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A novel fan-shaped cataract-microcornea syndrome caused by a mutation of *CRYAA* in an Indian family

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Purpose: The molecular characterization of an Indian family having 10 members in four generations affected with a unique fan-shaped cataract-microcornea syndrome.
Methods: Detailed family history and clinical data were recorded. A genome-wide screening by two-point linkage analysis using more than 400 microsatellite markers in combination with multipoint lod score and haplotype analysis was carried out. Mutation screening was performed in the candidate gene by bi-directional sequencing of amplified products.
Results: The cataract-microcornea locus in this family was mapped to a 23.5 cM region on chromosome 21q22.3. Direct sequencing of the candidate gene *CRYAA* revealed a heterozygous C>T transition resulting in the substitution of the highly conserved arginine at position 116 by cysteine (R116C).
Conclusions: This study provides the report of mapping a locus for syndromal cataract (cataract-microcornea syndrome) on 21q22.3. The mutation observed in *CRYAA* in the present family highlights the phenotypic heterogeneity of the disorder in relation to the genotype, as an identical mutation has previously been reported in an American family with a different type of cataract. The "fan-shaped cataract" observed in the present family has not been reported before.

Congenital cataract is a common, major abnormality of the eye that causes nearly one-third of childhood blindness. Its incidence is estimated to be 2.2 to 2.49 per 10,000 live births [1,2]. Nearly one-third of the cases show positive family history [3]. Although all Mendelian modes of inheritance have been reported for it, autosomal dominant is the most common [4]. Congenital cataract may occur as an isolated anomaly, in association with other eye anomalies, or associated with systemic diseases. At least 35 loci have been reported to be linked with various forms of congenital and developmental cataracts [5].

Microcornea-cataract syndrome (OMIM 116150) is characterized by the association of congenital cataract and microcornea, without any other systemic anomaly or dysmorphism. Many families showing microcornea-cataract syndrome have been described [6-12]. The inheritance pattern in these families has been reported as autosomal dominant and the cataract type most often as bilateral posterior polar. Salmon et al. [10] have reported posterior subcapsular and anterior polar types as well, in cases with microcornea-cataract syndrome. Recently, there was a single report of gene mapping for congenital cataract-microcornea syndrome, in a UK family, having nuclear type cataract with anterior and posterior polar opacities, at 22q11.2-12.2, and mutation identified in *CRYBB1* [12].

We came across an Indian family in which all probands had autosomal dominant inheritance of microcornea-cataract syndrome. The affected individuals showed a unique "fan-shaped" cataract. Linkage analyses using more than 400 microsatellite markers mapped the locus to a 23.5 cM interval on chromosome 21q22.3. Sequencing of all exons of the αA-crystallin-encoding candidate gene, *CRYAA*, which lies within the critical region, showed a C>T change resulting in the substitution of a highly conserved arginine by cysteine (R116C). Previously the same change had been identified in an autosomal dominant congenital cataract (ADCC) family having 13 members affected with an entirely different, type of cataract. Only five of these affected individuals had microcornea in conjunction with nystagmus and microphthalmia. Other common eye anomalies in this family included amblyopia, strabismus, and glaucoma [13]. The present study provides the first report of a *CRYAA* mutation leading to cataract-microcornea syndrome in all affected members of the family. Also the phenotype "fan-shaped" cataract observed in this family is unique and has not been reported before.

METHODS

Family description: We analyzed, clinically and genetically, a large four-generation family (case number 612; Figure 1) of Indian origin having microcornea-cataract syndrome. The detailed ophthalmologic examination, which included slit-lamp examination and photography of the lenses from 19 individuals of the family (all family members except individuals I-1, I-2, and III-10), confirmed 10 members were affected with bilateral cataracts and the remaining nine were unaffected. The family history revealed that in affected individuals the lens opacities were visible since birth. Five affecteds (individuals

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II-2, II-4, II-5, III-1, and III-7; Figure 1) were operated for bilateral cataract in early childhood and had intraocular lens (IOL) implantation. All affected members had microcornea in association with bilateral cataract.

Phenotype description: The cataract type was diagnosed as bilateral, fan-shaped cataract. The opacity was round, oval, or triangular with irregular margins, about 3 mm wide, topped by triangular opacities (Figure 2). In one of the opacities, the base of the triangular opacity coincided with the edge of the fetal nucleus. The other four triangular opacities extended beyond the edge of the fetal nucleus. All affected individuals had microcornea in association with congenital cataract, as the corneal diameter measured in each affected was less than 10 mm in both horizontal and vertical meridians (average normal diameters are 12.6 and 11.7 mm, respectively). Apart from congenital cataract and microcornea, no other ocular anomaly such as microphthalmia, ambylopia, strabismus, or glaucoma was detected in any of the affected members. No posterior capsular, polar, or cortical opacities were observed in any of the affected individuals.

Genotyping and linkage analysis: Informed consent was obtained from each individual studied. This study was approved by the ethics review board of the Guru Nanak Dev University, consistent with the provisions of the Declaration of Helsinki. Blood was drawn and DNA prepared by standard methods. For a genome-wide search, more than 400 microsatellite markers with an average distance of 10 cM were used. DNA samples of all 19 individuals (10 affected and nine unaffected, including three spouses) were analyzed. Microsatellites were amplified in singleplex reactions by "touch-down" PCR in PTC-225 cyclers (MJ-Research, Watertown, MA) using fluorescently labeled primers as described by Vanita et al. [14]. PCR products for 10-12 microsatellite markers were pooled together with regard to their size range and labeling so as to electrophorese these collectively on 96-capillary automated DNA sequencers (MegaBACE 1000; Amersham, Freiburg, Germany). Genotyping was done with the use of the software Genetic Profiler v. 1.5 (Amersham). Autosomal dominant inheritance with a disease gene frequency of 0.0001 and complete penetrance of the trait was assumed. To use as much data as possible, recombination frequencies were considered equal between males and females [15]. Two-point linkage analysis was carried out with MLINK from the Linkage program package [16] and multipoint analysis was carried out using GeneHunter [17].

Mutation analysis: Mutation screening was carried out in the exonic regions of candidate gene *CRYAA* that lies in the mapped critical interval. Three pairs of primers were designed (primer details available on request) from intronic regions to amplify the coding regions and splice sites of *CRYAA* (GenBank NM_000394 and NC_000021).

Genomic DNA from two affected and one unaffected individuals were amplified. Amplification was carried out in 25 μl reactions containing 50 ng genomic DNA, 10 pmoles of each forward and reverse primers, 200 μM dNTP, 10X PCR buffer, 1.5 mM $MgCl_2$, and 0.25 U Taq DNA polymerase (AmpliTaq Gold; ABI, Foster City, CA). PCR conditions con-

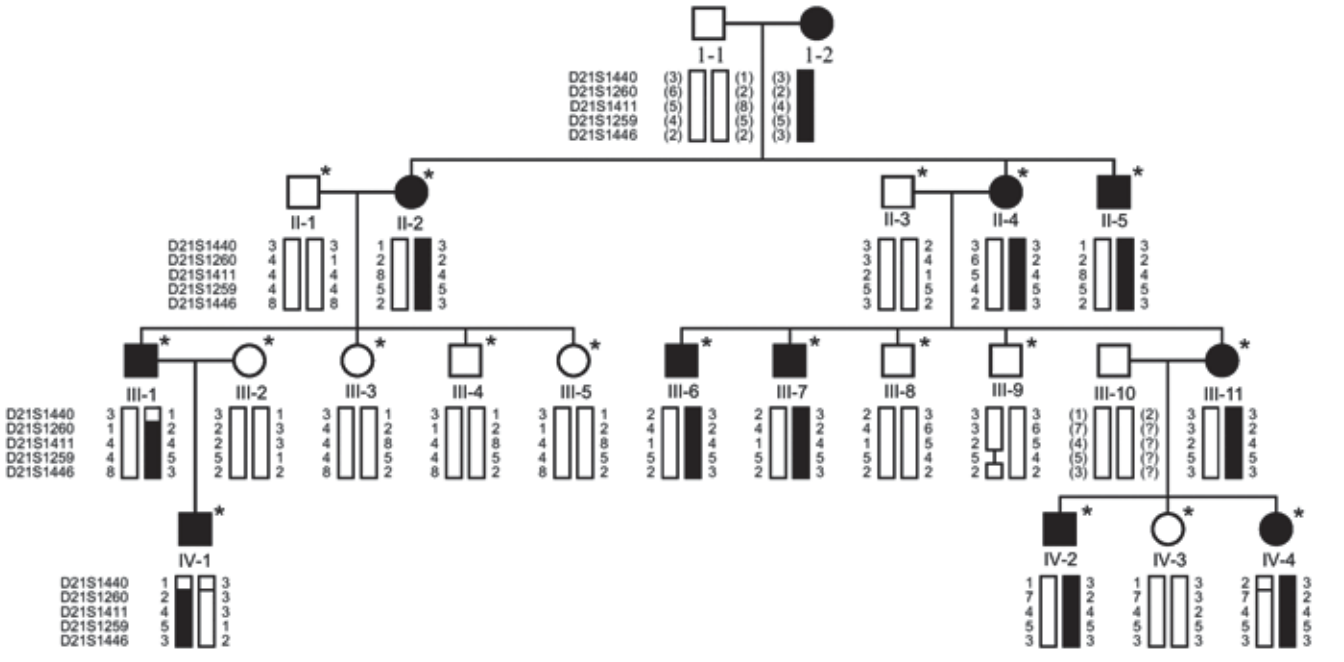

Figure 1. Haplotype analysis of the cataract-microcornea family (case number 612) with chromosome 21 markers. Sequence of markers is from centromere to telomere. Blackened bars indicate the affected haplotype. Inferred haplotypes are in parentheses. Only one haplotype each could be determined in I-2 and III-10. A decisive recombination event in III-1 placed the disease locus distal to D21S1440. Blackened symbols represents affected individuals, open symbols indicate unaffected members, and asterisks designate those who received ophthalmologic examinations.

sisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles, each consisting of denaturation at 95 °C for 45 s, annealing for 30 s at 57 °C, an extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. PCR products were purified using PCR products purification kit (QIAquick; Qiagen, Valencia, CA). Purified PCR products were sequenced bidirectionally with ABI BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems) for a 10 µl final volume, containing 5.0 µl purified PCR product, 4.0 µl BigDye Terminator ready reaction mix, and 3.2 pmoles of primer. Cycling conditions were as follows: 95 °C for 2 min., 25 cycles at 95 °C for 30 s, 52 °C, for 15 s, and 60 °C for 4 min. The sequencing reaction products were purified by the isopropanol precipitation method (ABI), suspended in 10 µl of formamide (Hi-Di-Formamide; ABI), denaturated at 95 °C for 5 min, and electrophoresed on an ABI 3100 Genetic Analyzer. Sequencing results were assembled and analyzed using the SeqMan II program of the Lasergene package (DNA STAR Inc., Madison, WI).

Restriction endonuclease analysis: DNA from 10 affected and nine unaffected individuals of the family were amplified for exon 3, which contains the identified mutation, using the *CRYAA* exon 3 primer pair and were subjected to restriction digestion with *Bts*I (New England Biolabs, Beverly, MA) at 37 °C overnight as per recommendations of the suppliers. The resulting products were analyzed on a 2.2% agarose gel. In addition, 100 unrelated controls were also tested by *Bts*I.

RESULTS

Linkage analysis: Linkage analysis was carried out with genomic DNA samples from 10 affected and nine unaffected family members. In a genome-wide scan using more than 400 microsatellite markers, we obtained 2-point lod scores of 2.833 at θ=0.000 with marker D21S1260 and 1.906 at θ=0.000 with marker D21S1259. Further analysis with more markers in this region on chromosome 21 gave a maximum lod score of 3.657 at θ=0.000 with marker D21S1411 (data not shown). Multipoint analysis carried out with chromosome 21 markers also supported linkage in this region with a maximum lod score of 3.546 at D21S1411 (data not shown).

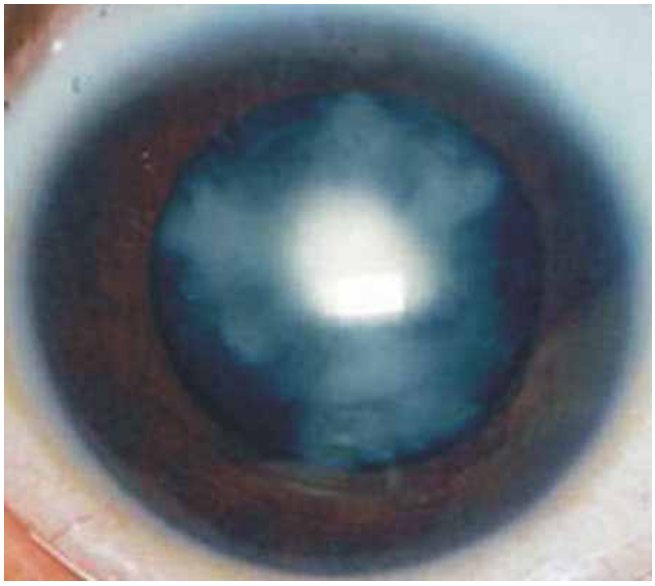

Figure 2. Lens photograph of a six-year-old patient who had cataract-microcornea syndrome. The opacity appeared as round, oval, or triangular with irregular margins, about 3 mm wide, topped by triangular opacities with base out. The cataract phenotype gives the appearance of a fan.

Haplotypes analyses: Haplotypes were constructed for the analyzed markers on chromosome 21 (Figure 1). A recombination event was detected in the affected individual (III-1) at D21S1440. No recombination event was observed in any of the affected individuals distal to D21S1440, indicating that the disease locus lies between D21S1440 and 21qter. Thus the results of both multipoint and haplotype analyses place the cataract locus in a region on chromosome 21q22.3 that is at least 23.5 cM in length.

Mutation detection (candidate gene analysis): Sequencing of the exonic regions of *CRYAA*, which lie within the mapped critical interval, showed a heterozygous change, a C>T substitution (Figure 3A) at the first base of codon 116, in two affected individuals. This change replaces the codon for an evolutionarily highly conserved arginine at amino acid position 116 of the CRYAA protein with a cysteine codon (R116C). This nucleotide change further created a *Bts*I restriction enzyme site, which showed complete cosegregation with the disease phenotype in all affected subjects, but in none of the unaffected members of this family (Figure 3B). This nucleotide change was not observed in 100 control subjects (data not shown).

DISCUSSION

The present study provides the first report of mapping a locus for cataract-microcornea syndrome characterized by a "fan-shaped" cataract on 21q22.3 in a family of Indian origin. All the affected members of the four-generation family showed a unique "fan-shaped" cataract combined with microcornea. To our knowledge, such a phenotype has not been reported before. There is only one previous report of a dominant X-linked sea fan-shaped cataract phenotype, however without microcornea [18]. For cataract-microcornea syndrome recently, there is a report of gene mapping and identification of mutation in *CRYBB1* at 22q11.2-12.2 in a UK family having nuclear type cataract [12].

In the present study, mutation analysis in the candidate gene, *CRYAA*, at 21q22.3, revealed a C>T substitution of codon 116, replacing the evolutionary highly conserved arginine by cysteine (R116C). This change was observed only in the affected individuals and not in the unaffected family member or in 100 control subjects. We, therefore, assume this change as the disease-causing mutation, rather than a polymorphism.

The same change (R116C) has previously been reported to result in autosomal dominant zonular type congenital cataract (ADCC) in a North American pedigree by Litt et al. [13]. Five of the 13 affected individuals had congenital nystagmus and congenital microphthalmia (corneal diameter about 9.00 mm). Other common anomalies were amblyopia, strabismus, and glaucoma. Despite an identical mutation, the clinical phe-

notype is clearly different. Since the American family is not of Indian origin (Michael Litt, personal communication, June 2005), the C>T transitions at the first base of codon 116 in both families occurred independently, perhaps indicating a hotspot for the mutation resulting from the instability of CpG dinucleotides.

The α-crystallins (αA- and αB-crystallin) comprise up to 40% of the protein mass of the lens, and belong to the small heat shock protein family (sHSPs). These function as molecular chaperones. They facilitate the correct folding of other lens proteins, and are of extreme importance in keeping lens proteins in a functional state [19]. It has been reported that α-crystallin chains have a propensity to keep their net charge conserved throughout evolution [20]. Arg116 has been found to be highly conserved in 28 species of mammals, chicken, and frog [20]. An alignment of αA-crystallin sequences obtained from public databases demonstrated that Arg116 is conserved in 69 different species (not shown). By site-directed spin labeling, Berengian et al. [21] showed the presence of buried salt bridges in the core of αA-crystallin and Arg116 to be present in a β-strand structure, near a subunit interface forming a buried salt bridge. The R116C change is assumed to disrupt an existing salt bridge environment and hence the native structure of αA-crystallins [13]. The consequence of the substitution of Arg116 by cysteine (R116C) is a reduction in chaperone-like activity, and the ability to exchange subunits with wild type complexes and a ten fold increase in membrane binding capacity in comparison with the wild-type form [22].

Apart from the crystallins, many other proteins also play an important role in maintenance of lens cell homeostasis such as membrane channels comprising “thin” and “gap” junctions [23]. The mapped critical interval at 21q22.3 also contains *COL18A1*, which is associated with Knobloch syndrome, characterized by vitreoretinal degeneration and congenital occipital encephalocele or scalp defects [24,25]. Thus, in cataract, one major gene seems to be affected, but variants in other genes involved in development, growth, and maintenance of lenses might modify its pathogenesis, explaining the poor correlation between a particular phenotype and the affected gene involved in human and mouse cataracts [26]. Even the same allelic mutation can lead to phenotypically different types of cataracts, as in the present case [27].

Based on tight association of cataract and microcornea in the present family, and on the basis of expression of *CRYAA* in the anterior eye segment as well, it appears that apart from the lens, αA-crystallins might also play a role in the normal development of the anterior segment of the eye. Interestingly, Graw et al. [28] reported a dominant mutation in the mouse *Cryaa* gene, showing nuclear cataract in association with microphthalmia due to the replacement of Val with Glu at codon 124 (V124E), which is close to the human mutation at amino acid position 116.

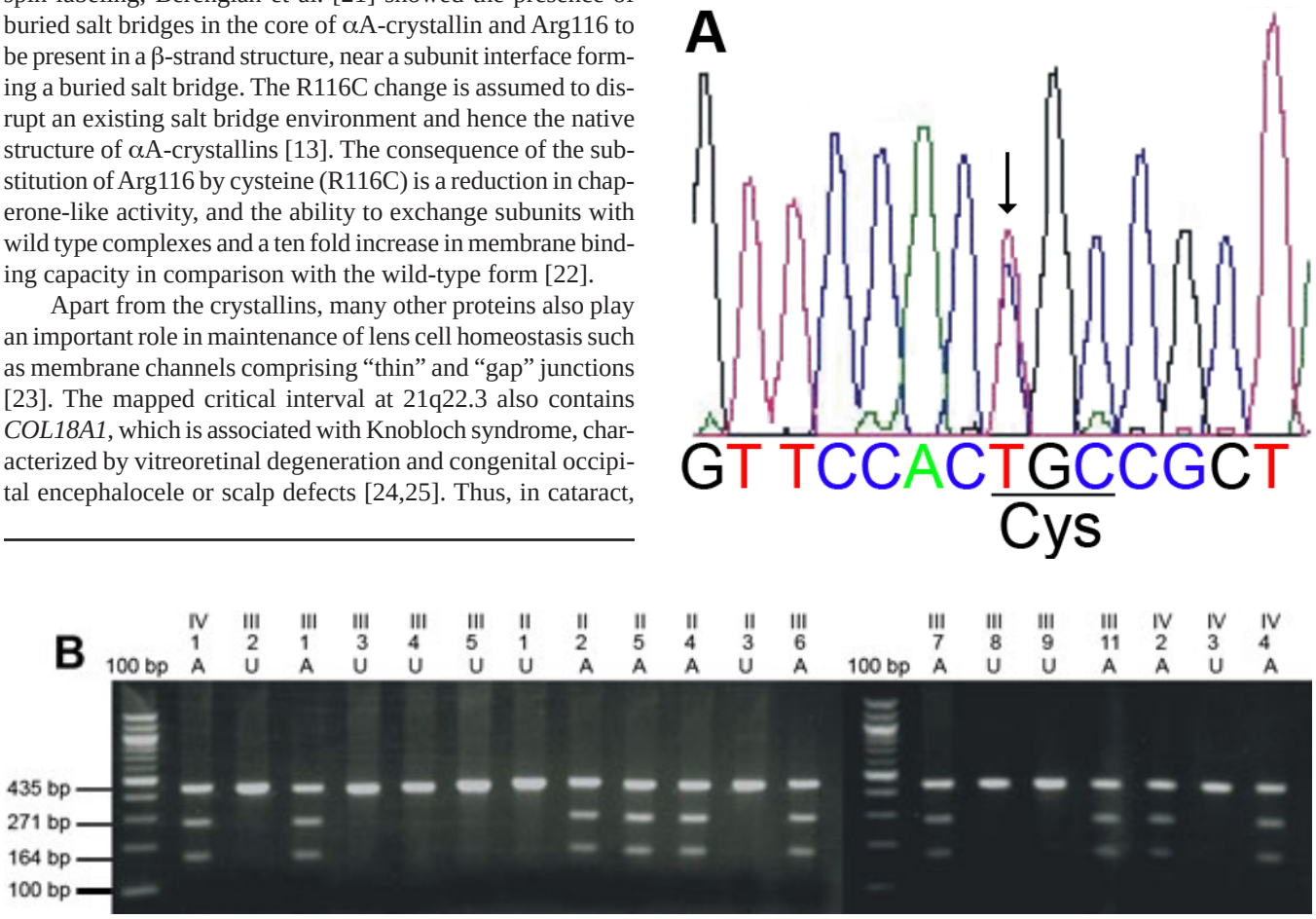

Figure 3. DNA sequence and restriction endonuclease analysis of *CRYAA* in an affected individual. **A**: Electropherogram showing a part of *CRYAA* sequence (forward strand) of an affected member (IV-1; Figure 1). A heterozygous C>T substitution at the first base of codon 116 (CGC-TGC), resulting in substitution of arginine-116 by cysteine (R116C) in the affected individual’s sequence is indicated by an arrow. **B**: Restriction endonuclease analysis shows creation of a novel restriction enzyme site for *Bts*I because of the C>T substitution in codon 116 of *CRYAA*. Affected individuals (marked A and with numbers at top as given in pedigree; Figure 1) show three bands following restriction digestion with *Bts*I (164 bp, 271 bp, and an undigested band of 435 bp) on 2.2% agarose gel. All the unaffected members (marked as U and with individual numbers at top) show only a single undigested band of 435 bp. The first and forteenth lanes show a 100 bp molecular weight marker.

In summary, this study describes a novel "fan-shaped" cataract phenotype with microcornea in all affected members, linked to an α-crystallin (*CRYAA*) gene. Furthermore, the association of R116C mutation in *CRYAA* with zonular type cataract and fan-shaped cataract in a United States and an Indian family, respectively, highlights the phenotypic heterogeneity of congenital cataracts.

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