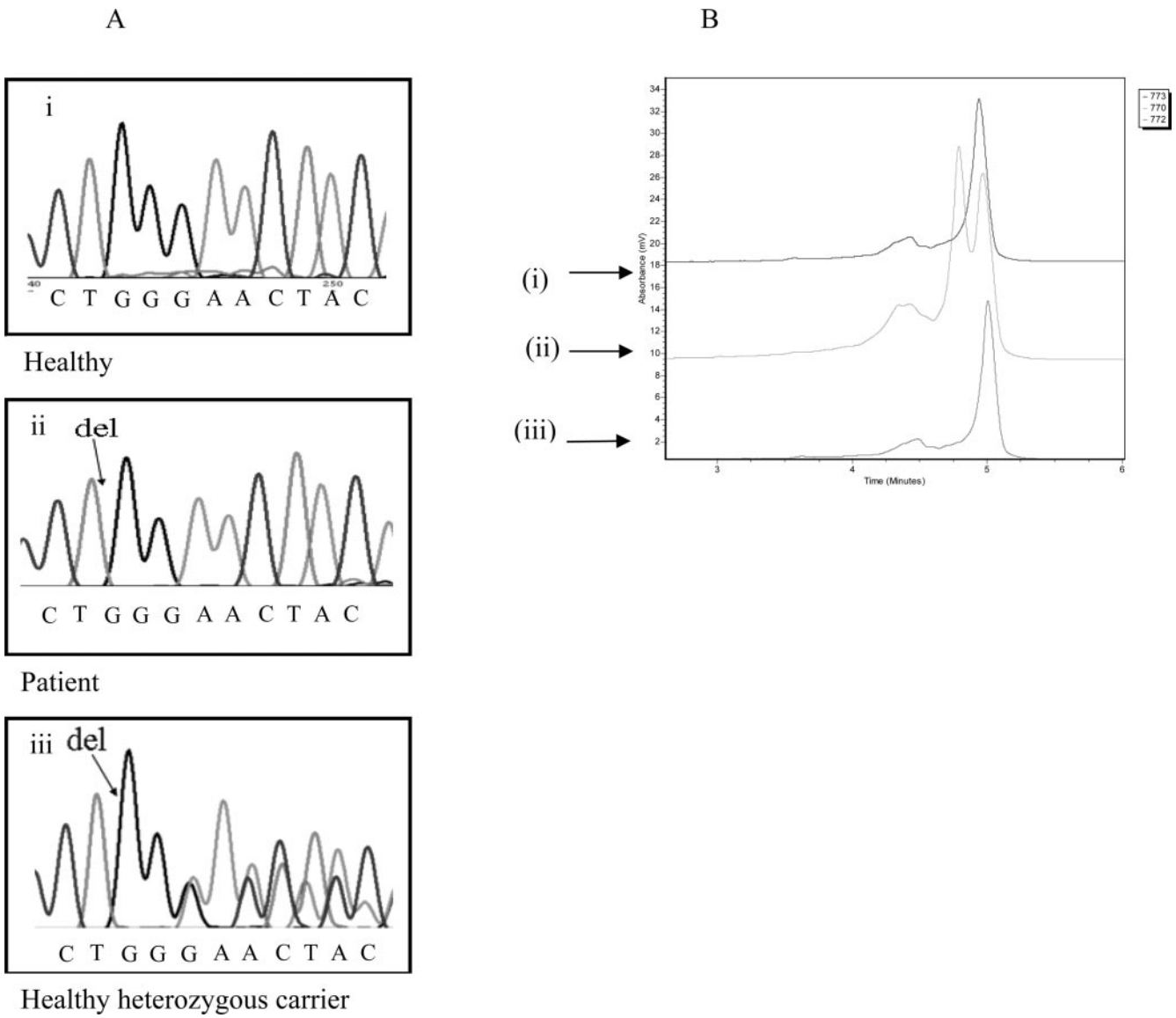

FIGURE 3. (**A**) Sequencing of *CRYBB1* (GenBank accession number gi: 21536279) in nonaffected, carrier, and affected individuals. (**Ai**) Healthy individual, (**Aii**) affected individual, and (**Aiii**) carrier. (**B**) dHPLC depicting (**Bi**) an unaffected individual, (**Bii**) a heterozygous carrier of the mutation, and (**Biii**) a patient with congenital cataracts.

RESULTS

Two large Israeli Bedouin extended families from the Negev desert, presenting with autosomal recessive congenital bilateral nuclear cataracts, were studied. Figure 1 depicts the two pedigrees. At the time of study, 14 patients existed, and linkage to 32 candidate genes was sought (Table 1). Twenty-one unaffected family members were also tested to generate haplotypes with two polymorphic markers flanking each gene. Linkage was ruled out for all loci tested, except for the *CRYBB1* locus (results not shown). Studies using polymorphic markers at the *CRYBB1* locus, shown in Figures 1 and 2, demonstrated that all patients in family 1 (Fig. 1A) were homozygous for a haplotype of three microsatellite markers flanking *CRYBB1* and that all their nonaffected parents were heterozygous carriers of that haplotype. In family 2 (Fig. 1B), there were several crossover events at that locus in the various branches of this larger kindred, with several individuals marrying more distant relatives. Yet, in affected individuals of kindred 2, there was consistent homozygosity for the same allele of *D22S1167* (that is immediately adjacent to *CRYBB1*), as in affected individuals of kindred 1 (Fig. 1A). A significant lod score of 6.57 at $\theta = 0$ was obtained for *D22S1167*, a polymorphic marker residing within 0.1 cM of two β-crystallin genes, *CRYBB1* and *CRYBA4*, known to encode proteins expressed in lens tissue. No mutations were found in *CRYBA4*. However, as shown in Figure 3A, an identical homozygous delG168 mutation in exon 2 of *CRYBB1* was demonstrated in affected individuals of both families, generating a frameshift (and missense protein sequence) as of amino acid 57 and a stop codon causing truncation after amino acid 107. dHPLC studies (Fig. 3B) showed that all affected individuals of both families were homozygous for the mutation, all parents of affected individuals were heterozygous for the mutation, whereas none of 100 unrelated Bedouin individuals from southern Israel carried the mutation.

DISCUSSION

Congenital cataracts frequently cause blindness in children. In this work, an autosomal recessive form of congenital cataracts

was found in two unrelated inbred Bedouin families. Linkage analysis studies of candidate genes led to the identification of a founder mutation in the *CRYBB1* gene encoding β-crystallin, a protein required for maintaining lens transparency. This protein is expressed prenatally in the lens tissue. To date, autosomal recessive cataracts have been associated with mutations in five genes (*CRYAA*, *CRYBB3*, *HSF4*, and *GCNT2* for congenital cataract and *LIM2* for presenile cataract),[2,19–21,25] as well as with three additional loci: 3p22,[26] 9q13,[21] and 19q13.[27] It should be noted that βB2-crystallin dominant mutations were shown to be associated with congenital cataracts, yet for at least for one of these mutations, the phenotype in a homozygous mutant individual was significantly more severe than in heterozygotes.[28]

In humans, α-crystallin is encoded by two closely related genes: the αA- and αB-crystallin genes. The β-crystallin family consists of four acidic (A) and three basic (B) protein forms.[29] Defects in crystallin genes have previously been shown to be associated with human cataract formation, mostly as a dominant trait.[8] Mackay et al.[16] demonstrated that congenital cataract can result from a dominant mutation in exon 6 of the β-crystallin gene, *CRYBB1*. It is of interest that both the Q155X mutation in *CRYBB2*[12] and the G220X mutation in *CRYBB1*[16] disrupt the fourth Greek key motif, probably causing instability of the molecule.[16] Willoughby et al.[30] published evidence of another dominant mutation in *CRYBB1* associated with the phenotype in conjunction with microcornea. This mutation, also in exon 6 of the gene, generated an X253R change in the protein sequence, leading to elongation of the C-terminal extension of the protein. Although it was suggested that this elongation interferes with β-crystallin interactions, this was not proven experimentally.[30] Heredity in all these cases was dominant.

In the present study, we demonstrate a recessively inherited congenital cataract condition caused by a frameshift mutation in *CRYBB1* that fully abrogates the C-terminal extension. βB1-crystallin comprises approximately 9% of all soluble crystallins in the human lens.[31] Loss of the terminal arms can either increase or decrease dimerization of the β-crystallins.[32] It seems likely that the C-terminal extensions affect higher order aggregation, although the evidence of this is indirect, being the preferential occurrence of βB1-crystallin in β-high peak on size-exclusion chromatography.[33]

The mutation characterized is distinct from those reported by Mackay et al.[16] and Willoughby et al.[30] It occurs within the same gene, but results in a recessive trait rather than a dominant one. The two previously described *CRYBB1* mutations,[16,30] both in exon 6 of the gene, were such that caused alterations in the extension region (Greek key motif) of the protein, probably leading to instability of the molecule.[16] In contrast with the previously described *CRYBB1* mutations, the mutation we demonstrate is in exon 2 of the gene and abrogates the protein very near to its N terminus (at amino acid 57 of the original 252-amino-acid protein). Thus, the extension region of the molecule (encoded by exon 6 of the gene) is nonexistent in the mutant protein and it is thus likely that the mutant protein cannot exert any dominant effects through dimerization with wild-type molecules. There is a strong possibility that the frameshift and premature truncation lead to nonsense-mediated decay[34] and thus no protein product, which would be consistent with a recessive phenotype.

References

1. Graw J. Congenital hereditary cataracts. *Int J Dev Biol*. 2004;48: 1031–1044.
2. Pras E, Frydman M, Levy-Nissenbaum E, et al. A nonsense mutation (W9X) in CRYAA causes autosomal recessive cataract in an inbred Jewish Persian family. *Invest Ophthalmol Vis Sci*. 2004;41:3511–3515.
3. Kramer P, Yount J, Mitchell T, et al. A second gene for cerulean cataracts maps to the beta crystalline region on chromosome 22. *Genomics*. 1996;35:539–542.
4. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystalline gene CRYAA. *Hum Mol Genet.* 1998;7:471–474.
5. MacKay D, Ionides A, Kibar Z, et al. Connexin46 mutations in autosomal dominant congenital cataract. *Am J Hum Genet*. 1999; 64:1357–1364.
6. Ring BZ, Cordes SP, Overbeek PA, Barsh GS. Regulation of mouse lens fiber cell development and differentiation by the Maf gene. *Development*. 2000;127:307–317.
7. Wistow GJ, Piatigorsky J. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Ann Rev Biochem*. 1988;57:479–504.
8. Graw J. The crystallins: genes, proteins and diseases. *Biol Chem*. 1997;378:1331–1348.
9. Hoehenwarter W, Klose J, Jungblut PR. Eye lens proteomics. *Amino Acids*. 2006;30:369–389.
10. Slingby C, Clout NJ. Structure of the crystallins. *Eye*. 1999;13:395–402.
11. Fu L, Liang JJ. Alteration of protein-protein interactions of congenital cataract crystalline mutants. *Invest Ophthalmol Vis Sci*. 2003; 44:1155–1159.
12. Brady JP, Garland D, Duglas-Tabor Y, Robison WG Jr, Groome A, Wawrousek EF. Targeted disruption of the mouse alpha A-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein alpha B-crystallin. *Proc Natl Acad Sci USA*. 1997;4:884–889.
13. Litt M, Carrero-Valenzuela R, LaMorticella DM, et al. Autosomal dominant cerulean cataract is associated with a chain termination mutation in the human beta-crystallin gene *CRYBB2. Hum Mol Genet.* 1997;6:665–668.
14. Ferrini W, Schorderet DF, Othenin-Girard P, Uffer S, Heon E, Munier FL. CRYBA3/A1 gene mutation associated with suture-sparing autosomal dominant congenital nuclear cataract: a novel phenotype. *Invest Ophthalmol Vis Sci*. 2004;45:1436–1441.
15. Vanita Sarhadi V, Reis A, Jung M, et al. unique form of autosomal dominant cataract explained by gene conversion between beta-crystallin B2 and its pseudogene. *J Med Genet*. 2001;38:392–396.
16. Mackay DS, Boskovska OB, Knopf HL, Lampi KJ, Shiels A. A nonsense mutation in *CRYBB1* associated with autosomal dominant cataract linked to human chromosome 22q. *Am J Hum Genet*. 2002;71:1216–1221.
17. Reddy MA, Bateman OA, Chakarova C, et al. Characterization of the G91del CRYBA1/3-crystallin protein: a cause of human inherited cataract. *Hum Mol Genet.* 2004;13:945–953.
18. Billingsley G, Santhiya ST, Paterson AD, et al. CRYBA4, a novel human cataract gene, is also involved in microphthalmia. *Am J Hum Genet.* 2006;79:702–709.
19. Riazuddin SA, Yasmeen A, Yao W, et al. Mutations in betaB3-crystallin associated with autosomal recessive cataract in two Pakistani families. *Invest Ophthalmol Vis Sci*. 2005;46:2100–2106.
20. Forshew T, Johnson CA, Khaliq S, et al. Locus heterogeneity in autosomal recessive congenital cataracts: linkage to 9q and germline HSF4 mutations. *Hum Genet.* 2005;117:452–459.
21. Yu L-C, Twu Y-C, Chou M-L, et al. The molecular genetics of the human I locus and molecular background explain the partial association of the adult i phenotype with congenital cataracts. *Blood*. 2003;101:2081–2088.
22. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv Ophthalmol*. 2004;49:300–315.
23. Harel T, Goldberg Y, Shalev SA, Chervinski I, Ofir R, Birk OS. Limb-girdle muscular dystrophy 2I: phenotypic variability within a large consanguineous Bedouin family associated with a novel FKRP mutation. *Eur J Hum Genet*. 2004;12:38–43.
24. Fishelson M, Geiger D. Exact genetic linkage computations for general pedigrees. *Bioinformatics.* 2002;18(suppl 1):S189–S198.

25. Pras E, Levy-Nissenbaum E, Bakhan T, et al. A missense mutation in the LIM2 gene is associated with autosomal recessive presenile cataract in an inbred Iraqi Jewish family. *Am J Hum Genet.* 2002; 70:1363–1367.
26. Pras E, Pras E, Bakhan T, et al. A gene causing autosomal recessive cataract maps to the short arm of chromosome 3. *Isr Med Assoc J*. 2001;3:559–562.
27. Riazuddin SA, Yasmeen A, Zhang Q, et al. A new locus for autosomal recessive nuclear cataract mapped to chromosome 19q13 in a Pakistani family. *Invest Ophthalmol Vis Sci*. 2005;46:623–626.
28. Litt M, Carrero-Valenzuela R, LaMorticella DM, et al. Autosomal dominant cerulean cataract is associated with a chain termination mutation in the human beta-crystallin gene CRYBB2. *Hum Mol Genet.* 1997;6:665–668.
29. Brackenhoff RH, Elliot GS, Schut FH, et al. A device for quantitative measurement of the opacity of small animal lenses: application to *Xenopus laevis. Exp Eye Res*. 1992;54:631–634.
30. Willoughby CE, Shafiq A, Ferrini W, et al. CRYBB1 mutation associated with congenital cataract and microcornea. *Mol Vision*. 2005;11:587–593.
31. Lampi KJ, Ma Z, Shih M, et al. Sequence analysis of betaA3, betaB3 and betaA4 crystallins completes the identification of the major proteins in young human lens. *J Biol Chem*. 1997; 272:2268–2275.
32. Sergeev YV, Wingfield PT, Hejtmancik JF. Monomer-dimer equilibrium of normal and modified beta A3-crystallins: experimental determination and molecular modeling. *Biochemistry*. 2000;39: 15799–15806.
33. Berbers GA, Boerman OC, Bloemendal H, de Jong WW. Primary gene products of bovine beta-crystallin and reassociation behavior of its aggregates. *Eur J Biochem.* 1982;128:495–502.
34. Hentze MW, Kulozik AE. A perfect message: RNA surveillance and nonsense-mediated decay. *Cell*. 1999;96:307–310.