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Published in:
Genomics

DOI:
10.1006/geno.1997.4892

Citation for published version (APA):

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Celsr1, a Neural-Specific Gene Encoding an Unusual Seven-Pass Transmembrane Receptor, Maps to Mouse Chromosome 15 and Human Chromosome 22qter

Anna-Katerina Hadjantonakis,*1 W. John Sheward,† Anthony J. Harmar,† Louis de Galan,‡ Jan M. N. Hoovers,‡ and Peter F. R. Little*2

*Wolfson Laboratories, Department of Biochemistry, Imperial College of Science, Technology and Medicine, South Kensington, London SW7 2AZ, United Kingdom; †MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Morningside Park, Edinburgh, United Kingdom; and ‡Institute of Human Genetics, University of Amsterdam, Academic Medical Centre, Amsterdam, The Netherlands

Received June 24, 1996; accepted June 30, 1997

We have identified Celsr1, a gene that encodes a developmentally regulated vertebrate seven-pass transmembrane protein. The extracellular domain of Celsr1 contains two regions each with homology to distinct classes of well-characterized motifs found in the extracellular domains of many cell surface molecules. The most N-terminal region contains a block of contiguous cadherin repeats, and C-terminal to this is a region containing seven epidermal growth factor-like repeats interrupted by two laminin A G-type repeats. Celsr1 is unique in that it contains this combination of repeats coupled to a seven-pass transmembrane domain. As part of the characterization of the Celsr1 gene, we have determined its chromosomal map location in both mouse and human. The European Collaborative Interspecific Backcross (EUCIB) and BXD recombinant inbred strains were used for mapping Celsr1. Clones in the mouse, and fluorescence in situ hybridization was used to map human Celsr1 cosmid clones on metaphase chromosomes. We report that Celsr1 maps to proximal mouse Chromosome 15 and human chromosome 22qter, a region of conserved synteny. Reverse transcriptase–polymerase chain reaction analysis and in situ hybridization were used to determine the spatial restriction of Celsr1 transcripts in adult and embryonic mice. The results presented here extend our previous finding of expression of the Celsr1 receptor in the embryo and show that expression continues into adult life when expression in the brain is localized principally in the ependymal cell layer, choroid plexus, and the area postrema.

INTRODUCTION

The Celsr1 gene (Cadherin EGF LAG seven-pass G-type Receptor, also referred to as ME2) encodes an orphan seven-pass transmembrane G-protein coupled receptor (GCR) with homology to the peptide hormone binding group of receptors, family B (Kolakowski, 1994; available on the public database http://receptor.mgh.harvard.edu).

The extracellular domain of Celsr1 contains two regions each with homology to distinct classes of well-characterized motifs found in the extracellular domains of many cell surface molecules. The most N-terminal region contains a block of contiguous cadherin repeats and C-terminal to this is a region containing seven epidermal growth factor (EGF)-like repeats (Davis, 1990) interrupted by two laminin A G-type (LAG) repeats (Pathy, 1992). Celsr1 is unique in that it contains this combination of repeats coupled to a seven-pass transmembrane domain. Outside of the repeat motifs, Celsr1 is most highly related to EMR1 (Baud et al., 1995) and CD97 (Hamann et al., 1995), which are unusual members of family B of seven-pass transmembrane G-protein coupled receptors (Kolakowski, 1994). Celsr1, EMR1, and CD97 differ from other B family GCRs in that they have large N-terminal extracellular domains containing multiple EGF-like repeats, but Celsr1 is unique in also possessing cadherin repeats in its extracellular domains. A full description of the structure of Celsr1 and its expression during early embryogenesis will be presented elsewhere (A.-K.H. and P.F.R.L., manuscript in preparation).

A number of G-protein coupled receptors are altered in several human genetic disorders and mouse mutants (Coughlin, 1994). Examples of these include the Melanocyte Stimulating Hormone receptor mutated in various coat color mutants including yellow (e), sombre (Es), and tobacco darkening (Etob) (Robbins et al., 1993), the growth hormone releasing hormone receptor mutated in little mice (Lin et al., 1993; Godfrey et al., 1993), and the endothelin B receptor mutated in the piebald-lethal mouse mutant and Hirschsprung's dis-
ease in humans (Hosoda et al., 1994; Puffenberger et al., 1994). The expression of Celsr1 is spatiotemporally restricted during mouse embryonic development (A.-K.H. and P.F.R.L., manuscript in preparation) and we wished to analyse gene expression in the adult to address the possible relationship of Celsr1 to existing mutations by establishing the chromosomal location of the Celsr1 gene in mouse and humans.

**MATERIALS AND METHODS**

DNA preparation and analysis. DNA preparations were carried out by standard methods detailed in Little (1987) and Sambrook et al. (1989). Radioactive signals were visualized either by standard autoradiography or by the use of a PhosphorImager and ImageQuant software (Molecular Dynamics).

Isolation of mouse cDNA clones. The ME2(19) and ME2(2) clones were isolated from a mouse 8.5 days post coitum (d.p.c.) embryonic cDNA library constructed in λgt10 (Farnher et al., 1987). ME2(2) and ME2(19) together comprise a 2407-bp cDNA whose sequence has been deposited in GenBank (Accession No. AF006014). The cDNA includes sequences from the middle of transmembrane region two (TMII) to the N-terminus (approximately 510 amino acids) and an additional 878 bp from the 3' untranslated region, which is 1811 bp in total length. The full Celsr1 cDNA sequence of >11 kb will be published elsewhere.

Isolation of human cosmid clones. The human Celsr1 cosmid clones were isolated by screening a human cosmid library constructed in pCos2EMBL (Ehrich et al., 1987) at reduced stringency using the ME2(19) mouse cDNA clone.

RT-PCR detection of transcripts. Total RNA was prepared from various adult mouse tissues using the acid guanidinium-phenol-chloroform procedure (Chomczynski and Sacchi, 1987). One microgram was used for reverse transcription using an oligo(dT) primer in a total volume of 30 μl. Five microliters of the first-strand cDNA was subjected to PCR under standard buffer conditions with Taq polymerase (Promega). The Celsr1 gene-specific primers used were PLKH23 (5'-TTTGCTCTTCTCTGCTCGTTC-3') and PLKH24 (5'-CAAAGCTCCTAAATCGGTTACCTCC-3'). The HPRT primers were taken from Koopman (1993): HPRT1a (5'-CCTGCTGGATTACATTACATTAAAGCAGACTG-3') and HPRT1b (5'-GTCAAGGGCATA-TCCAACAAACAAAC-3'). PCR amplifications were subjected to a "hot start" followed by 35 cycles of 93°C, 30 s; 60°C, 1 min; 72°C, 1 min; with one final extension step at 72°C for 10 min. The Celsr1 PLKH23/PLKH24 primer pair is directed against transmembrane domains I to IV and does not yield any products with genomic DNA. Sequencing

**FIG. 1.** In situ hybridization to Celsr1 transcripts in the midgestation mouse embryo. Whole-mount in situ hybridization of an antisense Celsr1 cRNA probe to (A) a transversely cut 12.5-d.p.c. embryo showing localized gene expression in the CNS and (C) the face of a 12.5-d.p.c. mouse embryo showing expression in the nascent eyelid (arrowheads) and prospective whisker follicles. Sense strand controls (B and D).
and hybridization to authentic Celsr1 cDNA clones showed that the amplified product was specific for the Celsr1 locus (data not shown).

Recombinant inbred (RI) strain mapping. DBA/2J and C57BL/6 inbred strains of mice were purchased from Olak through the Imperial College Central Biomedical Services (CBS) and maintained at the CBS unit (Biochemistry Department, Imperial College). DNA from the 26 BXD recombinant inbred strains was purchased from The Jackson Laboratory DNA Resource (Bar Harbor, ME). Linkage was determined by comparing the strain distribution pattern (SDP) for alleles at the locus of interest with those already typed in the series. Analysis of the SDPs was carried out using the RI manager computer program (Manly and Elliot, 1991) and by Dr. Ben Taylor (The Jackson Laboratory), who compared our SDP to all others in his BD database (Taylor, 1989).

EUCIB mapping. A facility for genetic mapping of the mouse genome (Breen et al., 1994) available from the UK Human Genome Mapping Project Resource Centre (Hinxton, Cambridge) was used. A Taq RFLV was obtained at the Celsr1 locus between Mus spretus and C57BL/6 using the ME2(2) clone as a probe.

Prometaphase chromosomes. High-resolution chromosomes from peripheral lymphocytes were obtained according to a double synchronization technique (Ronne, 1985).

Fluorescence in situ hybridization (FISH). Probes were biotinylated by nick-translation, according to the manufacturer’s specifications (Life Technologies nick-translation kit). Chromosomal in situ hybridization, posthybridization washes, Q-banding using DAPI/actinomycin D, and probe localization were performed as described previously (Hoovers et al., 1992).

Whole-mount in situ hybridizations. Mice were killed by cervical dislocation and the uteri removed by standard procedures (Hogan et al., 1986). Processing and whole-mount in situ hybridizations were performed essentially as described previously (Conlon and Rossant, 1992; Wilkinson, 1992).

Radioactive in situ hybridization to adult brain sections. In situ hybridization on sections of adult mouse brain was carried out using a modification of a method described previously (Sheward et al., 1995). In brief, adult mice (F1 hybrid of strains C3H/HeH and 101/H bred in the Animal House, Department of Pharmacology, University of Edinburgh) were killed by an overdose of sodium pentobarbital and the brains removed and frozen (–35°C) rapidly in isopentane. Serial coronal sections (10 μm) were cut on a cryostat, fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 5 min, acetylated, dehydrated, and delipidized, before hybridization at 50°C for 18–20 h with sense or antisense strand transcripts in embryonic and adult tissues. radioactivity in situ hybridization to embryos, radioactive in situ hybridization to sections of adult tissues, and RT-PCR analysis to identify the sites of expression of Celsr1 transcripts in embryonic and adult tissues.

Whole-mount in situ hybridization analysis of 11.5-d.p.c. embryos detected significant levels of Celsr1 transcripts in the neural tube, brain, lung epithelium, and nascent eyelid (Fig. 1) as well as other ectodermally derived regions. A complete expression analysis of embryos prior to 11.5 d.p.c. will be published elsewhere (A.-K.H. and P.F.R.L., manuscript in preparation).

PCR was performed on first-strand cDNA prepared from various mouse adult tissues (Fig. 2) using the PLK23/PLK24 primer pair. Products from Celsr1 transcripts were only detected in adult mouse brain, spinal cord, and eye RNAs, suggesting that the ectodermal-specific expression observed in embryos is maintained during adult life.

To refine the location of Celsr1 transcripts in the adult brain, we used in situ hybridization to sections (Fig. 3). Expression of the Celsr1 mRNA was seen chiefly in the ependymal cells lining the lateral, third, and fourth ventricles of the brain and the central canal of the spinal cord as well as in the choroid plexus and area postrema. Specificity of the hybridization was controlled by hybridization of similar sections with the sense strand riboprobe: no labeled cells were seen in any of these brain regions.

Mapping of Celsr1 in the Mouse Genome

We have mapped Celsr1 in the European interspecific backcross (EUCIB) (Breen et al., 1994) and the

RESULTS

Expression of Celsr1 in Embryonic and Adult Mouse Tissues

Celsr1 is a large (>11 kb) and low-abundance transcript, and as a consequence we have been unable to detect a signal on Northern blots containing up to 10 μg poly(A) RNA isolated from either dissected embryonic or adult tissues. Baud et al. (1995) reported similar difficulties in detection of EMR1 transcripts. We have instead used a combination of whole-mount nonradioactive in situ hybridization to embryos, radioactive in situ hybridization to sections of adult tissues, and RT-PCR analysis to identify the sites of expression of Celsr1 transcripts in embryonic and adult tissues.

Whole-mount in situ hybridization analysis of 11.5-d.p.c. embryos detected significant levels of Celsr1 transcripts in the neural tube, brain, lung epithelium, and nascent eyelid (Fig. 1) as well as other ectodermally derived regions. A complete expression analysis of embryos prior to 11.5 d.p.c. will be published elsewhere (A.-K.H. and P.F.R.L., manuscript in preparation).

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Mapping of Celsr1 in the Mouse Genome

We have mapped Celsr1 in the European interspecific backcross (EUCIB) (Breen et al., 1994) and the
C57BL/6J × DBA/2J (BXD) RI strain series (Taylor, 1989).

The EUCIB panel was screened by hybridization using the ME2(2) partial cDNA, which covers an area of the transcript containing part of the cytoplasmic tail and 3' untranslated region (UTR). A TaqI polymorphism was identified between the parental M. spretus and Mus musculus strain C57BL/6. Celsr1 was typed for 94 mice: 40 (43%) scored as homozygotes (C57BL/6 band only), and 54 (57%) scored as heterozygotes (both C57BL/6 and M. spretus bands present). The data placed Celsr1 at 48.9 cM on mouse chromosome 15. Celsr1 does not recombine with D15Mit72 in the eight animals scored with this marker. Celsr1 is thus distal to the anchor locus D15Mit30, with the closest distal marker to Celsr1 being D15Mit159 at position 49.5.

To obtain a more comprehensive map location for the Celsr1 gene in the mouse, we analyzed it in a second cross, the BXD RI strains. The BXD RI strains were analyzed by hybridization using two different partial cDNA clones, ME2(2) and ME2(19) as probes. Scal and PstI (ME2(19)) and Stul (ME2(2)) polymorphisms were identified in the C57BL/6 and DBA/2J progenitor strains. SDPs were generated by scoring each strain for the inheritance of the B or D type allele; all three SDPs generated were identical. Comparison of the Celsr1 SDP with those of other loci typed in this BXD cross suggests linkage to Cyp2d (4/22 recombinants, 6.2 ± 3.9 cM) and Pdgfb (5/25 recombinants, 7.1 ± 4.1 cM) on mouse chromosome 15. Cyp2d and Pdgfb are inseparable in the BXD RI series since they have an identical SDP for the mice scored for both markers. Prior to the analysis of Celsr1 in this cross, the marker order determined for this region of mouse chromosome 15 was centromere–D15Mit1–Pdgfb/Cyp2d–Celsr1–Pmv-42–Hox-C(D15Mit16)–Spt-2, then we can place Celsr1 distal to Pdgfb/Cyp2d and proximal to Pmv-42 (7/26 recombinants, 11.29 ± 6.1 cM) (Table 1).

Mapping of Celsr1 in the Human Genome

Having determined the map position of the gene in the mouse, we decided to obtain mapping information for the human orthologous locus. Reduced stringency screening of a human cosmid library with ME2(2) and ME2(19) mouse cDNA clones resulted in the isolation of six overlapping cosmids that represent part of the human Celsr1 locus. We determined the chromosomal location of the human Celsr1 locus by FISH using two cosmid clones, ME2HC6 and ME2HC20. In a sample size of 25 metaphases, signals were obtained to the long arm of one of the G group autosomes, either chromosome 21 or 22 (Fig. 5A). To resolve this ambiguity, the two cosmids were additionally hybridized to a trisomy 21 karyotype, and only two signals were observed (Fig. 5B); thus the human Celsr1 locus maps to chromosome 22q13.3. These data are consistent with the conserved synteny between the region of mouse chromosome 15 containing Celsr1 and the distal end of human chromosome 22 (Bucan et al., 1993).

**DISCUSSION**

The Celsr1 gene encodes a novel developmentally regulated seven-pass transmembrane protein whose N-
The terminal extracellular domain contains motifs that are recognized as mediators of protein–protein interactions. The structure of the Celsr1 protein, its putative G-linked signaling properties, and the spatiotemporally restricted expression suggest that it is a receptor involved in contact-mediated communication. Its expression during mouse embryonic development is confined to ectodermal derivatives.

Our present results also show that the expression of Celsr1 mRNA continues in the adult brain, where expression was seen mainly in the ependymal cell layer of the cerebroventricular system and in the choroid plexus and the area postrema. Our results do not exclude the possibility of other sites of expression in the brain at lower levels than we were able to detect by in situ hybridization. The function of the Celsr1 receptor in the mouse brain will remain unclear until the identification of the endogenous ligand; however, the localization is consistent with a possible role in sensory processing. The presence of Celsr1 transcripts in sites that are highly vascularized and/or in direct contact with cerebrospinal fluid (CSF) suggests that the receptor may be involved in the regulation of secretion of a number of neurohormones into CSF and may also play a role in signal transduction between blood, CSF, and neuronal tissue. The choroid plexus may constitute a paracrine system (Stylianopoulou et al., 1988), and it is possible that the Celsr1 receptor is involved in cell–cell signaling at this site in the adult brain; the ependymal layer of the ventricles and the circumventricular organs are sites that are known to contain a variety of hormones and neurotrophic factors and their receptors, including vasopressin (Jurzak et al., 1993; Kato et al., 1995), insulin-like growth factor I/II (Hynes et al., 1988; Marks et al., 1991; Stylianopoulou et al., 1988), Notch2 (Higuchi et al., 1995), and nerve growth factor (Timmusk et al., 1995).

The map position of Celsr1 in the mouse suggests that it lies within the same region as three known mutations whose phenotypes are compatible with the early developmental and neural-specific expression of this gene; Blind (Bld), stargazer (stg), and waggler (wag). It has been shown that wag is allelic to stg (Sweet, 1993). stg has been shown to map proximal to D15Mit69 and D15Mit70 (V. Letts and W. Frankel, pers. comm.). Both these markers give 4/19 recombinants with Celsr1 in the EUCIB backcross analysis, placing them proximal to D15Mit107. Celsr1 therefore cannot be a candidate gene for stg or wag.

Bld is a semidominant mouse mutant that has been mapped to mouse chromosome 15 with respect to the coat color markers underwhite (uw), belted (bt), and Caracul (Ca). The gene order is centromere–uw–28.2 ± 5.1 cM–Bld–14.9 ± 2.7 cM–bt–11.8 ± 3.2 cM–Ca (Teicher and Caspari, 1978). This extremely crude mapping places Bld in the same general region as Celsr1: the precision of the mapping is insufficient to allow us to determine whether Celsr1 is a candidate for Bld. Nevertheless, the expression pattern of Celsr1,
FIG. 5. Mapping of the human Celsr1 locus to chromosome 22qter. FISH of cosmid ME2HC20 on (A) normal karyotype and (B) trisomy 21 karyotype. Cosmid ME2HC6 gave similar results. White arrowheads indicate Celsr1-specific signals. The other marker used was a centromeric probe for chromosome 11.

when compared to the Bld phenotype, makes it an attractive potential candidate. Bld homozygotes are embryonic lethals that die during early development at around 8 d.p.c., with the major phenotypic defect occurring during gastrulation. Bld heterozygotes are born blind because they fail to develop complete eyelids, and as a consequence mice are born with open eyes, leading to damage of the cornea (Watson, 1968). Celsr1 expression in the nascent eyelid is an interesting feature of later stages of embryogenesis (Fig. 2). We have been unable to analyze the Celsr1 gene in Bld mice since the mutant is extinct. The possibility thus remains that Celsr1 is a candidate for Bld.

In humans, deletions of 22q13.3 are associated with a number of phenotypic defects including developmental delay, hypotonia, and dysmorphic facial features (Neslinger et al., 1994). This region overlaps with the map position of Celsr1. Both Bld and the 22qter deletion syndrome phenotypes are very specific and coincident with the spatiotemporal expression of the Celsr1 gene. In humans, it will be important to establish the relationship of Celsr1 to the complex deletion syndrome by high-resolution mapping. In the mouse, a null mutant created by an ES cell-mediated germline mutation will be required to gain insight into the role of the Celsr1 receptor in embryogenesis.

ACKNOWLEDGMENTS

We thank Anna-Maria Frischauf for the gift of a human cosmid library; Brigid Hogan for the gift of a mouse 8.5-d.p.c. cDNA library; Ben Taylor for his help with the analysis of the Celsr1 SDP in the BXD RI series; Maria Breen and Mary Kelly at the UK Medical Research Council Resource Centre for help with the EUCIB analysis; and Verity Letts and Wayne Frankel for communicating results prior to publication. This work was supported by a UK Medical Research Council HGMP directed program studentship (A.-K.H.) and the Wellcome Trust.

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