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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

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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* cDNA 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 (Geiger and Ayalon, 1992), 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 (Patthy, 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 (*Eso*), 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 Hirchsprung's dis-

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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′-TTTGTCCTTCTCTCGCTCGTTC-3′) and PLKH24 (5′-CAAAGCTCCAAATCAGGGTATCC-3′). The *HPRT* primers were taken from Koopman (1993): HPRT1a (5′-CCTGCTGGATTACATTTACATTAAAGCACTG-3′) and HPRT1b (5′-GTCAAGGGCATATCCAACAACAAAC-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

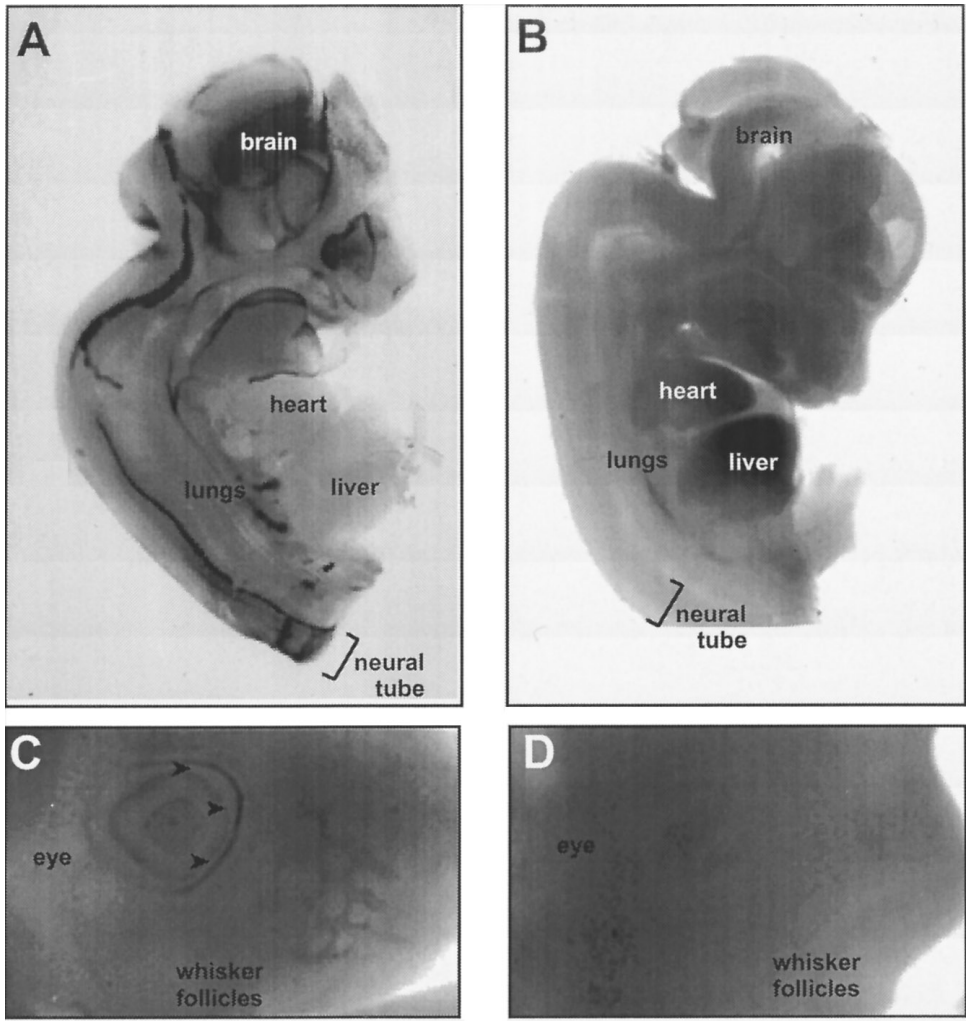


FIG. 1. *In situ* hybridization to *Celsr1* transcripts in the midgestation mouse embryo. Whole-mount *in situ* hybridization of an antisense *Celsr1* cRNA probe to (**A**) a transversely cut 12.5-d.p.c. embryo showing localized gene expression in the CNS and (**C**) the face of a 12.5-d.p.c. mouse embryo 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 BXD 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*I 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 pentobarbitone 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 *Celsr1* riboprobes labeled with ^{32}P. For riboprobe synthesis the plasmid containing ME2(19) was digested with *Xho*I and transcribed with T3 RNA polymerase; for a control the plasmid was linearized with *Bam*HI and transcribed with T7 RNA polymerase to generate the sense-strand probe. After hybridization, sections were washed in 4× SSC at room temperature, followed by 2× SSC at 37°C and digestion with RNaseA. After further washes in 1× SSC at 50°C and 0.1× SSC at 60°C, sections were dehydrated in increasing concentrations of ethanol, dried under vacuum, and dipped in photographic emulsion (Ilford K5 Nuclear emulsion, diluted 1:1 with distilled water). Exposure was for 10 weeks after which slides were developed, fixed, and counterstained with 1% aqueous pyronin.

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.

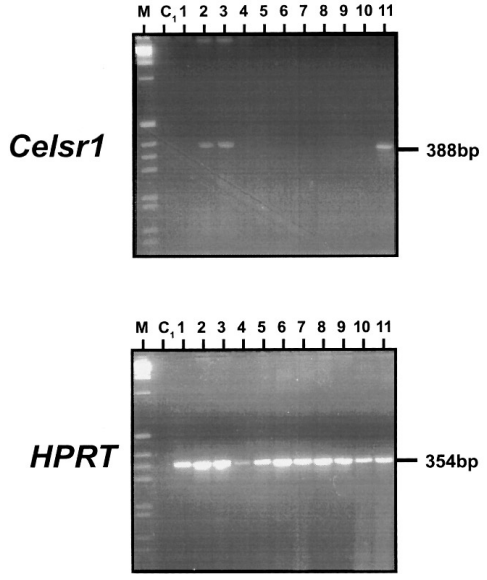


FIG. 2. RT-PCR analysis of *Celsr1* expression in adult mouse tissues. Lung (lane **1**), brain (lane **2**), spinal cord (lane **3**), kidney (lane **4**), liver (lane **5**), spleen (lane **6**), heart (lane **7**), muscle (lane **8**), thymus (lane **9**), intestine (lane **10**), eye (lane **11**). **C$_1$**, no *Taq* polymerase control. **M**, the marker is a Life Technologies kilobase ladder.

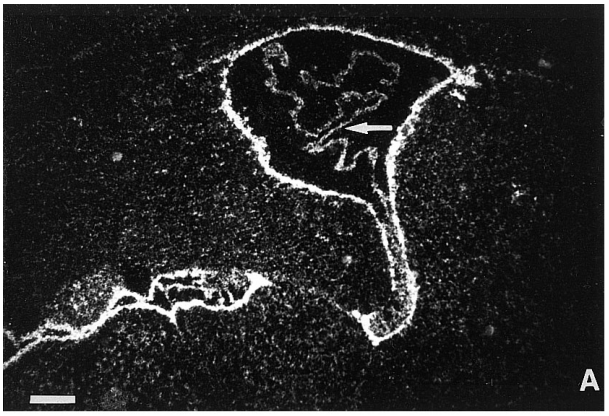
Whole-mount *in situ* hybridization analysis of 11.5-d.p.c. embryos detected significant levels of *Celsr1* transcripts in the neural tube, brain, lung epithelium, and nascent eyelid (Fig. 1) as well as other ectodermally derived regions. A complete expression analysis of embryos prior to 11.5 d.p.c. 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 PLKH23/PLKH24 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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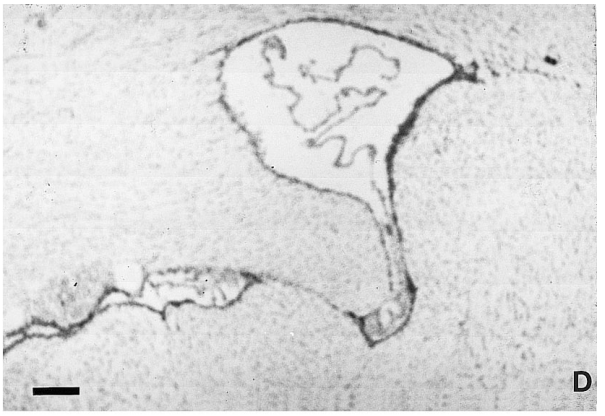
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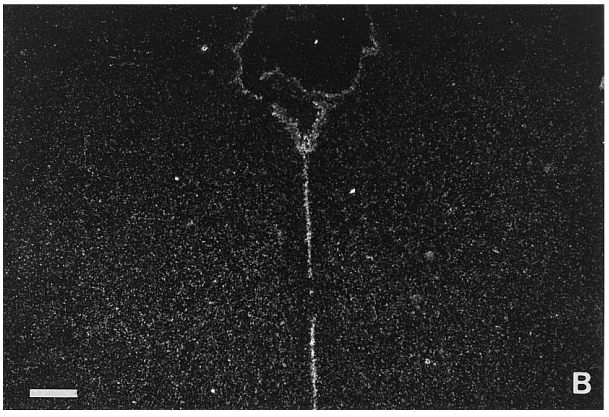
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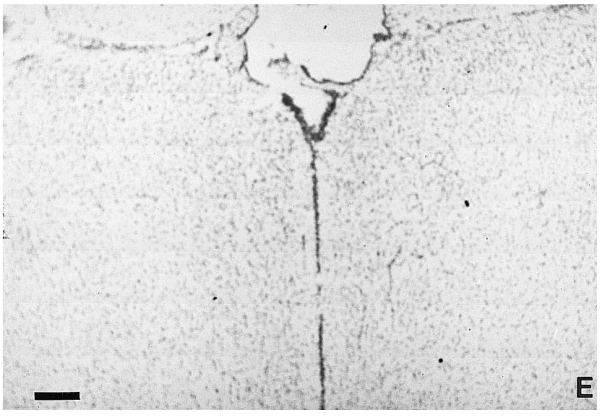
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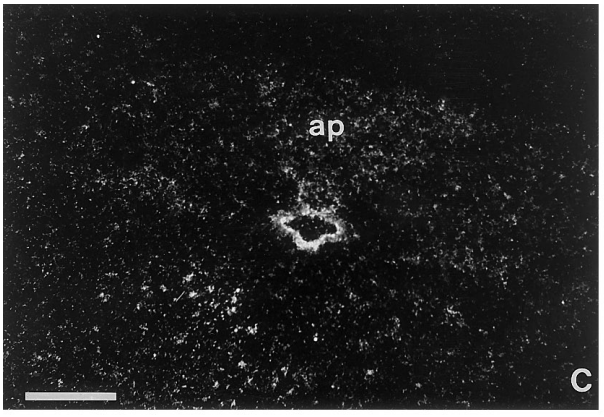
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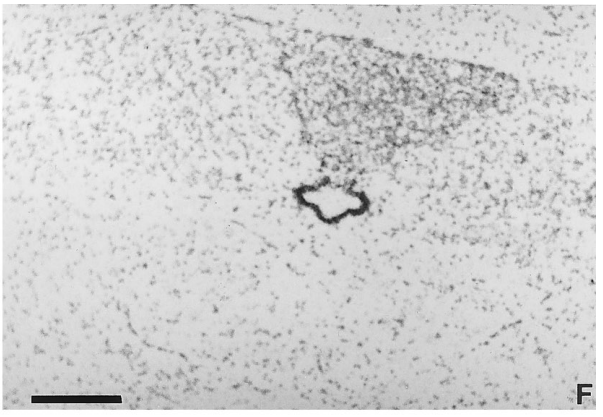
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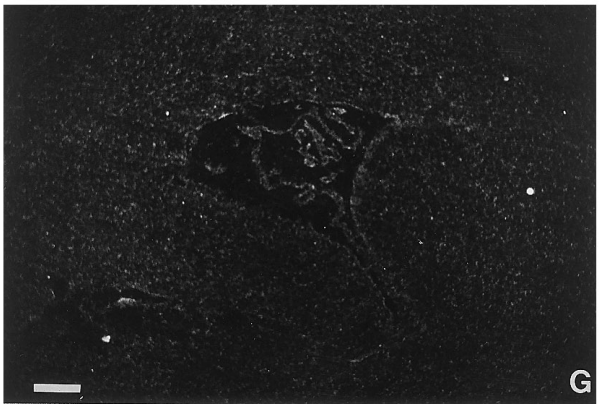
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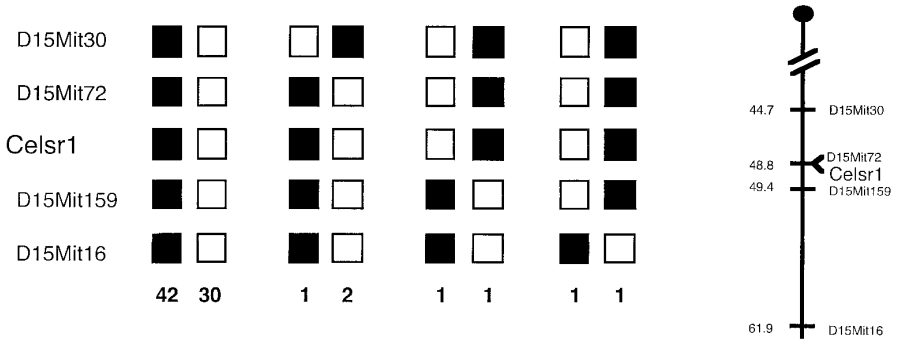

FIG. 4. Inheritance of *Celsr1* in the EUCIB. The segregation patterns of *Celsr1* and its closest flanking and anchor markers are shown on the left. Each column represents the genotype of backcross progeny. The black boxes represent the presence of a homozygote, the white boxes represent the presence of a heterozygote. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome linkage map showing the location of *Celsr1* in relation to linked loci is shown on the right.

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 *Taq*I 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 or *M. spretus* 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 (Fig. 4). *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. *Sca*I and *Pst*I (ME2(19)) and *Stu*I (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*–*Spt-2*–*Hox-C*(*D15Mit16*)–*Pmv-42* (B.Taylor, pers. comm.). Placing *Celsr1* on this map would have implied 10/25 recombination events with the flanking marker *Spt-2*. However, if we suggest that the gene order in the distal part of mouse chromosome 15 is in fact 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). Thus the predicted map positions of *Celsr1* in both the BXD and the EUCIB crosses are in agreement.

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-

FIG. 3. *In situ* hybridization of *Celsr1* transcripts in coronal sections of adult mouse brain. Dark-field photomicrographs of *Celsr1* transcripts show expression in (**A**) the ependymal cell layer of the lateral ventricle and the choroid plexus (arrow), (**B**) the ependymal cell layer of the third ventricle, and (**C**) the ependymal cell layer of the fourth ventricle in the area postrema (ap); (**D**–**F**) light-field photomicrographs of the same brain areas. (**G**) Dark-field photomicrograph of a coronal section of the brain showing the absence of labeled cells in the ependymal cell layer of the lateral ventricle after hybridization with a sense strand probe. Scale bar, 200 μm.

TABLE 1

Recombinant Inbred Strain Mapping of *Celsr1* in the 26 BXD Strains

HSA	Locus	Strain distribution pattern of *ME2* in the BXD RI strains																										
		Strains																										
		1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
22q	*Pdgfb*	B	D	D	B	D	D	B	B	D	D	—	B	D	D	D	D	D	B	D	B	D	D	B	D	D	B
22q11–qter	*Cyp2d*	B	D	D	B	D	D	—	B	D	D	—	B	D	D	D	D	D	B	D	B	D	D	B	D	—	—
				X							X				X						X						X
	Celsr1	B	D	B	B	D	D	B	B	D	B	D	B	D	B	D	D	D	B	D	D	D	D	B	D	D	D
			X						X			X						X				X	X		X	
	Pmv-42	B	B	B	B	D	D	B	D	—	B	B	B	D	B	D	D	B	B	D	D	D	B	D	D	B	D
				X																	X						
12q12–q13	*Hox-C*	D	B	D	B	D	D	B	D	D	B	B	B	D	B	D	D	B	B	D	B	D	B	D	D	B	D
															X												
	Spt-2	D	B	D	B	D	D	B	—	D	B	B	B	D	D	D	D	B	B	D	B	D	B	D	D	B	D

Note. Allelic designations for C57BL/6 and DBA/2J are B and D, respectively. Strains that were not scored are denoted by a dash. Crossovers are marked with an X. HSA indicates the human chromosomal assignment for the orthologous locus.

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*,

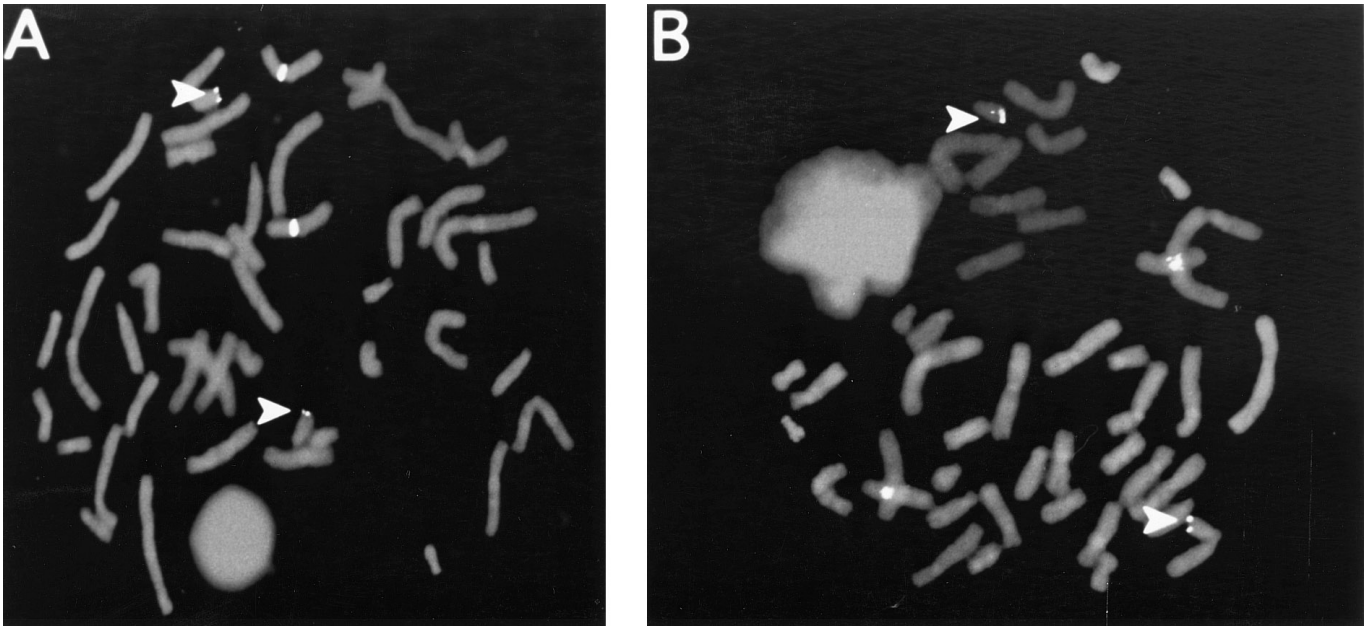

FIG. 5. Mapping of the human *Celsr1* locus to chromosome 22qter. FISH of cosmid ME2HC20 on (**A**) normal karyotype and (**B**) trisomy 21 karyotype. Cosmid ME2HC6 gave similar results. White arrowheads indicate *Celsr1*-specific signals. The other marker used was a centromeric probe for chromosome 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 (Nesslinger *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.

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