Locus for Autosomal Recessive Nonsyndromic Persistent Hyperplastic Primary Vitreous

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PURPOSE. To map the disease locus in a six-generation, consanguineous Pakistani family affected by nonsyndromic autosomal recessive persistent hyperplastic primary vitreous (arPHPV). All affected individuals had peripheral anterior synechiae and corneal opacities with variable degrees of cataract and a retrolenticular white mass behind the lens.

METHODS. Genomic DNA from family members was typed for alleles at more than 400 known polymorphic genetic markers, by polymerase chain reaction. Alleles were assigned to individuals, which allowed calculation of lod scores.

RESULTS. A maximum two-point lod score of 4.07 was obtained with marker D10S1225 with no recombination. Two recombination with marker D10S208 and D10S537 localized the disease within a region of approximately 30 centimorgans (cM). However, homozygosity across the region refined the arPHPV locus to 13 cM.


During embryonic development of the eye, the compartment between the retina and crystalline lens contains a vascular system (hyaloid artery) that provides the building blocks of nutrients for the developing eye. As the eye and its vascular system mature in the embryo, the hyaloid system regresses. Before this, during the fourth month of embryonic development, the hyaloid artery gives off retinal branches, and the proximal portion persists in the adult as the central artery of the retina.1 In some cases, the primary vitreous fails to regress in utero, resulting in a congenital anomaly often called persistent hyperplastic primary vitreous (PHPV),2 or persistent fetal vasculature (PFV). The latter, more general term includes all possible combinations of fetal vascular remnants remaining in both anterior and posterior segments of the eye.3 A study on childhood blindness and visual loss performed at an institution for visually impaired individuals in the United States showed that the 4.8% of blind persons had PHPV.4 Usually, it is unilateral, although bilateral cases have been described. Highly vascular mesenchymal tissue nurtures the developing lens during intrauterine life. In PHPV the mesenchymal tissue forms a mass behind the lens, and the lens subsequently becomes cataractous. PHPV is a congenital disorder with several different possible ocular manifestations, ranging from persistent pupillary membrane, Mittendorf dot, leukocoria due to cataract or a retrolental membrane, and Bergmeister papilla to congenital nonattachment of the retina and even microphthalmia.5-7 PHPV usually occurs as a nonheritable, unilateral eye disorder in an otherwise normal child.8 It has also been reported in association with other anomalies, including neurologic disorders,6 Walker-Warburg syndrome,7 trisomy 13,9 Norrie disease,10 tuberous sclerosis,10 and osteoporosis-pseudoglioma syndrome.11 Conditions that may mimic PHPV include familial exudative vitreoretinopathy (FEVR),12-13 incontinentia pigmenti, retinoblastoma, and retinopathy of prematurity.

Few reports have been published on inherited forms of isolated PHPV.12-15 Up to now, no locus for isolated PHPV has been mapped, and no candidate gene has been reported for this ocular condition in humans. We therefore sought to identify the first locus for isolated nonsyndromic PHPV by homozygosity mapping in a large inbred Pakistani pedigree. We report the linkage of a new autosomal recessive (ar)PHPV locus to 10q11-q21 by microsatellite mapping.

Patients and Methods

A six-generation, consanguineous Pakistani family with congenital blindness was ascertained. The family consisted of 5 affected (age range, 5-22 years) and 12 unaffected individuals. Consanguinity was present among the parents of all patients of three branches of the family (Fig. 1). An ophthalmologist clinically examined all patients and their normal family members. Both eyes of all affected subjects were blind and showed nystagmus. The youngest affected subject VI:2, aged 5 years, had perception of light in both eyes. Ultrasound scans revealed an axial length of within a range of 20 to 22 mm in both eyes of all patients. All the patients except the youngest (VI:2) had a shallow anterior chamber in both eyes. Peripheral anterior synechiae and corneal opacities were seen, with variable degrees of cataract and a retrolenticular white mass behind the lens. The iris was anteriorly displaced in all affected eyes.

Remnants of the hyaloid artery were seen with one vessel on the posterior side of the cataractous lens in the youngest subject (VI:2). There were retinal folds in one of her eyes, but no retinal detachment was seen at the time of examination, which was performed with the patient under general anesthesia. Grayish fibrous tissue protruding into the vitreous from the posterior side of the lens formed a tentlike structure, with its base located anteriorly and its tip posteriorly. Ophthalmic examination of the parents of the affected individuals revealed no evidence of PHPV, indicating an autosomal recessive mode of inheritance. Based on these clinical findings and the genealogical details, the disease segregating in this family was classified as congenital nonsyndromic arPHPV.

The protocol of the study conformed with the tenets of the Declaration of Helsinki. To perform a full genome search, using linkage analysis with microsatellite markers, we collected peripheral blood samples with informed consent from all the members of the family (Fig. 1). Samples were also obtained from 100 unrelated, normal Pakistani individuals to calculate the allele frequencies. Genomic DNA

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was extracted from whole blood using an extraction kit (Nucleon II; Scotlab Bioscience, Strathclyde, Scotland, UK). To identify the gene responsible for the disease in this family, we performed a whole-genome linkage analysis.

**Microsatellite and Linkage Analysis**

For linkage analysis, polymorphic microsatellite markers (Human MapPairs Set, ver. 8; Research Genetics, Inchinnan, Scotland, UK) were amplified by polymerase chain reaction (PCR). Three sets of markers were analyzed: those corresponding to the known loci of eye-specific transcription factor genes,16 a further 40 reported to define loci closest to the greatest number of expressed sequence tags, 17 and a set of 387 anonymous markers at 10- to 20-centimorgan (cM) intervals throughout the genome. PCR reactions were each performed in a 10-μl volume containing 1.5 mM MgCl₂, 0.4 mM of each primer, 200 μM dNTPs, 16 mM (NH₄)₂SO₄, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, and 1 U Taq DNA polymerase (Bio-Line, London, UK). Amplification was performed with an initial denaturation for 3 minutes at 95°C, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 7 minutes, and a final extension at 72°C for 7 minutes. The PCR products were separated on 8% to 10% nondenaturing polyacrylamide gels (Protogel; National Diagnostics, Edinburgh, Scotland, UK). The gels were stained with ethidium bromide and photographed under UV illumination.

On the first indication of linkage to chromosome 10q, the family was genotyped for more markers across the region. Alleles were assigned to individuals, and haplotypes of all family members were constructed (Fig. 1). The genotypic data were used to calculate the

![Figure 1](image-url)

**Figure 1.** Pedigree of an arPHPV-affected family with genotypic data for microsatellite markers analyzed. Circles: females; squares: males; filled symbols: affected individuals; open symbols: unaffected individuals; diagonal line through a symbol: deceased family member; double line between individuals: consanguinity; crosshatching: uninformative haplotypes.
two-point lod scores, using the Cyrillic (http://www.cyrillicsoftware.com) and MLINK (ftp://linkage.rockefeller.edu/software/linkage/) software programs. Allele frequencies were calculated from the normal, ethnically matched population. The phenotype was analyzed as an autosomal recessive trait with complete penetrance at a frequency of 0.0001 for the disease gene.

### RESULTS

Seventeen members of the family affected by arPHPV were typed for more than 300 polymorphic markers. Genomic DNA from each individual was initially genotyped for microsatellite markers for all the known eye developmental loci (RetNet and FIGURE 2. Schematic representation of chromosome 10, indicating the position of the arPHPV locus and relative locations of other known eye disorder loci and genes.

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* Marker with maximum score.

TABLE 1. Two-Point Scores of arPHPV-Affected Family for Chromosome 10q Microsatellite Markers

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references therein; available at http://www.sph.uth.tmc.edu/Retnet/disease.htm and provided in the public domain by the University of Texas Houston Health Science Center). Significant exclusion was observed for all the known loci. Subsequently, a genome-wide search was undertaken by using 250 polymorphic markers spanning the entire human genome at 20-cM intervals (Research Genetics). Significant linkage was obtained for markers on chromosome 10q. Two-point lod scores between PHPV and markers in this region (D10S208, D10S1220, D10S1221, D10S1225, GATA121A08, and D10S537) are summarized in Table 1. Positive lod scores ranging from 3.24 to 4.07 at θ = 0.00 were obtained for markers D10S1221, D10S1225, and GATA121A08. The maximum lod score of 4.07 was obtained for the marker D10S1225, with no crossover (Table 1).

The most probable disease haplotypes and the distal and proximal boundaries of the chromosomal interval containing the novel disease locus are shown in Figure 1. The proximal crossover was obtained in individual V:2 with marker D10S1220. However, because the marker D10S1220 was uninformative (shown crosshatched in Fig. 1), the next marker, D10S208, was considered to be the proximal flanking marker. Although the distal crossover was obtained with marker D10S537 in individual V:2, the critical disease region was flanked by markers D10S208 and D10S537, if we consider identity-by-descent in V:2. The estimated genetic distance between these two markers was approximately 30 cM. However, based on the recessive mode of inheritance, homozygosity in the disease region of approximately 13 cM was observed in all the patients in the family with microsatellite markers D10S1221, D10S1225, and GATA121A08.

The linkage data presented in this study suggest that a gene for arHPV is present at the proximal part of the long arm of chromosome 10, most likely at 10q11-q21 within the region of homozygosity of 13 cM (Fig. 2; genetic distances between markers are according to Marshmed genetic maps; Marshfield Laboratories, Marshfield, WI). Recently, a locus for nonsyndromic congenital retinal nonattachment has been reported on 10q21.19 Given that congenital retinal nonattachment is part of the spectrum of PFV,3 the fact that this condition was linked to an area within the disease interval for PHPV implicated in this study, may represent an example of allelic heterogeneity. The distal crossover with marker D10S537 excludes the human retinal G-protein–coupled receptor gene (RGR).20,21 expressed in the retina.

Other genes and loci reported on chromosome 10 that are associated with eye disorders include those for recessive Usher syndrome,22,23 recessive RPE degeneration, recessive gyrate atrophy, and recessive Refsum disease,24,25 as shown in Figure 2. This indicates that chromosome 10 is gene rich, especially in genes associated with ocular disorders.

This study describes the mapping of a first locus for isolated congenital nonsyndromic PHPV and defines the location of yet another novel developmental gene that may be critical in eye development.

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References


