Molecular Cell Biology Research Communications **2,** 103–110 (1999)
Article ID mcbr.1999.0161, available online at http://www.idealibrary.com on IDEAL®

Molecular Characterization and Genomic Mapping of Human IPM 200, a Second Member of a Novel Family of Proteoglycans

Markus H. Kuehn and Gregory S. Hageman[1]
Department of Ophthalmology and Visual Sciences, University of Iowa Center for Macular Degeneration, Iowa City, Iowa 52240

Received August 30, 1999

We report herein the characterization of the cDNA for a novel human chondroitin sulfate proteoglycan, designated IPM 200, and the chromosomal location of its gene, designated IMPG2. IPM 200 was isolated from the retinal interphotoreceptor matrix, a unique extracellular matrix that occupies the subretinal space between the apices of the retinal pigment epithelium and the neural retina. The cDNA contains an open reading frame of 3,726 bp that codes for a core protein with a deduced molecular weight of 138.5 kDa. The deduced IPM 200 core protein contains a putative transmembrane domain, two EGF-like repeats, numerous N- and O-linked glycosylation consensus sequences and one consensus sequence for glycosaminoglycan attachment. IMPG2 maps to human chromosome 3q12.2-12.3. Based on homologies within their amino acid sequences we propose that IPM 200 and a previously described human proteoglycan, IPM 150, form a new family of extracellular matrix glycoconjugates. © 1999 Academic Press

Key Words: **chondroitin sulfate proteoglycan; interphotoreceptor matrix; photoreceptor cells; retina.**

The outer segments of retinal rod and cone photoreceptor cells are encapsulated by the interphotoreceptor matrix (IPM). Functionally, the IPM mediates several processes that are crucial to photoreceptor function (1, 2). Significant among these functions are roles in retinal adhesion and the establishment and maintenance of a milieu suitable for photoreceptor survival (2–8). The IPM is an unique extracellular matrix in that it lacks many of the more typical constituents of extracellular compartments, such as elastin, collagens, and fibronectins. Many of the molecules that comprise the IPM are distributed heterogeneously. Most notably, the IPM surrounding human cone, but not rod, photoreceptor outer segments reacts with antibodies directed against chondroitin 6-sulfate glycosaminoglycans and the lectin peanut agglutinin (1, 9, 10). These structures, designated "cone matrix sheaths" (CMS), have been shown to participate in retinal adhesion (7, 11–15). Previous biochemical analyses documented the presence of two chondroitin sulfate proteoglycans in the IPM, designated IPM 150 and IPM 200, based on their apparent molecular weights following separation by SDS-polyacrylamide gel electrophoresis (1, 16).

The molecular relationship between IPM 150 and IPM 200 has not been elucidated fully. The results of preliminary amino acid sequencing analyses suggested that both proteoglycans might share a common core protein, since the amino-terminial sequences of both IPM 150 and IPM 200 were similar (17). Based on these data, we sought to determine whether: (i) both proteoglycans share a common core protein, differing only in the extent or nature of associated saccharides; (ii) IPM 150 and IPM 200 represent splicing variants of a single gene; or (iii) the two proteoglycans arise from two independent genes and share only partial homology to one other.

In an effort to address these questions we recently cloned the cDNA encoding the core protein of human IPM 150 (GenBank Accession Number AF047492) (18). Comparison of the IPM 150 cDNA to NCBI databases indicated that the region between nucleotides 1850 and 2080 of IPM 150 displays some homology with a cDNA, designated PG 10.2, that is expressed in the pineal gland and retina of rats (19). IPM 150 and PG 10.2 are clearly distinct proteins, however, differing from one another throughout most of their deduced amino acid sequences and in their overall molecular weights. In addition, PG 10.2 includes regions that are highly homologous to amino acid sequences we had obtained from a second human IPM proteoglycan, designated

[1] To whom correspondence should be addressed at University of Iowa Center for Macular Degeneration, Department of Ophthalmology and Visual Sciences, University of Iowa, 11190E PFP, 200 Hawkins Drive, Iowa City, IA 52240. Fax: (319) 335-7142. E-mail: gregory-hageman@uiowa.edu.

1522-4724/99 $30.00
Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

IPM 200. These observations suggested that PG 10.2 might represent the rat homologue of human IPM 200, rather than that of human IPM 150. Based on this contention, we utilized PG 10.2-derived cDNA probes to isolate and clone human IPM 200, and subsequently determined the chromosomal location of the human IPM 200 gene.

MATERIALS AND METHODS

Isolation of IPM 200 protein and amino acid sequencing. Aqueous-insoluble IPM was isolated from pig, monkey and human eyes and processed as described previously (33). Briefly, IPM was isolated, homogenized and digested with protease-free chondroitinase ABC (Seikagaku, Ijamsville, MD). IPM-associated proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to sequencing grade PVDF membrane (Immobilon P, Millipore, Bedford, PA). Membrane strips to which IPM 200 was bound, as visualized by staining of adjacent membrane strips with anti-chondroitin 6-sulfate antibody, were excised and subjected to direct NH_2-terminal amino acid sequencing by Edman degradation (38). In addition, gel strips containing IPM 200 were stained with Coomassie blue, excised, and subjected to an "in-gel" digestion protocol (39). The resulting tryptic fragments were isolated by reverse phase HPLC and their amino acid sequences determined. Obtained sequences were compared to the NCBI databases.

Isolation of human IPM 200 cDNA. In order to generate probes for the isolation of cDNA clones encoding human IPM 200, two primer pairs were synthesized based upon the rat PG 10.2 cDNA sequence (SN1: 5′-GACAGCTTTTGGATGCCAC-3′, AS1: 5′-ACCTGCCAATGTTGAGGTC-3′, amplification product spanning nucleotides 350 to 770, and SN2: 5′-AGCATTGTGGTGAACAGCAG-3′, AS2: 5′-CGGCATCTACAAATGGCT-3′, amplification product spanning nucleotides 3064 to 3430). Two PCR fragments were generated by reverse-transcription PCR of rat retinal RNA using these primer pairs. These fragments were utilized as probes to screen a human retinal λgt11 cDNA library (Clontech Laboratories, Palo Alto, CA) under low stringency conditions. Screening of 2×10^5 plaques resulted in the isolation of three clones. The cDNA inserts of these clones were amplified by PCR using primers to the regions flanking the insertion site of λgt11 (Sense: 5′-TGGCTGAATATCGACGGTTTCC-3′; Antisense: 5′-AGACCAACTGGTAATGGTAGCG-3′) and PFU polymerase (Stratagene, La Jolla, CA). The generated PCR fragments were subcloned into the pCRscript vector. The resulting clones, designated 107.1.1, 107.2.2, and 108.1.1, were then used as probes to rescreen the library; one additional clone, designated 121.2.1, was isolated and the cDNA insert was subcloned.

cDNA sequencing. The cDNA sequences of all subcloned cDNA fragments were determined by automated sequence analysis using an ABI 370 DNA sequencer. Both strands were analyzed at least twice using either vector-specific primers or custom oligonucleotide primers. The sequence presented here represents a consensus sequence from all cDNA clones obtained. Sequences obtained were compared with those in the NCBI databases using the BLAST algorithm (40) in the MacVector software package (Kodak International Biotechnologies, Inc., New Haven, CT).

Northern blot analyses. Total RNA was isolated from human retina, RPE/choroid, iris, and cornea collected within four hours of death using the RNeasy system (Qiagen, Valencia, CA). Prior to electrophoresis the samples were denatured at 55°C, for 15 min, in 6.5% formaldehyde and 50% formamide in MOPS running buffer (40 mM morpholinopropanesulfonic acid, pH 7.0, containing 100 mM sodium acetate and 10 mM EDTA). The RNA was then fractionated on agarose gels containing formaldehyde and subsequently transferred to nylon-based membranes by capillary transfer using 20× SSC (3 M NaCl and 0.3 M sodium citrate). RNA was cross-linked to the membranes and hybridized with ^{32}P-labeled DNA probes derived from clone 108.1.1 for detection of IPM 200 transcripts.

Radiation hybrid analysis. Radiation hybrid mapping was performed with the Genebridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, AL). Sense (5′-AAAAAGAAACAGCCTCTGGACCGCAG-3′) and antisense (5′-CAGCCTCTGCAACACTTTCATCTGGG-3′) primers were designed according to sequences spanning nucleotides 372 to 397 and 467 to 492 of the IPM 200 cDNA, respectively. 12.5 ng of each radiation hybrid clone DNA was then used as template in a 8.35 μl polymerase chain reaction (PCR) containing: 1.25 μl 10× buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 300 μM of each dNTP, 1 pmole of each primer, and 0.25 units *Taq* polymerase (Promega, Madison, WI). Samples were denatured for 5 min, at 94°C, and amplified in a DNA thermocycler for 35 cycles under the following conditions: 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec. After amplification, 5 μl of stop solution (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to each sample. Amplification products were electrophoresed on 6% polyacrylamide, 5% glycerol gels, at 50 W. Following electrophoresis, gels were stained with silver nitrate and scored for the presence of amplified product. The data were submitted to the Whitehead Institute Server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) for computation of the chromosomal location of the gene.

FISH analysis. In order to obtain a sufficiently large genomic DNA probe of the IPM 200 gene, the PCR primer pair indicated above was used to screen a

Monkey NH_2-Terminus	X I L F P N G V K I X P D E
Pig NH_2-Terminus	X X L F P N G V K I
Human NH_2-Terminus	X I L F P N G V K I X P
Human Peptide # 62	V L E F R
Human Peptide # 92	X(Q)Q E A X W E A
Human Peptide # 117	D Y I A E T L Q Q N F L
Human Peptide # 141-1	T V S S S E L S S P V
Human Peptide # 141-2	X Y A K E(P)V(K)X X E L

FIG. 1. Amino acid sequences obtained by directly sequencing native IPM 200 protein derived from pig, monkey, and human IPM. X represents positions for which no amino acid could be assigned and residues in parentheses could not be determined with certainty.

bacterial artificial chromosome (BAC) library of the human genome. After identification of a BAC clone containing IPM 200 sequence (BAC #340m10; Research Genetics, Huntsville, AL) its DNA was isolated, fractionated by enzymatic restriction, and the resulting fragments were subcloned. Subclones containing IPM 200 sequences were identified by hybridization with oligonucleotides and their identity was confirmed by partial sequence analysis. DNA from these subclones was labeled with digoxigenin dUTP and hybridized to normal metaphase chromosomes derived from PHA stimulated human lymphocytes in a solution containing 50% formamide, 10% dextran sulfate and 2× SSC. Specific hybridization signals were detected by incubating the hybridized slides with fluorescein-conjugated anti-digoxigenin antibodies followed by counterstaining with DAPI.

RESULTS

Amino acid sequencing of IPM 200. The amino-terminal sequences of IPM 200 collected from human, monkey, and pig, as well as the sequences of five internal human IPM 200 peptides were determined (Figure 1). The amino terminus and fragment #62 of human IPM 200 display some sequence similarities with the amino terminus of IPM 150 described previously (18), although several specific and consistent amino acid substitutions are noted. The sequences of human IPM 200-derived peptides #92, #117, #141-1, and #141-2 do not share homology to human IPM 150.

Cloning of human IPM 200. Regions of rat PG 10.2 cDNA that exhibit considerable sequence divergence from human IPM 150 cDNA were employed as probes to screen a human retinal cDNA library for clones encoding IPM 200. Four overlapping clones, designated 107.1.1, 107.2.2, 121.2.1, and 108.1.1, containing cDNA inserts of 4.8, 4.9, 1.2 and 2.2 kb respectively, were isolated (Figure 2). Sequences obtained from these clones were assembled to generate a 4,165 bp consensus sequence for human IPM 200 (Figure 3).

Translation of the human IPM 200 cDNA results in a deduced core protein with a molecular weight of 138.5 kDa. The deduced IPM 200 sequence contains both the amino terminal peptide as well as all five isolated internal fragments obtained by direct amino acid sequence analyses of native IPM 200 (Figure 3). Based on the amino terminal sequence data derived from native human IPM 200 protein, it appears that its first 82 amino acids may be removed during the maturation of the protein, leaving a mature core protein of 129 kDa. The deduced amino acid sequence features six consensus sequences for N-linked glycosylation (20) (located at amino acids 154, 301, 320, 370, 942, and 956) and 23 potential sites for O-linked glycosylation scattered throughout the central portion of the protein (21). Although there are several ser-gly dipeptide motifs within the core protein, only ser^{603}-gly^{604} fits the ser-gly-x-gly consensus sequence for glycosaminoglycan attachment and is located within a region containing several acidic residues, making it the most likely site for GAG attachment (22). The IPM 200 core protein is generally hydrophilic (23), except for a short hydrophobic NH_2-terminal region, presumably the signal sequence which is removed during the maturation of the molecule, and a hydrophobic region between amino acids 1097 and 1127 that might constitute a transmembrane domain (Figure 4). Additional features of IPM 200 include two EGF-like domains (24) located between amino acids 1014 and 1092. In contrast to IPM 150, no hyaluronic acid binding domains are present.

Ocular expression of human IPM 200. Northern blot analyses of RNA isolated from human retina, RPE/choroid, cornea and iris, using clone 108.1.1 as a probe, demonstrate that IPM 200 is expressed in the human retina, but not in the RPE/choroid, cornea, or iris (Figure 5). A single transcript, approximately 6.2 kb in size is observed, suggesting that spliced isoforms of IPM 200 do not exist in the human retina.

Chromosomal localization. Radiation hybrid analysis was performed to identify the chromosomal locus of the IPM 200 gene, herein referred to as IMPG2. The results of this assay indicate that the IMPG2 locus is on chromosome 3, between markers WI-3277 and NIB1880 (LOD >3.0) (Figure 6). This location has been confirmed by fluorescent *in situ* hybridization (FISH) analyses of human metaphasic chromosomes. Initial analyses indicated that IMPG2 probes hybridized to the proximal arm of chromosome three. In subsequent hybridizations, a genomic probe (previously mapped to band 3p21), was shown to cohybridize on the same chromosome as the IMPG2 probe (Figure 7). In this experiment, 60 of 80 metaphase cells examined exhib-

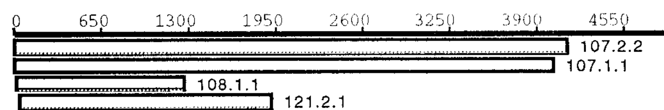

FIG. 2. Overlap of the isolated cDNA clones that encode the complete sequence of human IPM 200.

FIG. 3. Nucleotide and amino acid sequences of human IPM 200. Sequences confirmed by direct amino acid sequencing are boxed. The putative transmembrane domain is indicated by a single underline and the EGF-like domains by a broken line. The glycosaminoglycan attachment consensus sequence is indicated by a double underline. Potential O-linked glycosylation sites are marked by asterisks, and N-linked glycosylation consensus sequences are indicated by triangles. This sequence has been submitted to GenBank under the Accession Number AF173155.

ited specific labeling with both probes. Measurements of ten labeled chromosomes demonstrate that IMPG2 is located on chromosome 3q at a position which corresponds to band 3q12.2-12.3.

Alignment between the human IPM 200 and rat PG 10.2 amino acid sequences displays a high degree of sequence conservation. The similarity between the two molecules is most pronounced in the amino and carboxy terminal regions and weakest in their central regions. A significant degree of sequence conservation among mammalian species is further indicated by analyses of the amino termini of the two IPM proteoglycans derived from human, monkey and pig (Figure 8). However, distinct and conserved amino acid substitutions between the amino termini of IPM 150 and IPM 200 can be observed (e.g., AAs 3, 6, 10, and 13). These substitutions are conserved between the mammalian species examined.

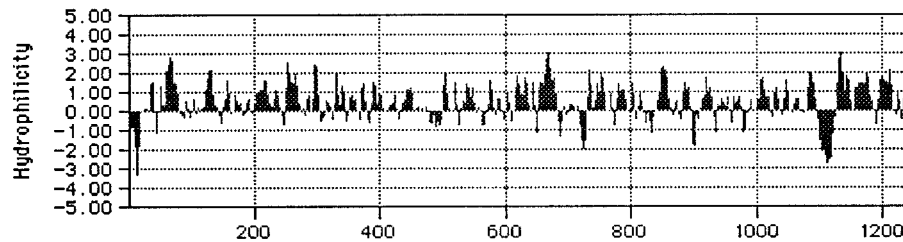

FIG. 4. Hydrophobicity plot of human IPM 200, indicating a possible transmembrane domain between amino acids 1097 and 1127.

Comparison of the human IPM 200 and IPM 150 amino acid sequences reveals little overall homology between the two molecules (Figure 9). However, both proteins contain a highly conserved region which spans from Leu^{899} to Phe^{986} of IPM 200 and is located immediately upstream of the EGF-like domains. A second conserved region begins at the presumed native NH_2-terminus (Ser^{82} of IPM 200) and extends to Pro^{129} of IPM 200 (Figure 9). The core proteins of IPM 150 and IPM 200 may assume a similar tertiary structure *in vivo.* Both molecules contain numerous cysteine residues in their NH_2-terminal regions, that may allow for the formation of globular domains. Additional globular domains, which include EGF-like domains, may be formed at the COOH-terminus of IPM 150 and in a region of IPM 200 which lies close to its putative transmembrane domain. Furthermore, there is a preponderance of consensus sequences for O-linked glycosylation sites in the central domains of the core proteins, whereas consensus sequences for N-linked glycosylation are more prevalent in the putative globular domains.

DISCUSSION

We report here the isolation, cloning and chromosomal localization of human IPM 200, which together with a previously described retinal proteoglycan, designated IPM 150, forms a novel family of chondroitin sulfate proteoglycans. Whereas both IPM 200 and IPM 150 are located within the retinal interphotoreceptor matrix, they are also expressed in several non-ocular tissues including brain and tonsillar tissue (19, 25, 40). IPM 200 appears to be the human homologue of PG 10.2, a cDNA originally isolated from the pineal gland of the rat (19). Both IPM 200 and PG 10.2 also display significant sequence homology to two domains of human IPM 150.

Biochemical analyses have demonstrated that the core protein of IPM 200 is heavily glycosylated (1, 16). In addition to one consensus sequence for glycosaminoglycan attachment, IPM 200 features six sites for potential N-linked glycosylation and contains at least twenty-three consensus sites for potential O-linked glycosylation. Glycosylation most likely accounts for the difference between the calculated molecular weight of the mature protein (approximately 130 kDa) and its observed molecular weight on SDS-PAGE gels following enzymatic removal of attached glycosaminoglycan moieties (approximately 200 kDa).

According to the predictions of the method of von Heijne, removal of the signal peptide is not likely to result in the loss of any amino acids past Ala^{27} (26). Yet NH_2-terminal amino acid sequencing of the native IPM 200 core protein from human, monkey and pig repeatedly identified ser^{83} as the first amino acid of the protein. One explanation is that IPM 200 is initially synthesized as a propeptide *in vivo* and is subsequently processed to yield a mature protein with ser^{83} as its amino terminus. It is also conceivable that the presumed amino terminus represents a site which is highly susceptible to proteolytic degradation that occurs either *in vivo* or during the isolation protocol.

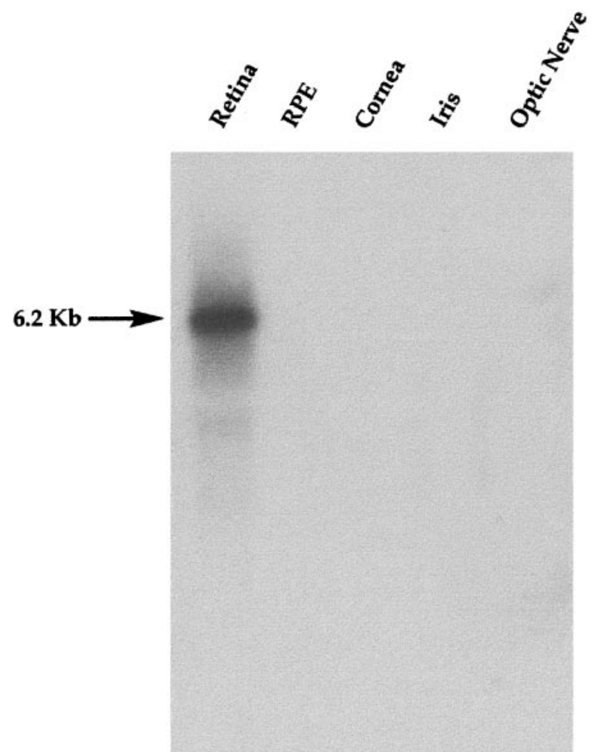

FIG. 5. Northern blot depicting IPM 200 transcripts in various ocular tissues. Only the neural retina expresses a 6.2-kb transcript that hybridizes with an IPM 200 cDNA probe.

Name	Dist.	Data Vector
FB17G2	78.5	1000001111111000111011000010110100111000111011101011101000010110000000001011201101002000011201
CHLC.GATA71D	20.9	0000011110011000111101000001111100111110110111001011101000110110010011101001000110021011110201
AFMA052YE5	8.4	0000010010011000111101000001100102011100110111001011101001110110010210002011001110021210102O1
WI-3277	5.2	0000010110011000111101000001101100210100110111001011101000110110010010001010001110021010102OO
IMPG2:	2.5	0000010110011000110102000001100100010100110010001011101000110112010010101012212110021010101OO
NIB1880	6.7	0000010110011000111101000001100100010100110010001011101000110110010010101010001100021010102OO
D3S1271	3.2	0000010010011000111101000001100100010100110010000011101000110110020010102010000000021010102OO
CHLC.GATA11F0	6.7	0000010010011000111101000001100100010100110010000011100000110110010210101011000000021010102OO
WI-5486	6.6	2000010000011000111101000000100100000000110010000011100000110110010010100011000000021010102OO
CHLC.GATA68D03	18.5	0000010010011000111101000000100100000000110010000011000000110120000010001021000000021010102OO

FIG. 6. Chromosomal localization of the IPM 200 gene as determined by radiation hybrid analysis and placement of the IMPG2 gene relative to the Whitehead Institute framework map for chromosome 3.

One of the aims of this investigation was to clarify the molecular relationship between IPM 150 and IPM 200. The data presented herein strongly suggest that these two proteoglycans are distinct, but related, members of a family of glycoconjugates that are encoded by two distinct genes. Comparison between the IPM 150 and IPM 200 amino acid sequences reveal that the core proteins of these two molecules differ from one another not only in their molecular weight, but also in their amino acid sequences. In addition, IPM 200 appears to contain a transmembrane domain which is not present in IPM 150. We present data demonstrating that the IPM 200 gene (IMPG2) is localized on chromosome 3q12.2-12.3, whereas the IPM 150 gene (IMPG1) has been previously localized to chromosome 6q13-15 (25, 27).

Data collected from previous NH_2-terminal amino acid sequencing and biochemical analyses initially suggested that the IPM 150 and IPM 200 core proteins might be closely related (17). Yet, based on the sequence data presented here, only two domains share considerable sequence homology between the two core proteins, the first in the region of their respective amino-termini and the second in a region toward their respective carboxy termini, immediately upstream of the EGF-like domains. Currently the function of these conserved regions is unknown. However, it is remarkable that the sequence conservation between IPM 200 and its rat homologue PG 10.2 is also particularly high in these regions.

Based on their deduced amino acid sequences, IPM 200 and IPM 150 might assume a similar configuration *in situ.* Several cysteine residues in the vicinity of their respective amino-termini might participate in the formation of globular domains. In addition, both molecules possess a central domain with numerous attachment sites for O-linked carbohydrate chains. In

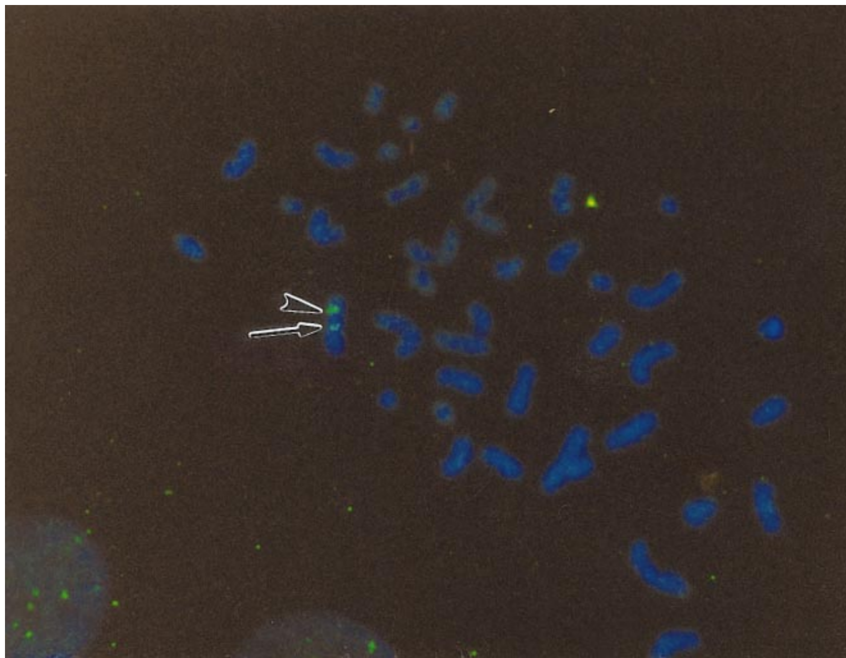

FIG. 7. Fluorescence *in situ* hybridization of a genomic fragment of IMPG2 to human chromosome 3 (arrow). Localization was confirmed by cohybridization with a second probe (arrowhead), known to localize to chromosome 3p21.

	Sequence
Rat PG 10.2	S I L F P N G V R I C P S D T V A E A V
Human IPM 200	S I L F P N G V K I C P D E S V A E A V
Monkey IPM 200	X I L F P N G V K I X P D E
Pig IPM 200	X I L F P N G V K I
Human IPM 150	S A F F P T G V K V C P Q E S M K Q I L
Monkey IPM 150	S A F F P T G V K V C P Q E S M K Q. I L
Pig IPM 150	X V F F P Y G V K V X P Q E S M K

FIG. 8. Alignment between human, monkey, and pig IPM 150 and IPM 200 amino termini and the deduced amino acid sequence of rat PG 10.2. Identical residues are boxed.

addition, one or two EGF-like domains are present in both molecules. This arrangement of domains is reminiscent of the lectican family of proteoglycans (28), however neither IPM 150 nor IPM 200 contain the lectin-like or immunoglobulin-like domains characteristic of the lecticans. Furthermore, the amino acid sequences of IPM 150 and IPM 200 and those of the members of the lectican family do not display any significant homologies. Thus, we propose that IPM 150 and IPM 200 constitute a new family of extracellular matrix proteins.

It has been demonstrated that the IPM chondroitin sulfate proteoglycans mediate retinal adhesion (7, 12). Both enzymatic (3, 4, 13) and metabolic (11) disruption of IPM glycosaminoglycans leads to retinal detachments and subsequent photoreceptor degeneration. However, the precise molecular mechanism which mediates retinal adhesion has not been elucidated. The IPM 200 core protein contains a stretch of hydrophobic amino acids which we propose may constitute a transmembrane domain. Thus, IPM 200 may represent a retinal anchor in the IPM adhesive complex. It remains to be determined whether IPM 200 interacts directly with a yet unidentified receptor on the surface of the retinal pigment epithelium, or whether additional "bridging" molecules, such as IPM 150, are involved in this process. One potential RPE-associated ligand for IPM 200 may be the vitronectin receptor ($\alpha v\beta 3$ integrin) which is present on the apical surfaces of both RPE and photoreceptor cells (14). The IPM 200 core protein lacks the typical vitronectin receptor binding RGD peptide, yet binding to this integrin can be mediated by several other mechanisms (29).

IPM 200 might also serve as a receptor for growth factors and/or function as a growth factor itself. It has been demonstrated that several growth factors, includ-

```
14  IFLQVQGT.KDISINIYHSETKDIDNP........PRNETTESTEKMYKMSTMRRIFDLAKH...RTKRSAFFPTGVKVCPQESMKQILDSLQAYYRLRV 101
16  IFVLIEGDFPSLTAQTYLS.IEEIQEPKSAVSFLLPEESTDLSLATKKKQPLDRR..ETERQWLIRRRRSILFPNGVKICPDESVAEAVANHVKYFKVRV 112

102 CQEAVWEAYRIFLDRIPDTGEYQDWVSICQQETFCLFDIGKNFSNSQEHLDLLQQRIKQRSFPDRKDEISAEKTLGEP...GETIVISTDVANVSLGPFP 198
113 CQEAVWEAFRTFWDRLPGREEYHYWMNLCEDGVTSIFEMGTNFSESVEHRSLIMKKLTY.....AKETVSSSE.LSSPVPVGDTSTLGDTTLSV...PHP 203

199 LT.............PDDTLLNEILDNTLNDTKMPTTERETEFAVLEEQRVELSVSLVNQKFKAELADSQSPYYQELAGKSQLQMQKIFKKLPGFKKIHV 285
204 EVDAYEGASESSLERPEESISNEI.ENVIEEATKPAGEQIAEF..........SIHLLGKQYREELQDSSSFHHQHLEEEFISEVENAFTGLPGYKEIRV 292

286 LGFRPKKEKDGSSSTEMQLTAIFKRHSAEAKSPAS.DLLSFDSNKIESEEVYHGTMEEDKQPEIYLTATDLKRLISKALEE.................. 365
293 LEFRSPKEND..SGVDVYYAVTF...NGEAISNTTWDLISLHSNKVEN....HGLVELDDKPTVVYTISNFRDYIAETLQQNFLLGNSSLNPDPDSLQLI 383

366 .......EQSLDV......GTIQFT...............DE.IAGSLP..........AFGPDTQSELPTSFAVITEDATL........SPE...... 411
384 NVRGVLRHQTEDLVWNTQSSSLQATPSSILDNTFQAAWPSADESITSSIPPLDFSSGPPSATGRELWSESPLGDLVSTHKLAFPSKMGLSSSPEVLEVSS 483

412 ..............LPP...........VEPQLETVDGAE.....HGLPDTSWSPPAMAST.........SLSEAPPFFMASSI.F...SLT....DQGT 464
484 LTLHSVTPAVLQTGLPVASEERTSGSHLVEDGLANVEESEDFLSIDSLPSSSFTQPVPKETIPSMEDSDVSLTSSP..YLTSSIPFGLDSLTSKVKDQLK 581

465 TDTMATDQTM.........LVPG.......LTIPTSDYSAISQLALGISHP.........PA..............SSD..........DSRSSAGGED. 514
582 VSPFLPDASMEKELIFDGGLGSGSGQKVDLITWPWSETSS.EKSAEPLSKPWLEDDDSLLPAEIEDKKLVLVDKMDSTDQISKHSKYEHDDRSTHFPEEE 680

515 .....................MVRHLDEM....DLSDTPAP.........................SEVPELSEY......VSV..PD........... 544
681 PLSGPAVPIFADTAAESASLTLPKHISEVPGVDDCSVTKAPLILTSVAISASTDKSDQADAILREDMEQITESSNYEWFDSEVSMVKPDMQTLWTILPES 780

545 .......HFLE....D...TTPVSA...........................................LQ........YI.......TTSSMTI 566
781 ERVWTRTSSLEKLSRDILASTPQSADRLWLSVTQSTKLPPTTISTLLEDEVIMGVQDISLELDRIGTDYYQPEQVQEQNGKVGSYVEMSTSVHSTEMVSV 880

567 A.P.KGRE..........LVVFFSLRVANMAFSNDLFNKSSLEYRALEQQFTQLLVPYLRSNLTGFKQLEILNFRNGSVIVNSKMKFAKSVPYNLTKAVH 654
881 AWPTEGGDDLSYTQTSGALVVFFSLRVTNMMFSEDLFNKNSLEYKALEQRFLELLVPYLQSNLTGFQNLEILNFRNGSIVVNSRMKFANSVPPNVNNAVY 980

655 GVLEDFRSAAAQQLHLEIDSYSLNIEPADQADPCKFLACGEFAQCVKNERTEEAECRCKPGYDSQGSLDGLEPGLCGPGTKE......CEVLQGKGAPCR 748
981 MILEDFCTTAYNTMNLAIDKYSLDVESGDEANPCKFQACNEFSECLVNPWSGEAKCRCFPGYLSVEERPCQS..LCDLQPDFCLNDGKCDIMPGHGAICR 1078
```

FIG. 9. Alignment between human IPM 150 (top) and human IPM 200 amino acid sequences. The entire IPM 200 amino acid sequence is not depicted. EGF-like domains are underlined by a single line and the presumed amino-termini are underlined by a double line. Conserved cysteine residues are indicated by triangles.

ing bFGF, aFGF, PEDF and TGF-β bind to glycosaminoglycans (30–32). bFGF and PEDF, in particular, reside within the human IPM (33, 34). It is also plausible that the EGF-like domains of the IPM 200 core protein support cell survival, akin to the demonstrated role of these domains in other molecules (35, 36).

The observed homology between IPM 150 and IPM 200 suggests that these proteoglycans comprise a new family of glycoconjugates that are conserved among mammalian species. Identification of the IPM 200 cDNA sequence and localization of the IMPG2 gene to chromosome 3q12.2-12.3 represent a significant steps towards elucidating the molecular organization and function of the IPM, and may prove to be helpful in elucidating the biology of other tissues as well. Currently, no hereditary ocular dystrophies or other diseases have been mapped to this locus. Yet, since IPM 200 represents a significant fraction of mRNA transcripts produced by retinal cells and since a number of retina-associated molecules are responsible for various retinal degenerations and dystrophies (37), IMPG2 is a plausible candidate gene for inherited ocular disorders that have not yet been mapped, such as age-related macular degeneration.

ACKNOWLEDGMENTS

The authors appreciate the assistance of Dr. Val Sheffield and his staff in the sequencing of IPM 200 clones, Kimberly Vandenburgh for assistance with the radiation hybrid assays and Dr. Robert F. Mullins for helpful discussions throughout the project. Work was supported in part by NIH EY06463, NIH EY11515, a Research to Prevent Blindness Lew R. Wasserman Merit Award, and an unrestricted grant to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, Inc.

REFERENCES

1. Hageman, G. S., and Johnson, L. V. (1991) *in* Progress in Retinal Research, (Osborne, N., and Chader, J., Eds.), pp. 207–249, Pergamon Press, Oxford.
2. Hageman, G. S., and Kuehn, M. H. (1998) *in* The Pigmented Retinal Epithelium: Current Aspects of Funtion and Disease (Marmor, M., and Wolfensberger, T., Eds.), pp. 417–454, Oxford Univ. Press, New York.
3. Yao, X. Y., Hageman, G. S., and Marmor, M. F. (1994) *Invest. Ophthalmol. Vis. Sci.* **35,** 744–748.
4. Yao, X. Y., Hageman, G. S., and Marmor, M. F. (1990) *Invest. Ophthalmol. Vis. Sci.* **31,** 2051–2058.
5. Li, A., Lane, W. S., Johnson, L. V., Chader, G. J., and Tombran-Tink, J. (1995) *J. Neurosci.* **15,** 385–393.
6. Hewitt, A. T., Lindsey, J. D., Carbott, D., and Adler, R. (1990) *Exp. Eye Res.* **50,** 79–88.
7. Hageman, G. S., Marmor, M. F., Yao, X. Y., and Johnson, L. V. (1995) *Arch. Ophthalmol.* **113,** 655–660.
8. Lazarus, H. S., Sly, W. S., Kyle, J. W., and Hageman, G. S. (1993) *Exp. Eye Res.* **56,** 531–541.
9. Hageman, G. S., and Johnson, L. V. (1987) *Curr. Eye Res.* **6,** 639–646.
10. Johnson, L. V., Hageman, G. S., and Blanks, J. C. (1986) *Invest. Ophthalmol. Vis. Sci.* **27,** 129–135.
11. Lazarus, H. S., and Hageman, G. S. (1992) *Invest. Ophthal. Vis. Sci.* **33,** 364–375.
12. Marmor, M., Yao, X. F., and Hageman, G. (1994) *Retina* **14,** 181–186.
13. Yao, X. Y., Hageman, G. S., and Marmor, M. F. (1992) *Invest. Ophthalmol. Vis. Sci.* **33,** 498–503.
14. Anderson, D. H., Johnson, L. V., and Hageman, G. S. (1995) *J. Comp. Neurol.* **360,** 1–16.
15. Hollyfield, J. G., Varner, H. H., Rayborn, M. E., and Osterfeld, A. M. (1989) *Retina* **9,** 59–68.
16. Tien, L., Rayborn, M. E., and Hollyfield, J. G. (1992) *Exp. Eye Res.* **55,** 297–306.
17. Kuehn, M. H., Mullins, R. F., and Hageman, G. S. (1993) *Invest. Ophthamol. Vis. Sci. Suppl.* **34,** 1201.
18. Kuehn, M. H., and Hageman, G. S. (1999) *Matrix Biol.,* in press.
19. Wang, X., Brownstein, M., and Young, W., III (1996) *Mol. Brain Res.* **41,** 269–278.
20. Kornfeld, R., and Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
21. Hansen, J. E., Lund, O., Engelbrecht, J., Bohr, H., Nielsen, J. O., Hansen, J.-E. S., and Brunak, S. (1995) *Biochem. J.* **308,** 801–813.
22. Bourdon, M. A., Krusius, T., Campbell, S., Schwartz, N. B., and Ruoslahti, E. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3194–3198.
23. Kyte, J., and Doolittle, R. (1982) *J. Mol. Biol.* **157,** 105–132.
24. Rees, D. J., Jones, I. M., Handford, P. A., Walter, S. J., Esnouf, M. P., Smith, K. J., and Brownlee, G. G. (1988) *EMBO J.* **7,** 2053–2061.
25. Kuehn, M., Stone, E., and Hageman, G. (1997) *Invest. Opthalmol. Vis. Sci. Suppl.* **38,** S599.
26. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690.
27. Felbor, U., Gehrig, A., Sauer, C., Marquardt, A., Kohler, M., Schmid, M., and Weber, B. (1998) *Cytogen. Cell Gen.* **81,** 12–17.
28. Ruoslahti, E. (1996) *Glycobiology* **6,** 489–492.
29. Hynes, R. (1992) *Cell* **69,** 11–25.
30. Andres, J. L., DeFalcis, D., Noda, M., and Massague, J. (1992) *J. Biol. Chem.* **267,** 5927–5930.
31. Cheifetz, S., Andres, J. L., and Massague, J. (1988) *J. Biol. Chem.* **263,** 16984–16991.
32. Alberdi, E., Hyde, C. C., and Becerra, S. P. (1998) *Biochem.* **37,** 10643–10652.
33. Hageman, G. S., Kirchoff-Rempe, M. A., Lewis, G. P., Fisher, S. K., and Anderson, D. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6706–6710.
34. Tombran-Tink, J., Shivaram, S. M., Chader, G. J., Johnson, L. V., and Bok, D. (1995) *J. Neurosci.* **15,** 4992–5003.
35. Panayotou, G., End, P., Aumailley, M., Timpl, R., and Engel, J. (1989) *Cell* **56,** 93–101.
36. Zhang, Y., Cao, L., Yang, B. L., and Yang, B. B. (1998) *J. Biol. Chem.* **273,** 21342–21351.
37. Farber, D. B. and Danciger, M. (1997) *Curr. Opin. Neurobiol.* **7,** 666–673.
38. Stone, K. L., McNulty, D. E., LoPresti, M. L., Crawford, J. M., DeAngelis, R., and Williams, K. R. (1992) *in* Techniques in Protein Chemistry, III (Angeletti, R. H., Ed.), pp. 23–34, Academic Press, San Diego.
39. Stone, K. L., and Williams, K. R. (1993) *in* A Practical Guide to Protein and Peptide Purification for Microsequencing (Matsudaira, P. T., Ed.), pp. 45–73, Academic Press, San Diego.
40. Altschul, S., Gish, W., Miller, W., Myers, E., and Lipman, D. (1990) *J. Mol. Biol.* **215,** 403–410.