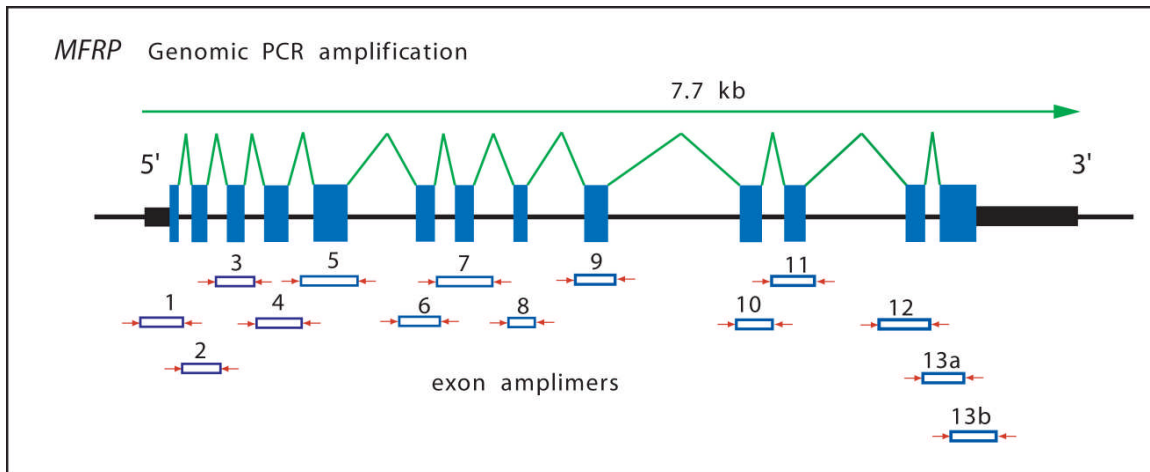


## Supporting Information

### 1. Primers used for PCR and sequencing of 13 MFRP exon coding regions.



**Fig. 7.** Coding exons of membrane-type, Frizzled-related protein (MFRP). Diagram showing genomic interval of MFRP, with noncoding mRNA sequence indicated by black rectangles, and coding sequence by solid blue rectangles. N-terminal end is on the left. Extent of exon amplimers indicated by open blue rectangles, with forward primers on the left and reverse on the right.

|             |                                  |
|-------------|----------------------------------|
| MFRP.01-For | CCAAGAACTGGTCTAGCCTGGCAGCCTT     |
| MFRP.01-Rev | TGGTGCTGGGTCTTAGGAGCACGATTCT     |
| MFRP.02-For | GCAAGGTAAGGACCACATAGAATCGTGCT    |
| MFRP.02-Rev | CCCCTTCTGTTGGGTATTCCTCATGCTGT    |
| MFRP.03-For | GAGGAATACCCAACAGAAAGGGCTTTCAA    |
| MFRP.03-Rev | GAGAGCGGCTCATGGAGTTTCATTCAA      |
| MFRP.04-For | AGCTCCTCTGAACGCCACCCTCCATCTTCT   |
| MFRP.04-Rev | GAGTTCAGAGGTCAAAAGGAGTGAGGTCCCT  |
| MFRP.05-For | GGACCTCACTCCTTTTGACCTCTGAACTCTA  |
| MFRP.05-Rev | TTAGCCCTTCTCCCTGCCACTCCCTGATTCT  |
| MFRP.06-For | CCAGTTTGGGGTTGAGAAAATAGGACTGCAA  |
| MFRP.06-Rev | TGGGTGGAGGGGAAGAAAGTGGACACTCAA   |
| MFRP.07-For | TTCCCCTCCACCCACTCCCAGTCTGTCAAA   |
| MFRP.07-Rev | GGGCCAAAGAATGACTGAGCAGGAAATGCTGA |
| MFRP.08-For | CCTAGCAAACCTCATGAGCCCTTCCACCTCT  |
| MFRP.08-Rev | CCCAAGTAGAAGGTAGTGTCTACCATGCCATT |
| MFRP.09-For | TTCCACAACCTCAGCCTGGAGGCTCAGGA    |
| MFRP.09-Rev | GAGAATGGAATGTGCTGGGCCGACATGGAA   |
| MFRP.10-For | AGGGCTGGTGCCCAGAACAGCTGTCTGCTTT  |
| MFRP.10-Rev | ATACCTACACCCCAAGTACCCCAAGAGTGT   |

MFRP.11-For ACAGACAAGGGCTCTGGACCCTGGTCA  
MFRP.11-Rev ACTGTGCAGTACGGCAGTAGGGTTCTGTGA

MFRP.12-For CATTGGACCCATGTACACACAGGACCGA  
MFRP.12-Rev AAATGCTGGTAGCAGGGCAGGCTTGTC

MFRP.13a-For GAGGTGGTAGAGGTCCTCAGCGTTACAA  
MFRP.13a-Rev AGACAGAGCGGCAAGGGGCAGAACACTGCCTA

MFRP.13b-For TGACAAGCCTGCCCTGCTACCAGCATTT  
MFRP.13b-Rev AGAGACCCTGCTGATGCTCCTTCCTTTGTT

## 2. Conservation at the Ile182Thr substitution site.



**Fig. 8.** Simplified rendering of X-ray structure of C1s backbone. This is an X-ray structure obtained from the National Institutes of Health/National Center for Biotechnology Information web site ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov); Protein Data Bank ID 1nzi, "Crystal Structure Of The Cub1-Egf Interaction Domain Of Complement Protease C1s" deposited by L. A. Gregory, N. M. Thielens, G. J. Arlaud, J. C. Fontecilla-Camps, and C. Gaboriaud, on February 18, 2003) Ile182 is highlighted in yellow.

In the available structure of C1QS, the corresponding Ile and Leu residues in the GIHL sequence are located in a  $\beta$ -sheet structure. Their hydrophobic side chains occupy an internal position in the subunit, filling a space between two closely apposed  $\beta$ -sheets. Both Ile and Leu are extremely conserved among genes containing this domain, with the only observed substitution being Ile>Val in a minority of cases. This extreme evolutionary conservation suggests the functional importance of having Ile, or a very similar hydrophobic residue at this site. The internal, hydrophobic location further suggests that substitution of the hydrophilic Thr could destabilize the protein structure.

**MFRP first cubilin domain, conserved 107 aa Ile182 marked in red**

CGLLSGPRGFFSSPNYPDPYPPNTHCVWHIQVATDHA**I**QLKIEALSIESVASCLFD  
 RLELSPEPEGPLLRVCGRVPPPTLNTNASHLLVVFVSDSSVEGFGFHAWY

**Complete protein sequence of polypeptide Chain A, C1S human serum complement subunit. The Ile182-homologous amino acid is indicated in red:**

EPTMYGEILSPNYPQAYPSEVEKSWDIEVPEGY**I**HLYFTHLDIELSENCAYSVQIISGDTEEGRLCGQ  
 RSSNNPHSPIVEEFQVPYNKLQVIFKSDFSNEERFTGFAAYVATDINECTDFVDVPCSHFCNNFIGGYF  
 CSCPPEYFLHDDMKNCGVN

**BLAST Protein Comparison of MFRP with ChainA, C1S**

The Ile82 site is marked in red, whereas other extremely conserved hydrophobic amino acids are in blue. All of these are involved in close contacts between the two  $\beta$ -sheets.

```

MFRP: 10  GFFSSPNYPDPYPPNTHCVWHIQVATDHAIQLKIEALSSIESVASCLFDRLEL--SPEPEG 67
      G  SPNYP YP      W I+V  + I L      L IE  +C +D +++      EG
C1S:  6  GEILSPNYPQAYPSEVEKSWDIEVPEGYIHLYFTHLDIELSENCAYSVQIISGDTEEG 65

MFRP: 68  PLLRVCGRVPPPTLNTNASH-----LLVVFVSD-SSVEGF-GFHAWY 107
      R+CG+      ++N H      L V+F SD S+ E F GF A+Y
C1S:  66  ---RLCGQ-----RSSNNPHSPIVEEFQVPYNKLQVIFKSDFSNEERFTGFAAY 112
  
```

**Evolutionary conservation of Ile182 in Cubilin domains of other human proteins.**

The location corresponding to **Ile 182** contains only an isoleucine or valine in nearly all Cubilin-type domains. The alignment shows amino acid position of part of the MFRP first cubilin domain (cub1) and the corresponding intervals of other human proteins. The second cubilin domain of MFRP shows considerable divergence, but conservation of this amino acid. Non-identity is indicated in lower case.

**Representative Alignments**

```

NP_113621 MFRP (cub1) 160 YPDYPPNTHCVWHIQVATDHAIQLKIEALSIESVASCLFD 200
NP_113621 MFRP (cub2) 317 YlqqYPhqllCtWHIsVpagHsIeLqfhnfSlEaqdeCkFD 357
NP_071317 CUZD1 170 YPkPhPelayCVWHIQVekDykIkLnfkeiflEidkqCkFD 210
NP_001072 CUBN 1407 fPnrYPPNkeCiWyIrtDpgssIQLtIhdfdvEyhsrCnFD 1447
NP_899234 OVCH1 436 YPdLYsNTrChWfIcapekHiIkLtfEdfavkfspnCiyD 476
NP_036596 TLL1 634 wPkeYPPNknCVWqvvpTqyrIsVKfEffeIEgnevCkyD 674
NP_036597 TLL2 480 YPdDyRPskeCVWrItVsegfhVgLtfqAfeIErhSCayD 520
NP_006122 BMP1 451 YPdDyRpskvCiWrIQVsegfhVgLtfqsfeIErhSCayD 491
NP_002584 PCOLCE2 180 Ypp----gisCsWHIiappDqvIaLtfEkfdlEpdyCryD 216
NP_002324 LRP3 62 wPlnYPPgtnCsWYIQgdrdmItIsfrnfdvEeshqCslD 102
NP_001725 C1s 28 YPqaYpseveksWdIeVpegygIhLyfthLdIElsenCaYD 68
  
```

### **3. Human MFRP cDNA clones in public databases.**

Below is a complete listing of all human MFRP cDNA clone sequences found in public cDNA or EST databases, as of April 16, 2005.

#### **A. Summary: Total of nine clones in GenBank and EST databases at NCBI.**

**One clone** from RPE cDNA library

**Six clones** from mixed fetal and adult eye tissues. All sources include RPE.

**Two clones** from brain (hippocampus).

Note that no clones are from neural retina cDNA libraries.

#### **B. List of cDNA library sources and sequence accession numbers:**

##### 1. Human Retinal Pigment Epithelium (Swaroop)

BI481236 clone [H2RPE-1382](#)

##### 2. Wistow RPE/Choroid

CA390202 clone [cs107g07](#)

CA389646 clone [cs100h03](#)

CA390714 clone [cs112g06](#)

##### 3. Fetal and adult eye tissues, including RPE. (Bonaldo *et al.*)

BM684116, BM719761 clone [UI-E-EJ1-aji-o-15-0-UI](#)

BU742804, BM929480 clone [UI-E-EJ1-aje-h-07-0-UI](#)

BM722889 clone [UI-E-EJ0-aie-o-07-0-UI](#)

##### 4. Sugano Hippocampus

BP251143 clone [HPR06485](#)

BI547497 clone [IMAGE:5262504](#)

cDNAs sources are accessed via accession numbers through NCBI/GenBank at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov).

#### **1. Human Retinal Pigment Epithelium (Swaroop):**

Buraczynska, M., Mears, A. J., Zarepari, S., Farjo, R., Filipova, E., Yuan, Y., MacNee, S. P., Hughes, B. and Swaroop, A. (2001) "Towards an expression profile of native human retinal pigment epithelium: Identification of a non-redundant set of more than 1100 genes" unpublished GenBank entry BI481236.

#### **2. Wistow RPE/Choroid**

Wistow, G., Bernstein, S. L., Wyatt, M. K., Farris, R. N., Behal, A., Touchman, J. W., Bouffard, G., Smith, D. and Peterson, K. (2002) Expressed sequence tag analysis of human RPE/choroid for the NEIBank Project: Over 6000 non-redundant transcripts, novel genes and splice variants. *Mol. Vis.* **8**, 205-220.

#### **3. Fetal and adult eye tissues, including RPE.(Bonaldo *et al.*)**

Bonaldo, M. F., Lennon, G. and Soares, M. B. (1996) Normalization and subtraction: two approaches to facilitate gene discovery *Genome Res.* **6**, 791-806.

#### 4. Sugano Hippocampus

Suzuki, Y., Yamashita, R., Shirota, M., Sakakibara, Y., Chiba, J., Mizushima-Sugano, J., Nakai, K. and Sugano, S. (2004) Sequence comparison of human and mouse genes reveals a homologous block structure in the promoter regions. *Genome Res.* **14**, 1711-1718