Genetic Heterogeneity for Recessively Inherited Congenital Cataract Microcornea with Corneal Opacity

Kamron Khan,^{1,2,3} Ahmed Al-Maskari,^{1,2,3} Martin McKibbin,^{1,2} Ian M. Carr,¹ Adam Booth,^{1,4} Moin Mohamed,^{1,5} Salina Siddiqui,^{1,2} James A. Poulter,¹ David A. Parry,¹ Clara V. Logan,¹ Anwar Hashmi,⁶ Tehseen Sabi,⁶ Hussain Jafri,⁷ Yasmin Raashid,⁸ Colin A. Johnson,¹ Alex F. Markham,¹ Carmel Toomes,¹ Aine Rice,^{1,2} Eamonn Sheridan,¹ Chris F. Inglehearn,¹ and Manir Ali¹

PURPOSE. To investigate whether three consanguineous families from the Punjab province of Pakistan, with affected members with recessively inherited congenital cataract microcornea with corneal opacity, are genetically homogeneous.

METHODS. An ophthalmic examination was performed on each family member to establish the diagnosis. The two largest families were analyzed by homozygosity mapping using SNP arrays. Linkage was confirmed using polymorphic microsatellite markers, and logarithm of odds (LOD) scores were calculated. Candidate genes were prioritized using the ENDEAVOUR program.

RESULTS. Autosomal recessive congenital cataract-microcornea with corneal opacity mapped to chromosome 10cen for family MEP57 and to either chromosomes 2ptel or 20p for family MEP60. For MEP57, the refined interval was 36.8 Mb flanked by D10S1208 (35.3 Mb) and D10S676 (72.1 Mb). For MEP60, the interval containing the mutation was either 6.7 Mb from the telomere of chromosome 2 to marker D2S281 or 3.8 Mb flanked by D20S906 (1.5 Mb) and D20S835 (5.3 Mb). Maximum multipoint LOD scores of 3.09, 1.94, and 3.09 were calculated at D10S676, D2S281, and D20S473 for families MEP57 and MEP60. Linkage to these loci was excluded for family MEP68. *SLC4A11* was excluded as a candidate gene for the observed phenotype in MEP60.

CONCLUSIONS. The authors have identified two new loci, one on chromosome 10cen and the other on 2ptel or 20p, that are associated with recessively inherited congenital cataract-microcornea with corneal opacity. This phenotype is genetically heterogeneous in the Pakistani population. Further genetic studies of this kind, combined with a detailed phenotypic description, will contribute to more precise classification criteria for anterior segment disease. (*Invest Ophthalmol Vis Sci*. 2011;52:4294–4299) DOI:10.1167/iovs.10-6776

Congenital cataracts are a major cause of childhood blindness in the developing world.^{1,2} There are many causes, and approximately one-third have a genetic basis. Most of the inherited forms that have been described are inherited in an autosomal dominant manner, though autosomal recessive and X-linked cases have also been reported.³ To date, 39 genetic loci have been mapped and 26 genes have been identified.⁴ The severity and morphology of congenital cataracts can vary between families even with the same mutation, reflecting differences due to genetic background and environmental factors. Cataracts can exist in isolation, alongside other ocular anomalies, or as part of a syndrome with systemic features. Microcornea is one of the ocular abnormalities that can occur with congenital cataract, highlighting the close interrelationship between the lens and the cornea during development.⁵ Corneal opacification is also another feature that can sometimes accompany this developmental abnormality.⁶ Genetic heterogeneity of the microcornea-cataract phenotype with dominant inheritance (CCMS; Mendelian Inheritance in Man [MIM] #116150) has already been documented.⁷ However, this combination of phenotype with corneal opacity and recessive inheritance has not been reported before. Reports on cataracts have tended to overlook corneal diameters and have instead focused on the sight-threatening cataract. For recessively inherited nonsyndromic congenital cataracts, mutations in seven genes have been implicated, and five other loci have been mapped. These are *CRYAA* (MIM +123580),⁸ *CRYBB1* (MIM +600929),^{9,10} *CRYBB3* (MIM *123630),¹¹ *HSF4* (MIM *602438),^{12,13} *GJA8* (MIM +600897),¹⁴ *GCNT2* (MIM *600429)¹⁵ and *LIM2* (MIM +154045)¹⁶ together with loci at 1p34 (MIM %612968),¹⁷ 3p22 (MIM %610019),¹⁸ 7q,¹⁹ 9q13 (MIM %605749)²⁰ and 19q13 (MIM %609376).²¹

Congenital corneal opacifications can either be primary, affecting the cornea alone, or they can be secondary, associated with anterior segment abnormalities.⁶ Secondary corneal opacities without nonocular manifestations are often diagnosed as Peters' anomaly and represent kerato-irido-lenticular dysgenesis as well as developmental anomalies of the iridotrabecular system. Some of these cases can be caused by mechanical damage or infectious agents, whereas others have a genetic

From the ¹Leeds Institute of Molecular Medicine, Leeds, United Kingdom; ²Eye Department, St. James's University Hospital, Leeds, United Kingdom; ³Peninsula Medical School, Plymouth, United Kingdom; ⁴St Thomas' Hospital, London, United Kingdom; the Departments of ⁵Ophthalmology and ⁶Obstetrics and Gynaecology, King Edward Medical University, Lahore, Pakistan; and ⁷Gene Technology Laboratory 146/1, Lahore, Pakistan.

³These authors contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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Corresponding author: Manir Ali, Leeds Institute of Molecular Medicine, Section of Ophthalmology and Neuroscience, Wellcome Trust Brenner Building, St. James's University Hospital, Leeds LS9 7TF, UK; medma@leeds.ac.uk.

FIGURE 1. Anterior segment images of affected members 2316 (aged 16 years) and 2318 (aged 8 years) from MEP60. Note that corneal opacity appears to be due to the sclera encroaching on the cornea. Given that 2316 has exotropia and 2318 shows esotropia, the strabismus is likely to be secondary to the underlying abnormality. The clinical descriptions are presented in Table 1.

basis. For example, dominant mutations in *FOXE3* (MIM *601094) have been described in patients with Peters' anomaly who have corneal opacity associated with cataract.^{22,23} Note that recessive *FOXE3* gene mutations give rise to a severe ocular phenotype, with patients having primary congenital aphakia and total sclerocornea.^{24,25} Other examples of the genetic basis of Peters' anomaly include recessive mutations in *CYP11B1* (MIM *601771), known to cause primary congenital glaucoma,^{26,27} and dominant mutations in *PAX6* (MIM *607108), known to be a cause of aniridia.^{28,29}

Here we present three consanguineous families from the Punjab province of Pakistan who have affected members with a general diagnosis of nonsyndromic congenital cataract microcornea with corneal opacity, which we have abbreviated to CCMCO. These are shown to be genetically heterogeneous, implicating two novel loci for this condition and proving the existence of yet further loci.

PATIENTS AND METHODS

Patients

The study was performed using a process approved by the Pakistan Medical Foundation in Lahore and the Leeds Research Ethics Committee. Participants gave their informed consent, in accordance with the tenets of the Declaration of Helsinki. Three multigenerational consanguineous pedigrees with a family history of poor vision were examined (Fig. 1, Table 1). All affected members had variable corneal opacities present from birth or very early in life, and each family history suggested that these changes were not progressive. They also had horizontal corneal diameters of <11 mm. Where a clinical view was possible, it showed cataract of the lens and a normal fundus appearance. The patients did not have any systemic problems or neurologic abnormalities. These patients fit broadly into a diagnosis of CCMCO. A

TABLE 1. Clinical Features of the Affected Patients Examined from the Three Consanguineous Families

Family	Member	Microcornea OD; OS (mm)	Cornea		Globe		Iris				Lens		Function	
			Vascularization	Opacity	Microphthalmia	Iridocorneal Adhesion	Iris Coloboma	Iridolenticular Adhesions	Iris Hypoplasia	Keratolenticular Touch	Cataract	Nystagmus	Vision	
MEP57	2298	+	—	c ^ p	+	p	—	—	—	—	?	+	PL	
	2299	+	—	c ^ p	+	p	—	—	—	—	?	+	PL	
	2301	+	—	c ^ p	+	p	—	—	—	—	?	+	PL	
	2303	+	—	c ^ p	+	p	—	—	—	—	?	+	PL	
MEP60	2316	7; 7	—	p > c	—	p	—	—	—	—	+	—	CF	
	2318	7; 8	—	p > c	—	p	—	—	—	—	+	—	CF	
	2319	+	+	p > c	—	p	—	—	—	—	+	+	CF	
	2362	+	+	p only	—	p	—	—	—	—	+	+	CF	
MEP68	2362	+	+	p only	—	p	—	—	—	—	+	+	CF	
	2363	+	+	p only	—	p	—	—	—	—	+	+	CF	

All patients had normal results on fundus examination except those from MEP57, in whom dense corneal opacity precluded retinal viewing. +, present; —, absent; ?, no clinical view due to anterior opacity; c, central; p, peripheral; CF, counting fingers; PL, perception of light.

blood sample was taken from both affected and unaffected family members, and genomic DNA was extracted according to standard procedures.

Homozygosity Mapping

DNA from affected persons in the two largest families, MEP57 and MEP60, were genotyped using single nucleotide polymorphism array (SNP; Affymetrix 6.0; AROS Applied Biotechnologies, Aarhus, Denmark). Regions of homozygosity were highlighted using identification software.³⁰

Linkage Analysis

Linkage was confirmed by analysis with fluorescence-labeled polymorphic microsatellite markers on a genetic analyzer (3130xl Genetic Analyzer; Applied Biosystems, Warrington, UK) using genotyping software (GeneMapper version 4.0; Applied Biosystems). LOD scores were calculated using multipoint analysis with the program SUPERLINK (<http://bioinfo.cs.technion.ac.il/superlink-online/>) assuming equal allele frequencies, a mutant allele frequency of 0.001, and 100% penetrance of mutant alleles with a recessive mode of inheritance.

DNA Sequencing

Specific primer pairs for the amplification of the coding regions and for the exon-intron boundaries of the *SLC4A11* gene on chromosome 20p have been described elsewhere.³¹ PCR products were digested (ExoSAP-IT; GE Healthcare, Chalfont, St. Giles, UK) and sequenced (BigDye Terminator, version 3.1, Cycle Sequencing Kit; Applied Biosystems) on the genetic analyzer (3130xl Genetic Analyzer; Applied Biosystems) according to the manufacturer's instructions.

Bioinformatic Analysis of Candidate Genes

Genes within the refined intervals were viewed as RefSeq Genes, which shows protein-coding and non-protein coding genes from the NCBI RNA reference sequences collection,³² using the human February 2009 assembly (hg19) of the UCSC Genome Browser (<http://genome.ucsc.edu/>).

Candidate genes were prioritized using the program ENDEAVOUR (<http://homes.esat.kuleuven.be/~bioiuser/endeavour/index.php>).³³ This software ranks the candidate genes based on their similarity, in terms of biological processes or diseases, to a set of training genes. The training genes—*BCOR*, *ERG*, *FOXC1*, *FOXE3*, *GJA1*, *GJA8*, *HCCS*, *PAX6*, *PITX3*, *RAX*, and *SOX2*—selected in our study were generated using the keywords cataract, microcornea, and sclerocornea.

Information regarding the top-ranked candidate genes and proteins was summarized using the GeneCards version 3 (<http://www.genecards.org/>) and Entrez (<http://www.ncbi.nlm.nih.gov/sites/entrez>) Web sites.

RESULTS

We examined three consanguineous families from the Punjab province of Pakistan with multiple affected members who had impaired vision (Fig. 1, Table 1). Microcornea and corneal opacification, caused by the sclera encroaching on the cornea, were the predominant and defining features for these families, with variable degrees of expression both between families and between persons of the same family. The ocular abnormalities were either severe enough to be noted by family members at birth or became apparent within the first year of life and did not progress thereafter. Where clinically visible, the lenses were cataractous but had normal contour and size. Eyes of affected persons had normal fundus appearance on indirect ophthalmoscopy and did not demonstrate any optic nerve or retinal colobomata or pigmentary retinopathy. We were unable to describe the lens or retinal status of family MEP57 because of the degree of corneal opacity and iris distortion from peripheral iridocorneal contact. As a group, however, the ante-

rior chambers were of near normal depth with no central keratolenticular touch or any obvious anterior capsular abnormality. These findings were consistent with a diagnosis of CCMCO, this being a description of the predominant features.

The absence of any obvious anterior segment abnormalities in the parents of the affected patients, the evident consanguinity, and the presence of multiple affected siblings within a family suggested a recessive pattern of inheritance. Therefore, we carried out whole genome SNP genotyping on genomic DNA from affected members of the two largest pedigrees, MEP57 and MEP60, to highlight regions of homozygosity. In MEP57, a single region of homozygosity was identified, but for MEP60, two regions were found. These regions were confirmed by linkage analysis using microsatellite markers.

For family MEP57, a shared homozygous region at chromosome 10 was identified in patients that segregated with the disease phenotype, as expected, in a recessive manner (Fig. 2). A maximum LOD score of 3.09 was established for the marker D10S567. The refined locus extends from D10S1208 (35.3 Mb) to D10S676 (72.1 Mb) and encompasses a 36.8-Mb (26.7-cM) interval that includes the centromere and contains 160 genes (Supplementary Fig. S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6776/-DCSupplemental>).

These genes were prioritized using ENDEAVOUR (Supplementary Table S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6776/-DCSupplemental>). The three top-ranked genes were *EGR2* (MIM *129010), *RET* (MIM +164761), and *NEUROG3* (MIM *604882). *EGR2* is a zinc finger transcription factor from the early growth response family important for the induction of cell differentiation, cell proliferation, and cell death in response to environmental stimuli. *EGR2* has been shown to be expressed in neural crest-derived Schwann cells that accompany peripheral nerves, and the presence of these cells has been demonstrated at the corneal limbus.³⁴ Defects in this gene are associated with various neuropathies.³⁵ *RET* is a member of the cadherin superfamily that encodes a receptor tyrosine kinase for transducing signals for cell growth and differentiation. *RET* plays a crucial role in neural crest development, and mutations in this gene are associated with multiple endocrine neoplasia type II that can have a corneal phenotype.³⁶ *NEUROG3* is a basic helix-loop-helix transcription factor that is expressed in the central and peripheral nervous system. *NEUROG3* has been shown to regulate retinal neurogenesis by inducing genes for early-born neurons while repressing those for later-born neurons.³⁷ Defects in this gene are a cause of congenital malabsorptive diarrhea.³⁸

For family MEP60, shared homozygous regions were identified on chromosomes 2ptel and 20p in the affected persons (Fig. 3). Maximum LOD scores of 1.94 and 3.09 were established for markers D2S281 and D20S473, respectively. The pathogenic mutation in this family lies either in a 6.7-Mb (14.1-cM) region extending from 2pter to microsatellite marker D2S281 and containing 20 genes (Supplementary Fig. S2A, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6776/-DCSupplemental>) or in a second 3.8-Mb (7.5-cM) interval spanning D20S906 (1.5 Mb) to D20S835 (5.3 Mb) and containing 55 genes (Supplementary Fig. S2B, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6776/-DCSupplemental>), including the *SLC4A11* gene (MIM *610206) (at 3.2 Mb). *SLC4A11* mutations have been described in patients with the anterior segment abnormality CHED2 (corneal hereditary endothelial dystrophy 2) (MIM #217700), which affects the cornea alone.³¹ DNA analysis of the coding sequence of the *SLC4A11* gene in affected members of the MEP60 pedigree failed to identify a pathogenic mutation, suggesting that another gene is likely to harbor the defect seen in this family.

These candidate genes from both regions were combined and prioritized using ENDEAVOUR (Supplementary Table S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6776/-DCSupplemental>). The three top-ranked genes were

FIGURE 2. Genetic analysis of the MEP57 pedigree. **(A)** Pedigree structure for MEP57. **(B)** Confirmatory microsatellite analysis around the locus on chromosome 10cen. The physical distance for each marker is based on the human February 2009 assembly (hg19) of the UCSC Genome Browser. The homozygous region in each of the affected members is highlighted in *gray*. ND represents no data available. Allele sizes are shown in base pairs. The multipoint LOD score for each marker is also represented.

SOX11 (MIM *600898), *MYT1L* (MIM *613084), and *PRNP* (MIM *176640). *SOX11* is an SRY-related HMG-box transcription factor that is abundantly expressed in the embryonic central and peripheral nervous system. Other Sox family members are important in lens development.³⁹ *MYT1L* encodes a neural-specific, zinc finger-containing transcription factor that plays a role in embryonic stem cell differentiation into functional neurons.⁴⁰ *PRNP* encodes the prion protein, a membrane glycosylphosphatidylinositol-anchored glycoprotein that tends to aggregate into rodlike structures. Mutations in this gene have been associated with Creutzfeldt-Jakob disease, fatal familial insomnia, Gerstmann-Straussler disease, Huntington diseaselike 1, and kuru. No specific ocular expression has been described, making this less likely to be involved in anterior segment embryogenesis.

The recessively inherited CCMCO phenotype observed in a third Pakistani family, MEP68, was excluded from the loci on chromosomes 10cen, 2ptel, and 20p, with significant negative LOD scores across all the regions, suggesting that there is at least one other locus to be identified for this condition (Fig. 4).

DISCUSSION

We studied consanguineous families from the Punjab province of Pakistan segregating a recessive form of kerato-irido-lenticular dysgenesis, which we have called congenital cataract microcornea with corneal opacification, or CCMCO. We expected that these families, having the same condition and being of the same ethnic and geographic origins, might have a mutation or mutations in the same gene, but instead we found considerable genetic heterogeneity associated with this phenotype. We describe new genetic loci implicated in this phenotype on chromosomes 10cen and either 2ptel or 20p. Candidate gene screening is underway to try to identify the pathogenic mutations. We also excluded linkage at these loci in a third family, suggesting further locus heterogeneity. As a consequence of this study, we can now offer accurate genetic counseling to the MEP57 family. Given that the detection of carriers is now possible and that consanguineous marriages are the cultural norm in these communities, we could, if the family

FIGURE 3. Genetic analysis of the MEP60 pedigree. **(A)** Pedigree structure for MEP60. **(B)** Confirmatory microsatellite analysis around the loci on chromosomes 2p and 20p. The physical distance for each marker is based on the human February 2009 assembly (hg19) of the UCSC Genome Browser. The homozygous region in each of the affected members is highlighted in *gray*. Allele sizes are shown in base pairs. The multipoint LOD score for each marker is also represented. ND, no data available.

FIGURE 4. Genetic analysis of the MEP68 pedigree. **(A)** Pedigree structure for MEP68. **(B)** Microsatellite genotyping for the regions on chromosomes 2ptel, 10cen, and 20p. Note the absence of linkage for affected members with CCMCO (highlighted in gray). Allele sizes are shown in base pairs. The multipoint LOD score for each marker is also represented.

desired, provide guidance on reducing the risk for more blind offspring in subsequent generations.

In terms of providing a clinical diagnosis, corneal opacification can sometimes prevent an accurate assessment of the underlying anterior segment structures (e.g., MEP57). This can usually be circumvented with the use of ultrasound biomicroscopy or optical coherence tomography. However, such techniques were not available in the rural setting in which these families lived, therefore considerable care was taken in describing the visible abnormalities as accurately as possible while accepting that some clinical details could not be commented on. An accurate clinical diagnosis is important for any subsequent genotype-phenotype correlations but may also have clinical implications. For instance, congenital cataracts associated with other forms of anterior segment dysgenesis carry an increased risk for glaucoma.⁴¹

By studying recessively inherited CCMCO, we hope to identify important proteins essential for normal function or structural development of the eye. It is not surprising that mutations in genes that lead to cataract formation can also lead to abnormalities of the anterior segment, since the development of the lens and the anterior segment structures are closely interdependent. The lens forms from surface ectoderm that invaginates to produce the lens vesicle, whereas the anterior segment structures are produced primarily from neural crest-derived cells, with the mesoderm and neuroectoderm also involved.^{5,42} However, lens transplantation studies have suggested that factors secreted by the anterior lens induce differentiation of the cornea and iris.^{43,44} Corneal opacity in the patients described in this article either could be secondary to a cataractous, but otherwise normally sized and shaped, lens or it could represent a primary phenotype independent of the lens cataract given that many severe cataracts are not associated with corneal abnormalities. Further studies of this condition will not only expand our knowledge of ocular disease pathogenesis but will also contribute to a more accurate classification of anterior segment conditions based on genotype-phenotype correlations.

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