# Determination of cDNA, gene structure and chromosomal localization of the novel human 17β-hydroxysteroid dehydrogenase type 7 [1]

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**Abstract We have identified human 17β-hydroxysteroid dehydrogenase type 7 (17β-HSD 7). The novel human cDNA encodes a 37 kDa protein that shows 78 and 74% amino acid identity with rat and mouse 17β-HSD 7, respectively. These enzymes are responsible for estradiol production in the corpus luteum during pregnancy, but are also present in placenta and several steroid target tissues (breast, testis and prostate) as revealed by RT-PCR. The human 17β-HSD 7 gene (*HSD17B7*) consists of nine exons and eight introns, spanning 21.8 kb and maps to chromosome 10p11.2 close to susceptibility loci for tumor progression, obesity and diabetes. The *HSD17B7* promoter (1.2 kb) reveals binding sites for brain-specific and lymphoid transcription factors corresponding to additional expression domains in hematopoietic tissues and the developing brain as identified by in silico Northern blot.**



*Key words:* 17β-Hydroxysteroid dehydrogenase; Prolactin receptor binding; Glioblastoma; Alternative splicing; Chromosome 10p11.2

## 1. Introduction

17β-Hydroxysteroid dehydrogenases (17β-HSDs) oxidize or reduce estrogens and androgens in mammals and regulate the biological potency of these steroids. Five types of 17β-HSDs have been described and analyzed in humans [1]. They differ in tissue distribution, kinetic parameters, substrate specificity, and the preferred direction of conversion. Further paralogs of the 17β-HSDs have recently been identified and await detailed characterization [2–5]. Most 17β-HSDs are widely distributed. Exceptions are the testosterone-producing 17β-HSD 3 which is testis-specific, and the recently cloned murine 17β-HSD 7 [3], an ovary-specific reductive enzyme. Rat and mouse 17β-HSD 7 are substantially upregulated in the ovary during pregnancy and at this time are responsible for the increasing levels of estradiol produced in the corpus luteum. Only very small amounts of mouse 17β-HSD 7 transcripts have been found in non-pregnant ovary, placenta, mammary gland, liver, kidney, and testis [3]. A special characteristic of rat 17β-HSD 7 is its association with the short form of the prolactin receptor. The function of this interaction is still unknown.

The tissue-specific expression pattern of 17β-HSD 7 in rodents suggests an important function in the maintenance of pregnancy, but also a role in tissues that are prone to develop endocrine-related cancers. The genomic structure of 17β-HSD 7 in mouse and rat has not yet been analyzed. To facilitate the functional analysis of 17β-HSD 7 in humans and the study of its possible role in human disease we present for the first time the complete coding sequence and genomic structure of human 17β-HSD 7 (gene name *HSD17B7*), as well as its chromosomal localization.

## 2. Materials and methods

### 2.1. Cloning of human 17β-HSD 7

By screening the human EST database at NCBI (http://www.ncbi.nlm.nih.gov) with murine 17β-HSD 7 cDNA, three candidate clones were identified and sequenced. These were used to design PCR primers for amplification of the complete coding sequence (1029 bp) of the human enzyme using ovary cDNA (Clontech, Heidelberg) as template. The forward primer (5′-TTTTGGATCCATGC-GAAAGGTGGTTTTGATCACCG-3′) contained a *Bam*HI restriction site (underlined), the reverse primer (5′-AAAAAAGCTTA-TAGGCATGAG-CCACTGAGCCTG-3′) a *Hin*dIII site (underlined) to facilitate subcloning.

### 2.2. Reverse transcriptase polymerase chain reaction (RT-PCR)

Expression of 17β-HSD 7 mRNA was checked by RT-PCR using the same primers. Human cDNA of ovary, testis, prostate, thymus, spleen and peripheral blood leukocytes were purchased from Clontech, cDNA from breast, placenta (kindly provided by Dr. F. Feuerhake) and liver hepatoma cell line HepG2 (ATCC, Manassas, USA) were prepared as described [6]. The concentration of templates was verified by PCR amplification of 324 bp of ribosomal protein 26S cDNA [7] with forward primer 5′-AATGGTCGTGCCAAA-AAGGGC-3′ and reverse primer 5′-TTACATGGG-CTTTGGTG-GGGG-3′ at the conditions described elsewhere [8].

### 2.3. Screening and characterization of human PAC clones

A genomic PAC library (female RPCI6 709) with four-fold coverage and an average insert length of 130 kb was obtained from the Resource Center, Primary Database of the German Human Genome Project at the Max-Planck-Institute for Molecular Genetics (Berlin, Germany). The library was screened with a randomly primed [$^{32}$P]dCTP-labeled probe corresponding to the full length of the human 17β-HSD 7 coding sequence. After hybridization filters were washed and exposed to Kodak X-Omat film for 12 h at −80°C as described [8]. Seven positive clones were detected and verified by a second PCR using primers in the 3′ region of the cDNA. The forward

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[1] The nucleotide sequence data reported in this paper have been submitted to the EMBL nucleotide sequence database with accession numbers: AF098786 (cDNA), AF126759–AF126767 (gene). Radiation hybrid mapping data were submitted to the EMBL RH database with accession numbers RH115827 and RH115828.
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*Abbreviations:* 17β-HSD, 17β-hydroxysteroid dehydrogenase; *HSD17B7*, gene of human 17β-HSD 7; SCAD, short-chain alcohol dehydrogenase


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primer (5′-CCAGGCTCAGTGGCTCATGC-3′) was placed at the end of the coding region, the reverse primer (5′-CCATGTACCCCA-GAATGTACAGAAGG-3′) was set complementary to the 3′ untranslated region, upstream of the polyadenylation signal resulting in a 370 bp PCR product. Out of three reconfirmed clones the one yielding the strongest PCR product (PAC128 = LLNLP709I05128Q2) was used for sequencing and determination of the genomic structure.

*2.4. Preparation of genomic DNA and sequencing*

Preparation of genomic DNA was performed with the Qiagen Maxi-Kit (Qiagen, Hilden, Germany). Sequencing was done by Dr. W. Metzger (Sequiserve, Vaterstetten, Germany) using standard protocols. To characterize the exon/intron organization of the *HSD17B7*, PAC128 was directly sequenced using primers derived from the cDNA sequence. Exon/intron boundaries were identified by alignments of

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TC TGA TTG GTG ACG GGT GAG GCG GCC CGA AAT CGT AGG ACT TCC GAA AGC AGC GGT GGC  -27

                                                    M   R   K   V   V   L   I   E
GTT TGC TTC ACT GCT TGG AAG TGT GAG TGC GCG AAG ATG CGA AAG GTG GTT TTG ATC ACC   24
cofactor binding site
G   A   G   G   G   I   G   L   A   L   C   K   R   L   L   A   E   D   D   E
GGG GCT AGC AGT GGC ATT GGC CTG GCC CTC TGC AAG CGG CTG CTG GCG GAA GAT GAT GAG   84

L   H   L   C   L   A   C   R   N   M   S   K   A   E   A   V   C   A   A   L
CTT CAT CTG TGT TTG GCG TGC AGG AAC ATG AGC AAG GCA GAA GCT GTC TGT GCT GCT CTG  144

L   A   S   P   T   T   A   E   V   T   I   V   P   V   D   V   S   N   L   Q
CTG GCC TCT CCC ACC ACT GCT GAG GTC ACC ATT GTA CCT GTG GAT GTC AGC AAC CTG CAG  204

S   V   F   R   A   S   K   E   L   K   Q   R   F   Q   R   L   D   C   I   Y
TCG GTC TTC CGG GCC TCC AAG GAA CTT AAG CAA AGG TTT CAG AGA TTA GAC TGT ATA TAT  264
NAG motif
L   N   A   G   I   M   P   N   P   Q   L   N   I   K   A   L   F   F   G   L
CTA AAT GCT GGG ATC ATG CCT AAT CCA CAA CTA AAT ATC AAA GCA CTT TTC TTT GGC CTC  324
predicted PRL receptor binding site
F   S   R   K   V   I   H   M   F   S   T   A   E   G   L   L   T   Q   G   D
TTT TCA AGA AAA GTG ATT CAT ATG TTC TCC ACA GCT GAA GGC CTG CTG ACC CAG GGT GAT  384

K   I   T   A   D   G   L   Q   E   V   F   E   T   N   V   F   G   H   F   I
AAG ATC ACT GCT GAT GGA CTT CAG GAG GTG TTT GAG ACC AAT GTC TTT GGC CAT TTT ATC  444

L   I   R   E   L   E   P   L   L   C   H   S   D   N   P   S   Q   L   I   W
CTG ATT CGG GAA CTG GAG CCT CTC CTC TGT CAC AGT GAC AAT CCA TCT CAG CTC ATC TGG  504

T   S   S   R   S   A   R   K   S   N   F   S   L   E   D   F   Q   H   S   K
ACA TCA TCT CGC AGT GCA AGG AAA TCT AAT TTC AGC CTC GAG GAC TTC CAG CAC AGC AAA  564
                active center
G   K   E   P   Y   S   S   A   K   Y   A   T   D   L   L   S   V   A   L   N
GGC AAG GAA CCC TAC AGC TCT TCC AAA TAT GCC ACT GAC CTT TTG AGT GTG GCT TTG AAC  624

R   N   F   N   Q   Q   G   L   Y   S   N   V   A   C   P   G   T   A   L   T
AGG AAC TTC AAC CAG CAG GGT CTC TAT TCC AAT GTG GCC TGT CCA GGT ACA GCA TTG ACC  684
                                putative transmembrane region
N   L   T   Y   G   I   L   P   P   F   I   W   T   L   L   M   P   A   I   L
AAT TTG ACA TAT GGA ATT CTG CCT CCG TTT ATA TGG ACG CTG TTG ATG CCG GCA ATA TTG  744

L   L   R   F   F   A   N   A   F   T   L   T   P   Y   N   G   T   E   A   L
CTA CTT CGC TTT TTT GCA AAT GCA TTC ACT TTG ACA CCA TAT AAT GGA ACA GAA GCT CTG  804

V   W   L   F   H   Q   K   P   E   S   L   N   P   L   I   K   Y   L   S   A
GTA TGG CTT TTC CAC CAA AAG CCT GAA TCT CTC AAT CCT CTG ATC AAA TAT CTG AGT GCC  864

T   T   G   F   G   R   N   Y   I   M   T   Q   K   M   D   L   D   E   D   T
ACC ACT GGC TTT GGA AGA AAT TAC ATT ATG ACC CAG AAG ATG GAC CTA GAT GAA GAC ACT  924

A   E   K   F   Y   Q   K   L   L   E   L   E   K   H   I   R   V   T   I   Q
GCT GAA AAA TTT TAT CAA AAG TTA CTG GAA CTG GAA AAG CAC ATT AGG GTC ACT ATT CAA  984

K   T   D   N   Q   A   R   L   S   G   S   C   L   .
AAA ACA GAT AAT CAG GCC AGG CTC AGT GGC TCA TGC CTA TAA TTCCAGCACTTTGGGAGGCCAAG 1049

GCAGAAGGATCACTTGAGACCAGGAGTTCAAGACCAGCCTGAGAAACATAGTGAGCCCTTGTCTCTACAAAAAGAAATA 1128

AAAATAATAGCTGGGTGTGGTGGCATGCGCATGTAGTCCCAGCTACTCAGAAGGATGAGGTGGGAGGATCTCTTGAGGC 1207

TGGGAGGCAGAGGTTGCAGTGAGCTGAGATTGTGCCACTGCACTCCAGCCTGGGTGACAGCGAGACCCTGTCTCAAAAT 1286

ATGTATATATTTAATATATATATAAAACCAGAGCTGACAATGACACTCTGGAACATTGCATACCTTCTGTACATTCTGG 1365

GGTACATGGATTTCTACTGAGTTGGATAATATGCATTTGTAATAAACTATGAACTATGAAAAAAAAAAAAAAAAAAA  1442
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Fig. 1. Protein and cDNA sequence of human 17β-HSD 7. The predicted amino acid sequence of human 17β-HSD 7 is given above its cDNA sequence. Exon boundaries are indicated by vertical black bars. Conserved motifs of the SCAD family are shaded dark gray. The predicted transmembrane region is shaded light gray. The putative prolactin receptor binding site is boxed. The in-frame stop codon upstream of the translation start site is given in boldface. The nucleotide sequence of human 17β-HSD 7 cDNA has been submitted to the EMBL nucleotide sequence database with accession number AF098786.

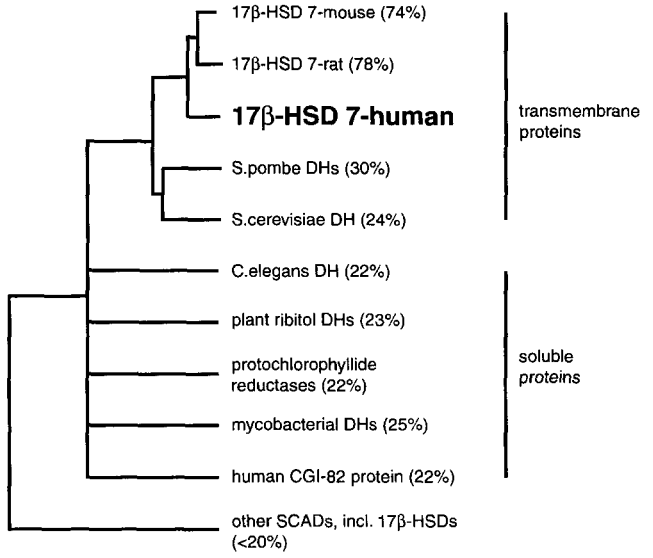


Fig. 2. Evolutionary tree of human 17β-HSD 7 and its relatives. The relationship among the transmembrane proteins was determined by a neighbor-joining analysis using the *C. elegans* dehydrogenase (GenBank accession number AAB37640) as outgroup. The branch lengths indicate the protein distances as evaluated by the Protdist algorithm of the Phylip package [12]. The details of the interrelationships of the other proteins were not determined. Therefore, branch lengths in this part of the diagram are arbitrary. Numbers in parentheses indicate percent amino acid identity compared to human 17β-HSD 7. GenBank accession numbers of representative proteins used for the analysis: mouse CAA75742; rat AAC52623; *S. cerevisiae* S64936; *S. pombe* CAA21246, BAA13878; mycobacteria CAA17300; *C. elegans* AAB37640; human CGI-82 AAD34077.1; ribitol dehydrogenase AAB63619.1; protochlorophyllide reductase Q01289; human 17β-HSD 3 NP000188; 11β-HSD1 P80365. DH: dehydrogenase.

cDNA and sequenced genomic fragments. The sizes of the introns were determined by long-range PCR using the Elongase System (Gibco Life Technologies, Berlin) with intron-spanning primers as described [8].

*2.5. Chromosomal mapping*

Genomic DNA of human-hamster radiation hybrid cell lines (Stanford G3 and GeneBridge GB4 panel) was purchased from Research Genetics (Huntsville, AL, USA). PCR typing of DNAs from the panels of hybrid cells was performed using primers designed from intron 8 and exon 9 (5′-GCTCTTTTACTAAGCCAGATTGATATTAGG-3′, 5′-ATGTCACC-ACACCCAGCTATTA-3′) which amplify a 565 bp PCR product. The results of the PCR reaction were assayed by 2% agarose gel electrophoresis. The resulting data were submitted to the Whitehead/MIT server at http://www-genome.wi.mit.edu (GB4) for multipoint analysis and the Stanford Human Genome Center server at http://www-shgc.stanford.edu (G3) for two-point analysis. The G3 data were further analyzed by multipoint analysis with the RHMAP 3.0 software package [9].

## 3. Results and discussion

*3.1. Cloning of the human 17β-HSD 7 cDNA*

Mouse 17β-HSD 7, the homolog of rat prolactin receptor-associated protein (PRAP), has been cloned recently [3]. Using the cDNA sequence of the murine enzyme we screened the human dbEST database at NCBI and identified initially three positive ESTs (GenBank accession numbers R83178, R92053 and H95413) which were ordered from the Resource Center, Primary Database of the German Human Genome Project at the Max-Planck-Institute for Molecular Genetics (Berlin, Germany). Only one of these clones (I.M.A.G.E clone IMAGp998K21369, GenBank R83178 [10]) contained the full length coding region, as confirmed by sequence comparison with the mouse and rat protein and the presence of an in-frame stop codon in the 5′ untranslated region. Unfortunately, this clone showed a point mutation leading to a premature stop signal at bp 248 from the start codon. Using the EST sequence data we designed PCR primers to clone the human 17β-HSD 7 from ovarian cDNA. PCR primers amplified the complete coding sequence plus restriction sites for

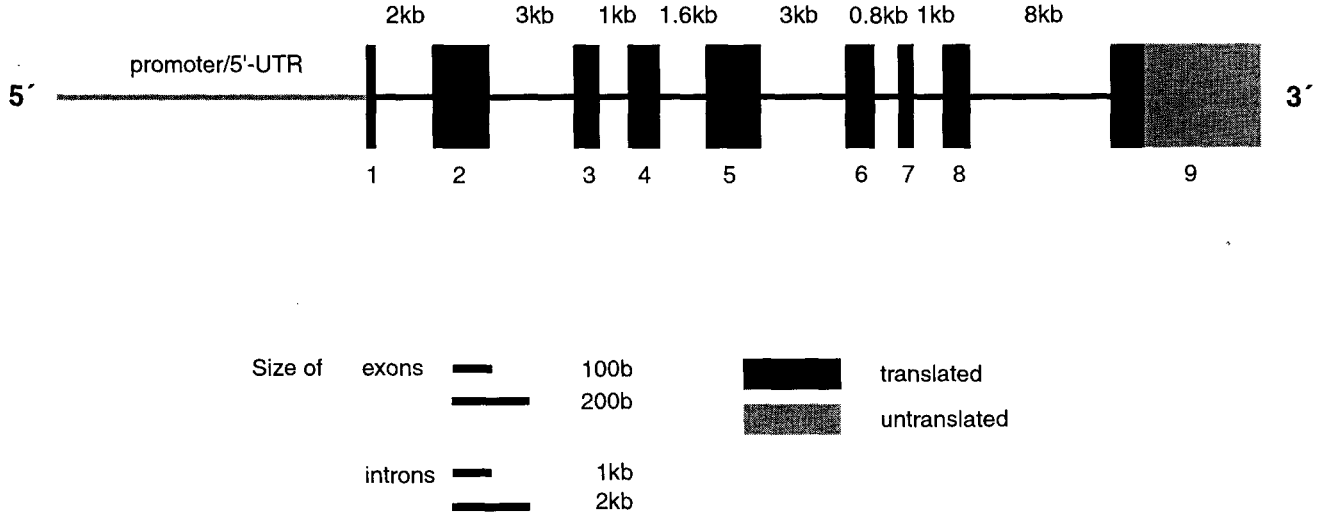


Fig. 3. Map of *HSD17B7* gene. Exons (boxes) and introns (black lines) are drawn to scale as indicated. The size of exon 1 was counted from the ATG codon. Exon 9 consists of translated (black box) and untranslated (gray box) regions and extends to the polyA site of the cDNA. Nucleotide sequences of the HSD17B7 gene have been submitted to the EMBL nucleotide sequence database with accession numbers AF126759–AF126767.

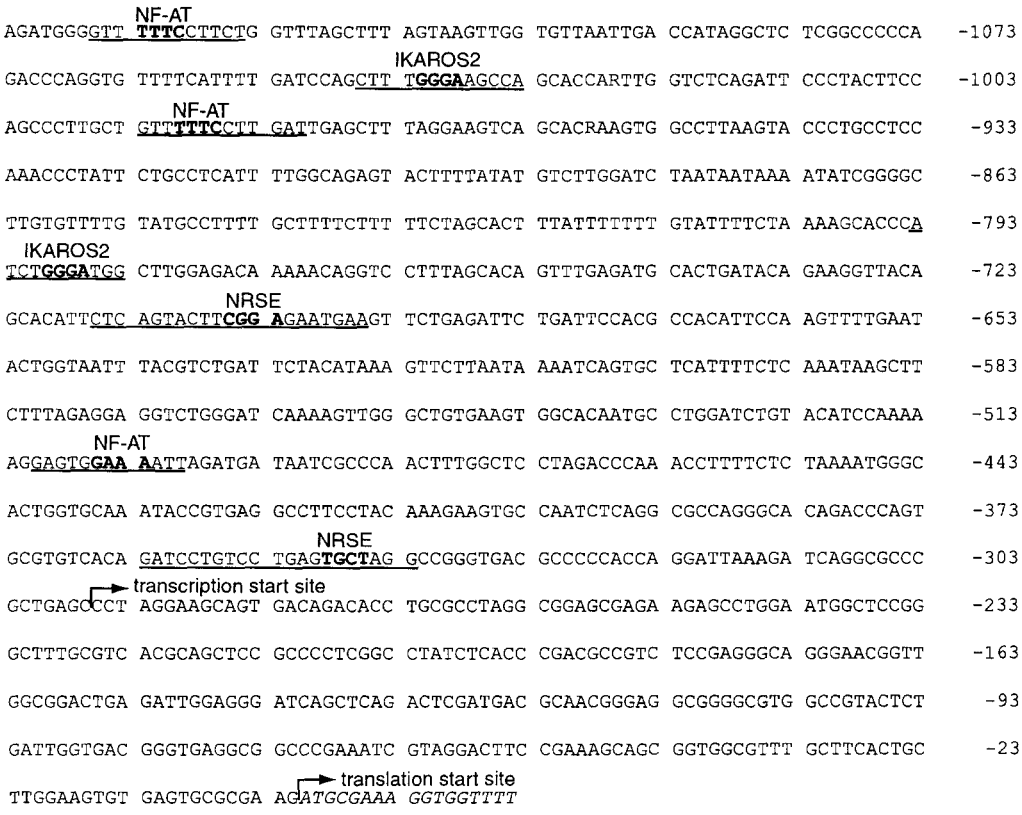


Fig. 4. Computer analysis of the HSD17B7 promoter sequence. The predicted transcription start site closest to the 5′ end of the transcript as known from the EST sequences and the translation start site are indicated by arrows. The coding sequence is indicated in italics. Predicted transcription factor binding sites are underlined. The core recognition sequences [15] are indicated in boldface. Nucleotide numbering is relative to the ATG start codon. NF-AT: nuclear factor of activated T-cells; NRSE: neuron-restrictive silencer element.

Table 1
Exon/intron organization of the *HSD17B7* gene

| Exon No. (size) | 5′splice donor | | Intron No. (size) | 3′splice acceptor | |
|---|---|---|---|---|---|
| 1 (34 bp) | A S S<br>GCT AGC AG | **gt**gaggcc... | I (2 kb) | ...cctaac**ag** | F I<br>T GGC ATT |
| 2 (203 bp) | K Q R<br>AAG CAA AG | **gt**atatct... | II (3kb) | ...aagcaa**ag** | F Q<br>G TTT CAG |
| 3 (92 bp) | F S R<br>TTT TCA AG | **gt**aatttt ... | III (1 kb) | ...tgtttc**ag** | K V<br>A AAA GTG |
| 4 (116 bp) | F I L<br>TTT ATC CTG | **gt**aaagaa ... | IV (1.6 kb) | ...tcaccc**ag** | I R E<br>ATT CGG GAA |
| 5 (194 bp) | N Q Q<br>AAC CAG CAG | **gt**aaggcc ... | V (3 kb) | ...gttcct**ag** | G L Y<br>GGT CTC TAT |
| 6 (104 bp) | I L L<br>ATA TTG CTA | **gt**aagtga ... | VI (0.8 kb) | ...ttccgc**ag** | L R F<br>CTT CGC TTT |
| 7 (56 bp) | E A L<br>GAA GCT CTG | **gt**atgtta ... | VII (1 kb) | ...ctttcc**ag** | V W L<br>GTA TGG CTT |
| 8 (98 bp) | T Q K<br>ACC CAG AAG | **gt**aaatgt ... | VIII (8 kb) | ...ggacct**ag** | M D L<br>ATG GAC CTA |
| 9 (520 bp) | | | | | |

The size of the first exon was counted from the initiating ATG and the end of exon 9 to the first A of the poly(A) tail of the cDNA.

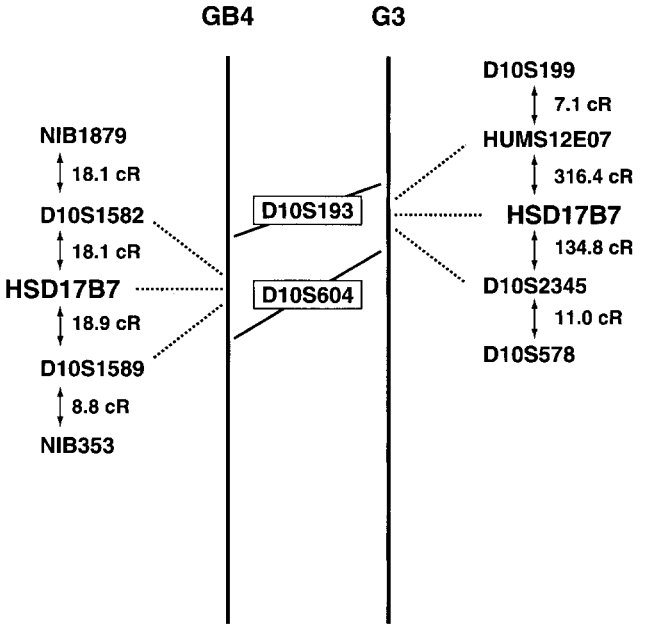


Fig. 5. Chromosomal localization of the *HSD17B7* gene. Mapping results from two radiation hybrid panels are compared. Two markers on the framework maps, D10S193 and D10S604, indicating the corresponding interval on GB4 and G3 maps are boxed. Distances on the GB4 and G3 maps are given in $cR_{3000}$ and $cR_{10000}$, respectively. Radiation hybrid mapping data were submitted to EMBL RH Database with accession numbers RH115827 and RH115828.

*Bam*HI and *Hin*dIII for subsequent subcloning. The resulting PCR product was ligated into the pCR 2.1 vector (Invitrogen, San Diego, CA, USA) and sequenced. It represented the full length coding sequence of the human 17β-HSD 7 (Fig. 1) and was devoid of the stop codon at position 248.

### 3.2. Phylogenetic analysis

Human 17β-HSD 7 is a member of the short-chain alcohol dehydrogenase (SCAD) family. Protein comparison using gapped BLAST [11] revealed high similarity between the mammalian 17β-HSD 7 enzymes and three transmembrane proteins from yeast (*Saccharomyces cerevisiae*, GenBank accession number S64936, *Schizosaccharomyces pombe*, numbers CAA21246, BAA13878). Phylogenetic analysis employing the Phylip package [12] showed that 17β-HSD 7 and the yeast proteins form a highly divergent group among a larger subfamily of the SCAD proteins (Fig. 2) which also includes the closest vertebrate homologue of 17β-HSD 7, an uncharacterized human protein identified by a comparative genomics approach (W.-C. Lin, 1999, GenBank number AAD34077). It was impossible to assign the 17β-HSD 7 to any of the HSD subgroups described earlier [13]. Therefore, 17β-HSD 7 is another example of convergent evolution towards 17β-HSD activity within the SCAD family. Sequence alignments (http://www2.ebi.ac.uk/clustalw) showed that compared to other SCAD domains the mammalian and yeast proteins share two unique features: a 28 amino acid insert following the conserved NAG motif (position 79–106) and a transmembrane region in the C-terminus detected by TMHMM1.0 [14] (Fig. 1). Comparison with the known 3D structures of SCAD domains revealed that the inserted 28 residues form a superficial loop close to the transmembrane domain. The spatial position and evolutionary conservation of this loop suggest an interaction with other membrane proteins. This loop is the best candidate for a domain responsible for binding to the prolactin receptor.

### 3.3. Structure of the HSD17B7 gene

We obtained seven clones representing genomic fragments of the *HSD17B7* gene by hybridization screening of a human genomic PAC library. Three of these were reconfirmed by PCR. PAC clone 128 was selected for direct sequencing with primers derived from the known cDNA. The 21.8 kb gene consists of nine exons and eight introns with a maximum size of 8 kb (Fig. 3 and Table 1). In all introns the consensus 5′ and 3′ splice acceptor sites GT and AG, respectively, were present. Among the closest relatives of the human 17β-HSD 7 as identified by phylogenetic analysis (Fig. 2) only the *Caenorhabditis elegans* dehydrogenase (GenBank number U80439) exhibits a comparable genomic organization consisting of eight exons. However, exon/intron borders differ widely in position and intron sizes are much smaller in the worm (total gene size $< 2.5$ kb).

### 3.4. Analysis of the 5′ untranslated region

To determine the most likely transcription start site we analyzed 1.2 kb of the genomic sequence upstream of the coding sequence, using a neural network. Three high-scoring transcription start sites were predicted. The most likely candidate is the one closest to the 5′ end of the transcript as deduced from the EST sequences (Fig. 4). An analysis of transcription factor binding sites by MatInspector Professional [15] identified recognition sequences for Ikaros2 and NF-AT, and two neuron-restrictive silencing elements, among others. Only binding sites with a core similarity of 100%

Table 2
In silico Northern blot for human 17β-HSD 7

| GB# | Start | End | Sequenced length | Source |
|---|---|---|---|---|
| T78371 | −95 | 352 | 447 | infant brain |
| AA317066 | −53 | 321 | 374 | retina |
| AA224260 | −51 | 399 | 450 | neuronal precursor |
| R83178 | −9 | 434 | 443 | fetal liver+spleen |
| R92053 | 415 | 851 | 436 | fetal liver+spleen |
| N77671 | 445 | 669 | 224 | fetal liver+spleen |
| AA811920 | 1365 | 523 | 842 | germinal B cells |
| H95413 | 575 | 1016 | 441 | fetal placenta |
| R92007 | 1398 | 1008 | 390 | fetal liver+spleen |
| AA506121 | 1423 | 1046 | 377 | breast |
| AA632360 | 1423 | 1051 | 372 | breast |
| H58818 | 1406 | 1058 | 348 | fetal liver+spleen |
| H72950 | 1422 | 1070 | 352 | fetal liver+spleen |
| H95380 | 1411 | 1079 | 332 | fetal placenta |
| AA743742 | 1423 | 1088 | 335 | germinal B cells |
| AI245405 | 1423 | 1089 | 334 | kidney |
| AI351558 | 1430 | 1122 | 308 | total fetus (8–9 weeks) |
| N58305 | 1423 | 1153 | 270 | fetal liver+spleen |
| R83179 | 1422 | 1202 | 220 | fetal liver+spleen |
| AA614782 | 1423 | 1240 | 183 | breast |
| AA037668 | 1423 | 1252 | 171 | pregnant uterus |
| AA047796 | 1422 | 1262 | 160 | retina |

EST sequences derived from human 17β-HSD 7 transcripts present in the dbEST database are shown. GB# corresponds to GenBank accession number. Start and end of the sequence are given relative to the start codon of the coding sequence of human 17β-HSD 7. The last column indicates the tissue source of the EST libraries.

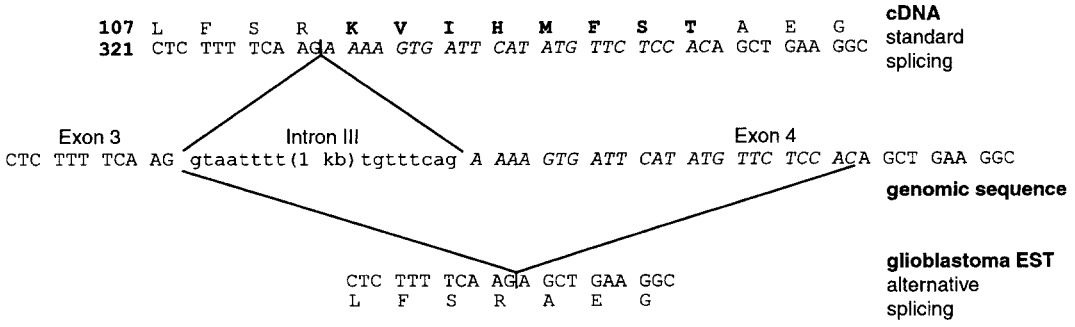


Fig. 6. Putative alternative splicing of the human 17β-HSD 7 transcript in a glioblastoma-derived EST (GenBank number AI589113.1). Alternative splicing at the 3′ end of intron 3 leads to a predicted deletion of eight amino acids in the prolactin binding region.

were evaluated. No recognition sequences for members of the steroid receptor superfamily were predicted (Fig. 4). The presence of several lymphoid and brain-specific transcription factor binding sites and the complete absence of steroid-responsive elements in the *HSD17B7* promoter were quite unexpected, as the tissue-specific expression of 17β-HSD 7 in mouse has been shown to be upregulated in response to increased estradiol levels during pregnancy and the $P450_{scc}$ inhibitor aminoglutethimide does disrupt murine 17β-HSD 7 expression [16]. Our data imply that the endocrine regulatory effect on 17β-HSD 7 might be indirect.

### 3.5. Mapping of human HSD17B7 gene to chromosome 10

Radiation hybrid mapping with the Stanford G3 and GeneBridge GB4 panels showed that the *HSD17B7* gene maps to chromosome 10p11.2 between markers D10S1582 and D10S1589 (Fig. 5). The radiation hybrid analysis with both GB4 and G3 pointed to a single location of *HSD17B7* in a pericentric region of high genetic plasticity [17,18]. Several human disorders were mapped to the same region: human prostate cancer and sarcoma are commonly associated with allelic loss in the chromosomal region 10p11.2 [19,20]. The mouse tumor progression locus 2 maps to a region on chromosome 18 syntenic with the human chromosome 10p11 [21]. In the glioblastoma multiforme cell line IN1434 chromosome 10 is disrupted at band 10p11–12.1 by translocation with chromosomes X and 15 [22]. Recently, susceptibility loci close to the *HSD17B7* locus were reported for hyperlipidemia [23], obesity [24], and type 1 diabetes [25]. The involvement of 17β-HSD 7 in the above disorders remains to be clarified.

### 3.6. Tissue distribution

Nokelainen et al. [3] have shown that rat and mouse 17β-HSD 7 are predominantly expressed in ovarian corpus luteum at days 8–18 of pregnancy whereas only weak expression is observed in non-pregnant ovary. The tissue distribution of the human enzyme was analyzed by RT-PCR using in silico Northern blot data for tissue pre-selection.

EST database searches with the full length human 17β-HSD 7 cDNA identified 22 EST sequences derived from the *HSD17B7* gene (Table 2). Most of the ESTs were obtained from normal tissue. One further EST (GenBank number AI589113.1) from a glioblastoma was obviously also derived from the *HSD17B7* gene but showed an internal 24 bp deletion compared to the cDNA. Comparison with the genomic sequence indicated that this EST might be the product of an alternative splicing reaction at the 3′ end of intron 3 leading to a predicted deletion of eight amino acids (Fig. 6) affecting the central part of the proposed prolactin receptor binding site. Truncation of the protein loop may lead to a loss of binding capacity, thus disrupting the functional interaction between 17β-HSD 7 and the short form of the prolactin receptor [16].

The results of the EST database search correspond closely to the expression pattern of 17β-HSD 7 seen in mouse and rat, e.g. ESTs were found in breast, the tissue the mouse enzyme has been cloned from [3]. The expression in pregnant uterus and placenta was also expected and confirmed by a strong signal in RT-PCR for the latter (Fig. 7). Absent are ESTs from ovary/corpus luteum, the tissue with the most abundant 17β-HSD 7 expression in mouse and rat, especially during pregnancy. However, RT-PCR revealed the presence of 17β-HSD 7 transcript in ovaries of non-pregnant women. Human 17β-HSD 7 like the rodent enzymes is also present in testis and prostate (Fig. 7).

17β-HSD activity has recently been shown for B and T lymphocyte 17β-HSD 1 [26]. Human 17β-HSD 7 ESTs were detected in germinal B cells and thymus but not in the peripheral leukocytes, indicating an involvement of the enzyme in hemopoiesis in addition to reported role of 17β-HSD types 1 and 4 [27]. This fits the prediction of Ikaros2 and NF-AT binding sites in the promoter region of 17β-HSD 7. In this context it is important to note the pronounced gender differences observed in autoimmune diseases and the immunomodulatory effects of both prolactin and estradiol [28].

Surprising was the large number of 17β-HSD 7 ESTs found in fetal liver. This was corroborated by RT-PCR in the liver cell line HepG2. The functional significance in this tissue remains unclear. Further unexpected regions of 17β-HSD 7 ex-

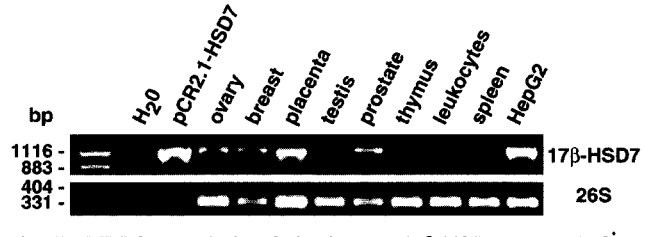


Fig. 7. RT-PCR analysis of the human 17β-HSD 7. cDNA from human tissues was amplified using primers specific for 17β-HSD 7 (1029 bp) or ribosomal protein 26S (324 bp) as described in Section 2. $H_2O$: negative control (no template); pCR2.1-HSD7: positive control (human 17β-HSD 7 cDNA in pCR2.1 vector); HepG2: human hepatoma cell line HepG2. Molecular mass markers are given on the left.

pression were detected in several neural tissues (retina, neuronal precursors and infant brain) and in a glioblastoma. 17β-HSD activity has been shown in the developing brain [29,30] and in the retina [31]. Transcripts of 17β-HSD 1–4 were detected in the human brain [32] and high levels of 17β-HSD 4-mRNA are present in chick retina [33]. In women brain tumor symptoms vary during pregnancy and the menstrual cycle [34] and altered levels of estradiol in brain tumors have been implicated in glioblastoma progression [35,36].

Analysis of the tissue distribution of 17β-HSD 7 transcripts suggests that in addition to its role in reproductive tissues the human enzyme is involved in brain steroid metabolism and might be an interesting new target for brain tumor treatment.

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