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Genetics

A locus for a human hereditary cataract is closely linked to the γ-crystallin gene family

N. H. LUBSEN*, J. H. RENWICK†, L.-C. TSUI‡, M. L. BREITMAN§, AND J. G. G. SCHOENMAKERS*¶

*Department of Molecular Biology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands; †Preventive Teratology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom; ‡Department of Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada, M5G 1XB; and §Division of Cancer Research, Harold Tanenbaum Department of Research, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, Canada M5G 1X5

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ABSTRACT **Within the human γ-crystallin gene cluster polymorphic *Taq* I sites are present. These give rise to three sets of allelic fragments from the γ-crystallin genes. Together these restriction fragment length polymorphisms define eight possible haplotypes, three of which (*Q*, *R*, and *S*) were found in the Dutch and English population. A fourth haplotype (*P*) was detected within a family in which a hereditary Coppock-like cataract of the embryonic lens nucleus occurs in heterozygotes. Haplotype *P* was found only in family members who suffered from cataract, and all family members who suffered from cataract had haplotype *P*. The absolute correlation between the presence of haplotype *P* and cataract within this family shows that the γ-crystallin gene cluster and the locus for the Coppock-like cataract are closely linked [logarithm of odds (lod) score of 7.58 at its maximum at $\vartheta = 0$]. This linkage provides genetic evidence that the primary cause of a cataract in humans could possibly be a lesion in a crystallin gene.**

Opacity of the lens, cataract, is the most common lens abnormality in humans. Often it is but one of the symptoms of a complex disease syndrome, and its primary cause must then be sought in the environment of the lens and not in the lens itself (1). When its occurrence is not associated with any other physical anomalies, as in some forms of hereditary cataract (for review, see ref. 2), it could be due to an inherent lens defect such as a dysfunction of the genes coding for lens-specific proteins. For example, the abundant water-soluble structural lens proteins, the α-, β-, and γ-crystallins, are thought to be involved in establishing the transparency of the lens and a mutation in one of these proteins might be expected to cause cataract. Unfortunately, the changes in physical properties of the lens proteins that occur during cataractogenesis are too complex to allow one to discern mutant proteins (3), and studies of cataractous lenses have therefore failed to provide direct evidence for the involvement of aberrant crystallins (or other lens-specific proteins). A direct contribution of crystallins to cataractogenesis has only been shown for coldcataract, which is due to a phase transition in the protein–water mixture in the lens (4).

A difference in the population of crystallin transcripts has been noted in two murine hereditary cataracts: the lens of the Philly mouse lacks a β-crystallin mRNA (5), while a premature cessation of γ-crystallin mRNA synthesis was seen in the lens of the Cat^{Fr} mouse (6). The interpretation of these results in terms of the molecular lesion in these cataracts is not straightforward, however, since both cataracts are thought to be osmotic ones (2, 6). The results of the studies of these (and other) murine hereditary cataracts once more illustrate that the primary lesion in hereditary cataract can only be identified genetically. With the cloning of most of the crystallin genes (for review, see refs. 7 and 8), this approach to the study of hereditary cataract has become possible: restriction enzyme fragment length polymorphisms (RFLPs) within or around a particular crystallin gene can be used to study linkage between a crystallin gene and a locus for a hereditary cataract. We report here direct genetic evidence for the involvement of mutant crystallin genes in hereditary cataract and show that the locus for a human autosomal Coppock-like cataract is closely linked to the human γ-crystallin gene cluster.

MATERIALS AND METHODS

Isolation, Restriction, and Hybridization of Genomic DNA. DNA was isolated from whole blood essentially as described by Bell *et al.* (9). Aliquots (10 μg) were digested with *Taq* I under the conditions recommended by the suppliers of the enzyme (Boehringer Mannheim), and the fragments were separated by electrophoresis on a 0.7% agarose gel and Southern blotted as described (10). The blots were hybridized with the ^{32}P-labeled 3.35-kilobase (kb) *Hin*dIII fragment containing the Gγ1-crystallin gene (11). Hybridization conditions were as described (10); after hybridization, blots were washed in 0.2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% $NaDodSO_4$ at 65°C for 1 hr.

RESULTS AND DISCUSSION

The human γ-crystallin genes are located in the 2q33–2q36 region of chromosome 2 (12–14) and are a family of seven closely homologous linked genes (10, 11). Six of these have been cloned and characterized: four have open reading frames and are likely to be active, while two are pseudogenes (refs. 10 and 11; Fig. 1). When a probe derived from a rat γ-crystallin cDNA (16) clone is hybridized to a *Taq* I restriction digest of human DNA, a complex pattern of bands with some RFLPs is obtained. A subset of these bands, including the RFLPs, hybridizes under stringent conditions with a probe derived from the Gγ1 gene (see Fig. 1). The use of this probe thus facilitates the analysis of the segregation pattern of these RFLPs (see, for example, Fig. 2). In the family examined here and in other families, three sets of allelic fragments are found: the 4.0- and 3.8-kb fragments (A1 and A2), the 2.5- and 1.5-kb fragments (B1 and B2), and the 2.3- and 1.7-kb fragments (C1 and C2). Of the eight possible haplotypes that could be formed by combination of these fragments, four are found within the pedigree shown in Fig. 3: A1.B2.C1 (haplotype *P*), A1.B2.C2 (haplotype *Q*), A2.B1.-C1 (haplotype *R*), and A2.B2.C1 (haplotypes *S*). Within this pedigree, an autosomal Coppock-like cataract occurs in heterozygotes (17, 18). All afflicted individuals carry haplo-

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Abbreviations: RFLP, restriction fragment length polymorphism; kb, kilobase(s).

¶To whom reprint requests should be addressed.

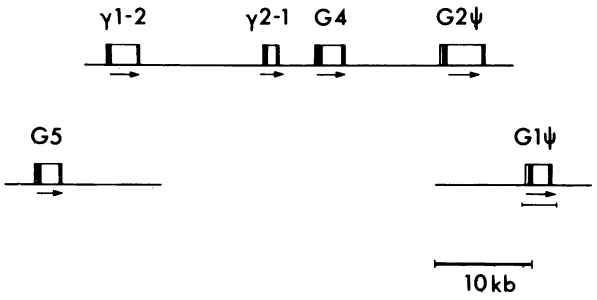

FIG. 1. Organization of the human γ-crystallin gene family. The organization of the six γ-crystallin genes as depicted here is based on the data of den Dunnen *et al.* (10) and Meakin *et al.* (11). The nomenclature of these authors has been maintained. The G5 and G1ψ genes have not yet been directly linked to the four-gene cluster. Their suggested location to the right and the left, respectively, of this cluster is based on the location of the orthologous genes in the rat (15). Heavy bars indicate exons; open bars denote introns. The direction of transcription is shown by arrows, and the region contained in the probe used is indicated by the bar.

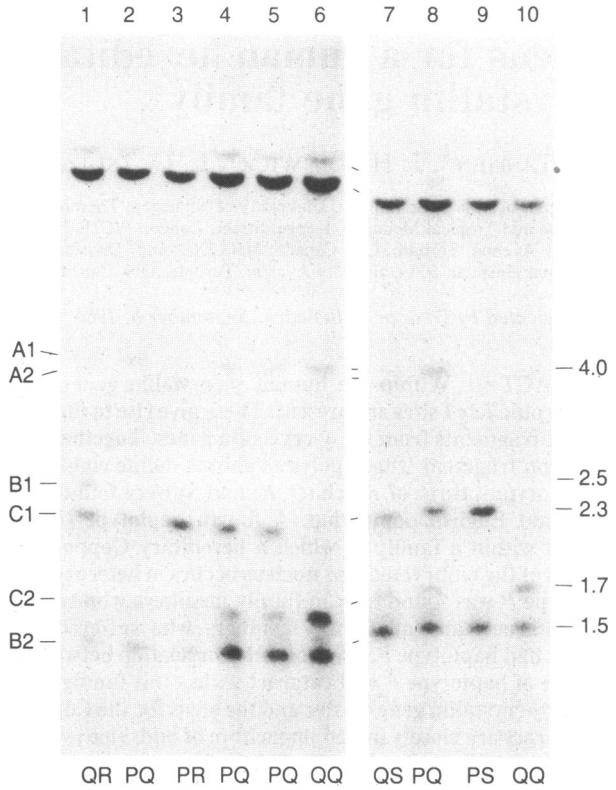

FIG. 2. Hybridization patterns of genomic DNA isolated from various family members. The RFLPs mentioned in the text are indicated on the left, while the size in kb of selected fragments is shown on the right. The individuals whose hybridization patterns are shown are identified in the pedigree (Fig. 3) by arabic numbers corresponding to the lane numbers. The haplotypes are indicated at the bottom of each lane.

type *P*, while none of the normal descendants does so. Thus, within this family, there is an absolute correlation between the presence of haplotype *P* and the presence of cataract. Although we have not detected haplotype *P* in any of the 26 normal Dutch and British individuals screened thus far, we have assumed for a (conservative) calculation of the logarithm of odds (lod) scores that haplotype *P* has a frequency of 0.1. The lod score is then 7.58 at its maximum at $\vartheta = 0$ (Table 1). A lower actual frequency of haplotype *P* increases the lod score (see Table 1). Thus, the locus for cataract in this family and the human γ-crystallin gene cluster are established to be closely linked, and the presence of haplotype *P* in a member of this family indicates a high probability that the allele for the Coppock-like cataract is also present. This could be used as a basis for prenatal diagnosis of this disease in this family.

The cloned copy of the γ1–2 gene contains an A2 sized *Taq* I fragment, while the polymorphic *Taq* I sites that engender the B1/B2 and C1/C2 RFLPs are probably located within and around the G2ψ gene, since the cloned copy of the G2ψ gene contains both a B1 and a C1 sized fragment (data not shown). Irrespective of their location, the polymorphic *Taq* I sites that define haplotype *P* are unlikely to disrupt a functional γ-crystallin gene, since haplotype *P* represents merely a particular combination of "normal" RFLPs. We could not detect any other RFLPs in this family by using several other restriction enzymes or rat γ-crystallin cDNA probes. Thus, we have detected no changes in the γ-crystallin gene organization that could be causally related to the occurrence of cataract. However, single base changes, small insertions, or

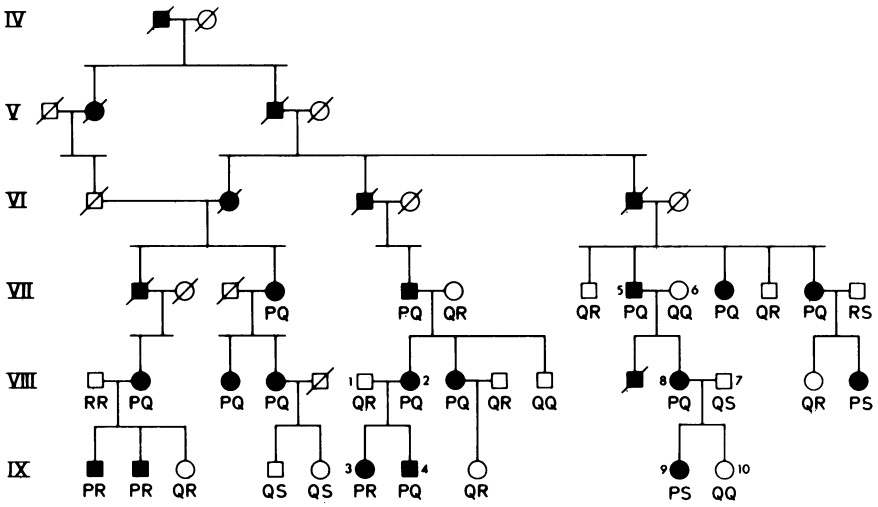

FIG. 3. Pedigree of the family studied. This family was first described in refs. 17 and 18 and the generations are numbered here in accordance with figure 50 of ref. 17. The pedigree has been simplified to show only the relationship between the members whose haplotypes (shown in capital letters) were determined. Solid symbols indicate individuals suffering from cataract; a slash through a symbol indicates that the haplotype of that individual was not determined. Arabic numbers identify the individuals whose genomic hybridization patterns are shown in the corresponding lanes of Fig. 2.

Table 1. Lod scores for various values of ϑ at two different frequencies of haplotype *P*

Frequency of *P*	ϑ				
	0.00	0.05	0.10	0.15	0.20
0.05	7.96	7.03	6.61	5.87	5.10
0.10	7.58	6.93	6.24	5.51	4.75

The lod scores were calculated assuming a frequency of haplotype *P* of either 0.05 or 0.1 for various values of ϑ as indicated by using the LINKAGE program (version 3.5 of Lalouel and Lathrop as modified by Ott).

deletions would have gone undetected in our experiments. It is noteworthy that haplotype *P* was found only in individuals afflicted with cataract. A recombination between γ-crystallin loci with haplotypes *Q* and *S* could have generated a locus with haplotype *P* and caused a dysfunction of a γ-crystallin gene. Similar events have been the cause of aberrant β-globin genes (19).

Although our study provides no direct evidence that the molecular basis for this cataract is indeed a lesion in the γ-crystallin genes, the phenotype of the cataract does support the suggestion that it is. It affects primarily the embryonic nucleus of the lens, is present at birth, and is virtually nonprogressive (17, 18). The development of this cataract thus correlates well with the time and place of the synthesis of γ-crystallins as determined in the calf and rat (20–22). The phenotype of this cataract is distinctly different from that of the murine eye lens obsolescence (*Elo*) mutation, which was suggested, on the basis of rather indirect genetic evidence, to be within a murine γ-crystallin gene (23). The disparity between the phenotypes suggests that further investigation of the *Elo* locus is warranted to show that this locus is indeed linked to the γ-crystallin locus.

The suggestion that the primary lesion in the Coppock-like cataract is in a γ-crystallin gene raises the intriguing question of how the product of a single mutant γ-crystallin gene can disturb the architecture of the lens even though normal γ-crystallins are present. Our working hypothesis is that a mutation in one of the amino acid residues involved in maintaining the highly symmetrical tertiary structure of the γ-crystallin protein (24) would distort the folding pattern of this protein and thereby also the regular short-range ordered packing, which is responsible for the transparency of the lens (25). An identification of the molecular nature of the lesion present in this family will show whether this is indeed the case and should thereby clarify the role of the γ-crystallins in the development and in the maintenance of the transparency of the lens and allow some insight into the changes in lens proteins that lead to cataract.

A number of linkage studies on hereditary cataract have been performed in humans (see, for example, refs. 26 and 27). The main conclusion from such studies is that hereditary cataract is a genotypically as well as phenotypically heterogeneous disease and only a few cases have yielded any information about the chromosomal location of a particular cataract: a total nuclear cataract has been linked to the Duffy blood group locus on chromosome 1 (28), another locus for congenital cataract might be linked to the locus for haptoglobin and located on chromosome 16 (29), while a reciprocal 2:14 translocation with breakpoints at 2p25 and 14q24 cosegregates with a locus for an anterior polar cataract (30). At this time, only a few crystallin genes have been mapped: the αA-crystallin gene is located on chromosome 21 (31), the βA1/3-crystallin gene is on chromosome 17 (L.-C.T. and M.L.B., unpublished data), and the γ-crystallin genes are located on chromosome 2 (12–14). Thus, at present, there is no genetic evidence for the possible involvement of mutant crystallin genes in hereditary cataract other than the Coppock-like cataract of the embryonic nucleus of the lens present in the family studied here. Further mapping studies of both the crystallin genes and the loci for hereditary cataract are needed to identify other cases in which a crystallin gene could be linked to a locus for congenital cataract.

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