even though twins in this subgroup are more likely to exhibit birth-weight differences (C. Derom, unpublished data). Of course, asymmetric splitting might occur occasionally but result in one or both fetuses being nonviable. Interestingly, there is marked female predominance in the MA-twin group, with a sex proportion of .23 (Derom et al. 1988). The reasons for this are unclear. It appears that female embryos are relatively delayed in early embryonic development (Pergament et al. 1994). Thus, female embryos could be somewhat less mature at the time of formation of the amnion, and thus splitting of female embryos may be more compatible with survival at this stage. The delay in early female development has been ascribed to the absence of a Y chromosome (Pergament et al. 1994). However, the process of X inactivation, since it may occur when there are ⩽10 cells in the embryo (Puck et al. 1992; Monteiro et al. 1998), might itself contribute to a slight delay in early female-embryo development.

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Refinement of the Locus for Autosomal Recessive Retinitis Pigmentosa (RP25) Linked to Chromosome 6q in a Family of Pakistani Origin

To the Editor:

“Retinitis pigmentosa” (RP) is the term used to define a clinically and genetically heterogeneous group of retinal degenerations primarily affecting the rod photoreceptors. RP is characterized by progressive loss of vision, initially manifesting as night blindness and reduction in the peripheral visual field and later involving loss of central vision (Bird 1995). Ophthalmoscopic examination typically reveals pigmentary disturbances of the mid-peripheral retina. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X-linked trait. Autosomal recessive RP (arRP) accounts for ~20% of all cases of RP, whereas sporadic RP, which is presumed to be recessive in most cases, accounts for a further 50% (Jay 1982).

Mutations causing arRP have been found in the genes encoding rhodopsin (Rosenfeld et al. 1992), in the α and β subunits of rod phosphodiesterase (Huang et al. 1995; McLaughlin et al. 1995), in the α subunit of the cyclic-GMP gated–channel protein (Dryja et al. 1995), and in the genes *RPE65* (Gu et al. 1997), *RLBP1* (Maw et al. 1997), *ABCR* (Martinez-Mir et al. 1998) and *TULP1* (Banerjee et al. 1998; Hagstrom et al. 1998). In addition, genetic linkage studies have identified arRP loci at 1q31-q32.1 (van Soest et al. 1994; Leutelt et al. 1995), 2q31-q33 (Bayes et al. 1998), and 16p12.1-p12.3 (Finckh et al. 1998). Recently, linkage of arRP to a region on chromosome 6q has been reported in several Spanish families (Ruiz et al. 1998). All the above are reference at the RetNet website.

We studied 20 members of a three-generation consanguineous Pakistani family in which RP segregated as an autosomal recessive trait (fig. 1). This pedigree contained 12 affected individuals. Examination of all af-

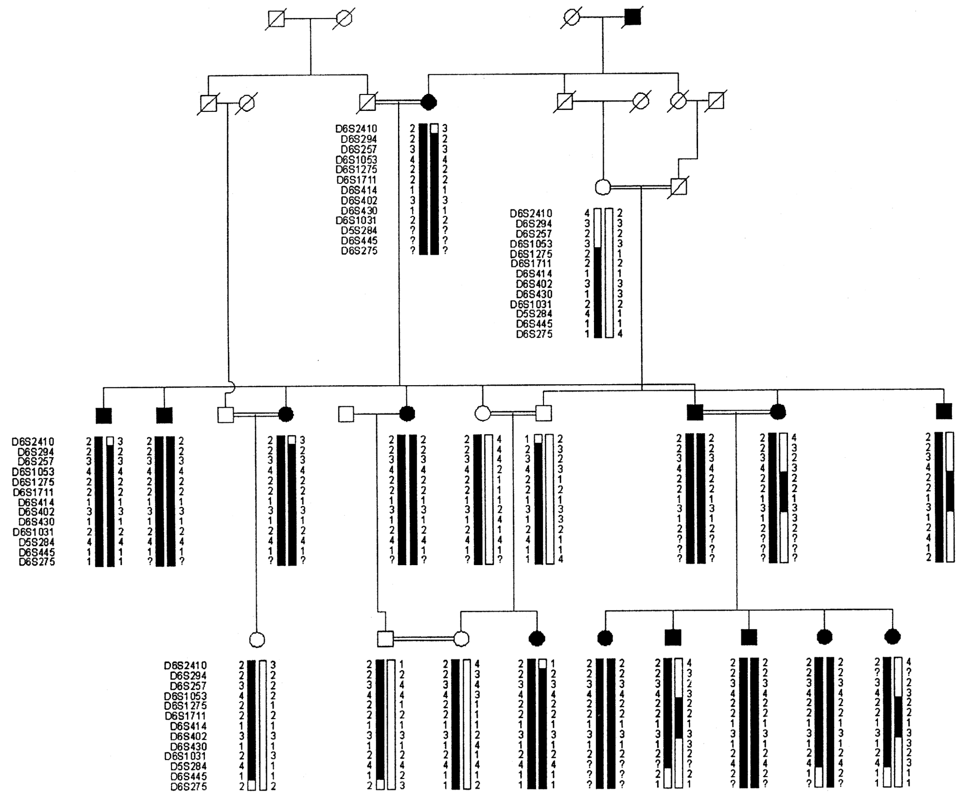

Figure 1 Family pedigree and haplotypes in the disease region of 6q. For each individual, the disease haplotype is shown as a blackened bar.

fected individuals revealed the typical clinical features of RP: pigmentary retinopathy associated with symptoms of night blindness and with the loss of peripheral visual fields. Affected subjects experienced night blindness, beginning at age ~25 years, and deterioration of visual acuity (central vision), beginning at age ~30 years. By age 55–60 years, many affected subjects had no perception of light in either eye.

Genomic DNA for linkage analysis was extracted from the peripheral blood of all 20 individuals in this pedigree (and from 50 unrelated normal individuals of Pakistani origin, who served as controls), by the Nucleon II extraction kit (Scotlab Bioscience). To identify the locus responsible for disease in this family, we performed homozygosity analysis. Genomic DNA from each individual was genotyped for microsatellite markers for all the known arRP loci (*RPE65* [1p31], *ABCR* [1p21-13], 1q31-q32.1, 2q31-q33, *RHO* [3q21-q24], *PDE6B*

Table 1

LOD-Score Calculations for Markers Used to Show Linkage in the Family

	LOD Score at θ =								
Marker	0	.01	.05	.1	.2	.3	.4	Z_{max}	θ_{max}
D6S2410	∞	1.38	1.95	2.03	1.74	1.17	.47	2.03	.1
D6S294	2.83	2.78	2.60	2.33	1.71	1.04	.37	2.83	0
D6S257	3.30	3.25	3.02	2.71	2.02	1.26	.48	3.30	0
D6S1053	3.30	3.25	3.01	2.69	1.98	1.21	.44	3.30	0
D6S1275	2.29	2.25	2.07	1.83	1.34	.82	.32	2.29	0
D6S1711	1.88	1.85	1.72	1.55	1.19	.77	.32	1.88	0
D6S402	1.88	1.85	1.72	1.55	1.19	.77	.32	1.88	0
D6S430	3.13	3.08	2.88	2.60	1.96	1.23	.47	3.13	0
D6S284	∞	−.52	.09	.26	.29	.19	.06	.29	.2
D6S445	∞	−3.06	−1.65	−1.03	−.43	−.14	−.02	−.02	.4
D6S275	∞	−.95	−.34	−.13	0	.01	.01	.01	.3

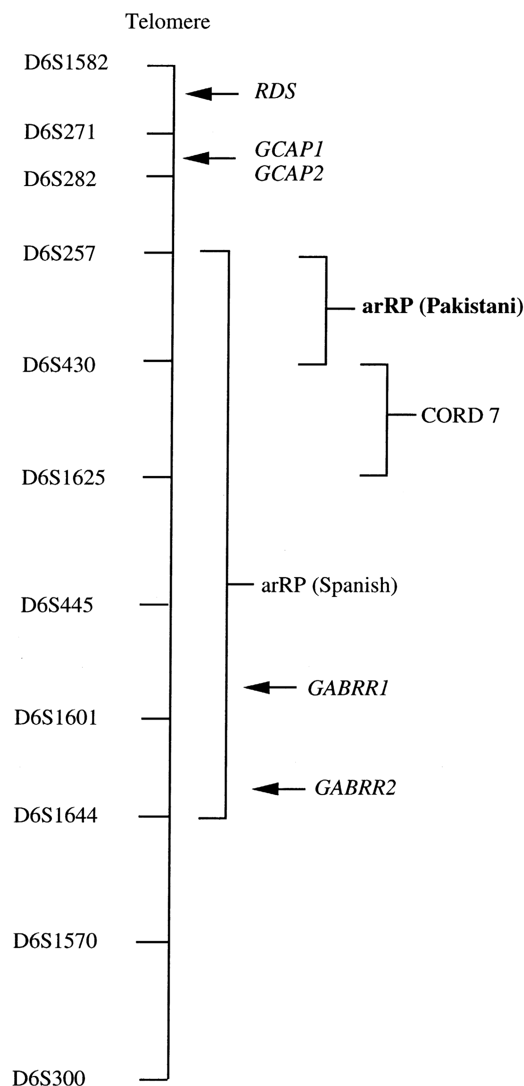

Figure 2 Boundaries of the chromosomal interval containing the 6q arRP gene, in relation to the critical intervals for other eye disorders mapping to this region. Our results indicate either that GABA-receptor candidate genes are no longer candidates for this phenotype or that there are in fact two arRP loci in close proximity on 6q.

[4p16.3], *CNGC* [4p14-q13], *PDE6A* [5q31.2-q34], *TULP-1* [6p21.3], 6cen-q15, *CRALBP* [15q26], and 16p12.1-p12.3). Subsequently, when evidence of linkage was obtained for the 6q locus, further polymorphic markers from this region were analyzed to determine whether analysis of recombinant individuals within the family would permit further refinement of the published disease interval. Marker order was determined from the Généthon sex-averaged genetic map (Dib et al. 1996). Primers were obtained from the MapPairs set (Research Genetics), or were synthesized commercially according to data from Genome Database.

PCR products were separated by nondenaturing PAGE (Protogel; National Diagnostics) and were visualized under UV illumination after being stained with ethidium bromide. Alleles were assigned to individuals, which allowed calculation of two-point LOD scores by the Cyrillic v2.01 (Cherwell Scientific) and MLINK software programs. Allele frequencies were calculated on the basis of data from the spouses in this family and from an ethnically matched control population. The phenotype was analyzed as an autosomal recessive trait, with complete penetrance and a frequency of .0001 for the disease allele.

Significant linkage initially was obtained for three markers on chromosome 6q (table 1). A maximum LOD-score (Z_{max}) value of 3.30 at recombination fraction (θ_{max}) 0 was observed for markers D6S257 and D6S1053 on chromosome 6q (table 1). Recombination events involving the centromeric marker D6S1053 and the telomeric marker D6S430 subsequently permitted refinement of the 6q arRP (RP25)-disease critical region, from the previously reported 16.1 cM (Ruiz et al. 1998) to 2.4 cM (fig. 1).

In their initial linkage report, Ruiz et al. (1998) have suggested, on the basis of a common haplotype for the region surrounding the GABA-receptor candidate genes (*GABRR1* and *GABRR2*) in two families with 6q-linked arRP, that the gene for 6q arRP lies in that region; they therefore have proposed the GABA-receptor candidate genes as candidates for this phenotype. The recombination events seen in the family that we studied exclude both *GABRR1* and *GABRR2* as the disease gene and exclude the area of homozygosity seen by Ruiz et al. (1998) from the critical disease interval.

Figure 2 shows the boundaries of the chromosomal interval containing the 6q arRP gene (RP25), in relation to the critical intervals for other eye disorders mapping to this region. If a single gene underlies arRP in both the Spanish families and the Pakistani family that we studied, then our results indicate that GABA-receptor candidate genes are no longer candidates for this phenotype. However, we cannot exclude the possibility that there are in fact two arRP loci located in close proximity on 6q, one in the Spanish population and one in the Pakistani population. There is no overlap between the locus that we report here and that of the dominant cone-rod dystrophy (CORD 7) (fig. 2).

The 2.4-cM critical interval defined by recombination events in the family that we studied contains no well-characterized candidate genes; however, four expressed sequence tags (ESTs) were identified from the human genome transcript map that are expressed in the retina, as were a further 29 ESTs that were of brain origin. These represent the best candidates available at this time. Further analysis of these cDNA clones will be needed before mutation screening in this family can be undertaken.

In the past, linkage to many of the loci identified as associated with RP (dominant or recessive) have been reported in single families. The identification of a family of Pakistani origin, in addition to the five Spanish families in which linkage to this locus has been reported by Ruiz et al. (1998), suggests that this may be an important gene in arRP, since the disease occurs in two different ethnic populations and in many different families that, according to haplotype analysis, are not ancestrally related.

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Electronic-Database Information

URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org/ (for primers)
Human Transcript Map, http://www.ncbi.nlm.nih.gov/SCIENCE96 (for retinal ESTs)
RetNet, http://www.sph.uth.tmc.edu (for linkage of arRP to chromosome 6q)

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