

The human cadherin-10 gene: complete coding sequence, predominant expression in the brain, and mapping on chromosome 5p13–14

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Abstract In a quest for novel cadherin gene family members in the human dbEST database, an interesting EST clone was identified and chosen for subsequent analysis. Using the technique of 5' rapid amplification of cDNA ends, we isolated the complete coding sequence and a large part of the UTRs of a novel gene. The sequence appeared to correspond to the human cadherin-10 gene, whose sequence was only partially known before. The expression pattern of this cadherin was found to be largely brain-specific, with additional expression in both adult and fetal kidney, and with minor expression in prostate and fetal lung. By FISH analysis the genomic location was determined at human chromosome 5p13–14, which is nearby the reported positions of the human cadherin-6, -12, and cadherin-14 (*CDH18*) genes. Cadherin-10 shows high relationship to the human cadherin-6 gene.

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Key words: Cadherin; Cell-cell adhesion; Expression pattern; Chromosomal localization

1. Introduction

Multicellular organisms depend for their structure and organization on their intercellular contacts. This was demonstrated in developmental studies and in the study of cancer, where loss of cell-cell adhesion contributes to the malignant character of tumor cells. These findings stimulated the characterization of a large number of cell-cell adhesion molecules, among which the cadherins.

Classical cadherins are calcium-dependent intercellular glycoproteins containing five extracellular cadherin repeats of about 110 amino acid residues with typically conserved sequences [1]. The extracellular cadherin domains bind Ca^{2+} , which is necessary for correct protein folding and normal functioning [2]. Additionally, most cadherins contain a transmembrane and a cytoplasmic domain. The cytoplasmic domains are often linked via the catenins to the cytoskeleton [3]. This structure is a general feature of most cadherins, notwithstanding the presence of several intermolecular differences [4].

Despite the large number of cadherins already identified, some of the cadherin-like sequences are still partial [5–9]. Their identification was based on the use of degenerate primers designed on well conserved sequences. In our study we aimed at the isolation of still new family members by *in silico* detection, i.e. screening of public domain databases contain-

ing data of expressed sequences (ESTs). This allowed us to identify at least ten new cadherin-like genes.

We report here the characterization of one of those novel family members, which turned out to be identical to the identified but only partially published human cadherin-10 cDNA [7]. We present here the complete coding sequence of this gene, a large part of the 5' untranslated region (UTR), and presumably its complete 3' UTR. Additionally, the expression pattern and chromosomal localization were determined. Furthermore, phylogenetic analysis using the cadherin-10 protein sequence demonstrated a close relationship to the previously reported mouse T2-cadherin [10].

2. Materials and methods

2.1. *In silico* detection and analysis

The public domain databases dbEST, Genbank and EMBL were screened with a simple 'cadherin' word search. All hits were analyzed by BLAST searches [11] and sequence alignments (SeqMan 3.57; Lasergene, DNASTAR, Madison, WI, USA).

Cadherin protein sequences used for phylogenetic analysis were deduced by *in silico* translation of the cDNA sequences: Genbank Acc. No. P12830 (human E-cadherin), P19022 (human N-cadherin), I34427 (rat cadherin-10), AB000512 (chicken cadherin-10), D31784 (human cadherin-6), D82029 (mouse cadherin-6), P55280 (rat cadherin-6), D42149 (chicken cadherin-6B), U69137 (mouse cadherin-T2), L34060 (human cadherin-8), and L34057 (human cadherin-12). A sequence editor was used to select the carboxy-terminal 265 amino acid residues of each translated product. Multiple sequence alignment was performed using CLUSTAL (MegAlign; DNASTAR) software.

2.2. DNA isolation, probe preparation and radioactive labeling

The cadherin-10 EST clone was obtained from the I.M.A.G.E. consortium UK-HGMP Resource Centre (Hinxton, UK). All DNA isolations of plasmid or PAC clones were carried out according to protocols supplied with Qiagen columns (Chatsworth, CA, USA) or with Magnum KB-100 columns (Genome System, St. Louis, MO, USA), respectively. DNA fragments were purified on agarose gel, extracted using a WIZARD DNA Clean-Up system (Promega, Madison, WI, USA), and radiolabeled with α - ^{32}P -dCTP (Amersham Pharmacia Biotech, Rainham, UK) using a Rad-Prime labeling kit (Gibco-BRL, Neu-Isenberg, Germany). For colony hybridizations, oligonucleotides were labeled using γ - ^{32}P -dATP (Amersham-Pharmacia Biotech) and T4 kinase (Gibco-BRL).

2.3. Nucleotide sequence analysis

Sequence reactions were performed using the dideoxy chain termination method with fluorescent detection on an ABI-PRISM-377 apparatus (Perkin Elmer, Foster City, CA, USA), using plasmid-specific primers, and various gene-specific walking primers. All primers were designed using Oligo v5.0 software (Medprobe, Plymouth, MN, USA) and synthesized by Gibco-BRL. All generated sequences were aligned using STADEN software pregap and gap4.0 [12].

2.4. PCR and RACE experiments

PCR reactions were performed on an MJ Research PTC-200 apparatus (MJ Research, Watertown, MA, USA). For every PCR reaction 2 mM of each dNTP (Sigma Chemical Co., St. Louis, MO, USA), 25 pmol of each primer, and 0.5 units of Taq DNA polymerase (Gib-

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co-BRL) was used in 1×PCR buffer (Gibco-BRL). Template DNA amounts were estimated to be 50–100 ng. The typical PCR temperature profile was 3 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at optimal annealing temperature, and 1 min at 72°C, followed by final extension at 72°C for 5 min.

RACE experiments were performed according to the Marathon cDNA amplification protocol (Clontech Laboratories, Palo Alto, CA, USA) using poly(A)⁺ selected (Micro-FastTrack, Invitrogen, San Diego, CA, USA) fetal brain RNA (Clontech). RACE PCR reactions were done in final volumes of 50 µl, using a Perkin-Elmer 2400 GeneAmp PCR system (Perkin Elmer) and the Advantage cDNA polymerase mix (Clontech). Primers for the first 5' RACE experiment were: MCB444 (5'-CACCACAGGCAAGAGATAGGTACTG-3') as GSP-1, and MCB445 (5'-TTTGGATTGACAGCAGCTAAACTGA-3') as GSP-2. The second 5' RACE experiment was carried out with primers MCB779 (5'-TGTGCCAACTGGGGAGGATTCA-3') as GSP-1 and MCB780 (5'-TCCCCGATAAGCCTCCCATCTG-3') as GSP-2. RACE products were cloned in pGEMT (Promega, Madison, WI, USA).

2.5. RNA isolation and Northern blot analysis

Total RNA was isolated using RNazol (WAK-Chemie-Medical, Bad Homburg, Germany). Fetal brain RNA was from Clontech. Most cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). For Northern blot analysis 15 µg total RNA was glyoxylated [13], size-fractionated on a 1% agarose gel and transferred to Hybond-N (Amersham). Hybridization of Northern blots with the insert of EST clone I.M.A.G.E. 32430 was performed according to Church and Gilbert [14]. The 285-bp cadherin-6-specific probe was obtained by standard PCR with genomic DNA as template, using primers MCB275 (5'-GAATCTTACCTTGTGCAG-3') and MCB276 (5'-CATCTTACTTCTTGGGT-3') at an annealing temperature of 54°C. For quantity and quality control of the RNA samples, Northern blots were stripped and checked by rehybridization with a GAPDH probe. A human RNA Master Blot (Clontech) was hybridized according to the manufacturer's protocol using Express-Hyb Hybridization solution (Clontech).

2.6. Isolation of the genomic PAC clone

Superpools and subsequent plate pools from the RPCII PAC library [15] were screened by PCR, using primers MCB363 (5'-AATCACTTCCAAGAGCCA-3') and MCB364 (5'-CAGGTTGCATATCACACC-3') at an annealing temperature of 54°C. This resulted in the plate number of a 384-well microtiter plate. PCR analysis of the pooled rows and columns of this plate yielded the coordinates of a positive well (31D12). Single colonies were purified and checked by PCR. A positive colony was grown and used for DNA isolation. Sequence analysis was done using primers MCB676 (5'-ATGCTGTCTCCACTTCACAA-3') and MCB364 on PAC DNA as template. About 820 bp of 3' UTR sequence was obtained, with 70 bp positioned 3' of the cDNA poly-adenylation site (data not shown). This excludes the possibility of having isolated a PAC clone specific for a processed pseudogene.

2.7. FISH analysis

FISH analysis using the human cadherin-10-specific PAC clone was performed according to standard procedures [16]. DNA of the PAC clone and centromere-5 probe was biotinylated using a BioNick kit (Gibco-BRL). Fluorescent images were captured by a Photometrics Image Point CCD camera (Photometrics, München, Germany) mounted on a Zeiss Axiophot microscope. Image processing and chromosome G-banding was obtained by reverse DAPI-banding, using the MacProbe v3.4.1 software (Perceptive Scientific International, League City, TX, USA). The chromosome 5 centromeric probe was a gift from Dr. F. Speleman (Department of Medical Genetics, University Hospital, Ghent, Belgium).

3. Results and discussion

3.1. Determination of the full-length cDNA sequence of cadherin-10

In an attempt to identify novel members of the cadherin superfamily, our database analysis revealed the existence of several putative cadherin-like genes. For one of these, the

representative clone I.M.A.G.E. 32430 was obtained from the UK-HGMP Resource Centre and sequenced. A BLAST-N search [11], with the sequence obtained for the 1450-bp insert of the EST clone, showed high similarity to the human cadherin-6 and chicken cadherin-10 cDNAs, but failed to indicate that it was identical to other already described human cadherin sequences.

Additional sequence data from the 5' end of the cadherin-like transcript was obtained after cloning of the products of a 5' RACE experiment using the gene-specific primers MCB444 and MCB445. The RACE clone sequences partially overlapped the previously obtained sequence, giving a full match in the overlapping area. However, they failed to reveal the presumed start codon, making further 5' RACE experiments obligatory. The remaining coding sequence and part of the 5' UTR were obtained by subsequent 5' RACE experiments using the primers MCB779 and MCB780.

BLAST-N searches of the resulting composed sequence of 3966 bp (Fig. 1, Genbank Acc. No. AF039747) revealed a sequence almost identical to the rat cadherin-10 cDNA (Acc. No. I34427; Suzuki et al., unpublished data). However, the latter sequence of 2490 bp is not complete as translation of its largest open reading frame (ORF) produces a protein of 618 amino acid residues, which should lack about 170 amino acid residues of the N-terminal. Within US patent 5597725-A a 730-bp, and therefore incomplete, sequence of the human cadherin-10 gene appeared to be identical to our sequence.

The cadherin-10 sequence encodes a 2367-bp ORF with a putative start codon on position 1036, and a TAA stop codon on position 3402. The start codon does not fully match the Kozak consensus sequence for a ideally translationable start codon [17]. However, we assume this codon to be the correct start site since a stop codon is present just upstream of the start codon, because of the absence of better start codons in the vicinity, and because of a similar position of the start codon of the highly homologous chicken cadherin-10 sequence [18]. The ORF encodes a 788 amino acid protein, with five extracellular cadherin repeats containing the characteristic conserved amino acid residues [1].

The mature cadherin-10 protein is most likely generated after cleavage of the 54 amino acid predomain. This domain contains a signal sequence for endoplasmic reticulum import, which is less well conserved between species. Chicken and human cadherin-10 differ by 46% (25 out of 54 amino acid residues) in the predomain, while the remaining part of the protein shows less than 5% difference (33 out of 735 amino acid residues). The mature protein would start with a glycine residue (Fig. 1) since this amino acid is preceded by a 4 amino acid proprotein cleavage signal (RQKR) characteristic for furin, a member of the subtilisin family of proprotein convertases [19]. This cleavage signal can be observed in various other cadherins, and is indeed recognized by these protein convertases [20].

Remarkably, in many cadherins a similar recognition site can be observed just cytoplasmic of the transmembrane domain. However, it is unclear whether this sequence can be actually cleaved as the convertases are not supposed to be present within the cytoplasm. One obvious experiment would be to mutagenize this putative proteolytic site and to look for differences in cellular behavior. Indeed, cleavage of this site would lead to a truncated cadherin molecule without cytoplasmic tail, and therefore with impaired cell adhesive capacities.

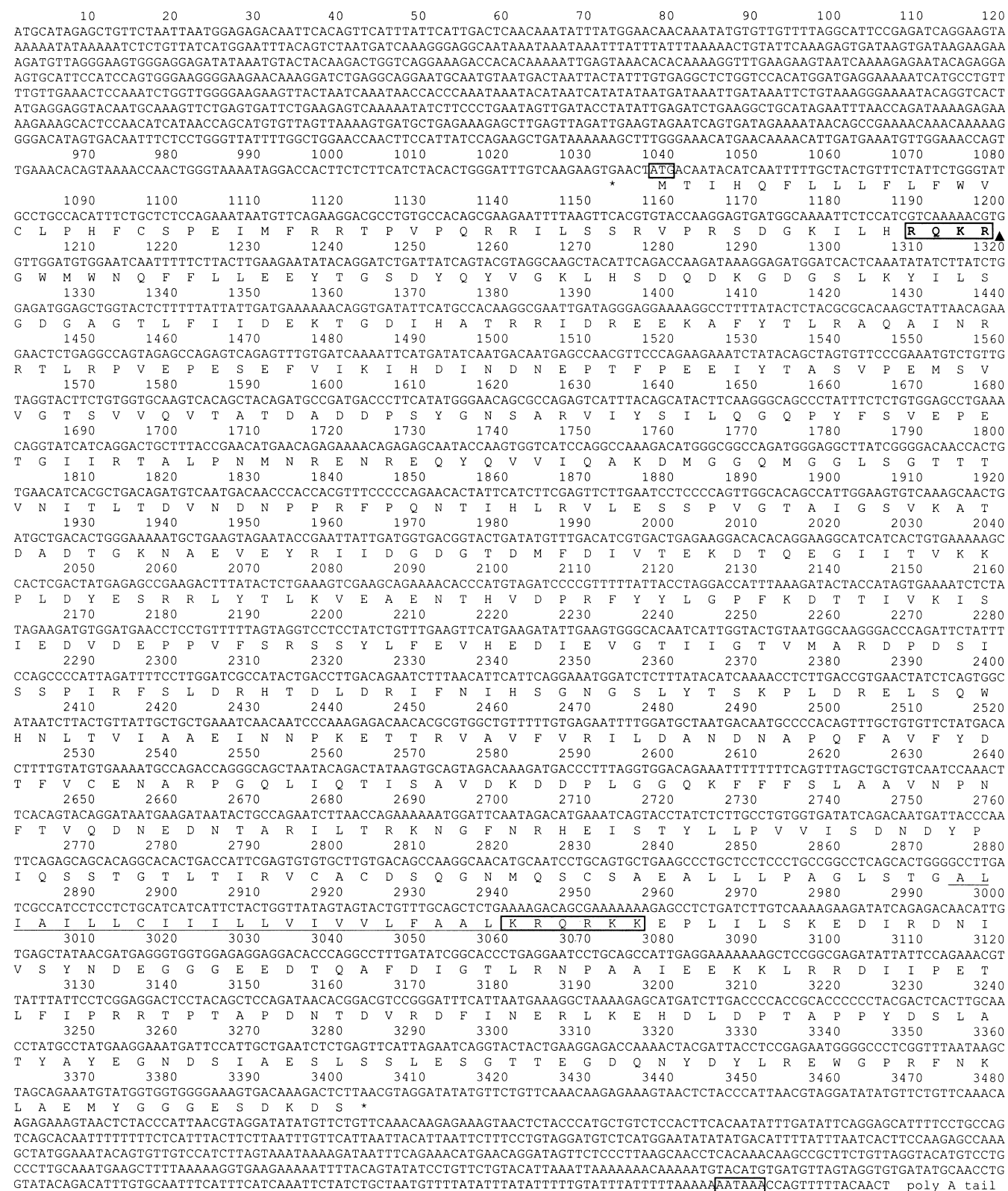


Fig. 1. cDNA sequence and predicted amino acid sequence of human cadherin-10. The possible proteolytic cleavage site of the precursor protein is indicated with a filled triangle. The recognition sequence is boxed and given in bold, while the putative other recognition sequence site is only boxed. The transmembrane region is underlined. The start codon and poly(A)-adenylation signal are boxed. Relevant stop codons are indicated by an asterisk. The Genbank accession number is AF039747.

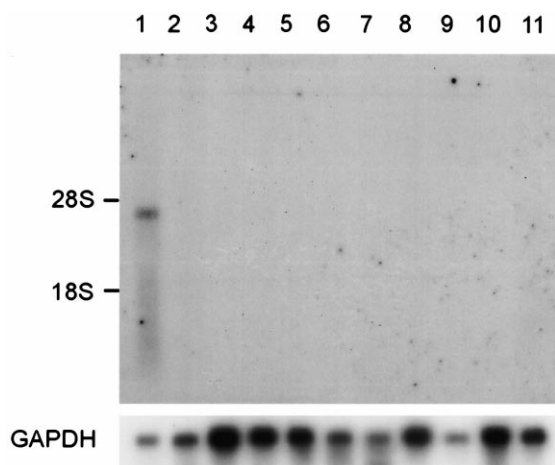


Fig. 2. Expression analysis of human cadherin-10. Upper panel: Northern blot of human cell lines. Markers are 28S and 18S ribosomal RNA. Lower panel shows signals of a control GAPDH hybridization on the same filter. Tissue RNA and cell lines are as follows: fetal brain (1), MCF7 breast carcinoma (2), B-CPAP thyroid carcinoma (3), GLC8 small-cell lung cancer (4), SW872 liposarcoma (5), SK-LMS-1 leiomyosarcoma (6), MKN45 gastric carcinoma (7), HOS osteosarcoma (8), SW1353 chondrosarcoma (9), SW620 colon carcinoma cells (10), and FS4 fibroblasts (11).

Although there are no observations so far that support cleavage at this site, the high evolutionary conservation of the sequence is intriguing (see also Fig. 5A).

3.2. Expression pattern of the human cadherin-10 gene

Northern blot analysis using total RNA of a variety of cell lines and also fetal brain was performed to determine the length of the cadherin-10 transcripts (Fig. 2). Cadherin-10 transcripts were only present in fetal brain total RNA. The length of the transcripts was estimated to be 3.6–4.0 kb. The obtained sequence of 3699 bp might therefore be complete.

In addition we investigated the human cadherin-10 expres-

sion using a panel of poly(A)+ selected RNA from different tissues spotted on a membrane. Our results (Fig. 3) indicate expression of cadherin-10 in almost all brain-derived tissues including pituitary gland (rows A and B, G1 and D4), and significant expression in adult and fetal kidney (Fig. 3; E1 and G3, respectively). Weak expression was demonstrated for medulla oblongata, prostate, lymph node, and fetal lung (Fig. 3). All other tissues, including some major components of the human body (liver, skin, skeletal muscle, heart), did not show detectable cadherin-10 expression.

An extensive study on the chicken cadherin-10 expression demonstrated its presence from an early stage, persisting until the hatching stage [18]. Furthermore, cadherin-10 expression reflected the neuromeric organization of the brain and was present in specific gray matter structures of all major divisions in the chicken brain. Our results indicate that cadherin-10 is also expressed in adult human brain, at least at the mRNA level.

It is noteworthy that the cadherin-12 (BR-cadherin) protein is exclusively expressed in human brain, although the mRNA has been observed in other tissues as well, suggesting post-transcriptional control [21]. It is therefore possible that the cadherin-10 expression we detected in fetal and adult kidney is also restricted to the mRNA level in these tissues.

Human cadherin-6 is a closely related family member of cadherin-10. This suggests possible cross-hybridization of the cadherin-10 cDNA probe used in the expression analysis with human cadherin-6 transcripts. DNA sequence comparison showed that about half of the probe has a 75% homology with the coding sequence of cadherin-6. The other part of the probe consists of the 3' UTR, with a very low homology to the cadherin-6 cDNA. Northern blot hybridization of two identical blots with fetal brain RNA, using either the cadherin-10 probe or the cadherin-6-specific probe, demonstrated non-identical sizes for the cadherin-10 and cadherin-6 transcripts, and therefore lack of cross-hybridization (data not shown). Therefore, it is very unlikely that our cadherin-10 probe also detects cadherin-6 transcripts in the multiple tissue master blot (Fig. 3). Moreover, we detected cadherin-10

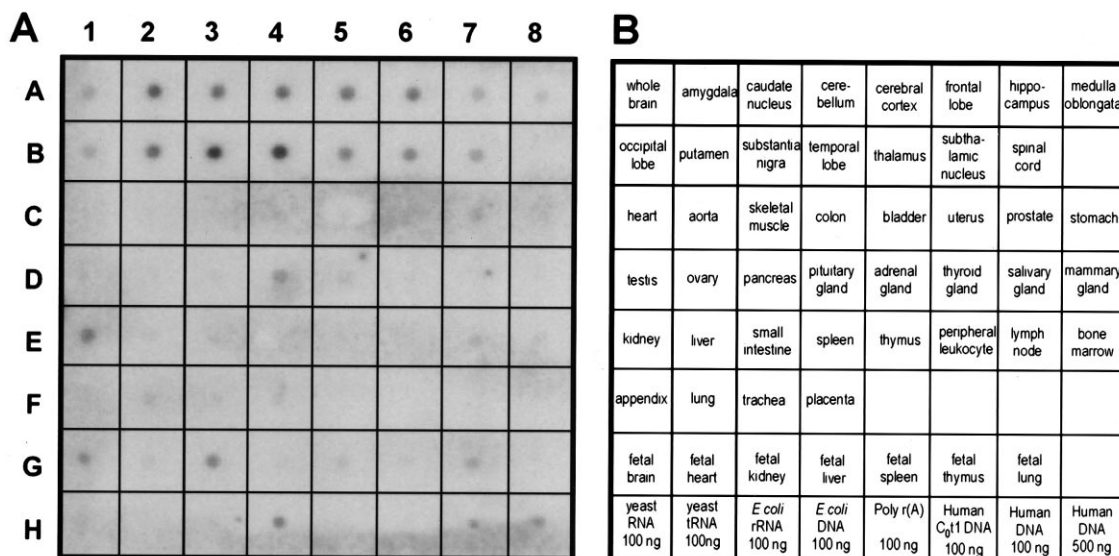


Fig. 3. A: RNA master blot hybridized with the same probe as in Fig. 2. RNA and control samples are as indicated in panel B.

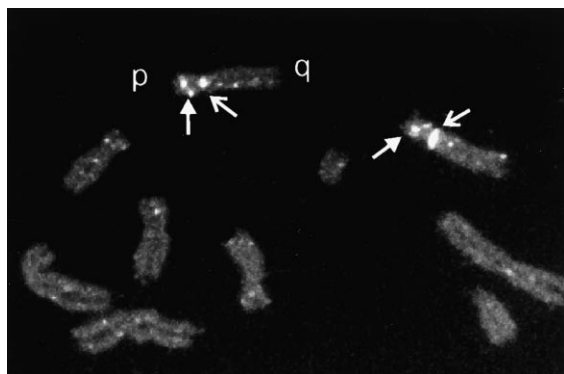


Fig. 4. Chromosomal localization of the cadherin-10 gene. Representative example of a FISH analysis using a cadherin-10-specific PAC clone. Open arrows mark the signals of the chromosome centromere 5 probe; closed arrows indicate the signal position of the cadherin-10-specific PAC probe. The long arm (q) and short arm (p) of one chromosome 5 are indicated.

mRNA in human lung and kidney by gene-specific RT-PCR (data not shown). This indicates that the expression patterns of both genes overlap to some extent. Cadherin-6 appeared to be present as protein in proximal renal tube epithelia, fetal kidney, and renal cell carcinomas [22]. In the latter tumors a correlation was observed between aberrant cadherin-6 protein expression (i.e. heterogenous or no expression) and poor prognosis [22]. Although the latter authors did not detect human cadherin-6 mRNA in the brain by Northern blot analysis, mouse cadherin-6 mRNA is present in specific brain areas [23]. The areas expressing cadherin-6 appeared to be synaptically connected and delineated restricted neuronal circuits [24]. Similar data were obtained for the chicken cadherin-6B protein. This molecule is highly related to human and mouse cadherin-6 and shows a restricted expression in specific neuronal circuits of the visual and cerebellar system of the chicken brain [25,26].

3.3. Chromosomal localization of the human cadherin-10 by FISH analysis

A cadherin-10-specific PAC clone was used in a FISH analysis on metaphase spreads of a healthy male donor. Each time specific signals (often double) were observed on the p-arm of a relatively large chromosome. Reverse DAPI-banding suggested the chromosomal localization to be 5p13–14, which was confirmed using a chromosome 5-specific centromere probe (Fig. 4). This is the same localization as described for the cadherin-12 gene [27], suggesting a potential clustering of these two cadherin genes.

Recently, the human cadherin-6 and cadherin-14 (*CDH18*) genes were assigned to chromosomal region 5p14–15 [28]. The method used was radiation hybrid mapping, which is rather accurate but does not always allow assignment to the correct chromosomal band. Therefore, it is conceivable that the human cadherin-10 gene is more tightly clustered to the three other cadherin genes than is indicated by the different chromosomal bands. The high overall sequence homology and gene clustering suggests that the cadherin-10 and cadherin-6 genes might be the result of a recent gene duplication.

3.4. Phylogenetic analysis of the human cadherin-10 gene

The deduced protein sequence of cadherin-10 was used for

screening of the database libraries (BLAST-P). The resulting hits were cadherin-6 and cadherin-10 proteins of various species. In addition, the mouse T2-cadherin, isolated from thymocytes [10], was identified as a possible orthologue.

Phylogenetic analysis was performed with that part of the protein sequences that was available for all sequences of interest, i.e. the last 265 amino acid residues encoded by the human cadherin-10 cDNA (containing part of the extracellular domain 5, the complete transmembrane region and cytoplasmic tail). This small region is similar to the known sequence of the mouse T2-cadherin. Corresponding regions of the following cDNAs were also included in the analysis: chicken and rat cadherin-10, human, mouse and rat cadherin-6, and chicken cadherin-6B sequences. Sequences of two more representatives of the atypical (type-II) cadherins, i.e. human cadherin-8 and cadherin-12, and of two classical (type-I) cadherins, i.e. human E-cadherin and N-cadherin, were also included. The results (Fig. 5), indicate the high conservation of our novel sequence with the chicken and rat cadherin-10, while other close family members (e.g. three mammalian cadherin-6 molecules plus chicken cadherin-6B) are clearly different. We therefore conclude that our cDNA sequence encodes the genuine human cadherin-10 gene, while the mouse T2-cadherin is most likely the mouse orthologue. Interestingly, the similarity between the human cadherin-6 and cadherin-10 proteins is large with respect to that seen between the classical E-cadherin and N-cadherin molecules. In addition, the two other analyzed human type-II cadherins (cadherin-8 and cadherin-12) show also a lower similarity. This might reflect a close evolutionary relationship between cadherin-6 and cadherin-10, and possibly partly shared functions.

The differences between the cytoplasmic domains of the human type-I classical cadherins (demonstrated here for E- and N-cadherin) and cadherin-10 are relatively large. For cadherin-10, this may suggest a weak binding activity towards the cytoplasmic protein β -catenin (or plakoglobin), resulting in weak cell adhesive properties. However, two recent papers indicate that also the related type-II cadherins, cadherin-6 [22] and cadherin-14 [29] (with a new gene signature *CDH18*, www.gene.ucl.ac.uk/nomenclature), are capable of binding β -catenin, suggesting a similar functionality of these cadherins in cell-cell adhesion, or even other functions.

An unexpected role for two classical cadherins, E-cadherin and N-cadherin, was suggested in a recent paper [30]. Functional disruption of these cadherins, by either blocking antibodies or adhesion-inhibiting polypeptides, prevented the induction of long-term potentiation in rat hippocampal slices. Combined with the reported expression of a variety of cadherins in mammalian brains (reviewed in [31]), this suggests that cadherins might play a role not only in establishing the brain structure but possibly also in forming the memory. It remains to be determined whether the human cadherin-10 has a function in cell-cell adhesion, and what its role in the human brain is.

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A

Gg10	1FF.SLA...AVNPNTVQDNEDNTARILTRKNGSRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDSRGNMQSCNAEALLPAGLS
Hs10	1FFFSLA...AVNPNTVQDNEDNTARILTRKNGSRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDSQGNMQSCNAEALLPAGLS
Hs6	1SLAPEAASGSNFTIQDNKDNTAGILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDHGNGMQSCNAEALLPAGLS
Hse	1	D.LPPNTSPFHAELTHCASANWTIOYNPTQESILPKP...MALEVGDYKINKIMDNQ...KDQVTLEVSVCDCEGAAG...VCRKAQPV EAGLQ
Hsn	1	P.LSPVT.....TKRNWITRLNGDFAQNLRIK...F.LPAGIYEVPTITIDSGNPPKSNISTLRVVCQCDSD...CTDVRIVGAGLG
Mm6	1SLAPEAASGSNFTIQDNKDNTAGILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDHGNGMQSCNAEALLPAGLS
Mmt2	1	DDVDRRSFSFSLA...AVNPNTVQDNEDNTARILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDSQGNMQSCNAEALLPAGLS
Rn10	1FFFSLA...AVNPNTVQDNEDNTARILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDSQGNMQSCNAEALLPAGLS
Rn6	1FSLAPEAASGSNFTIQDNKDNTAGILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDHGNGMQSCNAEALLPAGLS
Gg6b	1SLAPEAASGSNFTIQDNKDNTAGILTRKNGNRHEISTYLLPVVISDNDYPIQSSTGTLTIRVCACDHGNGMQSCNAEALLPAGLS
Hs12	1PEAALPKNFTVRDFRNTAGIETRENGSRROQELVLPVVIDESSYFVOSSTNTMTIRVCRCDSGDTILSNVEATFLPVGLS
Hs8	1LPPMVNPNFTIKKNEDNSLSILAKNGNRKQKEVYLLPTITIDSGNPPKSNISTLRVVCQCDSDGTVVOSCNVEAVVLEIGLS
Gg10	87	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Hs10	89	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Hs6	89	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Hse	91	IPAILGILGGTIALLLILLFRRRAVVKP...LIPPEDDTRDNVYYDEEGGEED...QDFDLSQLHRLDARPEVT...RNDVAPTLMSV
Hsn	81	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Mm6	89	SGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Mmt2	95	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Rn10	89	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Rn6	90	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Gg6b	89	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Hs12	85	TGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Hs8	87	MGALIAILLCTIILLVIVVLFALRRQRKKEP...LILSK...EDIRDNIYSYNDEGGGEEDTQAFDICTLRN...PAATEEKKLRDDIIPETLFI
Gg10	173	PR..RT..PSAPDNTDVRDFINORLKEHDSPPSAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDAS
Hs10	175	PR..RT..PTAPDNTDVRDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Hs6	175	PR..RT..PTARDNTDVRDFINORLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Hse	177	PRYLPRPA...NPDEIGNFIDENLKAADDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Hsn	173	PQYPPVRSAAHPGDLGDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Mm6	175	PR..RT..PTARDNTDVRDFINORLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Mmt2	175	PR..RT..PTAPDNTDVRDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Rn10	175	PR..RT..PTAPDNTDVRDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Rn6	175	PR..RT..PTARDNTDVRDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Gg6b	175	PR..RT..PTARDNTDVRDFINORLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Hs12	171	PR..QR..PPMEDNTDVRDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.
Hs8	175	PR..QGLAPVNGVDVDFINERLKEHDLDPPTAPPYDSLATYAYEGNDSVAESLSSLESSTTEGDQNDYDLREWGPRFNKLAEMYGGGES...DKDS.

B

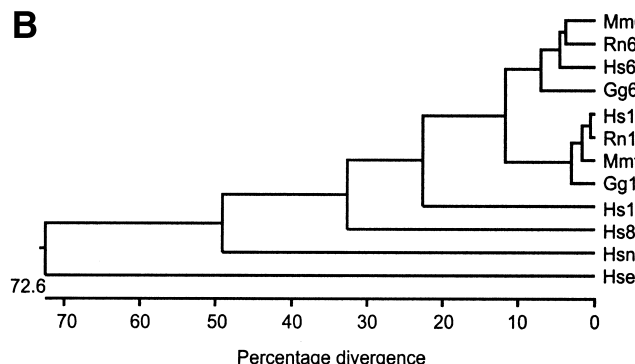


Fig. 5. Phylogenetic analysis of cadherin carboxy-terminal protein sequences, including these of *Gallus gallus* (Gg), *Homo sapiens* (Hs), *Mus musculus* (Mm), and *Rattus norvegicus* (Rn). The species indication is followed by the code of the cadherin subtype. A: Background staining is applied if similarity is present in more than 50% of the cases, i.e. black indicates identical amino acids, while gray areas mark residues with similar properties. Lack of background refers to a non-conserved protein sequence. Figure was generated using the Box-shade server (http://www.isrec.isb-sib.ch/software/BOX_form.html). B: Rooted tree view of the phylogenetic analysis.

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