A Nonsense Mutation (W9X) in CRYAA Causes Autosomal Recessive Cataract in an Inbred Jewish Persian Family

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PURPOSE. To identify the genetic defect causing autosomal recessive cataract in two inbred families.

METHODS. Linkage analysis was performed with polymorphic markers close to 14 loci previously shown to be involved in autosomal dominant congenital cataract. In one of the families a gene segregating with the disease was analyzed by single-strand conformation polymorphism (SSCP) and eventually sequenced.

RESULTS. Three polymorphic markers close to the CRYAA gene located on chromosome 21q segregated with the disease phenotype in one of the families, but not in the other. Sequencing of the CRYAA in this Jewish Persian family revealed a G-to-A substitution, resulting in the formation of a premature stop codon (W9X).

CONCLUSIONS. A nonsense mutation in the CRYAA gene causes autosomal recessive cataract in one family. This constitutes the first description of the molecular defect underlying nonsyndromic autosomal recessive congenital cataract. That there was no linkage to this locus in another family provides evidence for genetic heterogeneity. (Invest Ophthalmol Vis Sci. 2000;41:3511–3515)

Congenital cataracts are a major cause of blindness in infants, with an estimated incidence of 1 to 6 per 10,000 live births.1 At least a third of the cases are familial, most often inherited in a nonsyndromic autosomal dominant fashion. A wide phenotypic variability evident by the formation of opacities in different compartments of the lens, is consistent with the large number of distinct loci that have already been described in autosomal dominant congenital cataract. The crystallin proteins constitute 80% to 90% of the soluble proteins in the lens and are divided into three groups, \( \alpha \), \( \beta \), and \( \gamma \).2 The crystallins constitute a major component of the cellular cytoplasm needed for the transparency of the lens. This requires their refractive index to be relatively constant over distances approximating the wave length of the transmitted light, and therefore, maintenance of a high degree of short-range order among the crystallins is needed.3 Recently, some of the crystallins were also shown to have enzymatic activity.4 Because fiber cells in the central lens nucleus lose their nuclei during development, the crystallins in these cells do not turn over and thus must be extremely stable proteins.5 The transparency of the lens also depends on a variety of noncrystallin proteins, which include various enzymes such as those involved in the glutathione redox cycle and the mercapto acid pathway,5 and components of the lens cytoskeleton and membrane proteins such as MP-26, aquaporins, connexins, and N-cadherins.6 Each component of these systems could cause hereditary cataract.

Mutations in five different crystallin genes (or clusters) have been identified as the cause of dominant disease. These include: CRYAA,7 CRYAB,8–9CRYBA,10 CRYBB,11 and mutations within the \( \gamma \)-crystallin gene cluster at 2q34.12 Two gap junction gene mutations were identified, GJA8 at 1q21.113 and GJA3 at 13q11-12.14 In addition, in seven families the disease locus has been mapped by linkage studies, but the disease-causing gene has not yet been cloned.15–21 All together, 14 disease loci have been identified in autosomal dominant congenital cataract.

Despite the progress that has been made in understanding the molecular basis of autosomal dominant congenital cataract, no loci or genes in humans has been identified in the autosomal recessive form of the disease. An autosomal recessive cataract has been previously linked to the \( \alpha \)-blood group, but the chromosomal location of \( \alpha \) is not known.22 In this report, we present data showing that a nonsense mutation in the human \( \alpha \)-crystallin gene (CRYAA), is responsible for an autosomal recessive form of the disease in an inbred Jewish Persian family. In an additional Arab Muslim family we have ruled out this gene as the cause of the disease, thus providing evidence for genetic heterogeneity in the autosomal recessive form of the disease.

METHODS

Families and DNA Specimens

Families were recruited at the Sheba Medical Center, Tel Hashomer, and Sapir Medical Center, Kfar Saba, Israel. Partic-
participants gave informed consent to the study protocol, which was approved by the Institutional Review Board of the Sheba Medical Center, Tel Hashomer, and which conformed with the tenets of the Declaration of Helsinki. Disease was diagnosed in affected individuals within a few weeks after birth, and the patients were operated on during the first 3 months of life. No details of the pathologic findings in the lens were available. Ophthalmic examination of the parents and unaffected siblings in both families did not reveal any ocular abnormalities. Twenty milliliters of heparinized blood was obtained from each participant, and DNA was extracted using a commercial kit (Gentra Systems, Minneapolis, MN).

Genotyping and SSCP Analysis

Markers used in this study included: D11S1986, D11S1998 (for CRYAB); D21S2055, D21S226, and D21S1446 (for CRYAA); and D11S1997, D15S3669, D18S1653, D18S1659, D21S212, D21S226, D3S2146, D3S2176, D3S2111, D3S2178, D4S587, D4S592, D16S2624, D16S539, D17S1308, D17S2196, D17S2194, D17S2193, D17S21784, and D22S420 for the additional 12 loci described in autosomal dominant cataract.10–21 Amplification was performed in a 10-μl reaction volume containing 50 ng of DNA, 13.4 ng of each unlabeled primer, 1.5 mM each dNTP, and 0.08 μg 32P-labeled primer in 1.5 mM MgCl2 polymerase chain reaction (PCR) buffer, with 1.2 U Taq polymerase (Bio-Line, London, UK). After an initial denaturation of 5 minutes at 95°C, 31 cycles were performed at 94°C for 2 minutes, 52°C for 3 minutes, and 72°C for 1 minute, followed by a final extension time of 7 minutes at 72°C. Samples were mixed with 10 μl of loading buffer, denatured at 95°C for 5 minutes and electrophoresed on a 6% denaturing polyacrylamide gel. Single-strand conformational polymorphism (SSCP) analysis of the CRYAA gene was performed by amplifying exons 1, 2, and 3 using the primers 5'-CTCACGTTCCCCTGGTACCA-3' and 5'-GGCAGGGGGGGGGGGCGGGCG-3', respectively. The reaction was performed as described earlier. Samples were amplified using the conditions described for genotyping. Samples were mixed with 10 μl of loading buffer, denatured at 94°C for 5 minutes and electrophoresed on a 6% polyacrylamide-10% glycerol nondenaturing gel at 8 W for 16 hours.

Sequencing and Restriction Analysis

Exons 1, 2, and 3 were amplified as described and sequenced using an automated sequencing system (ABI Prism-310 Genetic Analyzer; Perkin Elmer–Applied Biosystems, Foster City, CA). The exon 1 mutation was confirmed by testing for the presence of a restriction site in the mutated allele. Exon 1 was amplified as described above and digested with HincII, to produce three fragments of 146, 51, and 56 bp in the mutated allele in contrast with the two fragments of 202 and 51 bp in the normal allele.

Linkage Analysis

Linkage was calculated with the LINKAGE (ver 5.1) package of computer programs, assuming an autosomal recessive model of inheritance, 100% penetrance in both sexes, and a gene frequency of 0.001. The marker order and distance, taken from published sources (http://cedar.genetics.soton.ac.uk/pub/), were as follows: 21qter-D21S2055-2.62 cm-D21S212-0.55 cm-CRYAA-0.24 cm-D21S1446-pter, 11qter-D11S1986-0.06 cm-CRYAB-6.81 cm-D11S1998-pter. Allele frequencies were also taken from published sources (http://www.gdb.org).

RESULTS

Using a candidate gene approach, we typed both families with polymorphic PCR markers surrounding the crystallin genes, gap junction genes, or loci to which autosomal dominant cataract has been mapped. Three markers on chromosome 21q (from top to bottom) D21S2055, D21S212, and D21S1446.

FIGURE 1. Family pedigrees and typings (in rectangles) for three markers on chromosome 21q (from top to bottom) D21S2055, D21S212, and D21S1446.
sequencing of the other two exons did not reveal any other changes.

This G-to-A change introduces a Hinfl restriction site in the mutated allele. We used this restriction site to test the other family members for the mutation and to test for its presence in the normal population. The three affected siblings in this family were found to be homozygous for this substitution, whereas the parents and the unaffected sibling were found to be heterozygous for it (Fig. 4). We did not find this change in the 70 normal chromosomes that we tested.

As is evident in Figure 1, in family 2 the disease did not segregate with chromosome 21 markers. Lod score calculations with the three markers located on both sides of this locus (Table 1), yielded negative results across the entire interval and ruled out the CRYAA gene as the disease-causing gene in this family. Because the α-crystallin protein is composed of the products of CRYAA and CRYAB, after excluding CRYAA as the gene responsible for the disease in family 2, we checked for linkage between CRYAB and two chromosome 11 markers located close to this gene in this family. The results, presented in Table 1, ruled out CRYAB as the disease-causing gene in family 2. We also excluded in this family the 12 additional loci involved in autosomal dominant cataract10–21 (data not shown).

**DISCUSSION**

In this study we showed that a nonsense mutation in the CRYAA gene is responsible for an autosomal recessive form of congenital cataract in an inbred Persian family. This constitutes the first description of the molecular defect underlying non-syndromic autosomal recessive congenital cataract in humans.

The α-crystallins that compose up to 50% of the total protein mass of the lens belong to the small heat shock protein family and function as chaperons.22 Molecular chaperons facilitate the correct folding of proteins in vivo and are of extreme importance in keeping these proteins properly folded and in a functional state.23 The small heat shock family protein is a group of closely related proteins that are induced by heat or hypertonic stress, bind to aggregation-prone proteins, and act as a reservoir of nonnative folding intermediates that are subsequently refolded by other chaperons.24 The α-crystallin is a multimeric protein composed from two gene products, αA and αB. CRYAA encodes the αA subunit, whereas αB is encoded by the CRYAB gene located on chromosome 11q. Thus, this multimeric protein is important in maintaining the transparency of the lens, possibly by ensuring that the complexes
formed by them and other proteins of the lens remain soluble.25

Brady et al.26 have produced a knockout mouse model for the CRYAA gene. In this animal model, the main disease was detected in the homozygote mice, who at the age of 10 weeks showed development of dense opacity of the lens, whereas no such opacities were detected in the heterozygote mice. In both the knockout mice and family 1, the mutation in the CRYAA gene resulted in a complete or an almost complete absence of the CRYAA protein in the homozygotes and in a recessive mode of inheritance. It seems as though the amount of protein produced by the normal allele in the family 1 heterozygotes and in the knockout mice heterozygotes is sufficient for the normal development and maintenance of the lens, whereas complete absence of the CRYAA protein results in cataract formation. In contrast, a missense mutation (R116C) in the gene described by Litt et al.,7 was inherited in an autosomal dominant manner. Heterozygosity for this mutation is sufficient to cause cataract, even in the presence of a normal second allele. The abnormal protein caused by the missense mutation may precipitate in the lens, induce the precipitation of other proteins, or interfere with the function of the normal allele (negative dominant effect).

In family 2 we excluded the CRYAA gene as the cause of the disease, thus providing proof for genetic heterogeneity in autosomal recessive congenital cataract. We also ruled out the CRYAB gene that encodes the 68 kDa subunit of the α-crystallin multimer and obviously constitutes an attractive candidate gene for recessive cataract, as well as the 12 other loci involved in autosomal dominant cataract. Ehling27 estimated that approximately 30 loci are involved in autosomal dominant human congenital cataract. In mice, 40 loci have already been mapped (http://www.ncbi.nlm.nih.gov/Homology/). The estimations about the number of loci involved in recessive congenital cataract are much smaller.27 As shown for the CRYAA gene in this study, mutations in other loci involved in dominant cataract could very well also account for the autosomal recessive form of the disease. It is also evident that not all the loci involved in human cataract have been mapped.

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References


