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A New Locus for Dominant "Zonular Pulverulent" Cataract, on Chromosome 13

Donna Mackay,[1] Alexander Ionides,[1] Vanita Berry,[1] Anthony Moore,[2] Shomi Bhattacharya,[1] and Alan Shiels[1,*]

[1]Department of Molecular Genetics, Institute of Ophthalmology, and [2]Moorfields Eye Hospital, London

Summary

Inherited cataract is a clinically and genetically heterogeneous disease that most often presents as a congenital autosomal dominant trait. Here we report the linkage of a new locus for dominant "zonular pulverulent" cataract (*CZP*) to chromosome 13. To map the *CZP* locus we performed molecular-genetic linkage analysis using microsatellite markers in a five-generation English pedigree. After exclusion of eight known loci and several candidate genes for autosomal dominant cataract, we obtained significantly positive LOD scores (*Z*) for markers *D13S175* (maximum Z [Z_{max}] = 4.06; maximum recombination frequency [θ_{max}] = 0) and *D13S1236* (Z_{max} = 5.75, θ_{max} = 0). Multipoint analysis gave Z_{max} = 6.62 (θ_{max} = 0) at marker *D13S175*. Haplotype data indicated that *CZP* probably lies in the centromeric region of chromosome 13, provocatively close to the gene for lens connexin46.

Introduction

Congenital or infantile cataract is a significant cause of visual impairment and blindness in childhood, with an estimated prevalence of 1–6/10,000 live births (Lambert and Drack 1996). As many as half of all congenital cataract cases may have a genetic cause either as part of a systemic disease (e.g., Lowe oculocerebrorenal syndrome) or as a nonsyndromic Mendelian trait (McKusick 1996). All three types of Mendelian inheritance have been reported for cataract; however, autosomal dominant transmission appears to be the most frequent (Merin 1991).

At least eight independent loci for autosomal dominant cataract have been genetically mapped, and these show considerable inter- and intrafamilial phenotypic variation (Francois 1982; Lund et al. 1992; Scott et al. 1994). Clinically distinct types of autosomal dominant cataract, however, have been closely linked genetically, raising the possibility of allelic heterogeneity. The Volkmann "zonular progressive" cataract (Eiberg et al. 1995) and a "posterior polar" cataract (Ionides et al. 1997) map to 1p, the Coppock-like "nuclear pulverulent" cataract (Brackenhoff et al. 1994) and a "polymorphic lamellar" cataract (Rogaev et al. 1996) map to 2q, and the Marner zonular progressive cataract (Eiberg et al. 1989) and a posterior polar cataract (Maumenee 1979) map to 16q. In contrast, clinically similar types of autosomal dominant cataract have been shown to be genetically distinct. "Cerulean" cataract has been mapped to 17q (Armitage et al. 1995) and 22q (Kramer et al. 1996), whereas "anterior polar" cataract has been linked to 17p (Berry et al. 1996) and also is associated with a balanced reciprocal 2;14 chromosome translocation (Moross et al. 1984). Finally, a "zonular sutural" cataract has been mapped to 17q (Padma et al. 1996), and a "zonular pulverulent" cataract has been linked to 1q (Renwick and Lawler 1963).

Clinical descriptions of inherited cataract, by use of a slit lamp, are based on the physical appearance and location of opacities within the lens. The term "zonular" is used to describe opacities that are confined to one or more discrete zones of the lens, other than at the poles (e.g., "lamellar," affecting a zone that encircles the center or nucleus of the lens). The term "pulverulent" refers to finely pulverized or powdery dustlike opacities that can be either zonular or widely dispersed throughout the lens. To obtain further insight into the etiology of autosomal dominant cataract, we performed linkage analysis on a five-generation family of English descent with zonular pulverulent cataract and identified a new locus on chromosome 13.

Subjects and Methods

Pedigree and Diagnosis

The cataract family was ascertained through the genetic database at Moorfields Eye Hospital, London. Autosomal dominant inheritance was supported by the presence of affected individuals in each generation,

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* Present affiliation and address for correspondence and reprints: Dr. Alan Shiels, Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, Campus Box 8096, 660 South Euclid Avenue, St. Louis, MO 63110.

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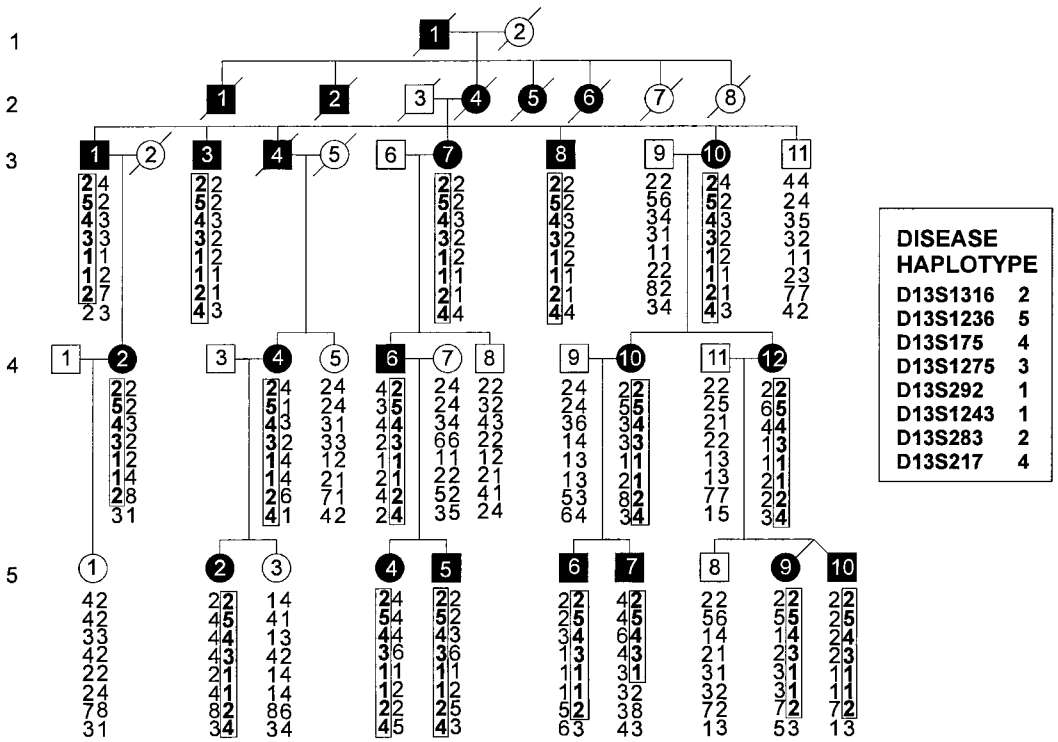

Figure 1 Abridged pedigree of the zonular-pulverulent-cataract family used in this study, showing the segregation of six chromosome 13 markers listed in descending order from the centromeric end. Squares and circles symbolize males and females, respectively; and unblackened and blackened symbols denote unaffected and affected individuals, respectively.

equal numbers of affected males and females, and male-to-male transmission (fig. 1). Sixteen affected and seven unaffected members of the family underwent a full ophthalmological examination by one of us (A.I.). The cataract, which was bilateral in all cases, consisted of fine dustlike opacities that varied considerably with respect to their zonular location in the lens. In some affected individuals the dustlike opacities formed a lamellar distribution with a clear peripheral cortex and minimal involvement of the central nucleus of the lens. Others had more widely spread dustlike opacities extending into the cortex, with no demarcation of the nucleus. In very mild cases, the dustlike opacities were clustered only around the anterior and posterior Y-shaped sutures of the lens nucleus. Hospital records indicated that usually the cataract either was present at birth or developed within the first few months of life, and there was no family history of other ocular or systemic abnormalities.

Genotyping

Blood samples, taken with informed consent and local ethical approval, were collected in Vacuette EDTA tubes (Greiner), and leukocyte genomic DNA was extracted by use of the Nucleon II kit (Scotlab Bioscience). Généthon microsatellite markers (Dib et al. 1996) were amplified by PCR in an Omnigene thermal cycler (Hybaid). PCR reactions (10 μl in 96-well microtiter plates) contained 100–200 ng genomic DNA, 1 × PCR buffer (50 mM KCL, 10 mM Tris-HCL pH 9.0, 0.15% Triton-X-100, and 1.5 mM $MgCl_2$), 250 μM of each dNTP, 2.5 pmol each of forward and reverse primers, and 0.5 U *Taq* DNA polymerase. For radiolabeled markers the forward primer was 5′-end labeled (37°C for 30 min) with [γ-^{32}P]ATP (Redivue, ~110 terabecquerels/mmol; Amersham) by use of 1 U T4 polynucleotide kinase (PNK; Promega)/μl in 30 μl of 1 × PNK buffer (70 mM Tris-HCL pH 7.6, 10 mM $MgCl_2$, and 5 mM DTT) prior to amplification. PCR conditions were 1 cycle at 94°C for 3 min, followed by 30 cycles at 94°C for 1 min, 50–55°C for 1 min, and 72°C for 1 min and then 1 cycle at 72°C for 5 min. Radiolabeled PCR products were separated on denaturing 6% polyacrylamide sequencing gels (National Diagnostics) by use of a SequiGen rig (BioRad) and were detected by autoradiography. Alternatively, unlabeled PCR products were separated on nondenaturing 8% polyacrylamide gels and were detected by ethidium bromide staining and UV photography.

Linkage Analysis

Pedigree and genotype data were collated by use of the LINKSYS (version 3.1) data-management package

Table 1

Two-Point *Z* Values for Linkage between *CZP* and Chromosome 13 Markers

Marker	Genetic Distance[a] (cM)	Z at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D13S1316	4.2	2.39	2.33	2.10	1.80	1.19	.60	.15	2.39	.00
D13S1236	3.2	5.75	5.65	5.26	4.75	3.64	2.40	1.05	5.75	.00
D13S175	1.4	4.06	3.97	3.64	3.21	2.28	1.55	.61	4.06	.00
D13S1275	2.6	3.07	3.02	2.79	2.49	1.85	1.16	.52	3.07	.00
D13S292	.1	2.17	2.14	1.99	1.80	1.37	.89	.40	2.17	.00
D13S1243	.1	−∞	1.02	1.95	2.14	1.91	1.36	.65	2.14	.10
D13S283	7.5	−∞	3.63	3.97	3.80	3.08	2.10	.93	3.97	.05
D13S217		−∞	−5.12	−1.87	−.67	.17	.34	.22	.34	.30

[a] Sex averaged, from Dib et al. (1996).

LS4 (Atwood and Bryant 1988), and linkage analysis was performed by use of the LINKAGE (version 5.1) package of programs (Lathrop et al. 1984). Allele frequencies for all markers were estimated on the basis of data from the normal spouses of affected family members. A gene frequency of .0001 and a full penetrance were assumed for the cataract locus. θ Values between males and females were assumed to be equal. Two-point *Z* values were calculated by use of the MLINK and ILINK subprograms. Multipoint *Z* values were computed by use of the LINKMAP subprogram run on the UK Human Genome Mapping Project (HGMP) Resource Centre computing facilities (Rysavy et al. 1992).

Results

For linkage analysis, 27 members of the pedigree (fig. 1), including 16 affected individuals, 7 unaffected individuals, and 4 spouses, were genotyped with microsatellite markers from the Généthon $(AC)_n$ map (Dib et al. 1996). We first excluded linkage of this cataract phenotype with the zonular pulverulent cataract locus on 1q, using markers *D1S305* ($Z = -3.96, \theta = .1$) and *D1S210* ($Z = -3.40, \theta = .1$), which flank the Duffy blood-group locus (Human Genome Database 1996). We then proceeded to exclude other known loci and candidate genes for autosomal dominant cataract (McKusick 1996) on 1p (Volkmann cataract and ζ-crystallin), 2q (γ-crystallin), 3q (phakinin), 11q (α2-crystallin), 12q (lens major intrinsic protein), 16p (μ-crystallin), 16q (Marner cataract), 17p (anterior polar cataract), 17q (zonular sutural cataract, βA3/A1-crystallin, and cerulean cataract type 1), 19q (lens integral membrane protein 2), 21q (α1-crystallin), and 22q (cerulean cataract type 2 and β-crystallin). We also excluded the remainder of chromosome 3 (γS-crystallin), all of chromosome 20 (filensin), and part of chromosome 13 (connexin46). In all, ~200 markers were used at 5–10-cM intervals across the candidate regions before we detected a significantly positive *Z* value for marker *D13S175* ($Z_{max} = 4.06$; $\theta_{max} = 0$), which cytogenetically has been mapped to band region q11 adjacent to the centromere of chromosome 13 (Human Genome Database 1996). The seven other Généthon markers in this region of chromosome 13 immediately were genotyped in the family; and the two-point *Z* values of all eight marker loci are summarized in table 1. The two-point Z_{max} was obtained with marker *D13S1236* ($Z_{max} = 5.75$; $\theta_{max} = 0$). Multipoint analysis using markers *D13S1236*, *D13S175*, and *D13S1243* (fig. 2) yielded $Z_{max} = 6.62$ ($\theta_{max} = 0$) at *D13S175*.

Haplotype analysis (fig. 1) detected one unaffected individual (5.3) and seven affected individuals (3.1, 4.2, 4.10, 5.6, 5.7, 5.9, and 5.10) who were obligate recombinants with the most distal marker, *D13S217*, in the region. However, only one affected individual (5.7) was recombinant for markers *D13S283* and *D13S1243*, which lie increasingly proximal to *D13S217*. No individuals recombinant for the cataract locus and the re-

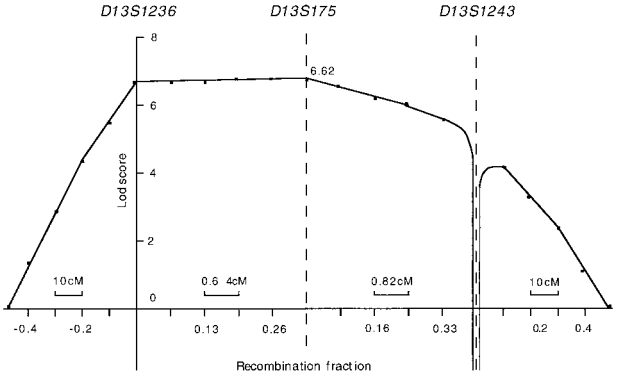

Figure 2 Multipoint linkage analysis between the *CZP* locus and chromosome 13 microsatellite markers, *D13S1236*-(3.2 cM)-*D13S175*-(4.1 cM)-*D13S1243*.

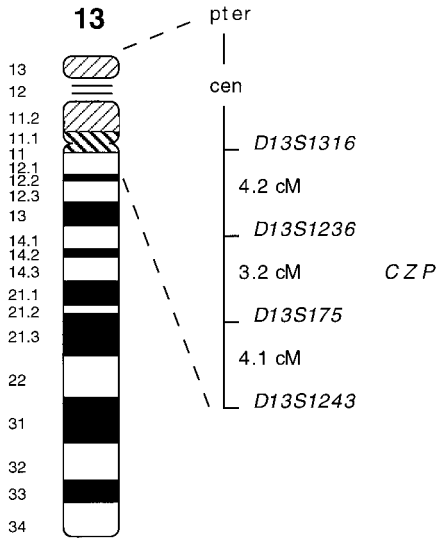

Figure 3 Idiogram of chromosome 13, showing the genetic distances (in cM) between both three microsatellite markers and the calculated position of the *CZP* gene, in the centromeric region.

maining markers in the 11.5-cM interval between *D13S1243* and *D13S1316* were observed. Chromosome 13 is one of five human chromosomes that are regarded as acrocentric—that is, lacking in a well-defined short arm—and *D13S1316* is the most proximal marker available from the Généthon map (Dib et al. 1996). Thus, the combined *Z*-value and haplotype data indicated that the cataract locus probably lies in the genetic interval between *D13S1243* and the centromeric end of chromosome 13—that is, *D13S1243*-(0.1 cM)-*D13S292*-(2.6 cM)-*D13S1275*-(1.4 cM)-*D13S175*-(3.2 cM)-*D13S1236*-(4.2 cM)-*D13S1316*-cen-13pter (fig. 3).

Discussion

Using molecular-genetic linkage analysis, we have localized a gene responsible for autosomal dominant zonular pulverulent cataract (*CZP*) to the centromeric region of chromosome 13. CZP is characterized by dustlike opacities showing considerable intrafamilial variation, and it shares certain clinical features with the zonular pulverulent cataract linked to 1q (Renwick and Lawler 1963), which first was described by Nettleship (1909). The linkage of *CZP* to 13cen has identified a new locus for zonular pulverulent cataract and brings the current number of mapped loci for autosomal dominant cataract in humans to at least nine, with no obvious candidate genes at four of these loci.

The crystallin genes encode >90% of the cytosolic protein in the lens and, therefore, provide likely candidate genes for inherited cataract. Indeed, the first cataract mutation to be proposed in humans involves novel activation of the γE-crystallin pseudogene, to give the pulverulent opacities that are confined to the central nucleus of the lens in the Coppock-like cataract (Brackenhoff et al. 1994). In addition, the βA3/A1 crystallin gene is a strong candidate for zonular sutural cataract on 17q (Padma et al. 1996), and a cerulean cataract maps close to the β-crystallin gene cluster on 22q (Kramer et al. 1996). Furthermore, dominant mutations in the βB2-, γE-, and ζ-crystallin genes have been associated with cataract in the Philly mouse (Chambers and Russell 1991), the eye lens–obsolescence mouse (Cartier et al. 1992), and the 13/N guinea pig (Rodriguez et al. 1992), respectively. However, the discovery that mutations in the gene for lens major intrinsic protein cause cataract in the mouse (Shiels and Bassnett 1996) supports the notion that lens genes other than those for crystallins also may be involved in human inherited cataract.

The *CZP* locus identified here was discovered during a candidate-gene search of chromosome 13, to which the gene for connexin46 (*CX46*) had been assigned previously (Schwartz et al. 1992). *CX46* is abundantly expressed in lens-fiber cells and at low levels in heart and kidney (Paul et al. 1991). Recently, the cytogenetic localization of *CX46* has been refined to band region q11-q12, close to the centromere of chromosome 13 (Mignon et al. 1996). Thus, *CX46* represents a strong candidate gene for *CZP* and indeed may be syntenic (Mouse Genome Database 1996) with the rupture of the lens-cataract gene on mouse chromosome 14 (Matsushima et al. 1996). It is also noteworthy that the lens *CX50* gene has been assigned to chromosome 1 (Church et al. 1995) and, therefore, represents an attractive candidate gene for the zonular pulverulent cataract that is linked to the Duffy blood group on 1q (Renwick and Lawler 1963). Moreover, disease-causing mutations in the human *CX32* and *CX43* genes have been shown to underlie X-linked Charcot-Marie-Tooth neuropathy (Bergoffen et al. 1993) and visceroatrial heterotaxia syndrome (Britz-Cunningham et al. 1995), respectively. Currently, we are sequencing the *CX46* gene for mutations in the *CZP* family and are identifying additional informative markers in the region, to enable fine mapping and, ultimately, positional cloning of the authentic cataract gene if *CX46* is excluded.

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