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SHORT COMMUNICATION

Refinement of the locus for autosomal recessive cone–rod dystrophy (CORD8) linked to chromosome 1q23–q24 in a Pakistani family and exclusion of candidate genes

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Abstract Cone–rod retinal dystrophy (CORD) characteristically leads to early impairment of vision due to the simultaneous involvement of both cone and rod photoreceptor cells. Several loci/genes have been identified for CORD, including the cone–rod dystrophy (CORD8) locus [OMIM#605549] identified for a Pakistani family. All members of this family underwent detailed clinical re-examination to determine the nature of the dystrophy. All affected individuals suffered from bilateral CORD8 with an autosomal recessive mode of inheritance. The CORD8 locus, mapped on chromosome 1q12–q24, consisted of a very large critical disease region of 21 cM. Analysis with more recently available microsatellite markers within the reported region showed heterozygosity with some of the new markers, and the crossovers lead to a refinement of the disease region from 21 to 11.53 cM. Mutation screening has excluded some of the candidate genes in the region. The disease phenotype of this family could be due to a mutation in a novel gene located within the refined CORD8 locus.

Keywords CORD8 · Phenotype · Cone–rod dystrophy · Visual acuity · Retinal dystrophy

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Introduction

Cone–rod retinal dystrophies (CORD) are characterized by the simultaneous involvement of both cone and rod photoreceptor cells (Berson et al. 1968; Heckenlively 1987; Moore 1992). Patients are affected from an early age and there is a progressive loss of visual acuity and color vision followed by night blindness and loss of peripheral vision. Later in life, vision is often reduced to the bare perception of light. In the early stages, typical fundus changes are macular atrophy and pigmentation, which become widespread as the disease progresses. Genetic studies have so far identified seven loci for cone–rod dystrophies on chromosomes 17q (Klystra and Aylsworth 1993), 19q (Evans et al. 1994), 18q (Warburg et al. 1991), 17p13 (Balciuniene et al. 1995; Payne et al. 1999), 6q (Kelsell et al. 1998a), 1q12 (Khaliq et al. 2000) and 8p11 (Danciger et al. 2001). Mutations in the *peripherin/RDS* (Nakazawa et al. 1994), *CRX* (Freund et al. 1997; Swain et al. 1997), *RetGC-I* (Kelsell et al. 1998b), *ABCR* (Cremers et al. 1998), *CNGA3* (Wissinger et al. 2001), *RPGRIP1* (Hameed et al. 2003) and *SEMA4A* (Abid et al. 2006) genes have been shown to cause both autosomal dominant and recessive cone–rod dystrophies.

We previously reported the mapping of an autosomal recessive CORD8 locus on chromosome 1q12–q24 in a consanguineous Pakistani family (Khaliq et al. 2000). The maximum LOD score, Z=4.22, was obtained for the marker D1S2635. Positive LOD scores

ranging from 2.13 to 3.97 at θ=0.00–0.20 were also obtained with several other markers. Recombination events defined the proximal and distal boundaries of the disease locus between markers D1S457 and D1S2681, respectively. All patients of the CORD8 family were homozygous for markers D1S498, D1S2635, D1S2771, D1S484 and D1S2768 that defined a very large region of approximately 21 cM. With the availability of several new markers in this region we have now refined the CORD8 critical disease interval to 11.53 cM. Important clinical observations are also reported in detail.

Materials and methods

One of the authors (KA) examined all of the patients and their unaffected family members (Fig. 1). Previous histories and clinical details were available for each patient from their childhood records. Fundoscopic examination using a Topcon (Tokyo, Japan) TRC 50× retinal camera was performed for all individuals affected. ERGs were recorded using LKC Technologies (Gaithersburg, MD, USA) EPIC 200 equipment.

For genotyping, polymorphic microsatellite markers were selected from the NCBI (http://www.ncbi.

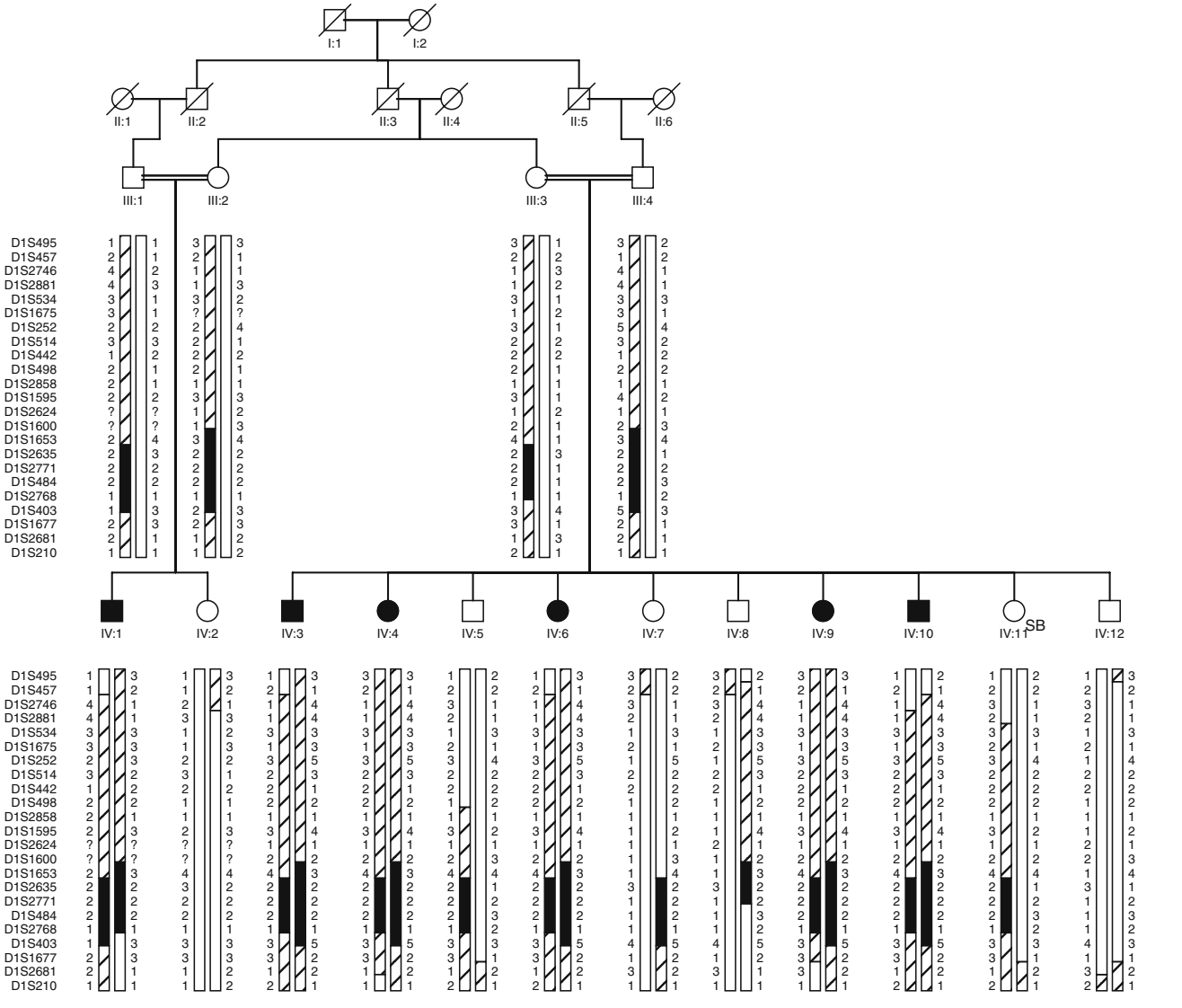

Fig. 1 Pedigree of the Pakistani autosomal recessive cone–rod dystrophy (CORD8) family with their genotypes. *Filled symbols* indicate affected individuals, *open symbols* indicate unaffected individuals, *hatched bars* indicate disease chromosomes and *filled regions* indicate disease-associated haplotypes

Table 1 Clinical details for the affected individuals in the Pakistani CORD8 family

Subjects	IV:3	IV:4	IV:9	IV:10
Age when examined (years)	44	42	32	30
Age of onset of disease (years)	12–14	12–16	12–14	12–15
Photophobia	+++	++	+++	+++
Epiphora	+++	+++	+++	+++
Loss of color vision	+++ Since childhood	+++ Since childhood	+++ Since childhood	+++ Since childhood
Visual acuity	PL+ HM+ Both eyes	Right PL+ Left PL–	PL– Both eyes	Counting fingers both eyes
Attenuation of arterioles	+++	+++	+++	+++
Ant. segment	Normal	Normal	–	Normal
Cornea	Clear	Clear	Keratoconus	Clear
Pupillary reaction	Sluggish	Less than normal	Poor	Less than normal
Choroidal vessels sclerosis	Visible	–	–	Not marked
Macular degeneration	+++	++	+++	+
Pigmentary distribution	Center+++ Periphery++	Center+++ Periphery+++	Center+++ Periphery++	Center++ Periphery+
Optic disc	Pale and waxy	Pale and waxy	Pale and waxy	Pale and waxy
Cataract	–	–	Posterior subcapsular changes	–

HM hand movements, *PL* perception of light

nlm.nih.gov), GDB (http://www.gdb.org) and Marshmed (http://www.research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html) genetic databases. Primers were synthesized for each microsatellite marker using an ABI 392 DNA/RNA synthesizer. Genomic DNA was amplified by polymerase chain reaction (PCR) and the products were separated on polyacrlyamide gels as described previously (Khaliq et al. 2000).

Mutation screening of the candidate genes *HPRP3* and *SEMA4A* in carriers was performed by direct DNA sequencing using the ABI377 DNA sequencer and the Prism Ready Reaction Sequencing Kit (PE-ABI). The sequences for specific *SEMA4A* gene primers used for mutation screening were the same as described previously (Abid et al. 2006). *HPRP3* gene primer sequences are given in Table 1 of the supplementary material.

Results and discussion

The clinical details (Table 1)revealed that the affected individuals had been suffering from loss of color vision, severe photophobia and epiphora in bright light since childhood. They usually preferred to stay in dimly lit environments and had suffered from progressive deterioration of vision since childhood. However, vigorous deterioration in central vision started at the age of 12 and there was a rapid loss of vision between the ages of 14 and 16 years.

Visual acuity among the patients varied from the bare perception of light, hand movement and counting fingers to complete blindness. Figure 2A–C shows the representative fundus photographs of selected patients. They reveal a high degree of fundus granularity and marked macular degeneration. Significant levels of attenuated arterioles with bony corpuscle types of retinal pigment epithelium disturbances were observed in all patients. Macular degeneration with choroidal sclerosis was prominent in patient IV:3 (Fig. 2A). This patient had typical RP bony spicule pigmentation along with sever maculopathy in both eyes. Peripheral pigmentary changes in individual IV:4 (Fig. 2B) were web-shaped. Pigmentary changes in the peripheral area were less marked in individual IV:10 (Fig. 2C). The anterior segment was normal in all affected individuals except in IV:9, who had keratoconus in both eyes with central scarring around the apex of the keratoconus and corneal thinning at the apex (Fig. 2D). The optic discs were pale and waxy in appearance, but intraocular pressure was normal in all affected individuals. Both photopic and scotopic full-field ERG amplitudes were extremely low, demonstrating the involvement of both cone and rod photoreceptors.

In order to narrow down the disease region, various databases were searched in order to identify polymorphic microsatellite markers within the distal and proximal ends of the homozygosity region. Five polymorphic microsatellite markers (D1S2858, D1S1595, D1S2624, D1S1600 and D1S1653) were selected at the proximal end between markers D1S498 and D1S2635. Three markers (D1S2844, D1S403 and D1S1677) were selected at the distal end between D1S2768 and D1S2681. Crossovers at the proximal end between

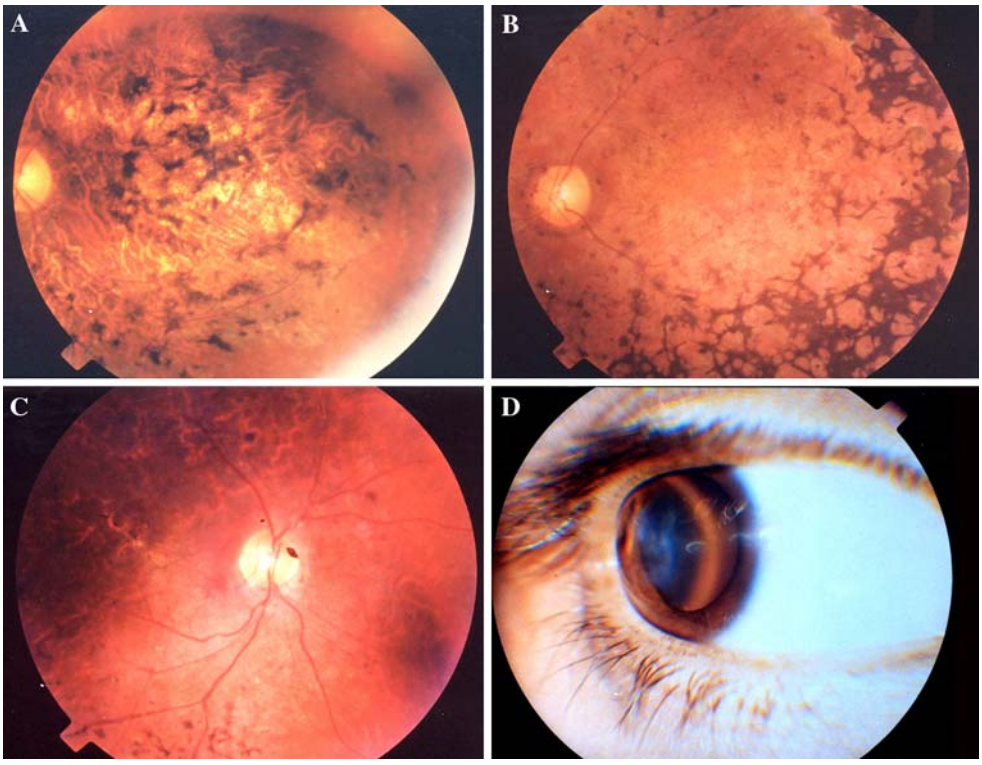

Fig. 2A–D Representative fundus photographs of affected individuals, IV:3 (**A**), IV:4 (**B**), IV:10 (**C**). Side view of the eye of individual IV:9 (**D**) showing *cone shaped* cornea (keratoconus)

markers D1S1600 and D1S1653 led to a reduction of 9.73 cM, while crossovers at the distal end with marker D1S1677 resulted in a further reduction of 3.48 cM. Thus the new critical disease region for CORD8 is flanked by microsatellite markers D1S1653 and D1S403, reducing it from 21 to 11.53 cM.

It has recently been shown that mutations in Semaphorin (*SEMA4A*) are associated with retinal degenerative diseases including cone–rod dystrophies (Abid et al. 2006). Therefore, bidirectional DNA sequencing for mutation screening of *SEMA4A* was performed using flanking intronic primers. No disease-associated gene mutation was found in *SEMA4*. Similarly, mutations in the human ortholog of the yeast pre-mRNA splicing factor 3 (*HPRP3*) have been shown to cause retinitis pigmentosa 18 (RP18) [OMIM#601414] (Chakarova et al. 2002) and this locus overlaps CORD8. Sequence analysis of *HPRP3* did not show any change in the patients. Another three genes *GNAT2, CRABP2* and *KCNJ10* within and around the disease region were excluded in a previous study (Khaliq et al. 2000). This suggests that another novel gene in the refined region may be responsible for the disease.

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