Localization of autosomal recessive congenital cataracts in consanguineous Pakistani families to a new locus on chromosome 1p

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Purpose: To identify the disease locus for autosomal recessive congenital cataracts in two consanguineous Pakistani families.

Methods: Two Pakistani families were ascertained, ophthalmologic examination including slit lamp biomicroscopy was performed on all members, blood samples were collected and DNA was extracted. A genome-wide scan was performed using 382 polymorphic microsatellite markers on genomic DNA from affected and unaffected family members. Two-point logarithm of odds (LOD) scores were calculated using the LINKAGE program package.

Results: All the affected individuals of family PKCC009 show bilateral membranous cataract, whereas the affected individuals of family PKCC039 show bilateral posterior sub-capsular cataract. Other ocular abnormalities include corneal opacities, microcornea and nystagmus in the affected individuals of PKCC009. Maximum two point LOD scores were obtained with D1S186 (4.14 at θ = 0), D1S432 (4.01 at θ = 0), D1S2892 (4.11 at θ = 0), and D1S2797 (4.07 at θ = 0) for family PKCC009 and with D1S496 (4.73 at θ = 0), D1S2892 (4.34 at θ = 0), D1S3721 (4.83 at θ = 0), and D1S2797 (4.32 at θ = 0) for family PKCC039. The common linked region, 20.76 cM (20.80 Mb), is flanked by markers D1S2729 and D1S2890 and co-segregates with the disease in both families, placing the disease locus on chromosome 1p34.3-p32.2.

Conclusions: Linkage analysis of autosomal recessive cataracts in two consanguineous Pakistani families localizes a novel locus for autosomal recessive congenital cataract on chromosome 1p.

Congenital cataracts are one of the major causes of vision loss in children worldwide and are responsible for about one-third of blindness in infants [1,2]. Congenital cataracts can occur in an isolated fashion or as one component of a syndrome affecting multiple tissues. Non-syndromic congenital cataracts have an estimated frequency of 1-6 per 10,000 live births [3]. They vary markedly in severity and morphology affecting the nuclear, cortical, polar, or subcapsular parts of the lens or in severe cases, the entire lens with various types of opacity. Congenital cataracts can lead to permanent blindness by interfering with the sharp focus of light on the retina during critical developmental intervals.

Approximately one-third of congenital cataract cases are familial [4]. To date, 23 independent autosomal dominant cataract loci have been reported. Genes that have been previously reported to be associated with autosomal dominant congenital cataract include crystallins, connexins, beaded fiber specific proteins, aquaporin 0, and developmental and transcription factors. Conversely, fewer autosomal recessive cataract loci have been mapped. Thus far, eleven loci residing on chromosomes 1q21.1, 3p22-24.2, 6p23-24, 9q13-22, 16q21-22, 19q13.3, 19q13.4, 20p12.1, 21q22.3, 22q11.2, and 22q11.2 have been mapped. Of these loci, mutations in eight genes: gap junction protein alpha 8 (GJA8), glucosaminyl (N-acetyl) transferase 2 (GCNT2), heat shock transcription factor 4 (HSF4), lens intrinsic membrane protein 2 (LIM2), beaded filament structural protein 1 (BFSP1), αA-crystallin (CRYAA), βB1-crystallin (CRYBB1), and βB3-crystallin (CRYBB3) have been found with three of these also causing autosomal dominant cataracts [5-15].

Here we report two consanguineous Pakistani families with multiple members affected by autosomal recessive congenital cataract. Initially, a genome-wide search including exclusion of known cataract loci was completed. Linkage analysis provided evidence of a locus for autosomal recessive congenital cataract on chromosome 1p34.3-p32.2. In the genome-wide scan, a maximum two point lod scores of 3.81 and 4.07 at θ = 0 were obtained with markers D1S255 and D1S2797 for family PKCC009, respectively. Similarly, a maximum two point lod scores of 2.36 and 4.32 at θ = 0 were obtained with markers D1S255 and D1S2797 for family PKCC039, respectively. Fine mapping using markers from the Marshfield database localizes the diseased locus to a 20.76 cM (20.80 Mb) interval flanked by markers D1S2729 and D1S2890. This region is distinct from the autosomal dominant cataract loci described by Eiberg et al. [16] and Mackay et al. [17] as well as the cataract region reported by Ionides et al.[18], which overlaps the first two loci. Thus, there are at least three distinct cataract loci on chromosome 1p.
METHODS

Clinical Ascertainment: Seventy-five Pakistani families with nonsyndromic congenital cataract were recruited to participate in a collaborative study between the National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan and the National Eye Institute, Bethesda, MD to identify new disease loci that causes inherited visual diseases. This study was approved by Institutional Review Board (IRB) of the National Centre of Excellence in Molecular Biology and the National Eye Institute. The participating subjects gave informed consent consistent with the tenets of the Declaration of Helsinki. The ophthalmologic examination was performed at the Layton Rahmatullah Benevolent Trust Hospital, Lahore, Pakistan.

The families described in this study, PKCC009 (60009) and PKCC039 (60039) are from the North West Frontier Province and the Punjab Province of Pakistan, respectively. A detailed medical history was obtained by interviewing family members. Cataracts in affected individuals were either present at birth or developed during infancy. Medical records of clinical exams conducted with slit lamp biomicroscopy reported different phenotypes of cataract in affected individuals of PKCC009 and PKCC039. Affected individuals in family PKCC009 had membranous cataracts whereas clinical examinations suggested that affected individuals in family PKCC039 had posterior subcapsular cataracts.

Genotype Analysis: Blood samples were collected from affected and unaffected family members. Genomic DNA was extracted from white blood cells as described by Grimberg et al. [19]. Genotyping for all participating family members was performed using 5' fluorescently labeled microsatellite markers. A genome wide scan was conducted using the Applied Biosystems PRISM Linkage Mapping Set MD-10 Version 2.5 (Applied Biosystems, Foster City, CA) with markers spaced at average intervals of about 10 cM. PCR was conducted with an initial denaturing step of 94 °C for 8 min followed by 10 cycles of amplification at 94 °C for 15 s, at 55 °C for 15 s, and at 72 °C for 30 s; then 20 cycles at 89 °C of 15 s, at 55 °C for 15 s, and at 72 °C for 30 s. The final extension step was performed at 72 °C for 10 min followed by a final hold at 4 °C. PCR products were denatured at 95 °C for five min and then immediately placed on ice for 5 min. Amplified DNA fragments were analyzed on ABI 3100 DNA Genetic analyzer(s) and alleles were assigned using GeneScan (version 3.7; Applied Biosystems) and Genotyper Software (version 3.7; Applied Biosystems).

Linkage Analysis: Two-point linkage analyses were performed with the FASTLINK version of MLINK from the LINKAGE program package [20,21]. Maximum LOD scores were calculated using ILINK. Autosomal recessive cataracts were analyzed as a fully penetrant trait with an affected allele frequency of 0.001. The marker order and distances between the markers were obtained from the Marshfield database and the National Center for Biotechnology Information chromosome 1 sequence maps (National Center for Biotechnology, Bethesda, MD provided the information in the public domain). For the initial genome scan, equal allele frequencies were assumed whereas for fine mapping, allele frequencies were estimated from 100 unrelated and unaffected individuals from the North West Frontier province and the Punjab province of Pakistan.

Figure 1. Slit lamp photographs illustrating bilateral membranous cataract and bilateral posterior subcapsular cataract. Individual 9 (A) and Individual 10 (B) of PKCC009 illustrate bilateral membranous cataract and unilateral corneal opacity. C. Individual 19 of PKCC039 shows bilateral posterior subcapsular cataract.
### Table 1. LOD scores of chromosome 1p microsatellite markers

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<th>0.05</th>
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Two point LOD scores of chromosome 1p microsatellite markers for family PKCC009 (A) and PKCC039 (B). The asterisk indicates marker included in genome wide scan. LOD scores were calculated at different q-values for each marker with the FASTLINK version of MLINK from the LINKAGE program package. Maximum lod scores for each marker were calculated using ILINK.
RESULTS

The families described in this study, PKCC009 and PKCC039, are from the North West Frontier province, bordering Afghanistan and the Punjab Province of Pakistan, respectively. Cataracts in affected individuals were either present at birth or developed during infancy. Clinical examination conducted with slit lamp biomicroscopy reveals congenital cataract. Affected individuals of family PKCC009 exhibit bilateral membranous cataract (Figure 1A,B) accompanied by other ocular abnormalities including corneal opacity, microcornea, and nystagmus. Affected individuals of PKCC039 reveal posterior subcapsular cataract without other accompanying abnormalities (Figure 1C).

Linkage to known autosomal recessive cataract loci was initially excluded by haplotype analysis using closely flanking markers (data not shown). For family PKCC009, a genome wide scan yields lod scores greater than 1.0 for markers D1S450, D1S255, D1S2797, and D9S1776. Of these markers, only D1S450 and D9S1776 had closely flanking markers yielding large negative LOD scores thus excluding the linkage regions. D1S255 and D1S2797 are adjacent markers in the MD-10 mapping set, yielding lod scores of 3.81 and 4.07 at $\theta=0$, respectively. For family PKCC039 LOD scores of 2.36 and 4.32 at $\theta=0$ were obtained with markers D1S255 and D1S2797, respectively, during the genome wide scan. No LOD scores greater than 1.0 were obtained for family PKCC039 other than with chromosome 1p markers.

Fine mapping with closely spaced markers was carried out in both linked families in order to refine the region further. Maximum lod scores were obtained without recombination with D1S255 (3.81), D1S186 (4.14), D1S432 (4.01), D1S2892 (4.11), D1S3721 (3.73), D1S2713 (2.34), D1S2797 (4.07), D1S197 (3.98), and D1S2652 (1.61) for family PKCC009 and with D1S496 (4.73), D1S2729 (2.36), D1S255 (2.36), D1S186 (3.99), D1S432 (3.99), D1S2892 (4.34), D1S3721 (4.83), D1S2713 (3.99), D1S2797 (4.32), D1S197 (4.51) and D1S2652 (2.40) for family PKCC039 (Table 1).

Visual inspection of the haplotypes of both families supports the results of linkage analysis. As shown in Figure 2A,B, there is a proximal recombination in affected individual 16 of family PKCC009 at D1S199, individual 18 of family

![Figure 2](http://www.molvis.org/molvis/v13/a182/)
PKCC039 at D1S234, individual 19 of family PKCC039 at D1S513, and individual 14 of family PKCC009 at D1S2729. Similarly, there is distal recombination in individual 10 of family PKCC009 and individuals 14 and 19 of family PKCC039 at D1S2890. Taken together, these results strongly suggest that the disease locus cosegregates in a 20.76 cM (20.80 Mb) interval flanked by markers D1S2729 and D1S2890.

**DISCUSSION**

Here we report linkage of congenital cataract inherited as an autosomal recessive trait in two consanguineous Pakistani families to markers on chromosome 1p34.3-p32.2. The lack of significant lod scores other than chromosome 1p region during the genome scan in both families and homozygous alleles for all the affected individuals of both families for markers of 1p34.3-p32.2 along with lack of homozygosity in all the unaffected individuals strongly suggest that the cataract locus maps to the linked region, 20.76 cM (20.80 Mb), flanked by markers D1S2729 and D1S2890 on chromosome 1p34.3-p32.2.

Previously, the autosomal dominant Volkmann cataract (CCV) locus described by Eiberg et al. [16] and a second locus for autosomal dominant cataracts described by Mackay et al. [17] have been mapped to chromosome 1p. In addition, the cataract region reported by Ionides et al. [18] overlaps the first two with the highest lod score in the Volkmann region. In addition, an autosomal dominant zonular pulvertulent cataract locus (CAE1) has been mapped to chromosome 1q21-25 and shown to result from mutations in the gene for connexin 50, GJA8 [22]. During the genome wide scan, we found no evidence of linkage to these regions or other known cataract regions.

The affected individuals of family PKCC039 have posterior subcapsular cataract, whereas affected individuals of PKCC009 exhibit bilateral membranous cataract, which can be an end stage of several forms of severe cataract, usually associated with rupture of the lens capsule or absorption of the lens. This leads to the question: why families linked to the same locus would have different cataract phenotypes. One possible explanation is that distinct cataract phenotype results from modifying genetic and epistemic factors in families with the same mutation. Previously, it has been shown that a single mutation, i.e. Q155X mutation in CRYβ2, could result in cerulean cataract, Coppock-like cataract, and sutural cataract with punctate and cerulean opacities [23-25]. Another plausible explanation is that different alterations in the same gene can result in distinct phenotypes. Mutations reported for CRYβ1 strongly suggest that mutations in a single gene can not only result in different phenotypes but also can lead to different modes of inheritance [15,26]. Lastly, we can not rule out the possibility that both of these families have mutations in two different closely linked genes residing on the same locus.

Transparency and precise shape are distinctive features of the lens that are critical for proper light refraction. Elucidating the molecular mechanisms that maintain or disrupt lens transparency is a fundamental precursor for preventing cataract. The search for genes responsible for these phenotypically distinct congenital cataracts is in progress. As other ocular abnormalities such as corneal opacity, microcornea, and nystagmus were reported by the affected individuals of PKCC009 along with congenital cataract, the disease-causing gene may not be lens specific, although they are all consistent with early severe disruption of the lens. Identification of the mutations causing these cataracts is likely to lead to a better understanding of the mechanisms involved in the loss of transparency in the lens and to better conventional treatments for preventing this loss.

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