A Novel Locus for Autosomal Dominant Nuclear Cataract Mapped to Chromosome 2p12 in a Pakistani Family

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METHODS. Genomic DNA from family members was typed for alleles at more than 300 known polymorphic genetic markers by polymerase chain reaction. The lod scores were calculated by using two-point linkage analysis of the genotyping data.

RESULTS. The maximum lod score, 4.05, was obtained for the marker D2S2333. Proximal and distal crossovers were observed with markers D2S286 and D2S1790, respectively. These crossovers define the critical disease locus to an interval of approximately 9 centimorgans (cM).

CONCLUSIONS. Linkage analysis identified a novel locus for adNCat on chromosome 2p12 in a Pakistani family. A genome database analysis of the target interval is being undertaken to identify candidate gene(s) for the disease. (Invest Ophthalmol Vis Sci. 2002;43:2083–2087)

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utosomal dominant congenital cataract (adCat) is a clinically and genetically heterogeneous lens disorder. Cataract may occur as an isolated anomaly or as a component of a multisystem syndrome. As an isolated anomaly, congenital cataracts can occur sporadically, but many are familial. Although X-linked and autosomal recessive transmission of hereditary cataracts has been observed, the most frequent mode of inheritance is autosomal dominant with a high degree of penetrance.1 Cataract is one of the most common major disorders of the eye in infants and accounts for 10% to 30% of legal blindness in children. Cataract shows a wide variety of phenotypic and genotypic heterogeneity.2 Although there is no agreed nomenclature for the morphologic patterns of cataract, depending on the location, opacity, and appearance the following types of cataracts are generally described in the literature: anterior polar, posterior polar, nuclear, lamellar (zonular), coralliform blue dot, cortical, pulverulent, and total (available at http://www.ncbi.nlm.nih.gov/omim, provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD; accessed February 2002). For nonsyndromic dominant congenital cataract, at least 16 loci have been identified by linkage analysis on chromosomes 1q21-q25, 3 1pter-p36.13, 4 2q35-35.1 3q21-22.3, 5 6q22-q22.3, 6 12q15,7 13q11-11q5,8 14q24-pter,9 15q21-22,10 16q22 12 17q24 13 17q11-q12,14 17p13,15 20p12q12 16 21q22,17 and 22q18 (Eye Research Network, provided in the public domain by the Eye Research Institute, Oakland University, Rochester, MI; Kenneth P, Mitton, editor; available at http://ken.mitton.com/ern/lensbase.html).

Mutations in several genes are known to cause cataracts. These include the genes encoding lens connexins (CX50, 18,19 and CX46,20), crystallins (CRYAA, 21 22 CRYAB, 23 CRYBB, 24 CRYGC,25 and CRYGD26), lens-developmental protein PITX3, 27 the major intrinsic protein of lens fiber (MIP),28 and cytoskeletal protein CP49. 30 The connexins comprise a family of structural proteins that are important in the formation of gap junctions and play a vital role in the structure and function of the lens. Mutations in the crystallin genes that encode more than 90% of the lens cytoplasmic proteins have been identified as a cause of dominant cataracts. MIP is a member of the aquaporin 0 (AQP0) family of membrane-bound water channels.30

In this study we describe a large, four-generation, consanguineous Pakistani family affected by adCat (Fig. 1). Morphologically the cataract in all the affected members of this family was nuclear with a phenotype typical of early onset of disease. After exclusion of all the candidate genes that are known to be involved in the structure and function of the lens and all the known loci for adCat, a whole-genome search was performed. Linkage analysis provided evidence for a novel locus for autosomal dominant nuclear cataract (adNCat) on chromosome 2p12.

MATERIALS AND METHODS

Clinical Details

A four-generation consanguineous Pakistani family with cataract was ascertained. Based on family history and clinical diagnosis, the type of cataract was classified as congenital embryonic nuclear, with an autosomal dominant mode of inheritance. In each generation, either one or both parents were affected (Fig. 1). Previous records showed that cataract was either present at birth, or developed in infancy and that there was no family history of any other ocular or systemic abnormalities. Two individuals, III:3 and IV:4, had had bilateral cataract surgery before the age of 40 years. Detailed ophthalmic examination was performed on 20 family members. Analysis included slit lamp biomicroscopy and photography of the lens to record the cataract type. Twelve members of the family were found to be affected with bilateral cataract, whereas the remaining eight were unaffected. After correction of astigmatism, all affected individuals had adequate vision. For example, individual IV:5 achieved a corrected vision of 6/15 and 6/18 in the left and right eyes, respectively. Figure 2 shows photographs and schematic views of lens opacities in individuals III:4 (Figs. 2a, 2b) and IV:5 (Figs. 2c, 2d), aged 65 and 28 years, respectively. Slit lamp photographs of the left and right eyes of individual IV:5 are illustrated (Figs. 2e, 2f). The cataracts were bilateral, with equal degrees of opacity in the two eyes.
biomicroscopy of the lens showed that the opacity consisted of a large number of minute shiny particles with a distinct bluish hue in the fetal part of the nucleus. The cataracts were identical in appearance in all patients but were of various sizes in different subjects. Cataract was also progressive in nature and, as shown in Figures 2c and 2d, the changes were more prominent in the offspring(s) (IV:5) of a first-cousin marriage (Fig. 1: III:3 and III:4).

For genetic analysis, blood samples were collected from the whole family with voluntarily signed informed consent. The protocol of the study conformed with the tenets of the Declaration of Helsinki. Genomic DNA was extracted from whole blood using a commercially available extraction kit (Nucleon II: Scotlab Bioscience, Strathclyde, Scotland, UK). To calculate the allele frequencies, samples were also obtained from 100 unrelated, normal Pakistani individuals with no symptoms of cataract or any other disease of the eye.

**Microsatellite and Linkage Analysis**

For linkage analysis polymorphic microsatellite markers (Human Mapping Set, ver. 8; Research Genetics, Inchinnan, Scotland, UK) were amplified by polymerase chain reaction (PCR). PCR reactions were each performed in a 10 µl volume, containing 1.5 mM MgCl₂, 0.4 mM of each primer, 200 µM dNTPs, 1 U Taq DNA polymerase, and PCR buffer containing 16 mM (NH₄)₂SO₄, 67 mM Tris-HCl (pH 8.8), and 0.01% of the nonionic detergent Tween-20 (Bio-Line, London, UK). Amplification was performed with an initial denaturation for 5 minutes.
at 95°C, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 3 minutes. The PCR products were separated on 8% to 10% nondenaturing polyacrylamide gels (Protogel; National Diagnostics, Edinburgh, Scotland, UK). The gel was stained with ethidium bromide and photographed under UV illumination. Alleles were assigned to individuals, and haplotypes of all family members were constructed. On the first indication of linkage to chromosome 2p12, the family was further genotyped for more markers across this region (Fig. 1). The genotypic data were used to calculate the lod scores using the MLINK software program (ver. 5.2, ftp://linkage.rockefeller.edu/software/linkage/, provided in the public domain by Rockefeller University, New York, NY). Allele frequencies were also calculated of the normal, ethnically matched population. The phenotype was analyzed as an autosomal dominant trait with nearly complete penetrance (0.99) at a frequency of 0.0001 for the disease allele.

**Mutation Screening**

To examine the probability of the existence of the disease-associated mutations, exon-specific intronic primers were designed for the candidate gene for mammalian cytochrome c oxidase subunit Vb (COX5B, GenBank accession number M59250; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, and available at http://www.ncbi.nlm.nih.gov/Genbank). The gene contains four coding exons spanning a region of approximately 2.4 kb. Each exon was amplified at an annealing temperature of 55°C under standard PCR conditions. Primer sequences and their PCR product sizes are given in Table 1. The amplified exons were analyzed by mutation-detection electrophoresis (MDE) at 180 V on a commercial apparatus (model 600S; Hoefer, San Francisco, CA). The products of PCR amplification were purified and sequenced using a commercially available kit (Big Dye Terminator; PE Biosystems, Foster City, CA) and the products were analyzed on an automated DNA sequencer (model 377; PE Biosystems). All PCR products were sequenced in both forward and reverse directions.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer Sequence</th>
<th>PCR Product (bp)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F 5’-CTGGAGCTTGTTCCCGGAAG-3’ R 5’-GGGCCTGAATCTGAGACAG-3’</td>
<td>261</td>
</tr>
<tr>
<td>2</td>
<td>F 5’-GTCAGAGGGCCGCTGACG-3’ R 5’-GTCAGAGGGCCGCTGACG-3’</td>
<td>296</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
<td>F 5’-CCTGGAGCTTGTTCCCGGAAG-3’ R 5’-GGGCCTGAATCTGAGACAG-3’</td>
<td>547</td>
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</tbody>
</table>

**RESULTS**

Known loci for adCat were first excluded by linkage analysis using locus-specific microsatellite markers. Subsequently, a genome-wide search was performed, using a set of 296 polymorphic markers spanning the entire human genome at approximately 15- to 20-cM intervals (Human Mapping Set; Research Genetics). Linkage was obtained for markers located on chromosome 2p12. More markers in this region were analyzed to obtain proximal and distal crossovers to localize the disease region. Haplotypes for these markers are shown in Figure 1. Two-point lod scores between adNCat and the markers in this region are summarized in Table 2. The maximum lod score of 4.05 was obtained for the marker D2S2333 with no crossover. A proximal crossover was obtained for individual IV:8 with marker D2S286, and a distal crossover was obtained with marker D2S1790 in individuals III:5 and IV:11. These crossovers localized the disease gene within approximately 9 cM on chromosome 2p12.

Individuals III:3 and III:4 were cousins, and because the mode of inheritance of the disease is autosomal dominant, both appeared to carry the same disease chromosomes in a heterozygous state. Individual III:3 was assumed to have a parental...
crossover (Fig. 1). All their affected children inherited the disease chromosomes from both parents—that is, they were homozygous in the disease region. Further, there was a marked difference in the size and severity of the cataract between individuals that inherited a single disease chromosome (Figs. 2a, 2b) compared with those with two disease chromosomes (Figs. 2c, 2d).

DISCUSSION

This novel locus, close to the centromeric region of chromosome 2, is the third genetic locus reported to date for adNCat. The other two loci for nuclear cataracts have been reported on chromosome 1pter-1p36 in a large Danish family1 and on chromosome 12q15 in a white family of European descent in the United States.8

Similar to retinal disorders, these results show the genetic heterogeneity of cataract, and many genes appear to be involved for a particular phenotype. The γ-crystallin gene that has been associated with Coppelock-like cataract is also present on chromosome 2q35-35.26 However, it is far from this new cataract locus, and the distal crossover marker D2S1790 excludes the possibility of this gene’s being responsible for the disease haplotype in this family. The candidate genes in this disease region were searched from the genomic database (provided in the public domain by the Sanger Centre, Hinxton Hall, UK, and available at http://www.ensembl.org/). A candidate gene of mammalian cytochrome c oxidase subunit Vb (COX5B; GenBank accession number M59250) which is located on chromosome 2-region cen-q13 was screened for mutations.31 No disease-associated mutation was found, and thus the disease-causing gene for this novel locus is yet to be identified.

Acknowledgments

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References

5. Lubsen NH, Renwick JH, Tsui LC, Breuning ML, Schoenmakers JG. A locus for a human hereditary cataract is closely linked to the gamma-crystallin gene family. Proc Natl Acad Sci USA. 1987;84:489–492.


