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A Locus for Autosomal Recessive Congenital Microphthalmia Maps to Chromosome 14q32

David A. R. Bessant,[1] Shagufta Khaliq,[2] Abdul Hameed,[2] Khalid Anwar,[2] S. Qasim Mehdi,[2] Annette M. Payne,[1] and Shomi S. Bhattacharya[1]

[1]Department of Molecular Genetics, Institute of Ophthalmology, University College, London; and [2]Dr. A. Q. Khan Research Laboratories, Biomedical and Genetic Engineering Division, Islamabad, Pakistan

Summary

Congenital microphthalmia (CMIC) (OMIM 309700) may occur in isolation or in association with a variety of systemic malformations. Isolated CMIC may be inherited as an autosomal dominant, an autosomal recessive, or an X-linked trait. On the basis of a whole-genome linkage analysis, we have mapped the first locus for isolated CMIC, in a five-generation consanguineous family with autosomal recessive inheritance, to chromosome 14q32. All affected individuals in this family have bilateral CMIC. Linkage analysis gave a maximum two-point LOD score of 3.55 for the marker D14S65. Surrounding this marker is a region of homozygosity of 7.3 cM, between the markers D14S987 and D14S267, within which the disease gene is predicted to lie. The genes for several eye-specific transcription factors are located on human chromosome 14q and in the syntenic region of mouse chromosome 12. However, both *CHX10* (14q24.3), mutations of which give rise to CMIC in mouse models, and *OTX2* (14q21–22) can be excluded as candidates for autosomal recessive congenital microphthalmia (arCMIC), since they map outside the critical disease region defined by recombination events. This suggests that arCMIC is caused by defects in a novel developmental gene that may be important or even essential in eye development.

Introduction

Congenital microphthalmia (CMIC) is a common ocular malformation with a reported prevalence at birth of 1.5 per 10,000 (Kallen et al. 1996). Both the extreme form of CMIC, anophthalmia and more minor degrees of CMIC have been observed in the same family, indicating that they should be considered as related conditions (Warburg 1993).

Up to 80% of cases of CMIC occur as part of syndromes that include systemic malformations, especially cardiac defects, facial clefts, microcephaly, and hydrocephaly (Kallen et al. 1996). These include >100 genetic traits (autosomal dominant, autosomal recessive, and sex linked) and deletions or translocations of virtually all the chromosomes (Warburg 1993; OMIM).

Isolated CMIC is frequently associated with other ocular abnormalities, including coloboma and cataracts (Warburg 1993). It may be inherited either as an autosomal dominant (Pearce 1986; Vingolo et al. 1994), an autosomal recessive (Kohn et al. 1988; Zlotogora et al. 1994), or an X-linked trait (Stephens 1947). As yet, no locus for isolated CMIC has been mapped and no candidate gene has been shown to be responsible for this condition in man.

Sclerocornea (OMIM 269400) is a congenital malformation of the cornea, such that the boundary between the cornea and the sclera is obscured. Usually the involvement is limited to the peripheral part of the cornea, but it may extend to the entire cornea. The mild form is inherited as a dominant and the severe form as a recessive trait (Elliott 1985).

At least 14 transcription factors that are essential for normal mammalian eye development have been described, along with 32 other tissue-restricted transcription factors that are expressed at some stage in the developing or mature eye (Freund et al. 1996; Graw 1996). Some of the human genes are homologues of *Drosophila* eye-development genes (e.g., *eyeless:* PAX6; *sine oculis:* Six3; and *eyes absent:* Eya), or of genes that have been shown to give rise to microphthalmia in mouse models (*ocular retardation: CHX10;* and *microphthalmia:* MITF). Only two of these, *PAX6* and *MITF,* have been associated with ocular disease in man (Tassabehji et al. 1994; Hanson and Van Heyningen 1995).

None of these important eye-development genes has, however, been associated with isolated human CMIC.

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Address for correspondence and reprints: Dr. Shomi S. Bhattacharya, Department of Molecular Genetics, Institute of Ophthalmology, London, EC1V 9EL, United Kingdom. E-mail: sbhatach@hgmp.mrc.ac.uk

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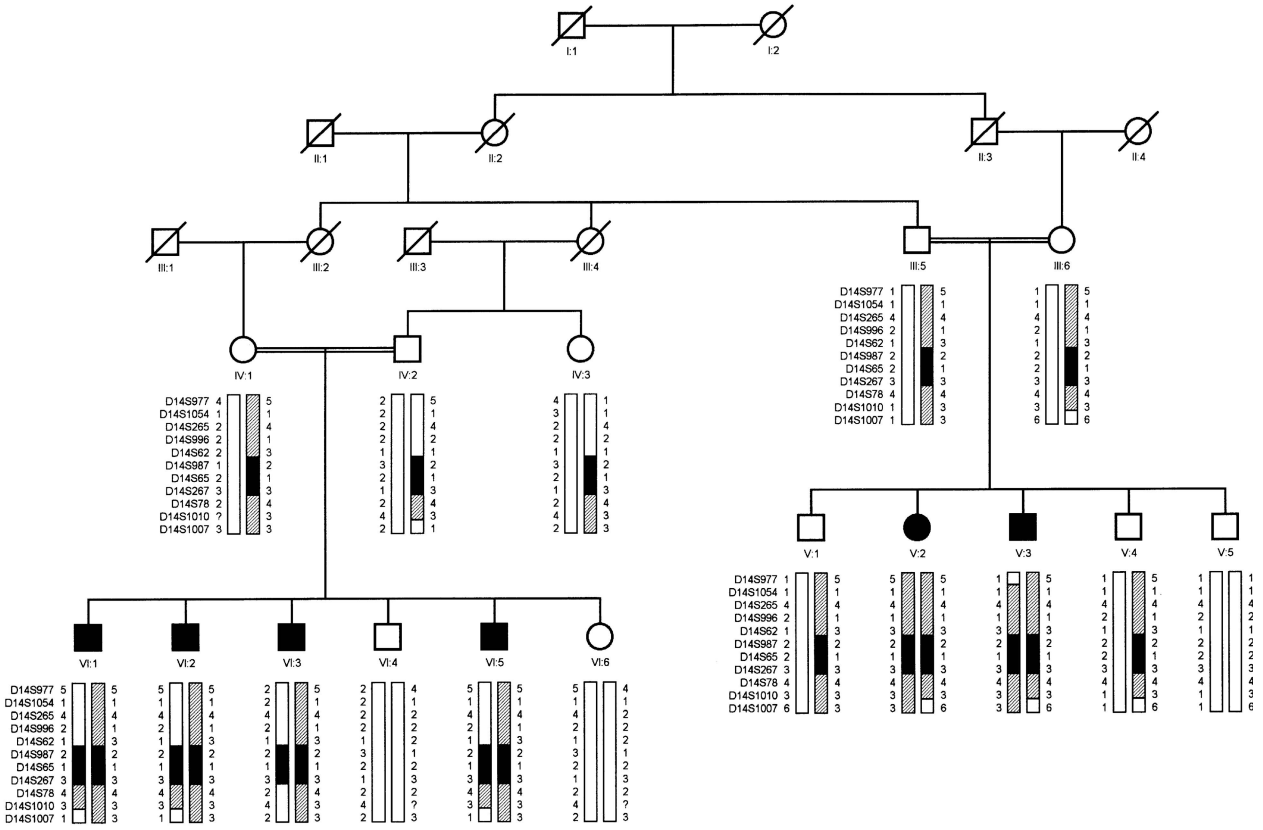

Figure 1 Pedigree of microphthalmia family, showing haplotypes for the polymorphic markers in the telomeric region of chromosome 14q. Marker order was determined from the Généthon sex-averaged genetic map (Gyapay et al. 1994). Recombination events in affected individuals III:4 (inferred from her children) and VI:3 define D14S62 and D14S78, respectively, as the flanking markers for the CMIC locus. The 7.3-cM region of homozygosity is indicated by black shading of the bars situated between the marker alleles. (Affected individuals are denoted by solid symbols.)

We set out, therefore, to identify the first locus for isolated CMIC by homozygosity mapping in a large inbred pedigree.

Patients and Methods

A five-generation consanguineous family in which six living members were affected by isolated CMIC was ascertained in Pakistan (fig. 1). Ophthalmologic examination of the parents of the affected individuals revealed no evidence of CMIC, indicating an autosomal recessive mode of inheritance. Clinical examination of the affected individuals revealed bilateral microphthalmia, sclerocornea, and nystagmus in all cases. No associated systemic abnormality or evidence of mental retardation could be found. Informed consent was obtained by local clinicians.

To identify the gene responsible for disease in this family, we performed a total genome linkage analysis. Three sets of markers were analyzed: those corresponding to the known loci of eye-specific transcription factor genes (including *PAX6*, *CHX10*, *MITF*, *Six3*, *Eya1*, *Eya2*, and *Eya3*); a further 40 reported to define loci closest to the greatest number of expressed sequence tags (ESTs) (Inglehearn 1997); and finally a set of anonymous markers selected from the 1994 Généthon sex-averaged genetic map at 10–20-cM intervals throughout the genome (Gyapay et al. 1994). Primers were obtained from the MapPairs set (Research Genetics) or were synthesized commercially according to data from the Genome

Table 1

Two-Point LOD Scores between CMIC and Markers on Chromosome 14q32

	LOD Score at Θ =				
Marker	0	.1	.2	.3	.4
D14S62	−.95	1.00	.77	.45	.14
D14S987	2.54	2.08	1.58	1.03	.46
D14S65	3.55	2.79	1.97	1.11	.34
D14S267	1.67	1.36	1.03	.69	.34
D14S78	−∞	1.12	.98	.68	.31

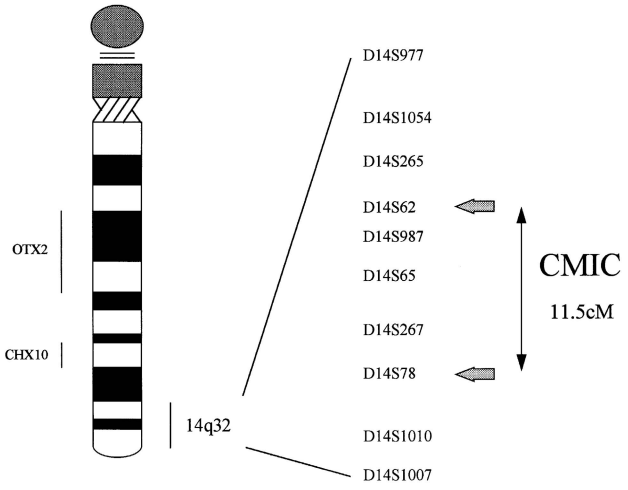

Figure 2 Schematic diagram of chromosome 14, indicating the position of the CMIC locus in 14q32 in relation to the FISH mapped loci for *OTX2* (14q21-q22) and *CHX10* (14q24.3). The markers shown are those that form the haplotypes in fig. 1, and their spacing is an indication of the genetic distance between them. The horizontal arrows indicate the boundaries of the critical genetic interval for CMIC as defined by recombination events.

Data Base (Johns Hopkins University [http://gdbwww.gdb.org/]).

Nonradioactive PCR was performed in a 10-ml reaction with 300 ng of genomic DNA, 10 pmol of each primer, 200 mM dNTPs, 1.5 mM $MgCl_2$, and 1 unit of *Taq* DNA polymerase (Promega) in a buffer provided by the manufacturer. A three-stage PCR consisting of 35 cycles of 94°C, 50°–62°C, and 72°C, each for 1 min, was used. The amplified products were then separated by electrophoresis on 6%–8% nondenaturing polyacrylamide gels (Protogel, National Diagnostics) and stained with ethidium bromide. Linkage analysis was performed with the LINKAGE version 5.1 software package (Lathrop and Lalouel 1984).

Results

Sixteen members of the family were typed for over 150 polymorphic markers. Significant exclusion was obtained for all the eye-specific transcription factor loci, for the *Inglehearn* marker set, and for all other markers except those located on chromosome 14q32. Haplotypes for polymorphic markers in this region are shown in figure 1. Two-point LOD scores between CMIC and markers in this region (D14S62, D14S987, D14S65, D14S267, and D14S78) are summarized in table 1. The maximum LOD score of 3.55 was obtained for the marker D14S65.

A centromeric recombination event involving the polymorphic marker D14S62 can be inferred in individual III:4 from the fact that both her children (IV:2 and IV:3) have a recombination at this point. A telomeric recombination event involving the marker D14S78 is observed in affected individual VI:3. These two crossovers define the disease locus region as an 11.50-cM interval between markers D14S62 and D14S78. This interval includes a region of homozygosity of at $\geqslant$7.3 cM between markers D14S987 and D14S267.

The centromeric recombination event observed in individuals IV:2 and IV:3 may involve a normal chromosome that carries identical alleles for the marker D14S987 to the chromosome carrying the CMIC mutation. This pattern is observed on the normal chromosome possessed by their uncle, individual III:5. While the exact location of this centromeric recombination event is, therefore, uncertain, it certainly involves D14S996 and D14S62.

Discussion

Homozygosity mapping in a large inbred pedigree has permitted us to identify the first locus for isolated CMIC at 14q32.

This region at the telomere of human chromosome 14q is syntenic with a region of mouse chromosome 12, which contains a number of transcription factors, some of which are important in eye development. The human homologues of these genes have been positioned by FISH mapping in chromosomal bands 14q21–q22 (*OTX2*) and 14q24.3 (*CHX10*), respectively (De Chen et al. 1990; Kastury et al. 1994). However, both *CHX10*, mutations of which give rise to microphthalmia in mouse models (Burmeister et al. 1996), and *OTX2* can be excluded as candidates for arCMIC, since they map outside the critical disease region defined by the observed recombination events (fig. 2).

The genes for two transcription factors, NF-E1 (YY-1) and delta-like precursor (DLK) map within the 11.5-cM critical region for arCMIC (Park and Atchison 1991; Laborda et al. 1993). NF-E1 is related to the *GLI-Krüppel* family of zinc-finger proteins, several of which (*GLI, GLI-2,* and *GLI-3*) are expressed in the developing mammalian eye (Freund et al. 1996). Both the NF-E1 and DLK proteins have been shown to be expressed in nonocular tissues and do not, therefore, appear to be good candidates for arCMIC, although the possibility of alternatively spliced transcripts being important in eye development cannot be excluded.

The human gene map at the National Council for Biotechnology Information lists 77 other ESTs situated within the arCMIC critical interval (http://www.ncbi.nlm.nih.gov/SCIENCE96/). At least 21 of these are derived from fetal brain or retinal cDNA libraries and might be considered as potential candidates for CMIC.

A second inherited eye disorder, autosomal dominant congenital cataract (OMIM 115650), has been reported in association with a translocation involving chromosome 14q32 (Moross et al. 1984; Miller et al. 1992), and it is possible that this may represent an example of allelic heterogeneity.

This article describes the first mapping of a locus for isolated congenital microphthalmia and almost certainly defines the location of a novel developmental gene that may be critical for eye development. The existence of a definite human phenotype associated with a defect of the arCMIC gene will make the search for this gene and the subsequent elucidation of its molecular biology particularly exciting.

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