

STRUCTURE AND CHROMOSOMAL LOCALIZATION OF THE HUMAN ANTI-MÜLLERIAN HORMONE TYPE II RECEPTOR GENE

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SUMMARY: Using the rat anti-müllerian hormone type II receptor (AMHR II) cDNA as a probe, two overlapping lambda phage clones containing the AMHR II gene were isolated from a human genomic library. Sequence analysis of the exons was performed and the exon/intron boundaries were determined. The coding region was found to consist of 11 exons, divided over 8 kb. The genomic structure resembles that of the related activin type II receptor gene. The AMHR II gene was mapped to human chromosome 12q12-q13. The results reported are essential for identification of AMHR II gene alterations in patients with persistent müllerian duct syndrome. © 1995 Academic Press, Inc.

Anti-müllerian hormone (AMH) is a member of the TGF β family of growth and differentiation factors (1). AMH plays a critical role during male fetal sex differentiation, by inducing regression of the müllerian ducts, the anlagen of the female urogenital tract (2-4). During fetal life, AMH is produced only by the testicular Sertoli cells, but postnatally this factor is also found in ovarian granulosa cells (5).

Previous studies have shown that members of the TGF β family exert their action via a heteromeric signalling complex that includes a type I and a type II receptor (6). Both receptors are transmembrane serine/threonine kinases. The type I receptors constitute a smaller subfamily, that is characterized by a specific cysteine spacing in the extracellular domain and a GS-rich juxtamembrane motif (7).

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Recently, the rat (8) and the rabbit (9) AMH type II receptor (AMHR II) cDNAs were cloned, as new members of the serine/threonine kinase receptor family. AMHR II mRNA is expressed in the mesenchymal cells surrounding the müllerian ducts, during the period of fetal development that the müllerian ducts respond to AMH (8). Furthermore, COS cells transfected with AMHR II cDNA are able to bind AMH (9). AMHR II mRNA was also found in fetal gonads, and in Sertoli cells and granulosa cells of adult rats, although the function of AMH in the gonads remains to be established (10,11).

Failure of AMH action leads to the Persistent Müllerian Duct Syndrome (PMDS), a rare form of male pseudohermaphroditism characterized by the presence of uterus and fallopian tubes in otherwise normally virilized males (12). In some cases, this phenotype is caused by a mutation in the AMH gene (13). However, the presence of bioactive AMH in other PMDS patients (14) indicates resistance of the müllerian ducts to AMH, possibly as a result of alterations in the AMHR II gene. A mutation in the AMHR II gene that may result in alternative splicing has recently been reported (15).

Herein, we present the genomic structure of the human AMHR II gene and its chromosomal localization.

MATERIALS AND METHODS

Library screening: Approximately 1.7×10^6 plaques of a phage lambda EMBL3 human CMLO (chronic myeloid leukaemia cell line) genomic DNA library were transferred in duplicate to Hybond-N⁺ nylon filters (Amersham International plc, Little Chalfont, UK), and screened with the full length 1.9 kb rat AMHR II cDNA as a probe (8). The probe was labelled with [³²P]-dATP (Amersham) by random-priming. Hybridization was done o/n at 42 C in 50% formamide, 5xSCC, 5x Denhardt's, 1% SDS, 50 mM Na₂HPO₄/NaH₂PO₄ (pH6.8) and 100 µg/ml denatured herring sperm DNA. The filters were washed in 2x SCC and 0.1% SDS at 42 C for 10 minutes prior to autoradiography.

Positive phage lambda clones: Four positive clones were identified on initial screening of the genomic library. These clones were purified to homogeneity by secondary and tertiary screening using the rat AMHR II cDNA probe. Phage DNA of 2 positive clones was isolated, digested with several restriction enzymes and analyzed by gel electrophoresis to orient the genomic DNA with respect to the lambda arms. The DNA was transferred to Hybond-N⁺ filters (Amersham) and probed with fragments of the rat AMHR II cDNA to determine the positions of the 5' and 3' ends of the gene, the overlap, and the coding regions. Fragments of the coding regions were subcloned into the appropriate sites of the vector pBluescript KS(+) (Stratagene, La Jolla, CA, U.S.A.) for sequence analysis.

DNA sequence analysis: DNA sequencing was performed on plasmid DNA by the dideoxy chain termination method, using M13 and human AMHR II primers. Sequence alignments were performed using the sequence analysis program DNAMAN (Lynnon Biosoft, Vaudrieul, Quebec, Canada) and Clustal V.

In situ hybridization to metaphase chromosomes: Phage DNA was labelled with biotin-14-dATP using the bio-NICK system (Life Technologies, Gaithersburg, MD, U.S.A.). The labelled DNA was precipitated with ethanol in the presence of herring sperm DNA. Precipitated DNA was dissolved and denatured at 80 C for 10 min followed by incubation

for 30 min at 37 C, and added to heat-denatured chromosome spreads where hybridization was carried out o/n in a moist chamber at 37 C. After posthybridization washing (50% formamide, 2 x SSC, at 42 C) and blocking with nonfat dry milk powder, the hybridized probe was detected using avidin-FITC (Vector Laboratories, Burlingame, CA, U.S.A.) with two amplification steps using rabbit-anti-FITC (Dako, Glostrup, Denmark) and mouse-anti-rabbit FITC (Jackson Immunoresearch, West Grove, U.S.A.). Chromosome spreads were mounted in antifade solution with blue dye DAPI.

RESULTS AND DISCUSSION

Isolation and characterization of the human AMHRII gene

A human CMLO genomic library was screened with the rat AMHRII cDNA as a probe. Four positive clones were identified and two of these clones were used to construct a restriction enzyme map of the human AMHRII gene. Restriction enzyme mapping, Southern blot analysis using specific regions of the rat AMHRII cDNA as a probe, and DNA sequencing revealed that the AMHRII gene consists of 11 exons and 10 introns, and spans approximately 8 kb (Figure 1).

All eleven exons of the human AMHRII gene, and the intron/exon boundaries were sequenced. The DNA and protein sequences of the deduced open reading frame and the DNA sequences of the intron-exon boundaries are shown in Figure 2. Exons 2-10 vary in length from 78 bp to 234 bp, and the coding regions of the first (exon 1) and last (exon 11) exons are 49 bp and 297 bp, respectively. These lengths are consistent with the observation that the length of coding exons usually does not exceed 300 bp (16). Introns 4 and 10 are relatively long, 3 kb and 1 kb, respectively, and contribute to approximately half of the gene. The other introns are much shorter, varying from 120 bp to 425 bp. All of the introns start with GT and end with AG, consistent with the donor/acceptor splice rule (17).

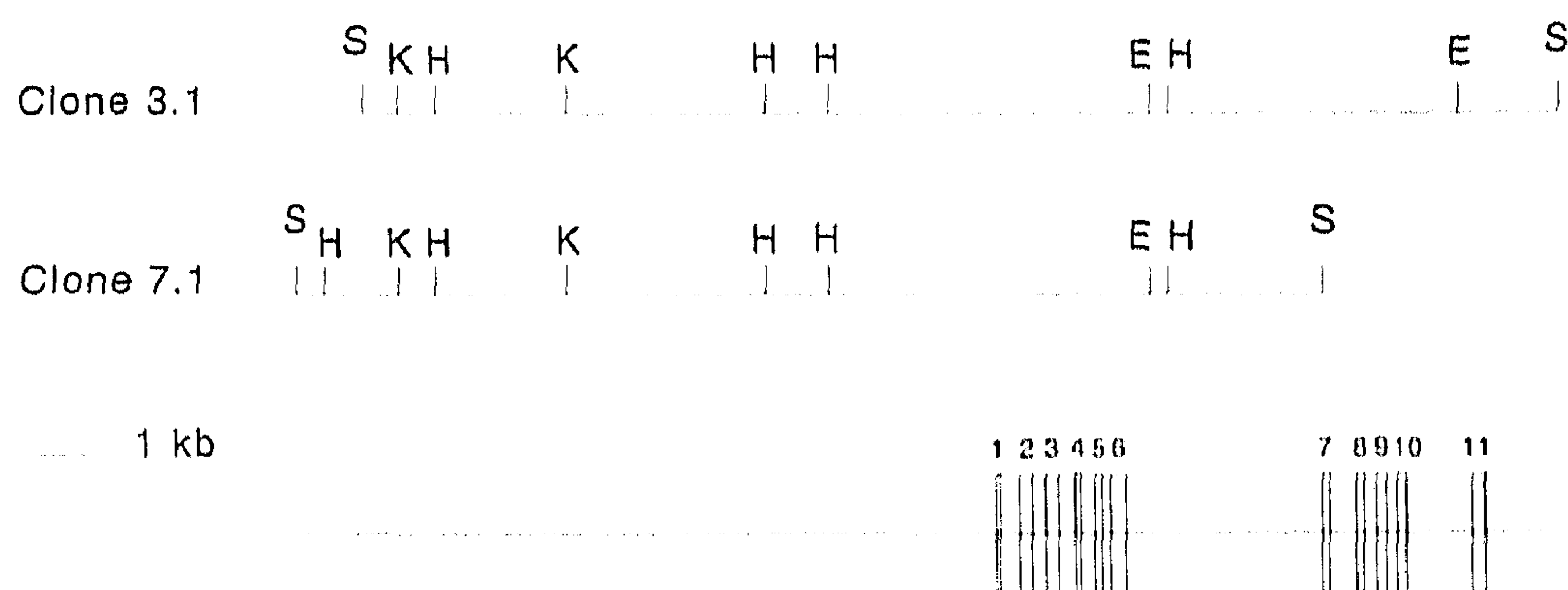


Figure 1. Structure of the human AMHRII gene. The overlapping phage lambda clones 3.1 and 7.1 are shown with the unique restriction enzymes that were used to construct a linear map. The relative length and localization of the exons (boxes) and introns is shown underneath (drawn to scale). E: EcoRI; H: HindIII; K: KpnI; S: Sall. The Sall sites are contained in the lambda EMBL3 arms and do not occur in the human AMHRII gene.

A

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                                             exon 1 <> exon 2
GGCTTATGCTCTTCTCCTTCTGCTGCTGCCATCCTCCAGCAAGATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAGGCCGAACCTGTGTGTT 120
M L G S L G L W A L L P T A V E A P P N R R T C V F 26

CTTTGAGGCCCTGGAGTGGCGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAA 240
F E A P G V R G S T K T L G E L L D T G T E L P R A I R C L Y S R C C F G I W N 66
                                             exon 2 <> exon 3
CCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCACCCCAGCCCTGGCTCCACTCT 360
L T Q D R A Q V E M Q G C R D S D E P G C E S L H C D P S P R A H P S P G S T L 106
                                             exon3 <> exon4
CTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCTG 480
F T C S C G T D F C N A N Y S H L P P P G S P G T P G S Q G P Q A A P G E S I W 146
                                             exon 4 <> exon 5
GATGGCACTGGTGCTGCTGCTGGGGCTGTTCCCTCCTCCTCCTGCTCGTGCTGGGCAGCATCATCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAG 600
M A L V L L G L F L L L L L V L G S I I L A L L Q R K N Y R V R G E P V P E P R 186
                                             exon 5 <> exon 6
GCCAGACTCAGGCAGGGACTGGAGTGTGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCAGGTAATCCGGGAAGGAGTTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAAGT 720
P D S G R D W S V E L Q E L P E L C F S Q V I R E G G H A V V W A G Q L Q G K L 226

GGTTGCCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAACTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGG 840
V A I K A F P P R S V A Q F Q A E R A L Y E L P G L Q H D H I V R F I T A S R G 266
                                             exon 6 <> exon 7
GGGTCCTGGCCGCCTGCTCTCTGGCCCCCTGCTGGTACTGGAAGTGCATCCCAAGGGTCCCTGTGCCACTACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCTGCGGATGGCACT 960
G P G R L L S G P L L V L E L H P K G S L C H Y L T Q Y T S D W G S S L R M A L 306
                                             exon 7 <> exon 8
GTCCCTCGCCCAGGGCCTGGCATTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCACCAGATCTGAGCAGCCAGAATGTGCTCATTTCGGGAAGATGGATC 1080
S L A Q G L A F L H E E R W Q N G Q Y K P G I A H R D L S S Q N V L I R E D G S 346
                                             exon 8 <> exon 9
GTGTGCCATTGGAGACCTGGGCCTTGCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCTCAAGGCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTA 1200
C A I G D L G L A L V L P G L T Q P P A W T P T Q P Q G P A A I M E A G T Q R Y 386

CATGGCACCAGAGCTCTTGGACAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGGCTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCA 1320
M A P E L L D K T L D L Q D W G M A L R R A D I Y S L A L L L W E I L S R C P D 426
                                             exon 9 <> exon 10
TTTGAGGCCTGACAGCAGTCCACCACCCTTCCAAGTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTGGCAGTGCAGGAGAGGAGGCGTCCCTACAT 1440
L R P D S S P P P F Q L A Y E A E L G N T P T S D E L W A L A V Q E R R R P Y I 466
                                             exon 10 <> exon 11
CCCATCCACCTGGCGCTGCTTTGCCACAGACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTGGGATGCAGACCCAGAAGCACGGCTGACAGCTGAGTGTGTACAGCAGCCCTGGC 1560
P S T W R C F A T D P D G L R E L L E D C W D A D P E A R L T A E C V Q Q R L A 506

TGCCTTGGCCCATCCTCAAGAGAGCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCACCTCTCTGCCAGAACTGTACTTCAATTCCCTGCCCCCTACCATTCTCCCTGTAGGCC 1680
A L A H P Q E S H P F P E S C P R G C P P L C P E D C T S I P A P T I L P C R P 546

TCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAAGGCCCTTGTTCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTGTAATATGCAGTTTATATCAGTTCTGACCAGTGACTT 1799
Q R S A C H F S V Q Q G ~ C S R N P Q P A C T L S P V * 573

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B

| EXON | (SIZE) | EXON 3' | INTRON | (SIZE) | EXON 5' | EXON | |
|---------|-----------|----------------------|------------------------|----------------|-------------------|----------------------|---------|
| exon 1 | (>49 bp) | GTG GAA G V E | gtaagt...intron | 1 (303 bp)... | tgggcctcag | CA CCC CCA A P P | exon 2 |
| exon 2 | (183 bp) | ATG CAA G M Q | gtgaat...intron | 2 (215 bp)... | catccatcag | GA GCC CGA G C R | exon 3 |
| exon 3 | (193 bp) | GCC CCA G A P | gtagcc...intron | 3 (264 bp)... | tgatgtccag | GT GAG TCC G E S | exon 4 |
| exon 4 | (78 bp) | ATC TTG G I L | gtacta...intron | 4 (212 bp)... | tctgttccag | CC CTG CTA A L L | exon 5 |
| exon 5 | (119 bp) | TTC TCC CAG F S Q | gtgccc...intron | 5 (120 bp)... | tccccagcag | GTA ATC CGG V I R | exon 6 |
| exon 6 | (234 bp) | CAT CCC AAG H P K | gtgagc...intron | 6 (~ 3 kb)... | gtttccccag | GGC TCC CTG G S L | exon 7 |
| exon 7 | (115 bp) | CAG AAT G Q N | gtgggt...intron | 7 (425 bp)... | tccccacag | GC CAA TAT G Q Y | exon 8 |
| exon 8 | (113 bp) | ATC ATG GAA I M E | gtgagt...intron | 8 (204 bp)... | tgctctccag | GCT GGC ACC A G T | exon 9 |
| exon 9 | (148 bp) | AGG CCT G R P | gtaagg...intron | 9 (165 bp)... | cttctccag | AC AGC AGT D S S | exon 10 |
| exon 10 | (147 bp) | TTT GCC ACA F A T | gtaaga...intron | 10 (~ 1 kb)... | ttccccccag | GAC CCT GAT D P D | exon 11 |
| exon 11 | (>297 bp) | | | | | | |

Figure 2. A Nucleotide and amino acid sequence of the deduced open reading frame. The arrowheads indicate the beginning (>) and end (<) of the respective exon. **B** Intron/exon boundaries in the human AMHRII gene. The nucleotide sequence for each intron/exon boundary and the size of each exon and intron are shown. The consensus acceptor/donor sequences are shown in bold.

Comparison of the deduced human AMHRII amino acid sequence to the sequences of the rat and rabbit receptors, revealed well-conserved sequences with few amino acid changes (Figure 3). The amino acid identity is approximately 80 %. In the AMH receptor the Ser³³⁵ in the sequence HRDLS is conserved in the three mammalian species investigated (Figure 3; see also (18)). This feature distinguishes the AMH type II receptor from the other members of the TGF β receptor family, which contain a lysine residue at that position. Another conspicuous characteristic of the AMHRII is the cysteine spacing in the extracellular ligand binding domain, which may reflect the evolutionary distance of the AMH-AMHRII pair to the other ligand-serine/threonine kinase receptor pairs.

Similar to the activin type II receptor (ActRII) (19) and the bone morphogenetic protein 2/4 type I receptor (BMPRI) (20) gene, the human AMHRII gene contains 11 exons. However, the AMHRII gene is remarkably small: 8 kb compared to >60 kb for the ActRII gene and 38.2 kb for the BMPRI gene. The different exons encode separate functional domains in the AMHRII protein molecule. The signal sequence is encoded by exon 1, the extracellular ligand binding domain by exons 2 and 3, and the transmembrane

| | | |
|-----------|-----|---|
| hAMHR II | 1 | MLGSLGLWALLP <small>T</small> AVEAPPNRRTCVFFFEAPG <small>C</small> RGSTKTLGELL <small>D</small> TGTELPR <small>A</small> IRCLYSRC |
| ocAMHR II | 1 | MLGTLGLWALLPAAVQAPPNRRTCVFFFEAPGVRGSTKTLGELL <small>D</small> AGPGPPRV <small>V</small> IRCLYSRC |
| rAMHR II | 1 | MLGTLGLW <small>T</small> LLPAA <small>A</small> QVSPNRRTCVFFFEAPGVRGSTKTLG <small>E</small> VV <small>D</small> AGPGPPK <small>G</small> IRCLYSH <small>C</small> |
| hAMHR II | 61 | CFGIWNL <small>T</small> Q <small>D</small> RAQVEMQGC <small>R</small> DSDEPGCESLHCDPSPRAHPSPGSTLFTCS <small>C</small> GTDFCNANY |
| ocAMHR II | 61 | CFGIWNL <small>T</small> R <small>D</small> Q <small>A</small> QVEMQGC <small>R</small> DSDEPGCESL <small>S</small> CDPSPRA <small>R</small> AS <small>S</small> GSTLFTCS <small>C</small> GA <small>D</small> DFCNANY |
| rAMHR II | 61 | CFGIWNL <small>T</small> H <small>G</small> RAQVEMQGC <small>L</small> DSDEPGCESLHCDP <small>V</small> PRAHPS <small>S</small> STLFTCS <small>C</small> GTDFCNANY |
| hAMHR II | 121 | SHLPPPG <small>S</small> PGTPGS <small>S</small> QGPQAAPGESIWMALVLLGLFLLLLLVLGSIILALLQ <small>R</small> KNYRV <small>R</small> GE |
| ocAMHR II | 121 | SHLPP <small>L</small> GGPGTPGPGQAAPGES <small>F</small> WMALALLGLVLLLLLLGGI <small>V</small> VALLQ <small>R</small> KAYRV <small>V</small> QSG |
| rAMHR II | 121 | SHLPP <small>S</small> GNRGAAPGQ <small>E</small> PQA <small>T</small> PGGPIWMAQ <small>L</small> LLGVFLVLLLSI--IILALLQ <small>R</small> KAC <small>R</small> VQGG |
| hAMHR II | 181 | PVPEPR <small>P</small> DSGRD <small>S</small> VELQ <small>E</small> LP <small>E</small> LCFSQVIREGGHAVVWAGOLQ <small>G</small> KLVAIKAFPPRS <small>V</small> VAQF |
| ocAMHR II | 181 | --PEPEP <small>D</small> SGRDCSEELP <small>E</small> LP <small>Q</small> LCFSQVIREGGHAAVWAGOLQ <small>G</small> ELVAIK <small>V</small> FP <small>R</small> RAVAQF |
| rAMHR II | 179 | SDPEPEP <small>G</small> SG <small>G</small> DCSEELP <small>E</small> LA <small>E</small> LR <small>F</small> SQVI <small>Q</small> EGGHAVVWAG <small>R</small> LQ <small>G</small> EM <small>V</small> VAIKAFPPRAVAQF |
| hAMHR II | 241 | QAERALYELPGLQ <small>H</small> DHIVRFITAS <small>R</small> GGPGR <small>L</small> LSGPLLVELELHPKGS <small>L</small> CHYL <small>T</small> OY <small>T</small> SDWGS |
| ocAMHR II | 239 | RAERALYELPGLQ <small>H</small> NH <small>V</small> VRFI <small>A</small> AGQGGPGLPSGPLLVELELHPKGS <small>L</small> C <small>Q</small> YLS <small>Q</small> H <small>T</small> SDWGS |
| rAMHR II | 239 | RAERAVY <small>Q</small> L <small>L</small> GLQHNHIVRFITAGQGGPGLPSGPLLVELEL <small>V</small> PKGS <small>L</small> CHYL <small>T</small> OY <small>T</small> SDWGS |
| hAMHR II | 301 | SLRMALS <small>L</small> AQGLAFLHEERW <small>Q</small> NGQYKPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLP <small>G</small> |
| ocAMHR II | 299 | SLRMALS <small>L</small> AQGLAFLHEERW <small>D</small> QYKPGIAHRDLSSQNVLIREDGSCAIGDLGLALVLP <small>G</small> |
| rAMHR II | 299 | SLRMALS <small>L</small> A <small>E</small> GLAFLH <small>G</small> ERW <small>D</small> QYKPGIAHRDLSSQNVLIRED <small>R</small> S <small>C</small> AIGDLGLALVLP <small>G</small> |
| hAMHR II | 361 | LTQPPAW <small>T</small> PTQP <small>Q</small> GPAIMEAGTQRYMAPELLDKTLDLQDWGMALRRAD <small>I</small> YSLALLLW <small>E</small> T |
| ocAMHR II | 359 | FAQPR <small>A</small> WAP <small>P</small> QPRGPAIMEAGTQRYMAPELLDK <small>S</small> LDLQDWGTALRRADVYSLALLLW <small>E</small> T |
| rAMHR II | 359 | LAQPPA <small>T</small> A <small>P</small> TQPRGPAI <small>T</small> EAGTQRYMAPELLDKTLDLQDWGTAL <small>Q</small> RADVYSLALLLW <small>E</small> T |
| hAMHR II | 421 | LSRCPDLRPDS <small>S</small> PPPFQLAYEAE <small>L</small> GN <small>T</small> PTSD <small>E</small> LWALAVQERRR <small>P</small> Y <small>I</small> P <small>S</small> T <small>W</small> R <small>C</small> FATDP <small>D</small> GL |
| ocAMHR II | 419 | LSRCPDLRPD <small>G</small> RPPPFQLAYEAE <small>L</small> GS <small>A</small> PT <small>T</small> CELWALAV <small>E</small> ERRR <small>P</small> D <small>I</small> P <small>S</small> SW <small>C</small> FATDP <small>G</small> GL |
| rAMHR II | 419 | LSRCS <small>D</small> LRPD <small>H</small> RPPPFQLAYEAE <small>L</small> GS <small>N</small> PS <small>A</small> CELWALAV <small>A</small> ER <small>K</small> R <small>P</small> N <small>I</small> P <small>S</small> SW <small>S</small> CS <small>A</small> TDP <small>R</small> GL |
| hAMHR II | 481 | RELLED <small>C</small> WDADPEARL <small>T</small> AECVQQR <small>L</small> AALAH <small>P</small> Q <small>E</small> SHFPF <small>E</small> SCPR <small>G</small> CPPLCP <small>E</small> D--CTSI <small>P</small> A |
| ocAMHR II | 479 | RELLED <small>C</small> WDADPEARL <small>T</small> AECVQQR <small>L</small> V <small>A</small> L <small>V</small> HP <small>Q</small> E <small>A</small> QP----CPEGR <small>P</small> HSH <small>P</small> EDWPPAP <small>A</small> P <small>A</small> |
| rAMHR II | 479 | RELLED <small>C</small> WDADPEARL <small>T</small> AECVQQR <small>L</small> AALAY <small>P</small> Q <small>V</small> ASSFP <small>E</small> SCP <small>Q</small> GC <small>P</small> ENY <small>P</small> -----A <small>P</small> A |
| hAMHR II | 539 | PTILPCR <small>P</small> Q <small>R</small> SACHFSVQ <small>G</small> PCSRNP <small>Q</small> PACTLS <small>P</small> V |
| ocAMHR II | 535 | PALLPGSP <small>Q</small> P <small>G</small> ACHFGVQ <small>G</small> L <small>C</small> SRNP <small>G</small> AACASS <small>D</small> V |
| rAMHR II | 533 | SAAFP <small>C</small> RP <small>Q</small> SS <small>C</small> LLSVQ <small>G</small> SG <small>S</small> KS----- |

Figure 3. Comparison of the human (hAMHR II), the rabbit (ocAMHR II) and the rat (rAMHR II) AMHR II amino acid sequences. Reversed script is used to highlight amino acid residues that are found at the same position in at least two of the three receptors.

domain is encoded by exon 4. The remaining exons 5-11 contain the sequence information for the kinase domain. When the gene structure of the receptors of the TGF β family is compared, the spacing of the exons and even the division of the functional protein domains into different numbers of exons indicates that the receptors show distant evolutionary relationship. Thus, this comparison provides no information on similarities in mechanism of action of the different serine/threonine kinase receptors.

Chromosomal localization of the AMHR II gene

Fluorescent *in situ* hybridization to metaphase chromosomes using the two isolated lambda phages, showed fluorescent signals at 12q12-13 (Figure 4). One of the clones (clone 3.1) also showed a signal at 9q, but this was not observed with clone 7.1 (not shown). Other genes in the 12q12/13 region include the Wingless-type MMTV integration site 1 gene (Wnt1), the Keratin 3 gene (Krt3), the retinoic acid receptor- γ gene (Rarg) and the Homeobox C cluster genes (Hoxc) (21). In the mouse, we mapped the mouse AMHR II

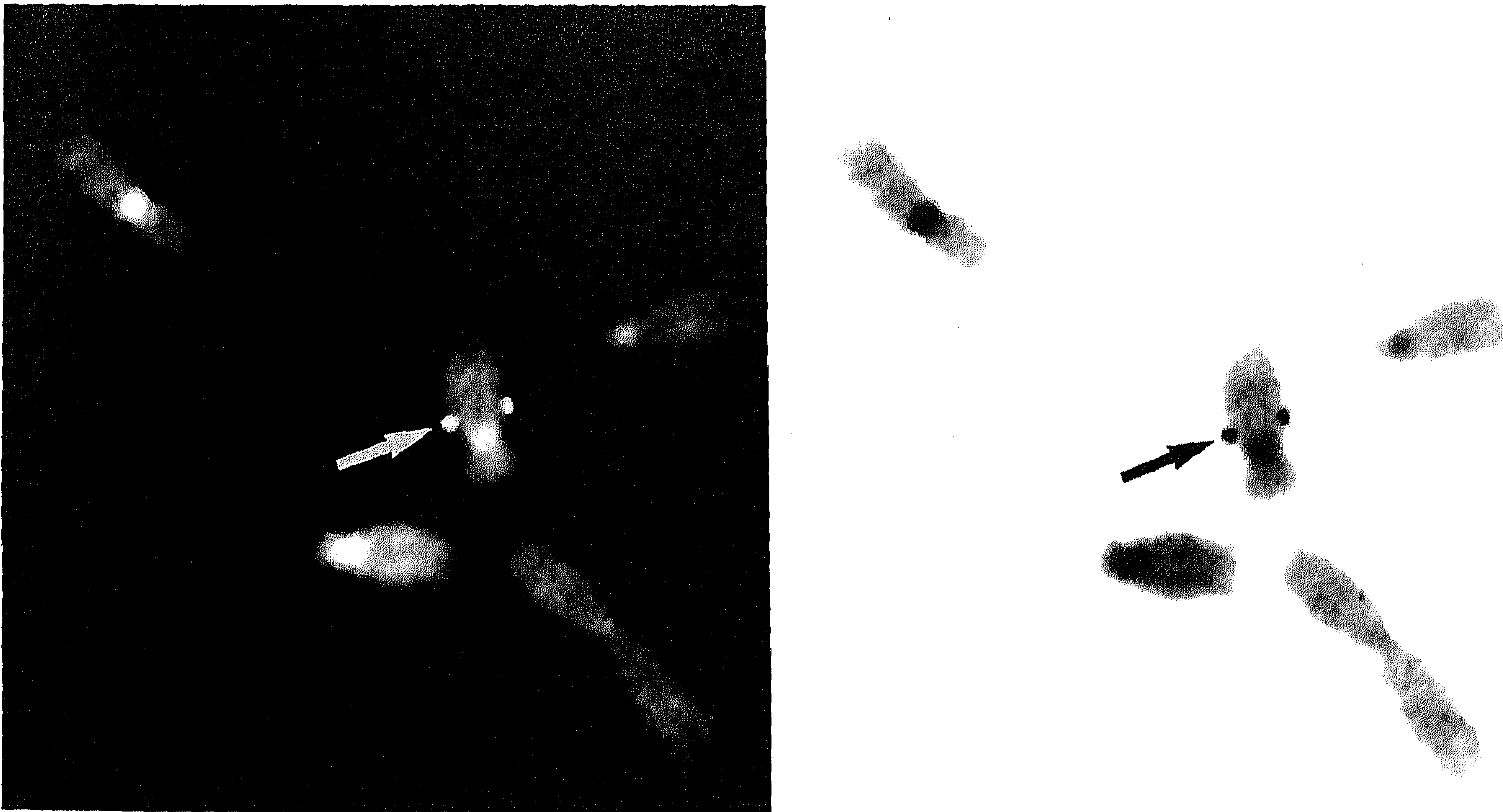


Figure 4. Chromosomal localization of the human AMHR II gene. The photomicrograph shows localization of the AMHR II probe on 12q12-q13 using fluorescence *in situ* hybridization (left panel). The identification of individual chromosomes was deduced from converted DAPI banding patterns (right panel). The hybridization sites are marked by arrows.

gene to chromosome 15F (result not shown). This region (15F(50-58)) is syntenic with the human chromosome 12, and contains mouse homologues of the genes mentioned above (22).

In conclusion, we have cloned and sequenced the human gene encoding the AMHR II. The AMHR II is well conserved between mammalian species. This information is essential to identify possible gene alterations in diseases involving the müllerian ducts.

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