Linkage of polymorphic congenital cataract to the γ-crystallin gene locus on human chromosome 2q33–35

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Received December 6, 1995; Revised and Accepted February 21, 1996

Cataract is one of the major causes of blindness in humans. We describe here an autosomal dominant polymorphic congenital cataract (PCC) which is characterised by wide variations in phenotype of non-nuclear lens opacities, even among affected members of the same family. PCC families included a large, unique pedigree (254 members, 103 affected individuals), and genetic linkage was conducted using a variety of polymorphic markers. Evidence for linkage was found for chromosome 2q33–35 with PCC mapping near D2S72 and TNP1. A tri-nucleotide microsatellite marker for γ-crystallin B gene (CRYG1) was found to co-segregate with PCC and yielded a maximum lod score of 10.62 at (θ = 0). A multipoint analysis demonstrated that the most probable location of the PCC gene was within an 8 cM genetic interval containing the γ-crystallin gene cluster. These data provide strong evidence of the existence of an autosomal dominant mutation for PCC in or near the γ-crystallin gene cluster. This defect is characterised by complete penetrance but variable expression of the cataract phenotype. Our study also suggests that non-nuclear human cataracts might be caused by some abnormality in γ-crystallin genes.

INTRODUCTION

Cataract is a major cause of blindness in human populations, and hereditary cataract is one of the leading causes of blindness in children (1,2). Some cataracts are associated with other abnormalities of systemic syndromes, and the increased risk for cataract is associated with various environmental factors (3–7). Many forms of hereditary congenital human cataracts have been described as isolated abnormalities, and over 60 cataract phenotype mutants of the mouse have been identified (7–13). Cataract types are classified principally into cortical, nuclear and anterior subscapular opacities. Different morphological variants, without an exactly defined phenotype, have also been identified and genetic heterogeneity even within one type has been suggested. Since 1963, suggestive or tight linkage, has been demonstrated for several forms of familial cataract (Coppock zonular pulverulent, posterior polar, cerulean, zonular central stellate and embryonal nuclear pulverulent) to different chromosomal loci (14–19). The opacities of the lens leading to broad variability in cataracts may be caused by different mechanisms. In many cases the cataractous lenses are characterised by loss of soluble crystallins (20,21). Therefore, crystallin genes or genes encoding enzymes modifying the crystallin proteins are candidates for analyzing inherited cataract mutations. Crystallin genes and pseudogenes have been mapped to 21q22.3, 11q22.3–23.1 (α-crystallins); 17q11.2-q12, 22q11.2-q13.1 (β-crystallins); 2q and 3 (γ-crystallins) regions (20,22–25).

Recently, we have identified families with non-nuclear polymorphic congenital cataract (PCC) (12,26). It is noteworthy that there may be high variability of location, colour, and number of opacities in affected members of the same pedigree, despite the strong evidence of a single genetic etiology. We evaluated protein and DNA markers randomly placed across the genome, as well as markers located near the above candidate genes, for linkage to the causative genetic defect for PCC.

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RESULTS

Polymorphic congenital cataract families

Polymorphic congenital cataract was identified in Turkmen populations by an epidemiologic survey of Middle Asian populations in the Former Soviet Union (12). Aboriginal populations of South Turkmen, which mainly consist of the ‘Nokhurli’ tribe, are characterised by complex ethnic origin, high endogamy and a high coefficient of inbreeding (>3%). A high incidence of two inherited diseases, polymorphic congenital cataract (PCC) and obesity, have been found in this population. The frequency of the PCC gene in this population is 0.26%, and that of the autosomal recessive obesity gene is 2.47%. These diseases were not associated with each other. In a survey of four different districts, 34 affected sibships with PCC were identified and investigated by ophthalmological examination. One unique large PCC pedigree (254 members, 103 affected individuals) and two smaller PCC pedigrees with no obvious relationship to other have been reconstructed. Blood and DNA samples were collected from as many members of the large and one of the smaller pedigrees as possible (Fig. 1). Apparently, PCC was transmitted through seven generations as an autosomal-dominant trait with virtually complete penetrance. The age of patients varied from a few months to 70 years. In all cases the cataract was congenital and the patients had no other inherited abnormalities. This cataract is characterised by partial opacity of the lens, which has a variable location on the periphery between the fetal nucleus of lens and the equator. The form of the opacity is irregular and looks similar to a bunch of grapes or a lump of cotton. The opacities can be located simultaneously in the different lens layers. The colour of opacities varies from shining crystal-like to snow-white. This cataract is different from nuclear cataracts and more similar to zonular or lamellar types of cataracts.

Linkage analysis

Since PCC displays some phenotypic features, such as zonular location of opacities for some individuals, which are similar to the zonular cataract (1q21-q23) (ref. 9,17), we initially tested the VNTR MUC1 marker at this locus and demonstrated negative lod scores for this marker. Subsequently, we embarked on a systematic genome search. Twenty four anonymous microsatellite and VNTR DNA markers and nine polymorphic protein markers demonstrating negative lod scores were excluded before the (TC)n microsatellite marker in the glucagon gene (Rogaev E.I. et al., unpublished) located on chromosome 2q (GCG) yielded positive lod scores ($Z_{\text{max}} = 1.21$, $\theta = 0.2$). Since the γ-crystallin gene cluster ($CRYG1$) is also located in this region of 2q, we studied additional markers near $CRYG1$ to confirm linkage and narrow the candidate region.

The genetic and physical map for these markers and γ-crystallin genes was built using several published reports and our own data which included a YAC contig [unpublished and (27–28)]. We found significant positive lod scores for several markers located at 2q33-35 (Table 1). One of these markers, $CRYG1$, showed no evidence of recombination ($Z = 10.62$, $\theta = 0.00$). These data were robust over a range of assumptions concerning disease allele frequency and marker allele frequencies from published data or determined from our study of Turkmen populations, and suggest that the location of the PCC gene is on chromosome 2q near $CRYG1$. Inspection of the
haplotypes demonstrated the segregation of the alleles with identical sizes for closely linked markers (population frequency of identical segregating alleles was 0.46 for CRYG1, 0.1 for D2S117 and 0.27 for D2S72 as estimated for populations of ‘nokhuri’ in pedigrees I and II. This is suggestive of a common ancestor for both pedigrees.

**DISCUSSION**

Many forms of congenital cataracts, including X-linked, autosomal-dominant and autosomal recessive forms, have been described. Progressive autosomal-dominant cataracts have also been reported, i.e., cerulean cataract with slow progression after birth (19) and Marner cataract with suggested ‘anticipation’ in successive generations (14). Strong evidence of linkage has been obtained for Coppock-like cataract (1q21–25), Coppock-like (2q33–36), Marner posterior polar cataract (16q22.1) and cerulean cataract (17q24) (refs 14,17–19). Recently two mutations in the galactokinase gene mapped to 17q24 chromosome have also been found in subjects with homozygous galactokinase deficiency and cataract (29). We describe a polymorphic congenital cataract which has phenotypic features distinct from any of the other cataracts reported to date. This cataract is characterised by a non-progressive and non-nuclear phenotype as well as zonular location of opacity for some individuals. In this respect it may be similar to the zonular or lamellar cataracts. Thus, as a first step we have excluded the Coppock cataract locus for PCC by analysis of the VNTR in the mucin gene (1q21–25). In subsequent analyses we demonstrated that the PCC gene is located on chromosome 2q32–35 within the cluster of the γ-crystallin genes. Linkage of nuclear Coppock-like cataract to an RFLP marker from a common ancestor for both pedigrees.

To date, no mutations in the coding regions of the γ-crystallin genes have been found for any form of human cataract. The single nucleotide frame shift deletion in exon 3 of the γ-E-crystallin gene has been found to be associated with eye lens obsolescence in the mouse mutant (murine Elo) (30). It has also been suggested that the sequence changes around and within the TATA box (TATAA→TATATA) of the γ-E-crystallin gene promoter might enhance the activity of the γ-E-crystallin pseudogene in the human nuclear Coppock-like cataract (31). On the other hand, reduced γ-crystallin transcripts in mutant lenses have been found for the murine Cat-2 dominant mutations linked to the γ-crystallin gene locus (32). It is likely that interaction between the crystallins is important for development and growth of the lens. It is conceivable that changes in the structure or expression of these proteins may cause a structural abnormality of the lens. Alternatively, some crystallins are thought to be derived from enzymes during the evolution of vertebrates [see review (20)]. Therefore, their enzymatic activity may still be implicated in the development of the lens and lens opacity. However, because the mouse genomic segment containing the γ-crystallin gene cluster on chromosome 1, which corresponds to the human 2q33-q36 locus, is a highly conserved region (33), other yet unknown members of the crystallin gene family, or other genes involved in the regulation of lens development have to be considered as potential candidate genes for PCC. In order to isolate the gene defect underlying PCC we have started to screen for mutations in the transcriptionally active γA, γB, γC and γD genes. Although it has been shown that the γE and γF crystallin sequences have no sufficient expression in human lens and belong to pseudogenes, the possible reactivation of these genes (31) in subjects with PCC should also be investigated.

**Table 1. Pairwise Lod scores of PCC with chromosome 2 markers**

| Locus | Recombination fraction (θ) | 0.00 | 0.01 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | Zmax | 0.0 |
|-------|---------------------------|------|------|------|------|------|------|------|------|-----|
| ApoB  | –∞                        | –33.83 | –17.03 | –10.00 | –3.76 | –1.09 | –0.03 | –0.03 | 0.40 |     |
| GCG   | –∞                        | –2.03  | 0.10  | 0.85  | 1.21 | 0.96 | 0.43 | 0.96 | 0.30 |     |
| D2S152| –∞                        | –2.41  | 2.58  | 4.07  | 4.29 | 3.20 | 1.53 | 4.29 | 0.20 |     |
| D2S117| –∞                        | 8.38   | 9.77  | 9.50  | 7.66 | 5.07 | 2.19 | 9.79 | 0.06 |     |
| D2S72 | –∞                        | 8.12   | 9.33  | 9.05  | 7.38 | 5.12 | 2.56 | 9.34 | 0.06 |     |
| CRYG1 | 10.62                     | 10.45  | 9.72  | 8.75  | 6.61 | 4.22 | 1.68 | 10.62 | 0.00 |     |
| TNP1  | –∞                        | 0.36   | 2.24  | 2.66  | 2.42 | 1.74 | 0.82 | 2.66 | 0.10 |     |
| D2S128| –∞                        | 3.59   | 5.26  | 5.59  | 5.02 | 3.67 | 1.91 | 5.59 | 0.10 |     |

Cumulative lod scores for PCC pedigrees 1 and 2 are displayed. The order and genetic map of these markers is ApoB-(170 cM)-GCG-(22 cM)-D2S152-(7 cM)-D2S117-(3 cM)-D2S72-(7 cM)-CRYG-(8 cM)-TNP1-(5 cM)-D2S128.
MATERIALS AND METHODS

Evaluation of patients and DNA collection

An epidemiological study of hereditary diseases was undertaken by expeditions of clinical geneticists to isolated populations in Middle Asia. A high incidence of congenital autosomal dominant cataract in one Turkmen population has been found (12). More than 100 patients in this isolated population were inspected by ophthalmologists. Pedigrees were constructed for most of these patients from interviews with relatives. Blood specimens were collected from more than 130 affected and normal individuals with a family history of cataract and, additionally, from 60 normal random individuals from the same population.

DNA was extracted from buffy coat of fresh blood samples collected with EDTA. Cells were lysed and proteins were digested in 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% SDS, 0.1 mg/ml proteinase K at 48°C overnight. Following extractions with phenol and chloroform, DNA was precipitated with ethanol and dissolved in sterile H2O.

Genotyping

Parentage of individuals from large pedigrees was verified by genotyping analysis of a large set of minisatellite markers (34) and locus-specific VNTR markers which were described previously. False maternity/paternity was found for one nuclear PCC family (three affected and two normal subjects) in the PCC I pedigree. These individuals were excluded from subsequent analysis. The genotype for each microsatellite or RFLP marker was determined with DMSO (35).

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Two-point and multipoint linkage analysis were performed using software (36–37).

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ACKNOWLEDGEMENTS

The authors would like to thank Drs Lap-Chee Tsui and M. Cartier for discussion, Drs W. Lukiw and A. Buzina for technical assistance. This work was supported partially by the grants from the Medical Research Council of Canada (PSTIH), Russian Human Genome Project (EKG and EIR) and Howard Hughes Medical Institute International Research Scholar Award (EIR).
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