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# Mapping of a Congenital Microcoria Locus to 13q31-q32

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## Summary

**Congenital microcoria is an autosomal dominant disorder characterized by a pupil with a diameter <2 mm. It is thought to be due to a maldevelopment of the dilator pupillae muscle of the iris, and it is associated with juvenile-onset glaucoma. A total genome search for the location of the congenital microcoria gene was launched in a single large family. We found linkage between the disease and markers located on 13q31-q32 ($Z_{max} = 9.79$; $\theta = 0$). Haplotype analysis narrowed the linked region to an interval <8 cM between markers D13S1239 proximally and D13S1280 distally.**

## Introduction

Inherited congenital microcoria, also referred to as "congenital miosis," is a rare bilateral condition (McKusick 1992 [OMIM 156600]) characterized by a small pupil (diameter <2 mm) that results from an underdevelopment of the dilator pupillae muscle of the iris (Holth and Berner 1923; Coulon et al. 1986; Simpson and Parson 1989). Iris transillumination defects are a constant feature. The pupil dilates poorly or not at all in response to topically administered mydriatic drugs. The disorder is transmitted as an autosomal dominant trait with complete penetrance and is associated with goniodysgenesis and glaucoma (Tawara and Inomata 1983; Coulon et al. 1986; Mazzeo et al. 1986; Toulemont et al. 1995). It is related to other congenital malformations of the anterior segment of the eye, such as Axenfeld-Rieger syndrome, congenital iris ectropion, iridocorneal endothelial syndromes, posterior embryotoxon, and congenital hereditary endothelial dystrophy, or Peter's anomaly. All of these conditions involve a developmental arrest, late in gestation, of anterior segment structures derived from neural crest cells (Shields 1983).

In 1964, Ardouin et al. described a large French family affected by congenital microcoria. In this article, we describe a linkage analysis of the same French family, with one branch added.

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## Material and Methods

### *Clinical Findings*

All affected family members had pupils with a diameter <2 mm bilaterally. In each case, the peripupillary region of the iris was faint in color and fleshy, and the iris periphery appeared washed out, flat, and transilluminable (Ardouin et al. 1964; Toulemont et al. 1995). More detailed descriptions of the clinical findings have been reported elsewhere (Toulemont et al. 1995). Recently, a statistically significant correlation has been demonstrated in this family between congenital microcoria, axial myopia, corneal astigmatism, and juvenile-onset glaucoma (Toulemont et al. 1995). Diagnosis of additional family members, more recently identified, was based mainly on the small pupil, always with a diameter <2 mm. Among the 31 affected individuals included in this study, 25 had myopia, 26 had astigmatism, and 9 showed evidence of glaucoma (intraocular pressure >21 mmHg and funduscopic findings of optic disc cupping). All patients with glaucoma also had myopia and showed chamber angle abnormalities. In some cases, glaucoma was diagnosed during the 1st decade of life. It was responsible for substantial loss of vision and acute pain and resulted in enucleation of one and both eyes for patients III-10 and II-3, respectively.

Autosomal dominant inheritance was highly likely because there were affected individuals in each generation, there was male-to-male transmission, and every affected member had an affected parent (fig. 1).

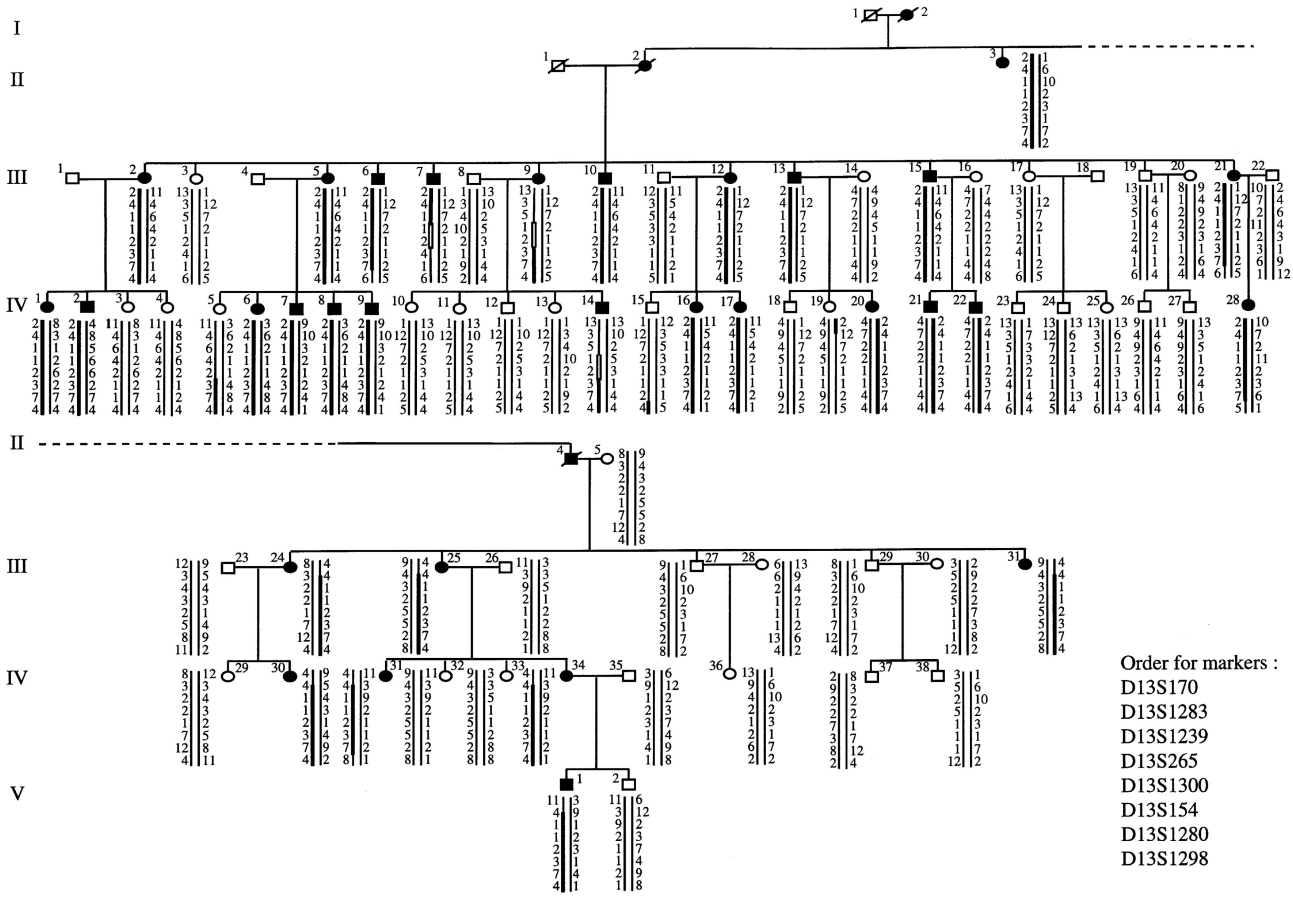


**Figure 1** Five-generation microcoria pedigree. Black symbols denote affected individuals; black bars denote the haplotype segregating with microcoria; and white bars indicate where the recombination event could not be determined unequivocally.

### *Genotyping*

Blood samples were collected from all consenting individuals (70), and DNA was prepared using standard methods. To analyze each microsatellite marker, oligonucleotide primers were synthesized on the basis of published sequences (Dib et al. 1996). The microsatellites were amplified from leukocyte DNA using a PTC100 (MJ Research) thermocycler. Each PCR was performed in a final volume of 20 $\mu$l containing 50 ng of genomic DNA, 0.25 $\mu$M of each primer, 1.5 mM $MgCl_2$, 125 $\mu$M each of dATP, dCTP, dGTP, dTTP, and 0.4 U *Taq* polymerase (Life Technologies). After incubation at 94°C for 5 min, samples were processed through 30 temperature cycles (denaturation at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 10 s). A final cycle had an extension step lasting 10 min. PCR products were separated by electrophoresis on standard denaturing polyacrylamide gels and were transferred to positive-charged nylon membranes (Pall). The membranes were hybridized overnight at 42°C with a primer labeled with [$\alpha$-$^{32}$P]-dCTP using terminal transferase (Boerhinger). DNA of Centre d'Étude du Polymorphisme Humain (CEPH) individual 1347 was included in each experiment as a control for allele-size estimation (Dib et al. 1996). After autoradiography, genotypes were determined in a double blind manner—independently by two persons who did not know the identities of the family members at the time of the gel reading.

### *Linkage Analysis*

Two-point disease-to-marker linkage analysis was conducted with the MLINK program, and multipoint analysis was conducted with the LINKMAP program using the software packages FASTLINK (version 2.3P) (Lathrop and Lalouel 1984; Cottingham et al. 1993) and VITESSE (O'Connell and Weeks 1995). The mode of inheritance of the disease was considered to be autosomal dominant with full penetrance. The gene frequency was set at .0001. The allele frequencies of the polymorphic markers and the genetic distances used in

**Table 1**

**Two-Point Linkage Data**

| | LOD Score at $\theta$ = | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Locus | .00 | .001 | .01 | .05 | .10 | .20 | .30 | .40 | $Z_{max}$ | $\theta_{max}$ |
| D13S170 | -infini | 2.47 | 6.26 | 8.24 | 8.39 | 7.29 | 5.39 | 2.88 | 8.39 | .10 |
| D13S1283 | -infini | 6.94 | 7.80 | 7.86 | 7.34 | 5.85 | 4.03 | 1.89 | 7.86 | .05 |
| D13S1239 | -infini | 11.75 | 12.55 | 12.35 | 11.47 | 9.20 | 6.48 | 3.32 | 12.55 | .01 |
| D13S265 | 9.79 | 9.78 | 9.63 | 8.99 | 8.16 | 6.35 | 4.35 | 2.15 | 9.79 | .00 |
| D13S1300 | 6.71 | 6.70 | 6.61 | 6.17 | 5.60 | 4.34 | 2.94 | 1.40 | 6.71 | .00 |
| D13S154 | -infini | 7.35 | 9.17 | 9.77 | 9.33 | 7.67 | 5.47 | 2.83 | 9.77 | .05 |
| D13S1280 | -infini | 8.66 | 10.47 | 10.98 | 10.41 | 8.48 | 5.99 | 3.00 | 10.98 | .05 |
| D13S1298 | -infini | −7.39 | −1.52 | 2.09 | 3.14 | 3.30 | 2.51 | 1.19 | 3.30 | .20 |

multipoint linkage analysis were as described elsewhere (Dib et al. 1996). These allele frequencies were deemed appropriate because they are based on CEPH pedigrees that are all of Caucasian origin, as is our family.

## Results

Compared with previous report of this family (Ardouin et al. 1964; Toulemont et al. 1995), we identified a new branch, the descendants of individual II-4 (fig. 1). Including this new branch, there were 78 members of the pedigree. Linkage analysis was based on 31 affected individuals, 27 unaffected individuals, and 12 spouses who were genotyped with microsatellite markers from the Généthon $(AC)_n$ map (Dib et al. 1996).

On the basis of accepted linkage criteria (Morton 1955), the disease gene was excluded from chromosomal regions to which other maldevelopment ocular disorders had been mapped, including 6p25 (iridogoniodysgenesis [IGDA] and familial glaucoma iridogoniodysplasia [FGI]) (Mears et al. 1996; Jordan et al. 1997), 1q21-31 (autosomal dominant juvenile glaucoma) (Sheffield et al. 1993), 4q25 and 13q14 (iridogoniodysgenesis with systemic features [IGDS] or Rieger syndrome) (Legius et al. 1994; Héon et al. 1995; Phillips et al. 1996), 15q15-q21, and 3p24.2-p25 (Marfan syndrome) (Sarfarazi et al. 1992; Collod et al. 1994). Subsequently, a genome-wide search for the location of the congenital microcoria locus was undertaken. Approximately 90% of the genome was excluded with 150 microsatellite markers (at intervals of ~30 cM) before significant linkage (LOD score >3) was demonstrated between the congenital microcoria trait and eight markers mapping to 13q31-32. The highest two-point LOD score ($Z_{max}$) obtained was 9.79 at a recombination fraction ($\theta$) of 0, with marker D13S265 (see table 1).

Haplotype analysis revealed recombination events in eight affected individuals (III-6, III-7, III-9, III-21, III-24, III-25, III-31, and IV-31) and three unaffected individuals (IV-5, IV-15, and IV-19) (fig. 1). If it is assumed that the disease-linked haplotype seen in II-3, III-2, III-5, III-10, III-12, III-13, and III-15 represents the ancestral haplotype, then a recombination event has occurred in individual II-4. He transmitted to his three affected daughters (III-24, III-25, and III-31) this recombinant haplotype.

According to haplotype analysis, family member II-2 was uninformative for markers D13S265 and D13S1300. As a consequence, we could not determine exactly where the recombination event occurred in her two affected children, III-7 and III-9 (fig. 1 and 2). Family member III-9 transmitted her recombinant haplotype to her single affected son, IV-14. Haplotype analysis indicated that the congenital microcoria locus probably is within an interval <8 cM flanked by the markers D13S1239 and D13S1280 (fig. 2). Multipoint analysis

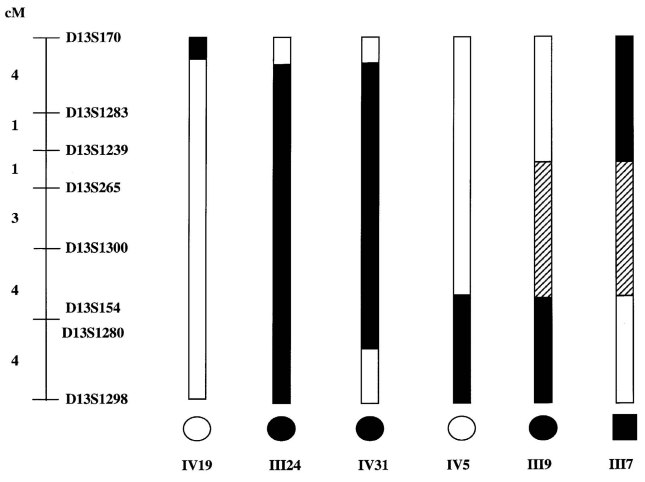


**Figure 2** Haplotype analysis illustrating recombination events between the disease locus and chromosome 13q markers. Key individuals are identified, with disease status indicated, at the bottom of the figure. Black bars denote the region cosegregating with the disease in this family. Hatched bars indicate the region where the recombination event could not be determined unequivocally and represent the most likely region for the congenital microcoria locus.

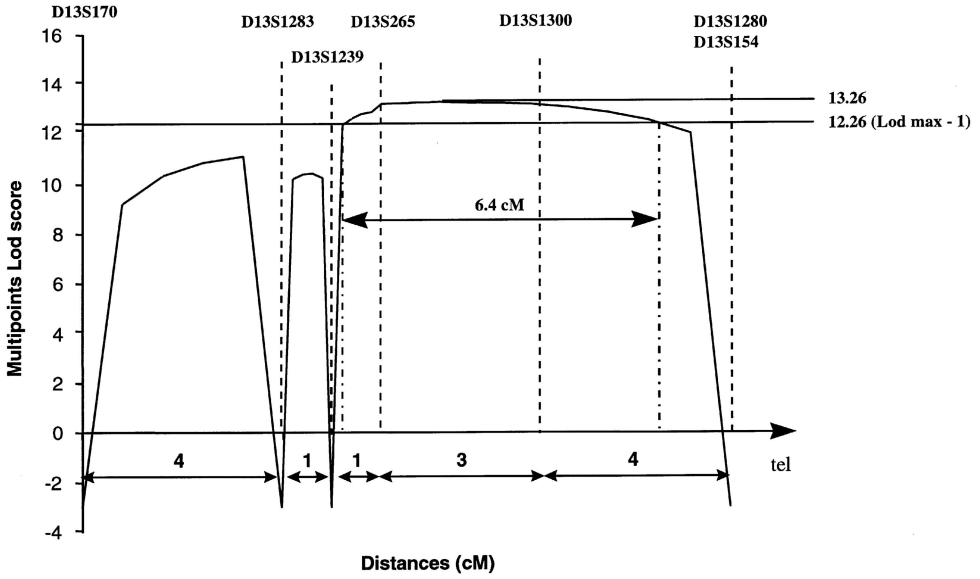


**Figure 3** Multipoint LOD scores between microcoria and seven different markers from 13q31-q32. Because of computer limitations, four overlapping four-point runs have been performed and compiled here. Double arrows indicate the 90% confidence interval for mapping of congenital microcoria.

between the disease and seven markers resulted in a $Z_{max}$ = 13.26 in a 3-cM interval between D13S265 and D13S1300. Using a $LOD_{max}$ − 1 threshold, the 90% confidence interval for congenital microcoria locus mapping is 6.4 cM (fig. 3).

## Discussion

We have mapped a gene responsible for congenital microcoria to chromosome 13q31-32, in an 8-cM region flanked by markers D13S1239 proximally and D13S1280 distally. Inherited congenital microcoria is clinically and genetically distinct from autosomal dominant IGDA or FGI, both of which have been previously mapped to 6p25 (Mears et al. 1996; Jordan et al. 1997). It differs also from autosomal dominant iris hypoplasia, IGDS, or Rieger syndrome on 4q25 (Legius et al. 1994; Héon et al. 1995; Phillips et al. 1996). Microcoria has never been observed in these syndromes. Furthermore, Rieger syndrome is associated with many systemic anomalies, in particular those involving developmental defects of the teeth and facial bones, whereas no facial abnormality nor any systemic anomaly is present in our family.

Congenital microcoria is characterized by an underdevelopment of the dilator pupillae muscle of the iris (Holth and Berner 1923; Coulon et al. 1986; Simpson and Parson 1989). The dilator and sphincter muscles of the iris, unlike other muscles, are derived from the neurectoderm. The dilator muscle is actually the anterior layer of the pigment epithelium of the iris. The sphincter begins its development at 4 mo and is well formed by 6 mo, although the dilator begins to develop at 6 mo and is completed only after birth. It has been suggested that the pigmentation of the epithelial layers is important for a normal development of the dilator and sphincter muscles of the iris (Badtke 1958; Agoston and Grof 1968). Furthermore, a defect in pigmentation has been suggested for individuals affected by congenital microcoria, since (1) they present a translucent peripheral iris (Ardouin et al. 1964; Polomeno and Milot 1979; Toulemont et al. 1995) and (2) a father affected with microcoria had three offspring with oculocutaneous albinism (Agoston and Graf 1968).

Genes mapped to chromosome 13q31-q32 include eukaryotic translation elongation factor 1 alpha-like 1 (EEF1AL1) (Bonaldo et al. 1994), excision repair gene (ERCC5) (Takahasi et al. 1992), G protein–coupled receptor 18 (GPR18) (Gantz et al. 1997), inhibitor of the interferon-induced dsRNA-activated protein kinase (PRKRI) (Korth et al. 1996), and propionyl coenzyme A carboxylase, alpha-chain (PCCA) (Kennerknecht et al. 1992). Interestingly, the dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2) (TYRP2) gene also maps to 13q31-32 (Sturm et al. 1994) and encodes for a protein (TRP-2) that acts, together with tyrosine related-protein 1 (TRP-1), at steps downstream of tyrosinase in the melanogenic pathway. Expressed sequence tags corresponding to the TYRP-2 gene have been mapped to a genetic interval bracketed by the markers D13S281 and D13S154 (Schuler et al. 1996).

These markers are unambiguously enclosed within the larger interval flanked by the markers D13S1239 and D13S1280 in which we have mapped the microcoria locus. TYRP-2 expression is restricted mainly to melanocytes and the retinal pigment epithelium (Steel et al. 1992). No mutation has yet been described in human TYRP-2 gene in patients affected with either oculocutaneous or cutaneous albinism.

We are currently undertaking fine mapping and identifying additional informative markers to enable, ultimately, positional cloning of the congenital microcoria gene. The identification and characterization of the gene responsible for this disorder should provide new insight into the ocular embryonic development and physiology. Other families with congenital microcoria have been reported (Polomeno and Milot 1979; Tawara and Inomata 1983). Linkage studies in these families and the future identification of the responsible gene will reveal whether this condition exhibits nonallelic heterogeneity.

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