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Identification, genomic structure, and screening of the vacuolar proton-ATPase membrane sector-associated protein M8-9 gene within the COD1 critical region (Xp11.4)

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Purpose: Our goal is to identify the gene responsible for X-linked cone-rod dystrophy (COD1) that has been localized to a limited region of Xp11.4.
Methods: A complete physical contig of the COD1 region was partially sequenced and subjected to BLAST searches to identify homologies with GenBank ESTs. ESTs were analyzed for overlapping or related cDNA sequences and retinal expression by PCR screening of multiple human retina cDNA libraries. RACE was performed to complete the missing 5' end of the transcripts. Transcripts were compared with genomic sequences to specify intron-exon boundaries. Genomic DNAs from COD1-affected males from 3 families were screened for mutations using direct PCR sequencing of the exons.
Results: The vacuolar proton-ATPase membrane sector-associated protein M8-9 (APT6M8-9) gene was identified within our critical region. We confirmed its retinal expression and its genomic location in our physical contig. Eight exons (with flanking intronic sequences) were characterized from partial cDNA sequence and genomic sequence data. An additional 5' end exon was identified using RACE. No mutations were found in the COD1-affected males.
Conclusions: The combination of disease mapping and information from the Human Genome project has enabled us to identify candidate genes within the COD1 region, including APT6M8-9 gene. We found no evidence that this gene is responsible for COD1 in our families, but it may be an important candidate for other diseases that have been mapped to this region of the X chromosome.

X-linked cone-rod dystrophy (COD1;) is a rare, progressive visual disease primarily affecting the cone photoreceptors. Affected males present with decreased visual acuity, myopia, photophobia, abnormal color vision, full peripheral visual fields, decreased photopic electroretinographic (ERG) responses and granularity of the macular retinal pigment epithelium (RPE). Complete atrophy of the macular RPE may develop late in life, accompanied by a progressive decline in visual acuity [1-3]. The degree of rod-photoreceptor involvement can be variable, with increasing degeneration as the disease progresses. While penetrance appears to be nearly 100%, there is variable expressivity, with respect to age of onset and severity of symptoms and findings [4].

COD1 was originally mapped to Xp11.3-21.1 in 1989 [3,5]. Additional evidence supporting this map location was presented by Bergen et al. [6,7], Meire et al. [8] and Hong et al. [4]. These linkage studies, however, were unable to resolve COD1 as an independent locus distinct from the retinitis pigmentosa (RP) 2 and RP3 loci and supported the hypothesis that COD1 could be an allelic variant of either RP2 or RP3 based on the chromosomal location as well as clinical phenotypes. Subsequent linkage studies by our group with the original families described by Jacobson et al. [3] and Hong et al. [4], as well as with a new large pedigree, refined COD1 as separate genetic locus to a limited region of Xp11.4, between the RP2 and RP3 loci, which is suitable for a combined positional and candidate gene screening methodology [9].

There are no known retina-specific genes within COD1 critical region, nor genes that are known to participate in the phototransduction process. During our effort to identify the gene responsible for COD1, we have identified sequences from within our physical contig, that matched ESTs corresponding to the transcript of vacuolar proton-ATPase membrane sector-associated protein M8-9 (APT6M8-9), previously reported in GenBank. Proton-translocating adenosine triphosphatases have been demonstrated to have important roles in energy conservation, secondary active transport, and cellular pH homeostasis in a wide range of cell populations [10]. As a potential candidate, we have characterized APT6M8-9 gene and screened for mutations in our COD1 families.

METHODS

COD1 Families: Sixteen members of a five-generation family (family 1), 36 members of a six-generation family (family 2), and 35 members of a six-generation family (family 3) were genotyped to map the COD1 locus between the RP2 and RP3 loci [9]. The clinical descriptions and diagnostic criteria for these families have been previously described [3,4,9,11]. The participation of family members in this study was approved

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by the University of Pittsburgh Biomedical IRB in accordance with OPRR (Office for the Protection from Research Risks) guidelines and informed patient consent was obtained prior to participation.

Identification of the APT6M8-9 transcript as a potential candidate: A complete physical contig of the COD1 critical region, comprised of PACs (P1-derived artificial chromosomes), BACs (bacterial artificial chromosomes) and YACs (yeast artificial chromosomes), has been characterized and has been used for high throughput sequencing and BLAST searches to identify homologies with ESTs and cDNAs in GenBank. The ESTs have been analyzed for overlapping or related cDNA sequences and particular attention was made to ESTs that were initially derived from a retina cDNA library.

Confirmation of the retinal expression and of the location in COD1 critical region: PCR was performed using gene specific primers (forward: 5' TCC TGT TGT TTT GCA GTT GG 3', reverse: 5' CTT GAA ACA GGC GAT TAC GG 3') to confirm its expression in human retina using three different human retina cDNA libraries, and its location in our region, using the genomic clones comprising the physical contig of the critical region. PCR products were confirmed by their expected mobility in polyacrylamide gels and then by direct sequencing of the observed bands. The size of the PCR product was expected to be 119 bp in the retina libraries, and 301 bp in the genomic clones because of the presence of a small intron (that was later identified as intron 4) between flanking exon primers.

Determination of full-length cDNA: Two different strategies were used to complete the missing 5' end and obtain the full-length cDNA. First, RACE PCR was performed with a retina cDNA library using 5' end cDNA reverse primer (5' CTT GAA ACA GGC GAT TAC GG 3') and vector primers. PCR conditions were: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 45 s of denaturation at 95 °C, and 4 min of annealing+extension (2 step PCR) at 65 °C. PCR was done in a 20 µl reaction volume containing 1X PCR buffer II, 1.5 mM $MgCl_2$, 200 µM each dATP, dCTP, dGTP, dTTP, 0.5 µM each forward and reverse primer, and 0.04 U/µl AmpliTaq Gold (Applied Biosystems, Foster City, CA). PCR fragments were excised and purified from the gel, cloned and amplified using pGEM-T easy vector system (Promega, Madison, WI) and electrocomp E. Coli cells (Invitrogen, Carlsbad, CA), followed by multiple miniprep preparations and purifications from each plate, and sequencing with ABI377. Second, RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE) [12,13] was performed on four different human tissue libraries (testis, heart, islets, and NMB7 cells), using the FirstChoice RLM-RACE kit, according to the manufacturer's instructions (Ambion, Austin, TX). Since the 5' end of the transcript was GC rich, both regular (Advantage cDNA Polymerase Mix; Clontech Laboratories, Palo Alto, CA) and GC-rich sequence optimized (Advantage-GC cDNA Polymerase Mix; Clontech Laboratories) polymerase mixes were used during the procedure. Primary PCR reactions were performed using the gene-specific primer (5' AAC TCG TTC CCC AAA ACA CC 3') and outer RNA adaptor primer, followed by the secondary PCR reactions of the diluted outer PCR products using the nested gene-specific primer (5' CAG GAG CAC GAC AAA CAC AG 3') and inner RNA adaptor primer. PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s of denaturation at 94 °C, 20 s of annealing at 58 °C, and 1 min of extension at 68 °C, and a final extension at 68 °C for 5 min. The inner PCR products were purified and cloned using standard cloning techniques [14], followed by multiple miniprep preparations and purifications from each plate, and sequencing with ABI377.

Determination of genomic structure: Two different strategies were used to determine the genomic structure. First, the cDNA transcript was compared with our fragmented, high throughput genomic sequencing data from our contig to specify intron-exon boundaries in silico, using Mac Vector 6.5 (Oxford Molecular Ltd., Oxford, England), AssemblyLIGN (Oxford Molecular Ltd.) and Sequencher 3.1 software (Gene Codes Corporation, Ann Arbor, MI). Second, since there was insufficient available genomic sequence in our high throughput sequencing data and from the Human Genome Project for the additional 5' end sequences that we identified, a vector-bubble PCR method [15] was used to determine the exon-intron boundaries. PAC and BAC clones that contained the candidate gene from our contig, were prepared and digested with restriction enzymes. Resulting fragments were then ligated to a vectorette (bubble) adaptor. PCR was performed using specific cDNA primers (5' GGT GAC GCG CTC GGA CTC 3'; 5' AGC GCG TCA CCT CCT CAC 3') and the vectorette primer. The resulting PCR products were cloned and sequenced with ABI377, to determine the genomic sequence.

Mutation screening in COD1 families: PCR primers flanking the exons were designed and confirmed, in order to generate PCR fragments that include also the intron-exon junctions for being able to determine splice site mutations. The exons from COD1 affected males (two from each family) and two unaffected males were amplified from leucocyte genomic DNA and screened for mutations by direct PCR sequencing in both strands with ABI377.

PCR conditions for genomic DNA amplification were: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 45 s of denaturation at 95 °C, 5 s of annealing at 56 °C, and 40-60 s (depending on the length of the PCR product) of extension at 72 °C, and a final extension at 72 °C for 10 min. PCR was done in a 50 µl reaction volume containing 1X PCR buffer II, 1.5 mM $MgCl_2$, 200 µM each dATP, dCTP, dGTP, dTTP, 0.5 µM each forward and reverse primer, and 0.04 U/µl AmpliTaq Gold (Applied Biosystems). After gel checking, PCR products were purified using QIAquick PCR purification kit (Qiagen, Valencia, CA) and used as template for sequencing reactions. For each sequencing reaction, template was combined with 3.2 pmol of primer and 4 µl of ABI Prism dRhodamine terminator cycle sequencing ready reaction kit (Applied Biosystems) to reach a final volume of 12 µl. Cycling conditions were performed as specified by the manufacturer. The sequencing reactions were precipitated with 70% ethanol containing 0.5 mM $MgCl_2$, resuspended in 4 µl of ABI loading buffer and were electrophoresed for 7 h. The sequenc-

ing results were analyzed with ABI Prism Sequencing Analysis 3.3 (Applied Biosystems) and Sequencher 3.1 software (Gene Codes Corporation).

RESULTS

BLAST analysis using high throughput genomic sequencing fragments from the COD1 region, enabled us to initially identify 12 ESTs (having greater than 95% homologies with our genomic sequence and originating from different tissues including retina), corresponding to a membrane sector-associated protein of vacuolar proton-ATPase. This transcript was initially represented by two cDNA sequences in GenBank: HSM800272 (AL049929.1,1884 nt) and APT6M8-9 (ATPase, H+ transporting, lysosomal (vacuolar proton pump) membrane

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        10        20        30        40        50        60        70        80        90       100
GTGGAGAAAGCGGCAGCTGTCCCAGCGGAAGCGACGAAGGGACGGGACCCGGGAGCCTGGACGAGTCCGAGCGCGTCACCTCCTCACGCTGCGGCTGTCG
       110       120       130       140       150       160       170       180       190       200
CCCGTGTCCCGCCGGCCCGTTCCGTGTCGCCCCGCAGTGCTGCGGCCGCCGCGGCACCATGGCTGTGTTTGTCGTGCTCCTGGCGTTGGTGGCGGGTGTT
                                                                M  A  V  F  V  V  L  L  A  L  V  A  G  V
       210       220       230       240       250       260       270       280       290       300
TTGGGGAACGAGTTTAGTATATTAAAATCACCAGGGTCTGTTGTTTTCCGAAATGGAAATTGGCCTATACCAGGAGAGCGGATCCCAGACGTGGCTGCAT
 L  G  N  E  F  S  I  L  K  S  P  G  S  V  V  F  R  N  G  N  W  P  I  P  G  E  R  I  P  D  V  A  A
       310       320       330       340       350       360       370       380       390       400
TGTCCATGGGCTTCTCTGTGAAAGAAGACCTTTCTTGGCCAGGACTCGCAGTGGGTAACCTGTTTCATCGTCCTCGGGCTACCGTCATGGTGATGGTGAA
L  S  M  G  F  S  V  K  E  D  L  S  W  P  G  L  A  V  G  N  L  F  H  R  P  R  A  T  V  M  V  M  V  K
       410       420       430       440       450       460       470       480       490       500
GGGAGTGAACAAACTGGCTCTACCCCCAGGCAGTGTCATTTCGTACCCTTTGGAGAATGCAGTTCCTTTTAGTCTTGACAGTGTTGCAAATTCCATTCAC
  G  V  N  K  L  A  L  P  P  G  S  V  I  S  Y  P  L  E  N  A  V  P  F  S  L  D  S  V  A  N  S  I  H
       510       520       530       540       550       560       570       580       590       600
TCCTTATTTTCTGAGGAAACTCCTGTTGTTTTGCAGTTGGCTCCCAGTGAGGAAAGAGTGTATATGGTAGGGAAGGCAAACTCAGTGTTTGAAGACCTTT
 S  L  F  S  E  E  T  P  V  V  L  Q  L  A  P  S  E  E  R  V  Y  M  V  G  K  A  N  S  V  F  E  D  L
       610       620       630       640       650       660       670       680       690       700
CAGTCACCTTGCGCCAGCTCCGTAATCGCCTGTTTCAAGAAAACTCTGTTCTCAGTTCACTCCCCCTCAATTCTCTGAGTAGGAACAATGAAGTTGACCT
S  V  T  L  R  Q  L  R  N  R  L  F  Q  E  N  S  V  L  S  S  L  P  L  N  S  L  S  R  N  N  E  V  D  L
       710       720       730       740       750       760       770       780       790       800
GCTCTTTCTTTCTGAACTGCAAGTGCTACATGATATTTCAAGCTTGCTGTCTCGTCATAAGCATCTAGCCAAGGATCATTCTCCTGATTTATATTCACTG
  L  F  L  S  E  L  Q  V  L  H  D  I  S  S  L  L  S  R  H  K  H  L  A  K  D  H  S  P  D  L  Y  S  L
       810       820       830       840       850       860       870       880       890       900
GAGCTGGCAGGTTTGGATGAAATTGGGAAGCGTTATGGGGAAGACTCTGAACAATTCAGAGATGCTTCTAAGATCCTTGTTGACGCTCTGCAAAAGTTTG
 E  L  A  G  L  D  E  I  G  K  R  Y  G  E  D  S  E  Q  F  R  D  A  S  K  I  L  V  D  A  L  Q  K  F
       910       920       930       940       950       960       970       980       990      1000
CAGATGACATGTACAGTCTTTATGGTGGGAATGCAGTGGTAGAGTTAGTCACTGTCAAGTCATTTGACACCTCCCTCATTAGGAAGACAAGGACTATCCT
A  D  D  M  Y  S  L  Y  G  G  N  A  V  V  E  L  V  T  V  K  S  F  D  T  S  L  I  R  K  T  R  T  I  L
      1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
TGAGGCAAAACAAGCGAAGAACCCAGCAAGTCCCTATAACCTTGCATATAAGTATAATTTTGAATATTCCGTGGTTTTCAACATGGTACTTTGGATAATG
  E  A  K  Q  A  K  N  P  A  S  P  Y  N  L  A  Y  K  Y  N  F  E  Y  S  V  V  F  N  M  V  L  W  I  M
      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
ATCGCCTTGGCCTTGGCTGTGATTATCACCTCTTACAATATTTGGAACATGGATCCTGGATATGATAGCATCATTTATAGGATGACAAACCAGAAGATTC
 I  A  L  A  L  A  V  I  I  T  S  Y  N  I  W  N  M  D  P  G  Y  D  S  I  I  Y  R  M  T  N  Q  K  I
      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
GAATGGATTGAATGTTACCTGTGCCAGAATTAGAAAAGGGGGTTGGAAATTGGCTGTTTTGTTAAAATATATCTTTTAGTGTGCTTTAAAGTAGATAGTA
R  M  D
      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
TACTTTACATTTATAAAAAAAAATCAAATTTTGTTCTTTATTTTGTGTGTGCCTGTGATGTTTTTCTAGAGTGAATTATAGTATTGACGTGAATCCCACT
      1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
GTGGTATAGATTCCATAATATGCTTGAATATTATGATATAGCCATTTAATAACATTGATTTCATTCTGTTTAATGAATTTGGAAATATGCACTGAAAGAA
      1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
ATGTAAAACATTTAGAATAGCTCGTGTTATGGAAAAAAGTGCACTGAATTTATTAGACAAACTTACGAATGCTTAACTTCTTTACACAGCATAGGTGAAA
      1610      1620      1630      1640      1650      1660      1670      1680      1690      1700
ATCATATTTGGGCTATTGTATACTATGAACAATTTGTAAATGTCTTAATTTGATGTAAATAACTCTGAAACAAGAGAAAAGGTTTTTAACTTAGAGTAGC
      1710      1720      1730      1740      1750      1760      1770      1780      1790      1800
CCTAAAATATGGATGTGCTTATATAATCGCTTAGTTTTGGAACTGTATCTGAGTAACAGAGGACAGCTGTTTTTTAACCCTCTTCTGCAAGTTTGTTGAC
      1810      1820      1830      1840      1850      1860      1870      1880      1890      1900
CTACATGGGCTAATATGGATACTAAAAATACTACATTGATCTAAGAAGAAACTAGCCTTGTGGAGTATATAGATGCTTTTCATTATACACACAAAAATCC
      1910      1920      1930      1940      1950      1960      1970      1980      1990      2000
CTGAGGGACATTTTGAGGCATGAATATAAAACATTTTTATTTCAGTAACTTTTCCCCCTGTGTAAGTTACTATGGTTTGTGGTACAACTTCATTCTATAG
      2010      2020      2030      2040      2050      2060      2070      2080      2090      2100
AATATTAAGTGGAAGTGGGTGAATTCTACTTTTTATGTTGGAGTGGACCAATGTCTATCAAGAGTGACAAATAAAGTTAATGATGATTCCAAAAAAAAAA
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Figure 1. APT6M8-9 cDNA sequence together with translation and exon-intron boundaries locations. The color scheme is as follows: black, HSM800272 partial cDNA sequence published in GenBank; blue, 5' end cDNA sequence identified by standard RACE; red, 5' end cDNA sequence identified by RLM-RACE; orange, nucleotides flanking an intron (exon-intron junction). The nucleotide at position 1012 was found to be an “A” in 8 people we have screened, the same as in the NM_005765 sequence from GenBank. However, the GenBank cDNA clones HSM800272 and AF248966 indicate a “G” at this location which encodes Arginine instead of Glutamine. Similarly, we found an “A” at position 1476 (3' UTR) which is indicated as a “G” in GenBank HSM800272 and AF248966 sequences.

sector-associated protein M8-9, NM_005765.1, 622 nt).

The expression of this gene was demonstrated in three independent retina cDNA libraries. The exact location of the gene was determined in our critical region using the genomic clones comprising our physical contig and was refined to be between DXS1368 and DXS993.

RACE experiments allowed us to identify an additional upstream 5' end sequence of 161 bp (Figure 1) including the presumptive start codon. Two ESTs were initially identified in GenBank (BE312617 and AA194554) that matched with this new sequence. Subsequently, several additional ESTs have been released and provide additional support for this new sequence. Additional upstream 5' end sequence (55 bp) was obtained (Figure 1) with RLM-RACE resulting in a composite mRNA transcript of 2100 bp total length, with the corresponding protein being 350 amino acids long. We observed different 5' end cDNA lengths in four different tissue libraries with the longest one (from testis) starting at -158 bp from the ATG translation initiation site (the A being nucleotide +1). However, the 5' end sequence preceding the presumptive start codon has a continuous open reading frame (ORF) that raises the possibility of another start codon that could be further upstream that would yield a single protein product. There are currently eight related mRNA sequences in GenBank (AL049929.1, Y17975.1, NM_005765.2, AF248966.1, XM_002904.2, XM_027535.2, BC010395.1, XM_027536.1) and the longest one is 2049 bp long (AF248966.1, tissue type: hypothalamus). An updated BLAST search in the human EST database of GenBank using the full-length cDNA sequence (2100 bp), revealed more than 400 EST hits from several different tissues, including eye (e.g., BF726753 from lens; W27971, AA053862, and H86728 from retina; and BG748332 from normal pigmented retinal epithelium). Some of the additional ESTs recently released in GenBank, also “partially” match with our additional 55 bp sequence obtained with RLM-RACE (e.g. BG529889 from testis).

Eight exons (with flanking intronic sequences) were identified using the published partial cDNA sequence and our high throughput genomic sequence data (Figure 1). These exons and intron-exon boundaries can also be located in the last three fragments of the working draft genomic sequence AC026156, which was submitted to GenBank subsequent to our characterizations. An additional exon, which was not present in the published sequence, was identified in the new 5' end sequence, and the flanking intronic sequences were determined using our genomic clones and bubble adapter method. We submitted the genomic sequence containing this new exon and flanking intronic sequences to GenBank (AF354120) and this sequence partially fits one of the gaps within the working draft genomic sequence AC026156. The corresponding contig sequence in GenBank, was first presented under the accession number NT_022618 and was recently replaced by NT_028412.

Nine exons and flanking intronic sequences were amplified and screened for mutations using the primers listed in Figure 2; no causative mutations were found by direct PCR sequencing.

EXON	BP	Forward primer	Reverse primer
1	195	TTCCCAGGTTACGTCCCTTC	AGGACCTCCCCAGGCACG
2	131	GCTAAGTCAGTGGTGAATGG	ACCTACGTTCGTTTGATGCT
3	132	TAGATGTTATTGGGGAGGTG	ACAGCTGACAAGGGAAAATG
4	96	CTTCCCACTTTGGTTCACATA	-
5	138	-	CAAGTAGTATAAGGAATGGGAGGC
6	54	GGCAAATAAAGATCTCTGGTG	CATGGGATCATAAATCTAGGG
7	150	TTAACTGGCTGTGTTTGACG	TTAGAGGAGCCCCAAAGAAG
8	120	GGACTTAAGCCATTCCACAA	TCATTCATGTCACCCTTAGGT
9	1077	CATCAGTGCAAGTGTCGTCT	GCCCAAATATGATTTTCACC
		TGACGTGAATCCCACTGTG	AGGAGGCAGCGAGAATAAAC

Figure 2. Primer pairs for the exons. Sizes of the exons (in basepairs) for APT6M8-9 gene and the forward and reverse primers (5'->3') generating PCR fragments including exons and flanking intron-exon junctions. Exons 4 and 5 were amplified together with incorporation of a small intron between the exon pairs. Exon 9 was amplified and sequenced using two primer pairs, producing two overlapping fragments.

DISCUSSION

The combination of mapping data, physical contig, and genomic sequence data enabled us to efficiently identify and initiate screening of candidates within the COD1 critical region, including the vacuolar proton-ATPase membrane sector-associated protein M8-9 (APT6M8-9). We found no evidence that this gene is responsible for the X-linked cone-rod dystrophy found in our three families, based on our analyses of the coding regions and intron-exon junctions.

RLM-RACE was developed as an improvement to the classic RACE technique in order to achieve complete representation of the 5' end of RNA transcripts [12,13]. This method is designed to amplify cDNA only from full-length capped mRNA, thus avoiding amplification from partial or degraded RNA transcripts. In our study, RLM-RACE provided us with more information from cDNA libraries to complete the missing 5' end of our candidate gene. There remains the possibility of additional 5' sequence for this transcript because of the presence of a single-continuous ORF extending through the entire 5' end of the transcript. This gene may undergo alternative splicing and/or have multiple transcription initiation sites. Our approach of using different tissue libraries increased our chances of detecting the largest possible transcript but we must consider the possibility that even longer versions may exist in some tissues that we have not evaluated.

Proton-translocating adenosine triphosphatases have been classified into three main categories, called F, P, and V [10]. The vacuolar type (V-ATPases) are the most recently identified category of this group of proteins. V-ATPases are the major electrogenic pumps of vacuolar membranes, and are also important for energizing animal plasma membranes [10,16,17]. V-ATPases have a complex modular protein structure with multiple subunits that are divided into transmembrane proton-conducting and extramembrane catalytic sectors [18]. Tissue or developmentally specific subunit isoforms have been identified [18,19]. These subunits are believed to have some functions in regulating the localization and/or the activity of the enzyme. The catalytic sector subunits have been named as subunits A through F, and the membrane sector subunits have

been named using the letter M followed by a number corresponding to the apparent molecular weight based on gel mobility [18].

V-ATPases are believed to be functional and fundamental in almost every eukaryotic cell [16,17]. They contribute to a wide spectrum of cellular functions by energizing a wide variety of organelles and membranes [17]. It has been already shown that they play important roles in male fertility, driving bone resorption in osteoclasts, generating the driving force for the accumulation of neurotransmitters into the synaptic vesicles, and energizing transport systems in kidney cells [17]. V-ATPases also seem to play important roles in ocular tissues. Deguchi et al. [20] reported that V-ATPases are essential for the acidification of the lumen of phagolysosomes and the subsequent degradation of rod outer segments in rat RPE cells. Wax et al. [21] demonstrated the importance of plasma membrane V-ATPase as a driving force for aqueous humor formation in rabbit ocular ciliary epithelium, thus contributing to the mechanisms controlling the regulation of intraocular pressure.

Due to the wide spectrum and important functions of V-ATPases, and the expression of APT6M8-9 in multiple tissues, this gene may be a putative candidate for other systemic and/or ocular diseases that map to this region of the X chromosome. Mutations in other vacuolar H(+)-ATPase subunits have recently been identified as a cause of autosomal recessive osteopetrosis and a cause of recessive distal renal tubular acidosis in humans [22-25]. The expression of APT6M8-9 in brain and its potential importance in neurotransmitter uptake and storage, may be also relevant for some X-linked forms of mental retardation (XLMR). XLMR is likely to be very heterogeneous with multiple loci along the X chromosome; out of 130 XLMR syndromes identified so far, 80 loci have been mapped, but only 25 genes have been cloned [26]. Moreover, at least 10 additional nonsyndromal XLMR (MRX) genes remain to be discovered, in addition to the seven already cloned, and this number is expected to increase [26]. Given its potential role in rod outer segment degradation, this gene may also be a candidate for one or more of the pedigrees in which XLMR segregates together with RP [27], as well as for some of the X-linked forms of RP for which no mutation in RP2 or RP3 has been found.

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