

Clinicopathologic Correlation and Genetic Analysis in a Case of Posterior Polymorphous Corneal Dystrophy

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- **PURPOSE:** To evaluate the clinical history, histopathology, and genetics of posterior polymorphous corneal dystrophy (PPMD) in a woman with a prominent retrocorneal membrane.
- **DESIGN:** Observational case report and genetic analysis of her family, UM:139.
- **METHODS:** Records were reviewed from a case and associated family members. The diagnosis of PPMD was based on clinical examination, immunohistochemical staining, electron microscopy, and screening of genetic markers from regions previously reported to be associated with PPMD.
- **RESULTS:** Over 17 years, the proband with PPMD had 25 ocular procedures performed for glaucoma, cataract, cornea, retina, and postoperative problems. A prominent

retrocorneal membrane grew onto the crystalline lens and intraocular lens (IOL). Histopathology revealed stratified epithelial-like cells on iris from an iridectomy and stratified corneal endothelium on a corneal button. Electron microscopy on the cornea revealed microvilli, tonofilaments, and desmosomes consistent with endothelial transformation, which was confirmed by positive anticytokeratin (CK) AE1/AE3 and CAM 5.2 immunoreactivity. Negative immunoreactivity in epithelium and positive in endothelium with anti-CK 7 supported the diagnosis of PPMD rather than epithelial downgrowth. Multiple relatives were affected with PPMD with apparent autosomal dominant inheritance, but surprisingly, the PPMD, congenital hereditary endothelial dystrophy 1 (CHED1) and CHED2 loci on chromosome 20 and the collagen, type VIII, α -2 (COL8A2) gene were excluded by linkage and haplotype analyses.

- **CONCLUSIONS:** We are unaware of previous PPMD reports describing the unusual feature of a retrocorneal membrane extending onto the crystalline lens and IOL. In addition, this family suggests another PPMD locus. (*Am J Ophthalmol* 2003;135:461–470. © 2003 by Elsevier Science Inc. All rights reserved.)

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POSTERIOR POLYMORPHOUS DYSTROPHY (PPMD), which is also referred to in the literature as posterior polymorphous corneal dystrophy (PPCD and PPD), is an autosomal dominant bilateral corneal dystrophy characterized by transformation of the endothelium into epithelial-like cells.¹ There is a wide clinical spectrum ranging from a benign clinical course of asymptomatic nonprogressive disease to relentless progression with serious visual disability from corneal edema and secondary glaucoma.² It is estimated that up to 40% of patients with PPMD have elevated intraocular pressure (IOP).³ Clinical findings include corneal endothelial vesicles, bands, diffuse

haze, iridocorneal adhesions, corectopia, and pupillary ectropion.³ Retrocorneal "glass-like" membranes have been described, and these membranes may extend across the angle and onto the iris.^{1,3-6} We describe a 52-year-old proband with PPMD who has the unusual complication of a retrocorneal membrane proliferating on the anterior surface of a posterior chamber intraocular lens (IOL) and crystalline lens. We also present evidence that PPMD in her family is not linked to the known PPMD (see Online Mendelian Inheritance in Man number, or OMIM#, 122000, which may be accessed at <http://www.ncbi.nlm.nih.gov/>), congenital hereditary endothelial dystrophy 1 (CHED1, OMIM# 121700) or CHED2 (OMIM# 217700) loci on chromosome 20,⁷ nor to the candidate PPMD genes, visual system homeobox gene 1 (VSX1, OMIM# 605020), which is localized within the PPMD locus,⁸ or collagen, type VIII, α -2 (COL8A2, OMIM# 120252), which is localized to 1p34.3-p32.3.⁹

METHODS

OPHTHALMOLOGIC EXAMINATION OF MEMBERS OF FAMILY UM:139 was performed as part of a protocol approved by the Institutional Review Board of the University of Michigan Medical Center. Blood samples from 26 family members were obtained after informed consent. Twenty-three of these family members were examined by at least one of the authors (S.E.M., M.T.S., or A.S.), and the medical records were obtained from the other three individuals' ophthalmologist. Participants were examined for the presence of unequivocally characteristic corneal endothelial abnormalities of PPMD.^{3,10} Subjects were classified as having PPMD if they exhibited any of the following corneal findings: vesicular, geographic or band-like lesions at the level of the Descemet membrane or posterior vesicles in at least one eye. For the three individuals not examined at our institution, the affected status was based on the clinical diagnosis of PPMD by the primary ophthalmologist. In addition, all examined subjects were evaluated for the presence of guttae based on excrescences visible on specular reflection of the posterior corneal surface. The clinicians who assigned the affected status had no genotype information.

Formalin-fixed paraffin-embedded sections of two surgical specimens from the left eye, the iris (February 27, 1992), and corneal button (September 17, 1998) were stained with hematoxylin-eosin. A portion of the cornea was prepared for transmission electron microscopy. Immunohistochemistry was performed on the cornea using anticytokeratin (CK) AE1/AE3 (Boehringer Mannheim, Indianapolis, Indiana, USA) and anti-CK CAM 5.2 (Bectin Dickinson, San Jose, California, USA) according to the manufacturers' instructions. Immunoperoxidase reactivity was detected by diaminobenzidine tetrahydrochloride according to a standard protocol. In addition, the

anti-CK7 monoclonal antibody (DAKO-CK7, clone OV-TL 12/30, DAKO Corp., Carpinteria, California, USA) was used.¹¹ The anti-CK7 monoclonal antibody was diluted 1:400, warmed to 60 C for 15 minutes, and then applied to the section for 1 hour at room temperature. The section was washed three times with DAKO Antibody Diluent (DAKO Corp.). The corneal sections were incubated with the secondary antibody conjugated to peroxidase using the Vectastain Elite ABC Kit (Vector Laboratories, Inc., Burlingame, California, USA) according to the manufacturer's instructions. Immunoperoxidase activity was detected with 3-amino-9-ethylcarbazole according to a standard protocol.

Genomic DNA was isolated from blood samples from 26 family members using Puregene (Gentra Systems, Minneapolis, Minneapolis, USA) according to the manufacturer's instructions. Microsatellite repeat markers were assayed by polymerase chain reaction using methods and parameters described previously.^{7,12-17} To evaluate linkage to the previously reported PPMD locus on chromosome 20q, eight markers were evaluated from D20S98 to D20S119, a region that contains the entire reported 19.73 cM PPMD interval between D20S98 and D20S108.⁷ Screening of these markers allowed us to evaluate linkage to the autosomal dominant CHED1 locus (also called adCHED) previously suggested to be allelic to the PPMD locus.¹⁴ The CHED1 locus lies in a 2.7 cM interval (D20S48-D20S471)¹⁴ located between D20S118 and D20S912. The VSX1 gene is reported to be mutated in two relatives with PPMD and lies between D20S912 and D20S195.^{8,15-17} Screening of the interval from D20S199 to D20S95 allowed us to evaluate linkage to the autosomal recessive CHED2 locus (also called arCHED) previously reported as mapping to a 6 cM interval between D20S113 and D20S882 on chromosome 20p.¹⁸ In addition, we screened DIS234 and DIS255, which flank COL8A2, to evaluate whether PPMD results from mutations in COL8A2. This gene is relevant because a Gln455Lys mutation has been reported in Fuchs endothelial corneal dystrophy and has been found in a woman and her father who both have PPMD.⁹

Single-point linkage analysis was performed by the method of log of the odds (LOD) scores,¹⁹ which compares the probability of the data assuming that the two loci are linked to each other at a hypothesized distance to the probability of the data if the two loci are actually unlinked. This project used the accepted standard value of a LOD score less than or equal to -2.0 as significant evidence against linkage and a LOD score greater than or equal to $+3.0$ as significant evidence in favor of linkage. The computer program MENDEL was used to carry out two-point linkage calculations to evaluate whether a single genetic marker is linked to a PPMD disease locus in this family.²⁰ Multipoint linkage analysis, which makes use of data from multiple genetic markers in a region simulta-

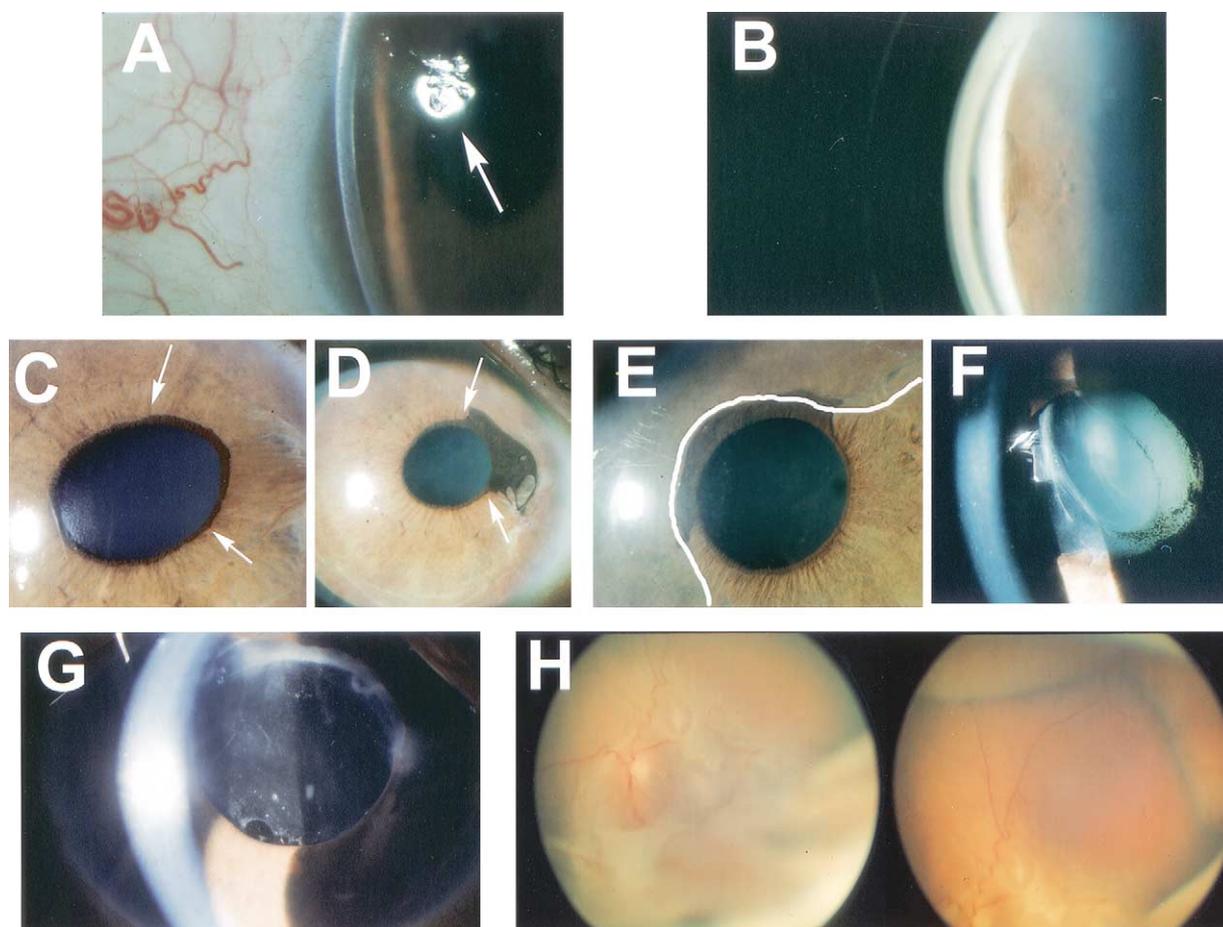


FIGURE 1. Photographs highlighting the 17-year history of our patient with posterior polymorphous corneal dystrophy (taken by our ophthalmic photography unit formerly directed by Professor Csaba Martonyi). (A) Vesicular lesion (white arrow) at the level of the Descemet membrane in the left eye. (B) Broad-based peripheral anterior synechiae of the nasal angle of the left eye showing 1 to 2 clock hours of open angle. (C, D) Progressive corectopia and ectropion uveae of the right eye approximately between the 1 and 4-o'clock hours (between the white arrows) from 1985 (C) to 1989 (D). (E) "Glasslike" membrane (curved white line) on the left iris and associated ectropion uvea from the 7 and 2-o'clock positions. (F) Two months following the first surgery, a trabeculectomy, on the left eye, this membrane proliferated on the crystalline lens. (G) Retrocorneal membrane nearly covers the surface of a posterior chamber intraocular lens in the right eye. (H) Proliferative vitreoretinopathy caused a tractional retinal detachment (left) with associated choroidal detachments (right) in the left eye.

neously, was performed using the Monte Carlo Markov chain method of Sobel and Lange,²¹ as implemented in the computer program SIMWALK2.²² Assumptions included 1% sporadic rate and 90% penetrance. Two different disease allele frequencies, 0.01 and 0.001, were tested with 0.001 presumed as the relevant disease allele frequency in the case of autosomal dominant inheritance and 0.01 presumed as the relevant disease allele frequency in the case of autosomal recessive inheritance. The autosomal dominant model was tested for both regions because of the observed mode of inheritance in this family and the mode of inheritance of the first reported PPMD locus⁷ and CHED1.¹⁴ We also tested a less likely model of autosomal recessive inheritance of prevalent recessive CHED2¹⁸ allele(s), presuming that the apparent autosomal dominant transmission of PPMD in this family could result from an

unusually high number of marriages of homozygous affected individuals to carriers. Marker allele frequencies were estimated by the method of Boehnke.²³ Lack of genotyping incompatibilities was determined through use of the method of O'Connell and Weeks²⁴ with the use of the program PedCheck and by the Monte Carlo Markov chain method of Sobel and Lange,²¹ as implemented in SIMWALK2.²²

For purposes of analysis, individuals were designated affected if they were clinically diagnosed with PPMD, as defined in methods, or unaffected if they had a normal ophthalmologic examination. Individuals with other ophthalmologic findings, such as guttae (individuals IV-2, IV-3, IV-15, and IV-17) and primary open-angle glaucoma (individual III-11) were considered to have an undesig-nated phenotype.

TABLE 1. Surgical Interventions in Proband to Manage Posterior Polymorphous Corneal Dystrophy, Secondary Glaucoma, and Other Ocular Complications

Date	Eye	Procedure
2/27/92	Left	Trabeculectomy and postoperative subconjunctival 5-FU injections
5/22/92	Left	Trabeculectomy with mitomycin C (0.4 mg/ml, 3.5 min)
12/22/92	Left	Phacoemulsification, no IOL placed
1/5/93	Left	Trabeculectomy with mitomycin C (0.5 mg/ml, 2.5 min)
2/23/93	Left	Molteno double plate implant
3/12/93	Left	Tube revision due to endothelial touch
3/30/93	Left	Pars plana vitrectomy for aqueous misdirection
11/24/93	Left	Lacrimal gland biopsy for nonspecific orbital inflammation
1/24/94	Left	Molteno implant removed due to orbital inflammation
2/8/94	Left	Nd:YAG contact transcleral laser cyclophotocoagulation
5/22/94	Left	Upper lid ptosis correction
10/4/95	Left	Argon/Nd:YAG laser peripheral iridotomy for iris bombé
12/12/95	Left	Repeat argon/Nd:YAG laser peripheral iridotomy for iris bombé
7/10/96	Left	Nd:YAG laser peripheral iridotomy and capsulotomy
11/7/96	Left	Pars plana vitrectomy and lysis of anterior chamber adhesions
4/24/97	Right	Thermal sclerostomy and postoperative 5-FU injections
5/22/97	Right	Trabeculectomy with mitomycin C (0.33 mg/ml, 3 min) and postoperative 5-FU injections
9/10/97	Right	Phacoemulsification with posterior chamber IOL, ab interno bleb revision, and postoperative 5-FU injections
10/15/97	Right	Nd:YAG posterior capsulotomy
12/16/97	Right	Trabeculectomy revision and postoperative 5-FU injections
2/24/98	Right	Baerveldt tube (350 mm ²) with scleral patch graft
5/20/98	Right	Nd:YAG “polishing” of anterior membrane on posterior chamber IOL
7/27/98	Right	Nd:YAG “polishing” of anterior membrane on posterior chamber IOL
9/17/98	Left	Penetrating keratoplasty
1/7/99	Left	Pars plana vitrectomy and membrane peeling

5-FU = 5-fluorouracil; IOL = intraocular lens; Nd:YAG = neodymium:yttrium–aluminum–garnet laser.

RESULTS

THE 52-YEAR-OLD PROBAND WAS FIRST EVALUATED AT THE University of Michigan in 1985 at the age of 38 years with an 18-month history of difficulty with vision and a 9-month history of corectopia in her left eye. Both eyes revealed corneal endothelial vesicles (Figure 1A), corectopia, iridocorneal adhesions, and broad-based peripheral anterior synechiae (PAS) (Figure 1B). Over the next 4 years, the corectopia and ectropion uveae progressed (Figure 1C, D), PAS increased, and IOP rose, requiring treatment with glaucoma medications. In February 1992, she underwent a trabeculectomy for secondary angle-closure glaucoma with an IOP of 45 mm Hg in the left eye. The peripheral iridectomy specimen was sent for histopathology. The filter failed, and subsequent surgical procedures are summarized in Table 1. Before the first surgery, a prominent retrocorneal membrane extended onto the left iris (Figure 1E) and 2 months following the trabeculectomy, the membrane was seen on the crystalline lens (Figure 1F). In December 1997, 3 months after uneventful cataract surgery and posterior chamber intraocular lens (IOL) placement in the right eye, a prominent membrane was visible on the anterior surface of the posterior chamber

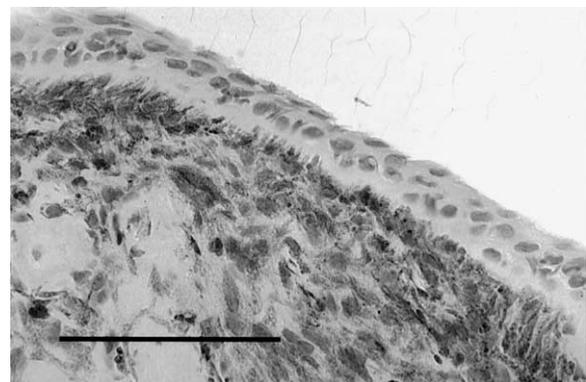


FIGURE 2. Stratified epithelial-like cells on anterior surface of left peripheral iridectomy specimen from the first surgery (hematoxylin–eosin; bar is 100 μ m).

IOL (Figure 1G). A “membranotomy” was performed twice with a neodymium:yttrium–aluminum–garnet (Nd:YAG) laser, but the membrane regrew, and no further laser procedures were advised.

Given the complicated glaucoma and retrocorneal membrane growth onto the IOL in the right eye, visual

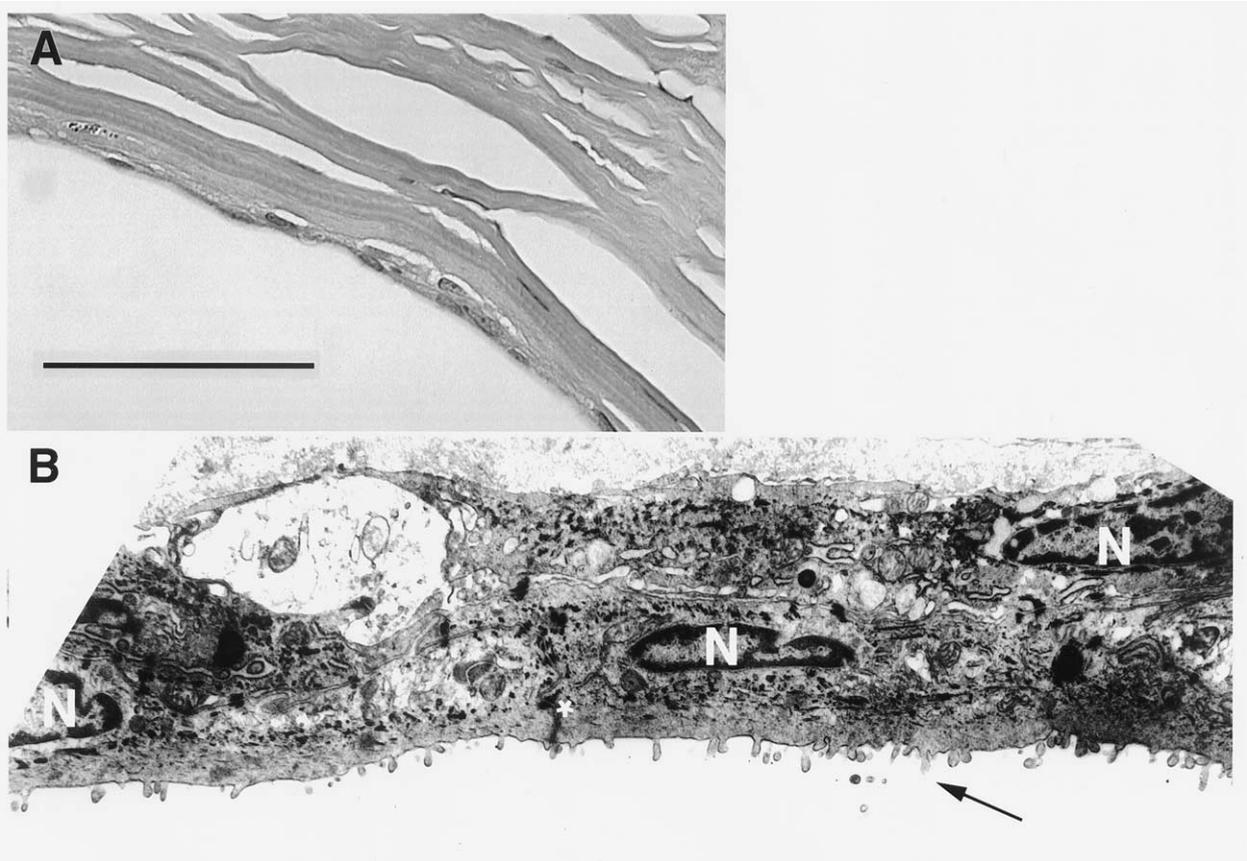


FIGURE 3. Light and transmission electron microscopy of left corneal button. (A) Light microscopy revealed area of the Descemet membrane covered by stratified endothelial cells (hematoxylin–eosin; bar is 100 μm). Endothelial surface is oriented down. (B) Transmission electron microscopy revealed stratified endothelial cells with nuclei (N), microvilli (arrow), and desmosomal attachments (asterisk) (final magnification, 7625 \times).

rehabilitation was pursued in the left eye, which was an aphakic eye and had bullous keratopathy and an epiretinal membrane. In September 1998, the proband had an uncomplicated penetrating keratoplasty. Two months postoperatively, she complained of a scotoma and had an IOP of 9 mm Hg on timolol 0.5% twice daily and dorzolamide 2% twice daily. There were peripheral choroidal detachments and a tractional macular retinal detachment due to progression of the epiretinal membrane (Figure 1H). The choroidal detachments did not resolve after treatment with systemic prednisone and stopping glaucoma medications. In January 1999, a repeat pars plana vitrectomy and membrane peeling was performed. Unlike typical proliferative vitreoretinopathy, this membrane was extremely tenacious and required a peripheral 360-degree retinotomy to separate it from the retina. The membrane spanned from the posterior segment to the inferior ciliary body and the posterior iris surface.

Seven months postoperatively, she had an episode of bilateral anterior uveitis with hypotony (4 mm Hg, right eye and 0–5 mm Hg, left eye) and nongranulomatous inflammation, which responded to topical and systemic

corticosteroids. In May 2002, her visual acuity was 20/100 right eye and light perception left eye. Her IOP was 5 mm Hg in the right eye without glaucoma medications. The anterior segment examinations were unchanged, and the right retina was attached.

An iris biopsy obtained from the first surgical intervention, trabeculectomy of the left eye, revealed stratified epithelial-like cells on the anterior surface of the iris (Figure 2). Given this histopathologic appearance, it was necessary to discriminate between epithelial downgrowth and PPMD. The left penetrating keratoplasty specimen obtained in September 1998 allowed us to resolve this important issue. This cornea revealed areas of stratified endothelium, which is a typical feature of PPMD (Figure 3A). On transmission electron microscopy, the endothelium revealed microvilli projecting into the anterior chamber and desmosomes with associated tonofilaments consistent with epithelial-like transformation (Figure 3B).

To further discriminate the epithelial-like corneal endothelium from epithelium, an immunohistochemical analysis was performed using anti-CK antibodies. Cytokeratins are water-insoluble proteins that form intracellular

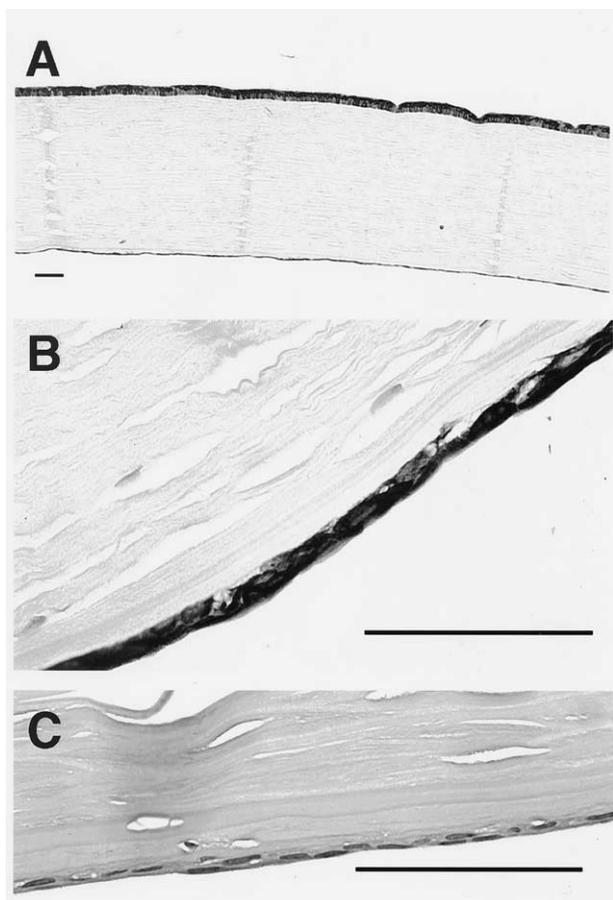


FIGURE 4. Anticytokeratin (CK) AE1/AE3 and CAM 5.2 immunoperoxidase staining with diaminobenzidine of left corneal specimen. (A) Corneal epithelium (top) and endothelium (bottom) stained positively for anti-CKs (bar is 100 μm). (B) Higher magnification of corneal endothelium (bar is 100 μm). (C) Negative control with no endothelial anti-CK staining (bar is 100 μm).

intermediate filaments in epidermis and most epithelial tissues,²⁵ including normal corneal epithelium, but not in endothelium.^{26,27} In contrast, corneas with PPMD stain positively in the endothelium with anti-CK.²⁶ Our case illustrated positive immunoreactivity for anti-CK AE1/AE3 and CAM 5.2 in both epithelium and endothelium (Figure 4). Although this result is consistent with PPMD, it does not eliminate the possibility of epithelial downgrowth in which the associated intraocular proliferative membrane would stain similar to the ocular surface epithelia. A marker that is expressed in corneal endothelium, but not epithelium, is desirable. Two such markers have been identified: 2B4.14.1,²⁸ a monoclonal antibody against human corneal endothelium and CK7, a 54-kD CK identified in an ovarian carcinoma cell line.²⁹ The 2B4.14.1 antibody stains normal corneal endothelium and not epithelium, but inconsistently stains the epithelial-like endothelium in PPMD.²⁸ In contrast, a comparative study of normal and diseased corneas, including PPMD, illus-

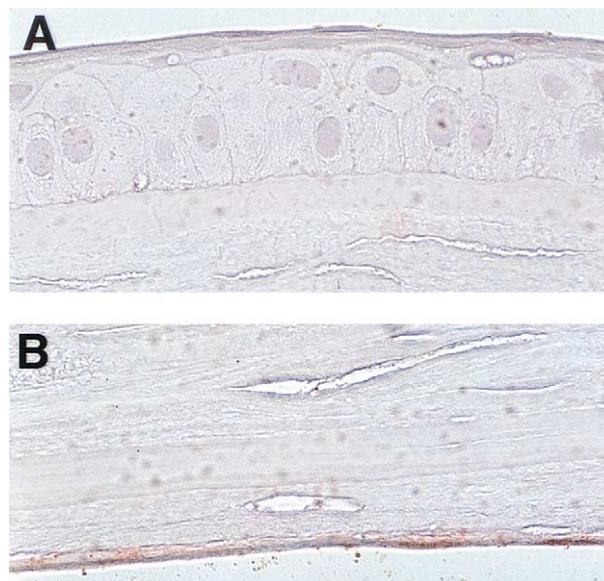


FIGURE 5. Anticytokeratin (CK)7 immunoperoxidase staining with 3-amino-9-ethylcarbazole of left corneal button (final magnification 2375 \times ; stained by Zong-Mei Bian, MD). (A) Epithelium revealed no staining. (B) Endothelium stained positively, as evident with reddish reaction product.

trated that a monoclonal anti-CK7 antibody stains only the endothelium of corneas and not the epithelium.¹¹ In our case, the corneal epithelium stained negatively, whereas the endothelium stained positively with anti-CK7 antibodies, supporting the diagnosis of PPMD rather than epithelial downgrowth (Figure 5).

The histopathology of the proliferative vitreoretinopathy membrane obtained from January 1999 revealed fibrocellular connective tissue containing pigmented epithelial cells, fibroblast-like cells, lens capsule, and stratified epithelial-like cells (not shown). Evaluation of anti-CK immunohistochemical staining was not possible due to the limited specimen.

A detailed family history covering six generations indicated that the proband is Caucasian with ancestry from both western and eastern Europe. A diagnosis of PPMD was confirmed in 12 relatives of the proband in three generations through ophthalmologic examination or evaluation of clinical records (Figure 6). Pedigree analysis of family UM:139 (Figure 6) supports an autosomal dominant inheritance with high, but incomplete penetrance and excludes X-linked and mitochondrial inheritance. In addition, the lack of any history of hereditary nephritis in UM:139 family clearly eliminates the possibility of the rare occurrence of PPMD associated with X-linked Alport syndrome.³⁰

Multipoint analysis revealed formal exclusion of the entire PPMD interval and both the CHED1 locus and VSX1 gene contained within it (Figure 7). Under an autosomal dominant model with disease allele frequency of

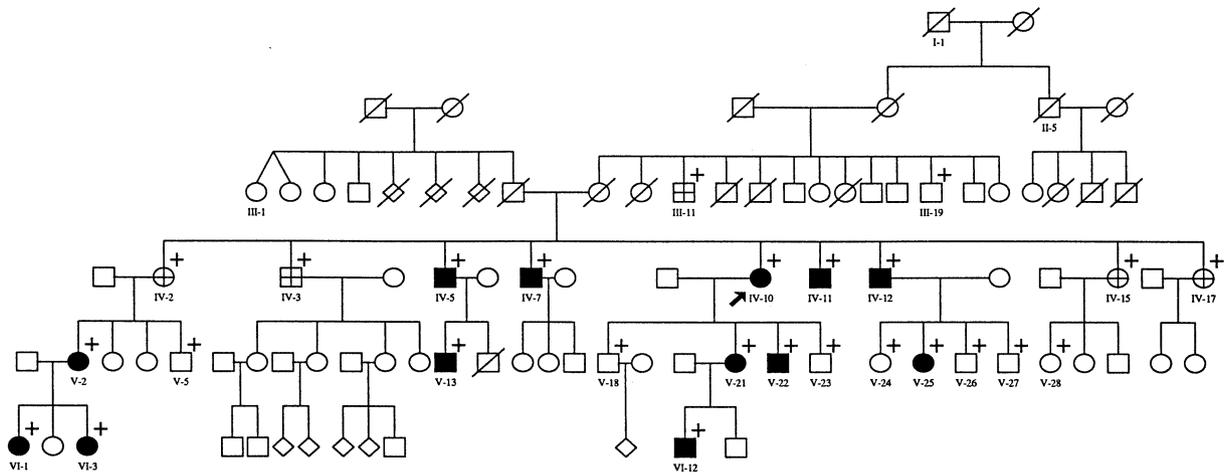


FIGURE 6. Pedigree of Family UM:139. Among those individuals for whom molecular data were generated for use in the analysis (marked with +), filled symbols are individuals with posterior polymorphous corneal dystrophy (PPMD), open symbols are individuals deemed unaffected with PPMD, and a cross within a symbol indicates individuals who were scored as having an indeterminate PPMD status for purposes of analysis. Open symbols not marked with a + are individuals for whom molecular data were not generated; they are free of PPMD by family report, but were not examined by us. Arrow indicates proband. Squares represent males; circles represent females. A diagonal line through a symbol indicates the individual is deceased. Other significant eye disease in the family includes angle-closure glaucoma in the proband (IV-10), primary open-angle glaucoma (III-11), and family report of glaucoma (II-5); blindness in one eye due to accident (I-1) and blindness in one eye due to cataract (III-3).

0.001, an “affecteds”-only multipoint analysis yielded LOD scores between -2.85 and -6.22 , where values below -2.0 are normally considered formal proof of exclusion.¹⁹ Other combinations of models and parameters tested all resulted in LOD scores below -2.0 across the entire PPMD interval.

Although no previous linkage of PPMD to the CHED2 locus has been reported, we also tested it because of the model proposed for a genetic relationship between CHED1 and PPMD.^{14,31} The most relevant combination of parameters for evaluating linkage to the CHED2 locus (autosomal recessive inheritance, disease allele frequency of 0.01, affecteds-only analysis) resulted in formal exclusion of the CHED2 locus with LOD scores ranging from -3.17 to -3.38 . Most other model-parameter combinations tested also resulted in formal exclusion with LOD scores below -2.0 , except for the test of autosomal recessive inheritance using information from the whole family with disease allele frequency of 0.001, which yielded LOD scores from -1.76 to -2.07 .

Because of a report that two affected members of one PPMD family have a mutation in COL8A2, we also tested for linkage to the relevant region of chromosome 1.⁹ When data from the whole UM:139 family were used under a model of autosomal dominant inheritance with disease allele frequency of 0.001, multipoint analysis excluded the region containing COL8A2 with LOD scores ranging from -4.35 to -6.02 . Although evidence against involvement of COL8A2 was less strong when considering data from affected individuals only, the LOD scores ranging from

-1.65 to -2.20 provide evidence against linkage of PPMD in this family to the region containing COL8A2.

For no combination of models and parameters tested was evidence found to favor linkage of PPMD in this family to the PPMD/CHED1 interval, which contains the VSX1 gene, the CHED2 interval, or the region containing the COL8A2 gene. Haplotype analysis, which allowed us to determine which alleles were likely to be traveling together on the same chromosome, supported our conclusion that alleles of PPMD, CHED1, CHED2, or COL8A2 are unlikely to be the cause of PPMD in this family.

DISCUSSION

ALTHOUGH PPMD IS USUALLY NONPROGRESSIVE, THERE have been several reported cases of aggressive disease, as in this case with retrocorneal membranes and glaucoma, which required penetrating keratoplasty and glaucoma procedures.^{1,5,6,32} Pathology specimens in previous reports have demonstrated the presence of retrocorneal membranes extending onto the anterior surface of the iris.^{1,4-6} We are unaware of previous PPMD reports of a retrocorneal membrane growing onto the anterior surface of the crystalline lens and the anterior surface of a posterior chamber IOL. It is striking that the patient had multiple surgeries in both eyes (see Table 1) with apparently accelerated growth of this membrane after each surgery. It is conceivable that inflammation during the postoperative period stimulated membrane growth. Given the appear-

Chromosome 20 Affecteds—Only Multipoint Exclusion Analysis
Disease Allele Freq = 0.001

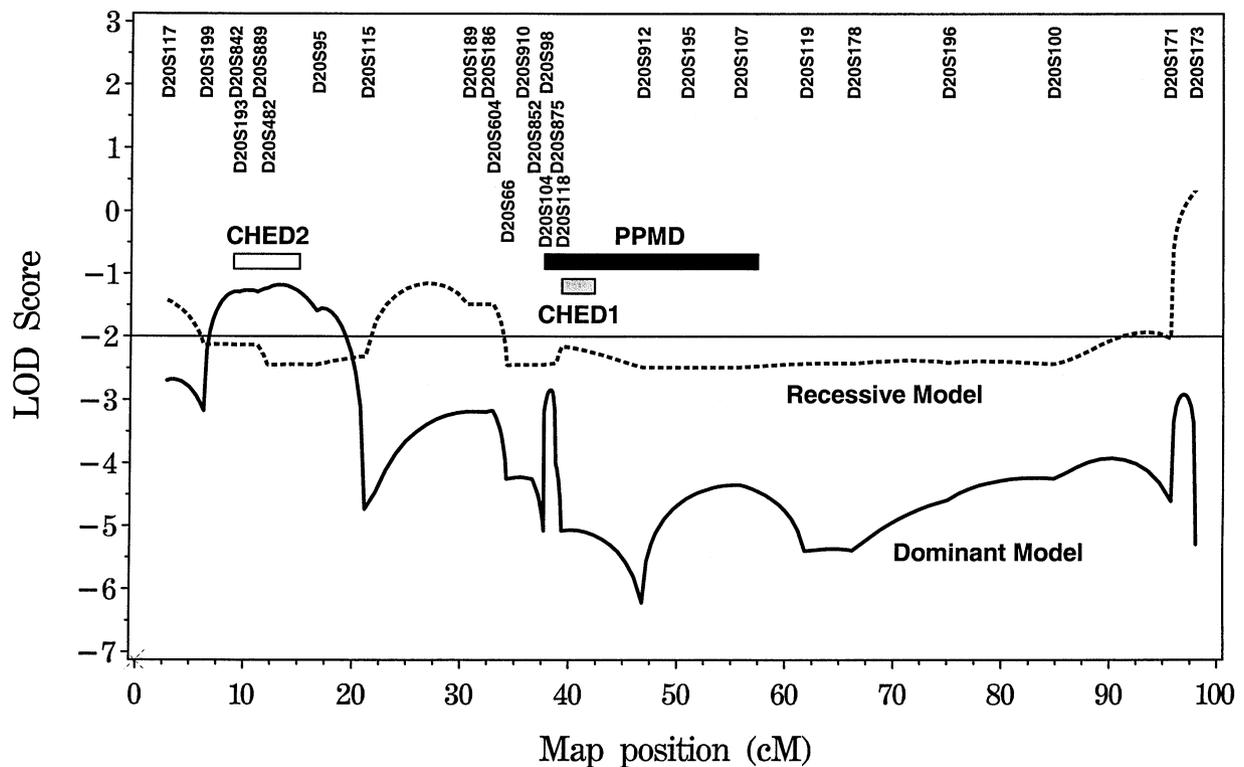


FIGURE 7. Results of UM:139 affecteds-only multipoint linkage exclusion analysis (disease allele frequency of 0.001) on chromosome 20. This diagram indicates the locations of the previously mapped loci of interest on chromosome 20, and charts the log of odds ratio (LOD) scores as indications of whether markers located along chromosome 20 can be excluded from being linked to posterior polymorphous corneal dystrophy (PPMD) in this family. Bars diagram the range of plausible locations of previously reported PPMD, autosomal dominant congenital hereditary endothelial dystrophy (CHED)1, and autosomal recessive CHED2 loci.^{7,14,18} The solid line represents the results of testing for linkage under an autosomal dominant model, which is the most relevant model to be tested when evaluating linkage to the autosomal dominant PPMD and CHED1 loci. The dotted line represents the results of testing for linkage under an autosomal recessive model, which is the most relevant model to be tested for linkage to the autosomal recessive CHED2 locus. Note that LOD scores remain below -2.0 when the model most relevant to a particular locus is being evaluated. Even when the less likely autosomal dominant model is tested across the autosomal recessive CHED2 region (solid line), substantially negative values are found and no positive LOD scores are observed within the CHED2 region. Marker positions and separations used in the analyses derive from the sex-averaged genetic distance from the top of chromosome 20 based on the Marshfield master map in MapView Build 28, Human Genome Sequence version (December 24, 2001).⁴³

ance of the retrocorneal membrane growing onto the IOL of the right eye, it is conceivable that the aphakic status in the left eye allowed the membrane to grow to the posterior segment in the absence of an IOL-capsule-anterior hyaloid barrier.³³

Other possible clinical diagnoses to consider for this case are epithelial downgrowth and iridocorneal endothelial syndrome (ICE). Epithelial downgrowth, a complication of intraocular surgery or trauma, may be diagnosed by clinical history, cytopathology of aqueous humor aspirate, immunoreactivity to certain cytokeratins, and reaction of the membrane to argon laser photocoagulation.^{34,35} In ICE, the clinical features of abnormal corneal endothelium,

PAS, and iris changes may be similar to PPMD,³² yet typical ICE differs from PPMD in being unilateral, nonfamilial, and more rapidly progressive.^{10,36}

The following five arguments support a diagnosis of PPMD over these other clinical entities. First, there was a 7-year history of progression of the retrocorneal membrane before any ocular surgeries or trauma. Second, the histopathology of the left iris specimen from the first ocular surgery demonstrated an epithelial-like membrane. Because no prior surgical intervention was performed, this specimen confirms cellular proliferation before surgical intervention. Furthermore, the histopathology of the cornea is consistent with the published findings of stratified

endothelium, microvilli, desmosomal attachments, and tonofilaments in PPMD.^{1,37-39} Third, this disease was bilateral. Fourth, the immunohistochemistry staining of her cornea demonstrated that the corneal endothelium expressed epithelial-like keratin markers (Figure 4).^{26,28} More important, however, the keratin marker CK7, which is positive for endothelium and negative for epithelium¹¹ was positive in our patient's corneal endothelium and negative in epithelium (Figure 5). Fifth, 13 family members in three generations were affected with PPMD indicating the expected autosomal dominant pattern of inheritance. Hence, the clinical course, family history, histopathology, and immunohistochemistry support the diagnosis of PPMD in our patient and not epithelial downgrowth or ICE.

Surprisingly, although our observation of autosomal dominant inheritance of PPMD in the UM:139 family was consistent with the previously reported PPMD locus on chromosome 20q,⁷ multipoint linkage analysis excluded this PPMD locus. Specifically, screening of chromosome 20 markers suggests that PPMD in the UM:139 family is not the result of disease-influencing allele(s) located at the PPMD locus, the CHED1 locus, or the CHED2 locus. In addition, the PPMD in this family is apparently not caused by the COL8A2 mutation previously reported in a woman and her father who both had PPMD.⁹ Exclusion of these loci in a family whose pedigree is strongly consistent with an autosomal dominant form of PPMD does not detract from the PPMD diagnosis but does suggest the existence of another PPMD locus, which could be identified through a genome scan.

In summary, the proband demonstrated a prominent retrocorneal membrane that extended onto the crystalline lens and anterior surface of the IOL, which has not been previously reported for PPMD. The genetic analysis of her family, UM:139, illustrates the genetic heterogeneity of PPMD. Insights into the pathogenesis of PPMD will undoubtedly result from genetic studies of this autosomal dominant corneal disease, as with recent genetic studies of other corneal dystrophies.⁴⁰⁻⁴² Identification of the PPMD genes will provide valuable tools with which to study the abnormal biology of the corneal endothelium in PPMD and to investigate the phenotypic variability of this corneal dystrophy.

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