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SHORT COMMUNICATION

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A novel locus of coralliform cataract mapped to chromosome 2p24-pter

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Abstract Congenital cataract is a common major abnormality of the eye, which can result in significant visual impairment or blindness in childhood. In this work, we studied four generations of a Chinese family that exhibited autosomal dominant coralliform cataract but no other ocular or systemic abnormalities. Members of the family were firstly genotyped with microsatellite markers at loci associated with congenital cataract on the reported regions of chromosomes 1, 2, 3, 10, 11, 12, 13, 15, 16, 17, 20, 21, and 22, but negative LOD scores were obtained. Following exclusion of these loci, a genome-wide scan was performed, and significant evidence of linkage was obtained for marker D2S2211 ($Z = 2.69$, $\theta = 0.00$). In multipoint analysis, a maximum LOD score 4.87 ($\theta = 0.00$) was reached between markers D2S2211 and D2S2164. Haplotype data indicated a coralliform cataract disease gene in a 26-cM interval at a novel disease locus 2p24-pter between D2S297 and D2S2268. No genes related to cataract in this region have been reported so far.

Keywords Autosomal dominant congenital cataract (ADCC) · Coralliform cataract · Linkage analysis · Genetic heterogeneity

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Introduction

Cataract refers to the loss of transparency in the lens of the eyes. During the critical phrase of vision forming, opacities of the neonatal lens can cause blurring of vision. Congenital cataract is a common major abnormality of the eye, which can result in significant visual impairment or blindness in childhood, accounting for an estimated one-tenth of clinical childhood blindness worldwide (Gilbert et al. 1993). The World Health Organization (WHO) has set itself the task, as a part of its international program, to eliminate avoidable blindness due to congenital cataract by 2020 (Thylefors 1998).

Congenital cataract shows a wide variety of phenotypic and genotypic heterogeneity. The relationship between morphology and genetic etiology is not yet clear. Morphological variations of congenital cataract are many and complex (Amaya et al. 2003), and no universal classification system has yet been adopted by researchers worldwide. At present, congenital cataract can be divided into several types according to their specific morphology based on location and appearance of lens opacities. Such categories include anterior polar, posterior polar, nuclear, lamellar (zonular), pulverulent, cerulean, total, cortical, polymorphic, sutural, aculeiform, and coralliform, etc. (Amaya et al. 2003; Francis et al. 2000; Reddy et al. 2004). Approximately one-quarter to one-third of congenital cataract is hereditary. Among the three types of Mendelian inheritance in congenital cataract, autosomal dominant appears to be the most significant. At least seventeen loci of autosomal

dominant congenital cataract (ADCC) have been mapped by linkage analysis on 13 human chromosomes. These loci are: 1p36 (Eiberg et al. 1995; Ionides et al. 1997), 1q21–q25 (Renwick and Lawler 1963), 2p12 (Khaliq et al. 2002), 2q33–q35 (Lubsen et al. 1987; Rogaev et al. 1996), 3q21–q22 (Jakobs et al. 2000), 10q24–q25 (Semina et al. 1998), 11q22.3–q23.1 (Berry et al. 2001), 12q13 (Bateman et al. 2000), 13cen-q13 (Mackay et al. 1997), 15q21–q22 (Vanita et al. 2001), 16q22.1 (Eiberg et al. 1988), 17p (Berry et al. 1996), 17q11.1-q12 (Padma et al. 1995), 17q24 (Armitage et al. 1995), 20p12-q12 (Yamada et al. 2000), 21q22.3 (Litt et al. 1998), and 22q11.2 (Hulsebos et al. 1995).

Cataract-related proteins that play vital roles in the structure and function of the lens include crystallins, lens connexin (Cx 50 and Cx 46), major intrinsic protein (MIP), cytoskeletal protein (CP49), and lens-developmental protein (PITX3). Crystalline genes are major candidates in relation to congenital cataract because of their abundant expression in the lens, and they can be divided into distinct evolutionary groups comprising two α-crystallins (CRYAA and CRYAB), which belong to the small heat shock protein family of molecular chaperones and nine β-/γ-crystallins (CRYBA1/A3/A4/B1/B2/B3 and CRYGC/D/S), which belong to the family of epidermis-specific differentiation proteins (He and Li 2000). Connexin genes express intercellular gap junctions that facilitate the cytoplasmic exchange of ions, second messengers, and small metabolites and preserve lens fiber cell homeostasis (Kumar and Gilula 1996). Major intrinsic protein is the most abundant junctional membrane protein in the mature lens. It minimizes extracellular space in the lens and facilitates the formation of specialized membrane junctions between fiber cells (Heymann et al. 1998; Michea et al. 1994). The interaction of the cytoskeletal proteins and crystallins in the cytoplasm determines the structural framework of lens cells, which is important for the maintenance of transparency in the lens (Matsushima et al. 1997). As transcription factors and diffusible growth factors, PITX3 and HSF4 play a key role in the complex development of the lens (Beebe 1994). So far, more and more mutations causing congenital cataract have been discovered.

In this study, we mapped the candidate gene to chromosome 2p24-pter with a four-generation Chinese family affected by autosomal dominant coralliform cataract.

Materials and methods

Clinical evaluations

Patients in this family who had no cataract extraction were given an examination, which included photography and slit-lamp microscopy of the lens, to record cataract type. Informed consent was then obtained for molecular analysis and clinical investigation of the family.

Gene scan

Blood samples were obtained, and genomic DNA was extracted from peripheral blood leukocytes using conventional methods. DNA samples were amplified with three-temperature touchdown PCR: 96°C for 12 min; followed by 14 cycles at 95°C for 30 s, 64°C for 1 min (with a temperature decrease from 64°C to 57°C by 0.5°C per cycle), 72°C for 1.5 min; followed by 30 cycles at 95°C for 30 s, 57°C for 1 min, and 72°C for 1.5 min; and a final extension at 72°C for 10 min, in a Gene Amp PCR System 9700 (PE Applied Biosystems, USA). Standard PCR reaction was preformed in a 5-μl volume containing 50 ng genomic DNA, 1 μM of each primer, 300 μM dNTP, 10 mM Tris–HCl (pH8.3), 50 mM KCl, 1.5 μM $MgCl_2$, 3 U Hotstart Taq. The PCR products were separated on 8% denaturing polyacrylamide gels in an Applied Biosystems 377 Sequencer. Genescan (version 3.7) and Genotyper (version 3.7) software packages (Perkin Elmer Corporation, USA) were used to generate genotypes. Dinucleotide repeat microsatellite markers were labeled with fluorescent dye.

Linkage analysis and haplotyping

Genotype data were stored in a family program. A two-point LOD scores (Z) was calculated using the MLINK subprogram of the LINKAGE package (version 5.1) with the following assumptions of a gene frequency of 0.0001, a penetrance of 100%, and equal allele frequencies. Multipoint analysis was computed using FASTMAP. Microsatellite markers, allele frequencies, and recombination distances between the marker loci were based on the Généthon linkage map. Family and haplotype data were processed using Cyrillic (version 2.1) software.

Results

Clinical data

The four-generation Chinese family consisted of 27 members, including 11 affected individuals, 7 unaffected individuals, and 9 spouses (Fig. 1). The affected members appear in four successive generations, the ratio of affected males to females is 1:1, and male-to-male transmission is also involved, which is consistent with autosomal dominant inheritance. The affected individuals in the family had visual handicaps but no family history of any other ocular or systemic abnormalities. The age of the affected individuals varied from 5 to 50 years. In all cases, the cataract

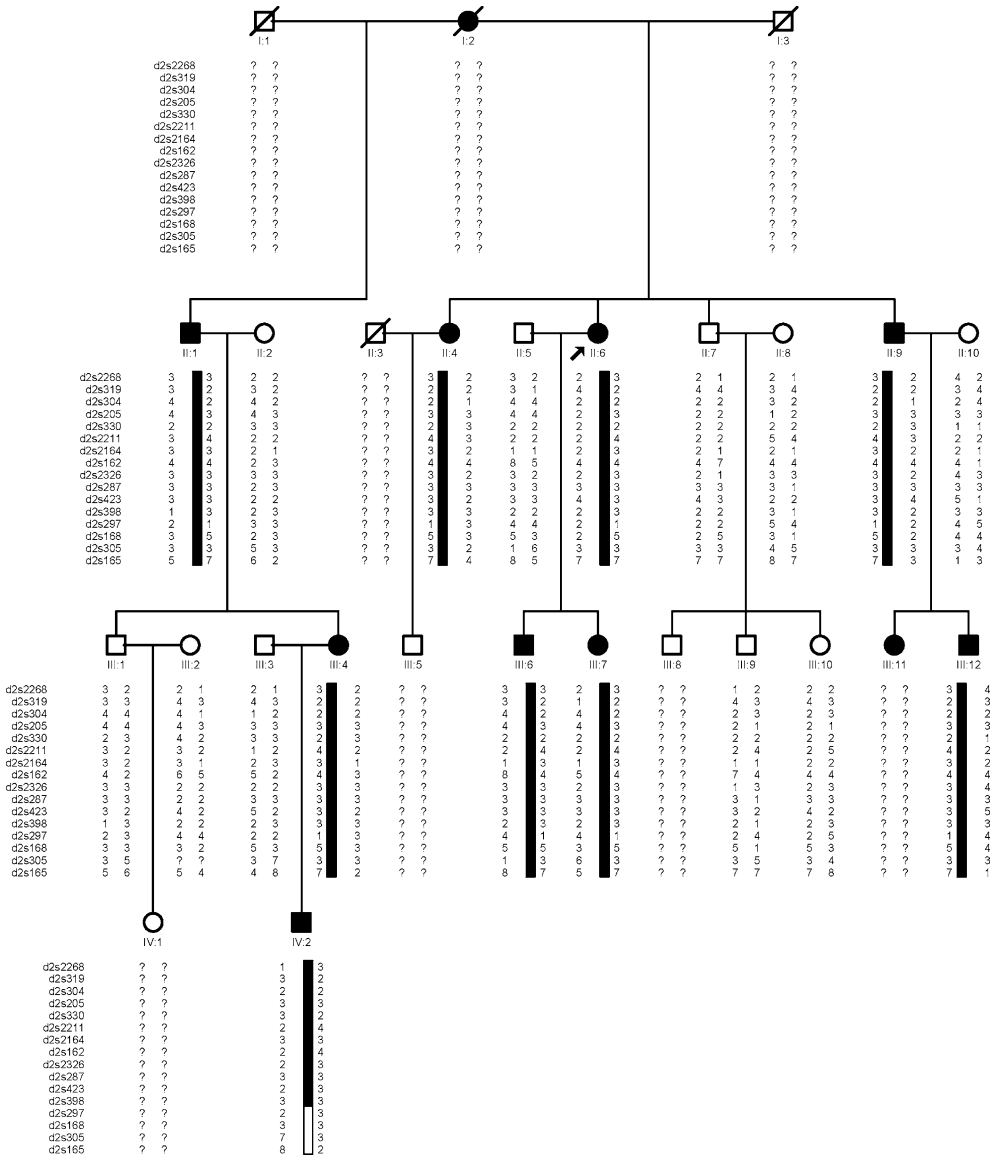


Fig. 1 Cataract family and haplotype analysis. *Squares* and *circles* symbolize males and females, respectively. *Filled symbols* denote affected status. The *dark bar* represents the presumed affected chromosome

was congenital, that is, dating from either birth or infancy. Throughout childhood and early adolescence, the affected individuals experienced mild reduction of visual acuity, followed by gradually decreasing visual acuity over time. Thus III:6, III:7, III:11 and III:12 had been obliged to discontinue their studies during elementary school because of slowly deteriorating vision. The proband II:6 underwent cataract extraction for both eyes at age 45. Subsequently, II:4 and all other affected individuals in the third generation of the family had surgery in the same hospital. After correction of astigmatism, all patients recovered adequate vision. Figure 3 shows the cataract type as disclosed by slit-lamp microscopy and photography of the lens. The cataract was bilateral and symmetric while the segments of the eyes were normal. The form of the opacity was irregular and looked similar to sea coral, with crystal clumps radiating from center to capsule. Based on family history and clinical diagnosis, the type of cataract was classified as autosomal dominant coralliform cataract.

Gene scan and linkage analysis

As a first step, we tested markers on known loci, which are related to genes involved in lens formation, metabolism, or opacification, for autosomal dominant congenital cataract in the family. However, the negative LOD scores obtained ruled out these chromosomal regions (data not shown). Subsequently, a genome-wide scan with a number of microsatellite markers spanning the entire human genome at approximately 10-cM intervals was performed. As a result, significant positive LOD scores were obtained through several markers located at 2p, with a maximum LOD score 2.69 at D2S2211 ($\theta = 0.00$). More markers in this region were analyzed to obtain

Table 1 Two-point linkage analysis between the disease gene in this family and the microsatellite markers on chromosome 2p

cM	Marker	LOD score at θ =								
		0.00	0.05	0.1	0.2	0.3	0.4	0.5	Z_{max}	θ_{max}
1.95	D2S2268	0.71	0.77	0.76	0.64	0.45	0.24	0.00	0.77	0.05
7.60	D2S319	2.09	1.89	1.68	1.23	0.75	0.29	0.00	2.09	0.00
10.04	D2S304	0.47	0.53	0.52	0.44	0.30	0.16	0.00	0.53	0.05
10.42	D2S205	2.09	1.89	1.68	1.23	0.75	0.29	0.00	2.09	0.00
14.10	D2S330	0.19	0.27	0.29	0.25	0.17	0.09	0.00	0.27	0.05
15.61	D2S2211	2.69	2.45	2.19	1.63	1.01	0.36	0.00	2.69	0.00
18.42	D2S2164	2.38	2.18	1.97	1.51	1.0	0.45	0.00	2.38	0.00
18.42	D2S359	2.39	2.17	1.94	1.44	0.89	0.37	0.00	2.39	0.00
20.03	D2S162	1.13	1.02	0.90	0.65	0.39	0.15	0.00	1.13	0.00
20.57	D2S2207	1.01	1.04	1.01	0.84	0.60	0.31	0.00	1.04	0.05
20.57	D2S2169	1.41	1.28	1.13	0.83	0.52	0.22	0.00	1.41	0.00
20.57	D2S2326	1.78	1.62	1.46	1.10	0.70	0.29	0.00	1.78	0.00
21.22	D2S287	−0.17	−0.04	0.02	0.05	0.04	0.01	0.00	0.05	0.03
22.10	D2S423	0.98	1.03	1.00	0.84	0.60	0.31	0.00	1.03	0.05
23.57	D2S398	−2.49	−0.67	−0.25	−0.03	−0.06	−0.10	0.00	0.00	0.5
26.52	D2S297	−1.60	1.0	1.06	0.86	0.50	0.12	0.00	1.06	0.1
27.06	D2S168	−2.95	0.06	0.31	0.41	0.29	0.11	0.00	0.41	0.2
38.87	D2S305	−0.39	−0.26	−0.17	−0.08	−0.03	−0.01	0.00	0.00	0.5
47.43	D2S165	−3.44	−0.67	−0.34	−0.07	0.03	0.05	0.00	0.05	0.4
54.96	D2S367	−7.00	−2.54	−1.61	−0.76	−0.35	−0.13	0.00	0.00	0.5

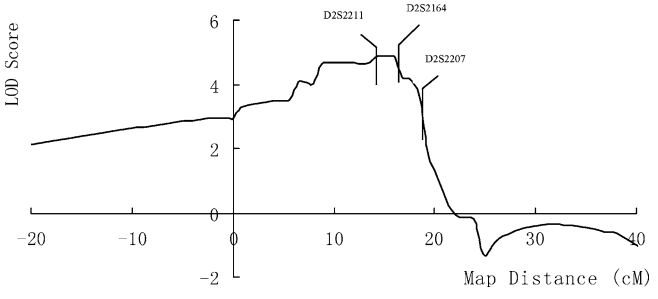
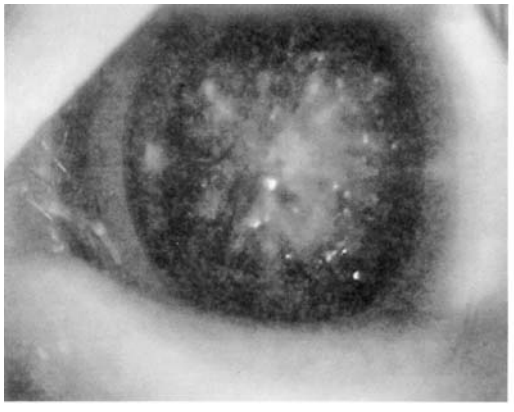


Fig. 2 Multipoint linkage analysis between D2S2268 and D2S367 on 2p24-pter

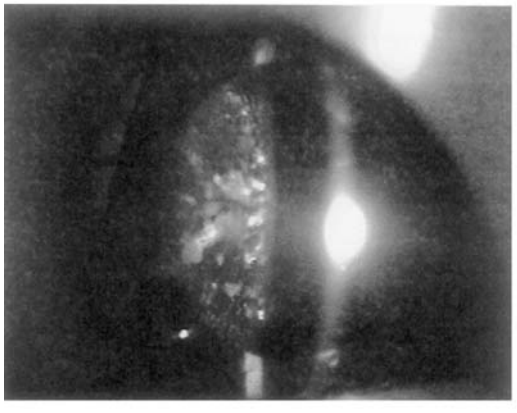
proximal and distal crossovers to localize the disease region (Table 1). Multipoint analysis using these markers yielded a maximum LOD of 4.87 between markers D2S2211 and D2S2164 (θ = 0.00) (Fig. 2).

Haplotyping

The disease-linked chromosome was identified by haplotype analysis in the affected individuals in the family (Fig. 1). The only informative recombination event was seen in individual IV:2 between markers D2S398 and D2S297, which lies approximately 10-cM proximal to D2S2211. However, no crossovers were observed for markers lying telomeric to D2S2268. Thus, the crossover suggested a critical region for possible disease gene location at approximately 26-cM between the markers D2S297 and the telomere on chromosome 2p, namely, 2p24-pter.



Photograph of coralliform cataract



Slit-lamp microscopy of coralliform cataract

Fig. 3 Photograph and slit-lamp microscopy of coralliform cataract in individual IV:2 from the family

Discussion

Coralliform cataract, derived from a phenotype with a resemblance to sea coral, was first reported in a patient in 1895 (Gunn 1895) and described in pedigrees as an autosomal dominant trait in 1910 (Harman 1910). Since then, there have been few reports worldwide, as clinical cases have been rare. Coralliform cataract is usually a central complex cataract that cuts across normal anatomical boundaries and is comprised of multiple coral-like white opacities. They radiate out bilaterally in an axial direction from the center of the lens in a fusiform or spindle-shaped fashion but never actually reach the capsule. The anatomical arrangement suggests that they may be due to a primary abnormal arrangement of lens fibers (Amaya et al. 2003).

Recently, a missense P23T substitution in *CRYGD* on chromosome 2q33-35 has been found in a four-generation Chinese family (Xu et al. 2004) and also in a three-generation Caucasian family (Mackay et al. 2004), both affected by coralliform cataract. However, no crystal-formation was observed by the recombinant P23T mutant expressed in cultured HLE B-3 cells, possibly because of the relatively low level of CRYGD protein compared with that in the lens (Mackay et al. 2004).

In our work, we have presented evidence for a coralliform cataract locus on chromosome 2p24-pter, suggesting that the disease gene in the family is localized in a 26-cM region between D2S297 and D2S2268. This novel locus is the second genetic locus reported to date for coralliform cataract in addition to the previously reported region on chromosome 2q33-35, and it represents a genetically distinct form. More and more human cataract genes have now been assigned to different chromosomal regions by the analysis of genetic linkage and abnormal chromosomes, but it remains unclear how many genes are actually involved in the formation of congenital cataract. More than 130 known genes lie within this new region (2p24-pter). It is not clear which gene in this region plays a role in the maintenance of either lens transparency or lens metabolism, but it will be searched in the genomic database for disease-related mutations screening.

In addition, the extensive homologies between the mouse and human genome provide a method for predicting the location of human congenital cataract genes. Although the phenotypes between the two species are different, the available mouse models are useful in suggesting candidate genes. Statistically significant data have been provided by mouse mutants, which have suggested novel candidate genes for human cataract formation, in understanding normal eye development and identifying certain mutations. More than 60 inherited congenital cataracts with some mapped have been described in mice (He and Li 2000). Among them, an autosomal dominant coralliform cataract (Coc) mutation, which has been mapped to chromosome 6 and is similar to a conserved region on human chromosome 3, has been reported in the mouse with small, round, irregularly shaped opacities in fetal nuclear-like corals (Kratochvilova and Favor 1988).

ADCC is not only a clinically heterogeneous group but also an example of genetic heterogeneity. Mutations in different genes have been reported to cause identical phenotypes, for example, the pulverulent cataract has been reported with mutations in six different genes and an additional locus implicated in AR disease (Reddy et al. 2004). On the other hand, an identical mutation can give rise to different phenotypes (Gill 2000). Heterogeneities occur not only between families but also within a single family and even between the two lenses of an individual patient. Our study further underlines the large degree of heterogeneity in cataract families as well as the difficulty of predictive testing for the molecular characterization of congenital cataract.

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