A New Locus for Autosomal Dominant Cataract on Chromosome 12q13

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PURPOSE. To map the gene for autosomal dominant cataracts (ADC) in an American white family of European descent.

METHODS. Ophthalmic examinations and linkage analyses using a variety of polymorphisms were performed; two-point lod scores calculated.

RESULTS. Affected individuals (14 studied) exhibited variable expressivity of embryonal nuclear opacities based on morphology, location within the lens, and density. This ADC locus to 12q13 was mapped on the basis of statistically significantly positive lod scores and no recombinations ($\theta_m = 0$) with markers D12S368, D12S270, D12S96, D12S359, D12S1586, D12S312, D12S1632, D12S90, and D12S83; assuming full penetrance, a maximum lod score of 4.73 was calculated between the disease locus and D12S90.

CONCLUSIONS. The disease in this family represents the first ADC locus on chromosome 12; major intrinsic protein of lens fiber (MIP) is a candidate gene. (Invest Ophthalmol Vis Sci. 2000;41: 2665–2670)

Cataracts in the pediatric population may be caused by intrauterine embroyopathies, single gene defects, and chromosomal rearrangements. Immunization programs have reduced the incidence of rubella, commonly associated with congenital cataracts; some congenital cataracts, particularly unilateral, are of unknown etiology. Hereditary congenital cataracts account for about one third of pediatric visual loss, and nonsyndromal autosomal dominant cataracts (ADC) are the most common.

Most ADC are congenital, and progression is common. Phenotypic variability has been documented among and within families. Generally, the cataracts are bilateral and are characterized on the basis of location, size, color, the presence or absence of refractility, and, most notably, shape. Despite attempts to clinically categorize hereditary cataracts, there is limited correlation of phenotypes with genetic loci.

ADC is genetically heterogeneous, and 13 loci for ADC have been identified on the basis of linkage analyses and gene mutations; hyperferritinemia, an additional locus, is a systemic disease of autosomal dominant cataracts without other symptoms. Several recent reviews of human cataracts and mouse models are available. We expanded our clinical study of an American white family of European descent with some members affected by ADC of embryonal nuclear and pulvulcent cortical forms; expressivity was variable. Using linkage analysis, we mapped the disease to 12q13.

METHODS

The family (ADC2) of European extraction (Fig. 1) was ascertained at the Ophthalmology Clinic of the Jules Stein Eye Institute, Department of Ophthalmology, UCLA School of Medicine, through the courtesy of Sherwin J. Isenberg, MD; clinical and negative linkage analyses have been reported. Informed consent in accordance with the Declaration of Helsinki and with the UCLA Institutional Review Board approval was obtained in all cases. Twenty-seven individuals participated in the study: 14 affected individuals and 13 unaffected individuals of whom 5 were spouses; no other diseases aside from age-related disorders were identified. Affected status was determined by pupillary dilation and evaluation of lenses by slit-lamp biomicroscopy or retroillumination in the field, or by a history of cataract extraction before senility (before 60 years of age; JBB); in this family, all aphakic patients were younger than 20 years of age at the time of venipuncture. In all patients except 1 and 6 (categorized originally as unknown based on an examination in the field), the phenotype was determined before genotyping.

Adults in the family reported that the cataracts were present from early in life (Fig. 2) and are presumed to be congenital based on the examinations of the proband and his sister. The proband (27) had an embryonal nuclear opacity in each eye with vacuolization; there was mild asymmetry. His affected father (24) had been unaware of his cataracts and had...
20/20 vision in each eye. Nystagmus was evident only in those individuals who had undergone cataract surgery early in life and by history had a delay of correction of refractive error.

Blood samples (between 7 and 30 ml depending on ease of venipuncture, level of cooperation, and size of the patient) were collected in EDTA, and genomic DNA was extracted. Markers were analyzed for linkage with the ADC2 locus several times as methodology evolved; candidate genes/regions were identified on the basis of expression within the lens or linkage with a chromosomal region in another family. Initially, linkage analysis was based on available polymorphic phenotypic blood markers; one individual’s Duffy (FY) genotype was found to be incorrectly coded and corrected in the present study. Lod scores for haploglobin (HP) linked to the CTM locus at 16q22.1; A-crystallin (CRYAA) on human chromosome 21q22.3, 18 B-crystallin (CRYAB) on 11q22.3-q23.1,20 A1 (formerly bA3/A1)-crystallin (CRYBA1)21 on 17q11.2-q12,22,23 and B2-crystallin (CRYBB2) on 22q11.2-q12.1,25,26 g-crystallin cluster (CRYG)27 on 2q33-q35, and z-crystallin (CRYZ)29 on 1p22-p31 were analyzed for linkage to the ADC2 gene using RFLPs. We screened for new RFLPs for the CRYAB, CRYBA1, and CRYBB2 genes in 10 normal and unaffected individuals. Markers for a mapped ADC locus on 17q13 were studied with STR marker loci.

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/)  
**Figure 1.** Pedigree of ADC family with haplotypes for the most relevant markers. Only members from whom blood was drawn are included. Solid circles and squares represent affected females and males, respectively; open circles and squares denote unaffected females and males, respectively. The proband is identified by an arrow. The box represents the disease haplotype inherited from the founder.
The marker loci were localized to chromosomal regions based on data from the Marshfield Institute for Molecular Genetics\(^{32}\) and the Genome Database.\(^{33}\) For linkage analyses, pedigree and genotype data were analyzed with LIPEX.\(^{34}\) Lod scores were calculated using published allele frequencies.\(^{35,35}\) A gene frequency of 0.0001 and penetrance at 1.0 and 0.9 were assumed for the cataract locus; two-point lod scores were calculated for a full range of \(\theta_m\) and \(\theta_l\) values.

**RESULTS**

Two male siblings (1 and 6), initially coded as unknown based on retroillumination in the field, were restudied by slit-lamp biomicroscopy and recategorized as affected. Both had punctate white opacities of the posterior cortical region, the posterior Y suture, and, to a lesser extent, the anterior cortex; individual 6 had small vertical linear opacities in the inferior cortical region.

We identified new RFLPs for the \(CRYBA1\) (PstI; 14.0 and 13.5 kb with frequencies of 0.6 and 0.4, respectively) and \(CRYBB2\) (DraI and PstI in linkage disequilibrium; DraI of 10.5 and 9.6 kb with frequencies of 0.55 and 0.45, respectively and PstI of 11.8 and 6.0/4.5 kb with frequencies of 0.55 and 0.45, respectively). No RFLPs for \(CRYAB\) were identified.

Two-point lod score(s) were less than \(-2.00\) \((\theta_m = \theta_l = 0.001)\) for crystallins \(CRYA4, CRYBA1,\) and \(CRYBB2\) as well as for markers flanking both \(FY\) and \(HP\) and were less than \(-1.8\) \((\theta_m = \theta_l = 0.001)\) for \(CRYG\) flanking markers; multipoint data excluded linkage with flanking markers for \(CRYZ\) gene. Using the pooling methods, regions of chromosomes 3, 8, 14, and 19 were excluded. Using the ABI Prism system, markers on chromosomes 1, 12, 13, 17, and 18 were studied and all excluded with the exception of D12S83 and D12S368 (\(Z_{\max} = 2.33\) and 3.76, respectively; \(\theta_m = \theta_l = 0\)); additional markers on chromosome 12 (National Jewish Resource Center, Denver, CO or Research Genetics, Huntsville, AL) were tested (Table 1). Assuming full penetrance, a maximum lod score of 4.73 \((\theta_m = \theta_l = 0)\) was calculated for marker D12S90; lod scores without recombinations extended telomeric from D12S368 to D12S83, a distance of 25 to 31 cM. Lod scores at 0.95 penetrance demonstrated linkage and were similar to those calculated with full penetrance; the maximum score for marker D12S90 was 4.62 \((\theta_m = \theta_l = 0)\).

**DISCUSSION**

Eight single genes have been implicated as causative for ADC to date each in a single family with the exception of the \(\beta A1\)-crystallin, gap junction protein \(\alpha-3\) (connexin43), \(\gamma D\)-crystallin, \(PAX6\), and the \(\beta\)-crystallin gene \((CRYBB2)\) all of which have been reported in two. A chain termination mutation in the \(\beta\)-crystallin gene \((CRYBB2)\) on chromosome 22\(^{36,37}\) a missense mutation in the gap junction protein \(\alpha-8\) gene \((connexin50; MP70)\)\(^{36}\) and a missense mutation in the human \(\alpha4\)-crystallin gene \((CRYAA)\)\(^{40}\) on chromosome 21 have been shown to cause ADC. Activation of the \(\gamma E\)-crystallin pseudogene \((CRYGEP1)\)\(^{14}\) on 2q33–q35 was reported as the basis of the Coppock-like cataract. Recently, Heon and colleagues\(^{42}\) restudied the family and found that the variation in the pseudogene \(CRYGEP1\), presumed to activate the gene, is a polymorphism and identified a missense mutation in a highly

**FIGURE 2.** Photographs of affected members of the family demonstrating phenotypic heterogeneity. Proband (27) had vacuoles in the embryonal nucleus (age, 1 month; A); his father (age, 38 years; 24) has a star-shaped opacity that does not alter vision (B). The proband’s aunt (age, 60 years; no specimen obtained) has a dense cataract in the embryonal nucleus (C).

Once linkage to available candidate genes was excluded, we initiated a genome-wide search using a pooling technique and, thereafter, a systematic approach using the ABI Prism Linkage Mapping Set (version 2; Perkin Elmer–Applied Biosystems, Foster City, CA), with end-labeled fluorescent primers as detailed in the user’s manual. For the pooling method, anonymous markers selected on the basis of predominant alleles in a DNA pool of affected family members compared with an unaffected pool, were amplified using the polymerase chain reaction (PCR).
conserved region of exon 2 of the γC-crystallin (CRYGC). Kannabiran and colleagues demonstrated a mutation of a donor splice junction (intron C) of the βA1-crystallin gene on 17q11.2-q12 as the basis for the ADC in an Indian family; we studied a large Brazilian family with ADC of variable morphology and found a new and different mutation at the same βA1-crystallin splice site (Bateman et al., unpublished data, 2000). Two-point mutations, a missense and a frame-shift, in gap junction protein α-3 (connexin46) on chromosome 13q11-12 have been reported in two families with granular opacities of the fetal nucleus and juvenile cortex. Two families with missense mutations of the γD crystallin (CRYGD) gene (2q33-q35) and disparate clinical features have been reported. Although mutations in the homeobox DNA-binding PAX6 gene usually cause aniridia and/or anterior segment dysgenesis, isolated cataracts have been documented. Hyperferritinemia, an autosomal dominant systemic disease characterized by elevated serum ferritin, congenital cataracts, and abnormal liver biopsy, is caused by mutations of the iron responsive element of ferritin L-subunit gene and may represent an additional locus.

There is considerable phenotypic variability in the ADC families that have been studied by linkage analyses. Curiously, similar forms of ADC have been mapped to different chromosomal regions, whereas disparate forms have mapped to the same locus. For example, embryonic/fetal and progressive sutural opacities in one family and stationary posterior polar cataracts in another have been mapped to chromosome 1p36.52,53 Recently, affected members of a family with cataracts were found to have the identical mutation as a family with Coppock-like cataracts. The cataracts in our family varied considerably among individuals, and correlation with genotype would not be feasible based on morphology.

The locus in family ADC2 is in the 12q13 chromosomal region based on linkage analysis and represents the first ADC locus on chromosome 12. There were no recombinations with 9 markers, and lod scores were over 3.0 with the exception of D12S83; differences are based on the number of informative matings. The region spans 25 to 31 cM, depending on which map was used (Table 1).

There are several eye-related genes in the 12q12-14.1 region. Retinol dehydrogenase 1 (RDH1) expressed in the retinal pigment epithelium was assigned to chromosome 1p36.32,54 Recently, affected members of a family with cataracts were found to have the identical mutation as a family with Coppock-like cataracts. The cataracts in our family varied considerably among individuals, and correlation with genotype would not be feasible based on morphology.

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<th>Marker</th>
<th>( Z_{\text{max}} )</th>
<th>( \theta_m )</th>
<th>( \theta_f )</th>
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Figure 3. Ideogram of chromosome 12 with linked markers and MIP. The underlined markers recombined with the ADC locus and identify the interval.
as an adhesion molecule\textsuperscript{62}, MIP probably maintains lens transparency by reducing interfiber space.\textsuperscript{73,74} The MIP mRNA is expressed in the lens vesicle early in embryogenesis and in the secondary lens fibers.\textsuperscript{63,65}

There are many candidate genes for ADC based on animal models, gene expression within the lens, and chromosomal localization in humans and other mammals. For example, mutations in the \textit{Crybb2}(\textit{BBB2}), \textit{Cryg} and \textit{\textgamma}-crystallin genes have been found to cause ADC in the Philly mouse,\textsuperscript{75} eye lens–obluescence mouse (ELO),\textsuperscript{76} and 13/N guinea pig,\textsuperscript{77} respectively. In the mouse, the homologous region of human chromosome 12q13 (region of our ADC2 locus) is chromosomal localization in humans and other mammals. For example, mu- tations in the \textit{Mip} (\textit{MP26}) cause cataracts in the cataract Fraser (CatFr),\textsuperscript{79,80} lens opacity mutations (Lop),\textsuperscript{80} and hydroptic fibers (Hfi) mice.\textsuperscript{81} In the CatF\textsuperscript{m} mouse, the most abundant \textit{Mip} mRNA transcript in the adult lens is truncated and is the result of a transposon-induced splicing defect that substitutes a long terminal repeat sequence for the carboxy-terminal exon of the gene;\textsuperscript{79} in this model, the water channel function is disrupted.\textsuperscript{72} In the Lop mouse, an amino acid substitution inhibits targeting of \textit{Mip} to the cell membrane.\textsuperscript{80} In the Hfi mouse, an exon 2 deletion in the transcript is associated with a cataract.\textsuperscript{81} \textit{MIP} is a candidate gene based on its close location to the cataract locus in our ADC2 family and the reported mutations in the mouse.

In conclusion, we have identified a new locus for ADC on chromosome 12q13. Affected members of this American family exhibit variable morphology with some opacities in the breamy nucleus and others in the cortex. \textit{MIP} is a candidate gene that we are analyzing in this family.

Acknowledgments

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References

33. The Genome Database (GDB). \textit{http://www.gdb.org/}.