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LETTER

The Human Reelin Gene: Isolation, Sequencing, and Mapping on Chromosome 7

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The mouse reelin gene (*Reln*) encodes a novel protein that, when mutated, results in the characteristic reeler phenotype. A key component of this phenotype is the extensive disruption of the organization of many brain structures. Reelin is believed to be an extracellular protein that controls neural cell positioning during brain development. The reelin gene is conserved in many vertebrate species, including humans. To study the role of the reelin homolog in human brain development, we have isolated and characterized the human gene (*RELN*). Like its murine counterpart, *RELN* is large, encoding an mRNA of ~12 kb. Overlapping cDNA clones containing the entire open reading frame were isolated and sequenced, revealing that the predicted mouse and human proteins are similar in size (388 kD) and that the amino acid and nucleotide sequences are 94.2% and 87.2% identical, respectively. Northern hybridization analyses revealed that *RELN* is expressed in fetal and postnatal brain as well as liver. The expression of *RELN* in postnatal human brain was high in the cerebellum. *RELN* was mapped to human chromosome 7q22, based on both fluorescence in situ hybridization studies and localization within a well-positioned yeast artificial chromosome (YAC) contig. The YAC contig also contains a number of genetic markers. Together, these studies provide the sequence information and genetic tools for performing more detailed analyses of *RELN* in an attempt to define its role in human brain development and possibly in human disease.

[The sequence data described in this paper have been submitted to GenBank under accession no. U79716.]

The mouse autosomal recessive mutation, reeler, is associated with a characteristic phenotype, which includes both pronounced neurological symptoms (e.g., dystonia, ataxia, tremors) and striking abnormalities in the architecture of the telencephalic and cerebellar cortices (for review, see Caviness and Rakic 1978; Goffinet 1984, 1990, 1992). The defect in reeler mice has been known for many years to perturb the migration of postmitotic neurons during formation of the normal multilayered (laminar) structures in the cerebral cortex, cerebellum, hippocampus, and several other subcortical regions. As a result, the reeler mouse has provided a useful model for abnormal corticogenesis.

The gene responsible for the reeler mutation, *Reln*, was identified recently (D'Arcangelo et al. 1995). Three different reeler alleles ($Reln^{rl}$, $Reln^{orl}$, and $Reln^{tg}$) have been defined at a molecular level (D'Arcangelo et al. 1995; Takahara et al. 1996). Each contains a partial deletion of the *Reln* coding sequence. The encoded protein is large, consisting of 3461 amino acids [derived from an open reading frame (ORF) of 10,383 nucleotides] and containing a deduced molecular mass of 388 kD. The reelin protein appears to be a novel extracellular matrix molecule containing an amino-terminal region with homology to F-spondin (Klar et al. 1992), as well as eight consecutive repeats, each with two related subdomains separated by an epidermal growth factor (EGF)-like motif (D'Arcangelo et al. 1995). Based on its predicted structure and pattern of expression in the mouse, reelin has been proposed to function as a neuron-specific extracellular protein that controls neuronal migration in the developing brain and stabilizes the architecture of laminar structures. Such a role is supported by immunological studies in the developing mouse employing the monoclonal CR-50 antibody (Ogawa et al. 1995), which recognizes reelin (D'Arcangelo et al. 1997).

Comparative human–mouse genome mapping has identified a region of conserved synteny be-

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tween human chromosome 7q and mouse chromosome 5 containing *Reln* (O'Brien et al. 1993; Bar et al. 1994; DeBry and Seldin 1996). We sought to characterize the human homolog of *Reln*, both to compare the sequences of the human and mouse genes and to establish its precise location in the human genome. Such studies should provide the foundation for defining the role of reelin in the human brain. Here, we report the isolation of a set of overlapping *RELN* cDNA clones and elucidation of the full-length coding sequence. In addition, we have determined the expression pattern of *RELN* and its precise location on human chromosome 7q22.

RESULTS

Conservation of the Reelin Gene in Vertebrates

To determine the degree to which reelin is evolutionarily conserved, Southern hybridization analysis using genomic DNA from several vertebrate species was performed with a *Reln* cDNA probe (corresponding to nucleotides 3268–3613; GenBank accession no. U24703). Hybridizing DNA fragments were detected in all samples under moderate stringency conditions (Fig. 1), indicating that *Reln*-related sequences are highly conserved among vertebrates. No hybridization was detected using insect or plant DNA (data not shown).

Cloning and Sequencing of *RELN*

The established sequence of *Reln* (D'Arcangelo et al. 1995) was used to design a series of PCR assays, a subset of which were found to amplify appropriately sized products from human DNA. The precise sequence of the resulting amplified products was established and used to design human-specific oligonucleotides, with the latter being used for "capturing" clones from a brain cDNA library (see Methods). Seven overlapping clones were isolated (these ranged in size from 2.2 to 4.8 kb and were ultimately found to account for >90% of the coding sequence). Two additional cDNA clones were obtained by hybridization-based screening of a human cerebellum cDNA library using the insert ends of two previously isolated clones as probes. The resulting set of overlapping *RELN* cDNA clones is depicted in Figure 2.

A subset of the cDNA clones (indicated in Fig. 2) were mixed and sequenced by a shotgun strategy to establish the sequence of expressed *RELN*. The resulting sequence (GenBank accession no. U79716) revealed an ORF of 10,380 nucleotides, which begins at a conserved, consensus sequence for translation initiation that contains the initial methionine codon (GGCGGCAUGG) (Kozak 1991) and ends at an in-frame stop codon. The 5′-untranslated region (UTR) contains a stretch of 10 repeated GGC triplets immediately proximal to the translation initiation site, whereas the 3′-UTR contains a consensus polyadenylation signal. The nucleotide sequence of *RELN* is 87.2% identical to that of *Reln*. The coding regions exhibit 88.2% identity, whereas the 5′ and 3′ UTRs analyzed are 72.4% and 80.4% identical, respectively.

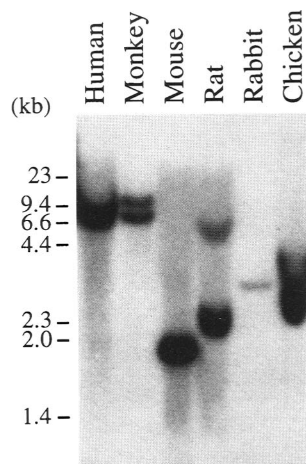

Figure 1 Evolutionary conservation of the reelin gene. EcoRI-digested genomic DNA from the indicated vertebrate species was subjected to gel-transfer hybridization with a ^{32}P-labeled *Reln* cDNA probe. The relative positions of molecular mass markers are indicated (in kb).

Deduced Amino Acid Sequence of *RELN*

RELN encompasses an ORF of 3460 amino acids that has a predicted molecular mass of 388 kD. The deduced human and mouse amino acid sequences are 94.2% identical, with the human protein being shorter by a single amino acid (Fig. 3). The amino terminus of both contains a signal peptide followed by a consensus cleavage site (von Heijne 1983, 1986). The signal peptide is less conserved between the human and mouse (66% identical) than the rest of the protein. There are no other transmembrane domains. Similar to the mouse, the human reelin protein has an amino terminus with significant homology to F-spondin (Klar et al. 1992), whereas the rest of the protein consists of eight consecutive repeats of 350–390 amino acids, each containing two related subdomains separated by an EGF-like domain of 30 amino acids. These conserved repeats exhibit no similarity with other known proteins.

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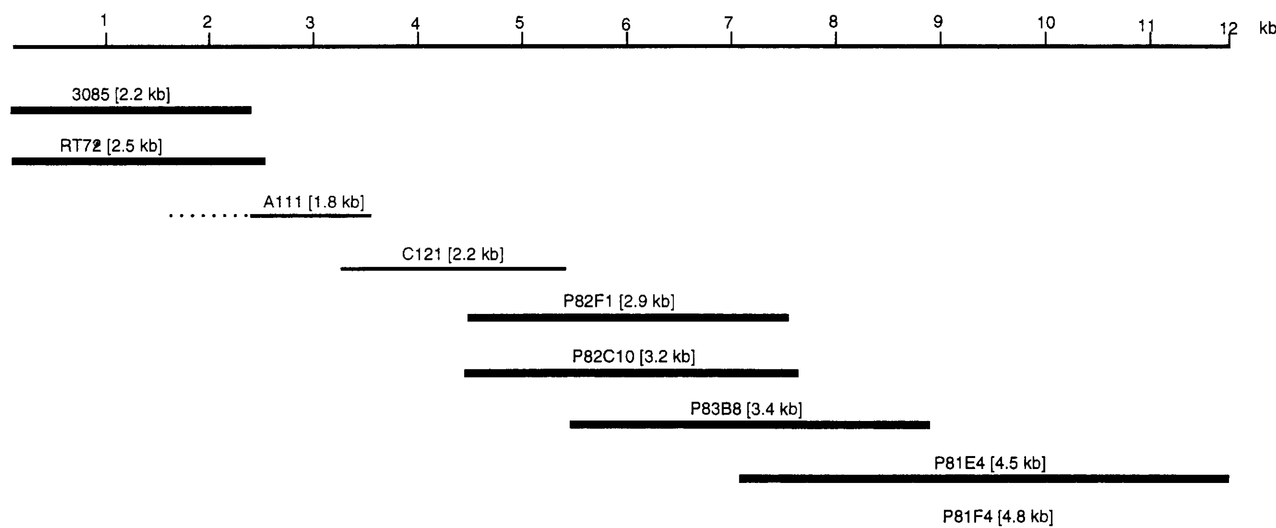

Figure 2 Contig map of *RELN* cDNA clones. The nine independent cDNA clones used to obtain the sequence of *RELN* are depicted. The seven clones indicated by thicker horizontal lines were isolated from a human brain cDNA library and used for shotgun sequencing. Clones C121 and A111 were isolated from a human cerebellum cDNA library, and the inserts of these clones were sequenced by successive walking with custom primers. The proximal end of clone A111 is chimeric (depicted as a dotted line).

Tissue-Specific Expression of *RELN*

Northern hybridization analyses revealed a tissue-specific pattern of expression for *RELN*. Hybridization of *RELN* cDNA probes to poly(A) RNA from various fetal and adult tissues revealed a transcript of ~12 kb in fetal and adult brain as well as liver, but not in any other tissue examined (Fig. 4; data not shown). The expression pattern of *RELN* was examined in more detail in adult brain. A *RELN* transcript was detected in all brain regions examined, with the highest levels observed in the cerebellum (Fig. 4). The high level of postnatal expression of *RELN* in the cerebellum is reminiscent of that reported in the mouse (D'Arcangelo et al. 1995).

Mapping *RELN* on Human Chromosome 7

Three PCR assays specific for sequence-tagged sites (STSs) within the *RELN* cDNA sequence (sWSS2926, sWSS3174, and sWSS3176) were developed and verified to amplify the appropriate product from human genomic DNA. These STSs were scattered across *RELN* (corresponding to nucleotides 1114–1294, 10,525–10,685, and 11,314–11,551). All three STSs were localized on a single yeast artificial chromosome (YAC) contig that was constructed as part of a larger effort to assemble a physical map of human chromosome 7 (Green et al. 1991, 1994, 1995; G.G. Bouffard, J.R. Idol, V.V. Braden, L.M. Iyer, A.F. Cunningham, L.A. Weintraub, J.W. Touchman, R.M. Mohr-Tidwell, D.C. Peluso, R.S. Fulton, et al., in prep.). The relevant portion of this YAC contig containing *RELN* is depicted in Figure 5. Although the exact size of the genomic interval containing *RELN* cannot be established based on the existing data, the smallest YAC containing all three *RELN*-specific STSs (yWSS2204) is 250 kb. Importantly, the assignment of *RELN* to the contig shown in Figure 5 allows its placement on the human genetic map, because of the presence of eight genetic markers that lie in close physical proximity to the gene. A summary of these genetic markers is provided in Table 1. One gene [MSS1 (sWSS1845), GenBank accession no. D11094] and two ESTs (sWSS3569 and sWSS3871; GenBank accession nos. G30782 and G30871, respectively) map immediately centromeric to *RELN*. Finally, *RELN* was found to map to band 7q22 based on fluorescence in situ hybridization (FISH) studies performed using YACs from the contig in Figure 5 as well as a P1 clone containing part of *RELN* (data not shown).

DISCUSSION

The study of mouse genetic mutations associated with profound phenotypes can provide valuable information about fundamental biological processes. Such insight can also be used to investigate the role of the corresponding genes in humans, both in healthy and disease states. In this paper, we have

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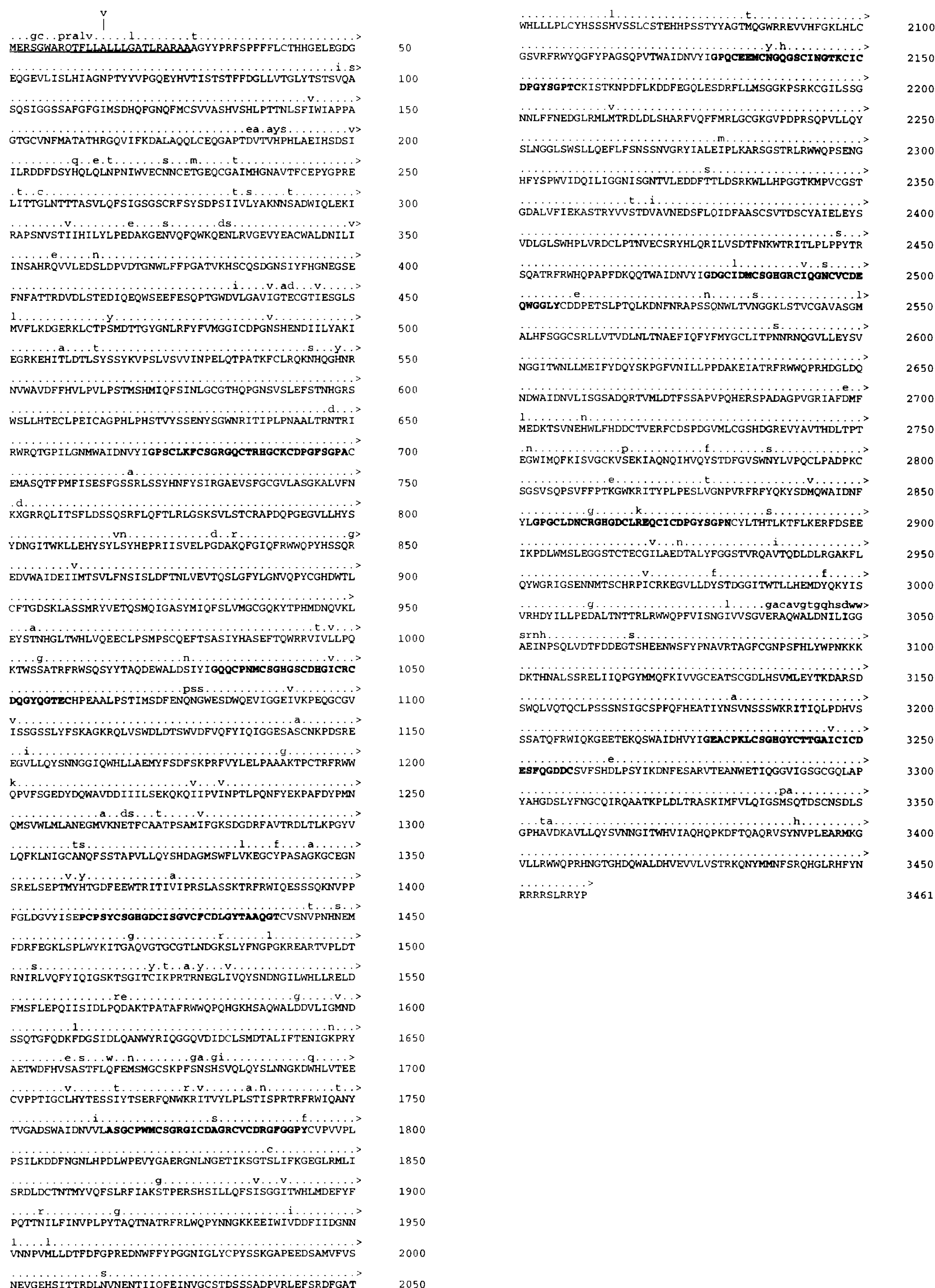

Figure 3 Predicted amino acid sequence of human reelin. Based on the cDNA sequence (GenBank accession no. U79716), the amino acid sequence of human reelin was deduced and aligned against the mouse protein (GenBank accession no. U24703; D'Arcangelo et al. 1995), as indicated on the *bottom* and *top* lines, respectively. Residues conserved between the two species are shown as a dot in the mouse sequence. The conserved EGF-like domains are shown in bold, and the signal peptide is underlined. The valine residue depicted above a vertical bar (near the beginning) is an insertion in the mouse sequence.

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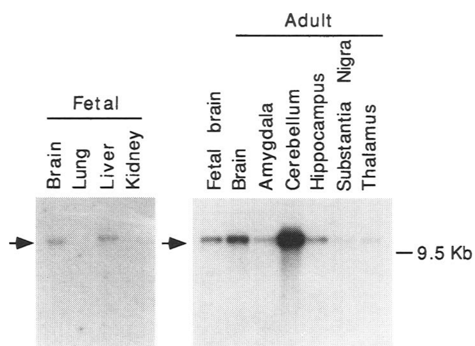

Figure 4 *RELN* expression pattern. Membranes containing poly(A) RNA purified from multiple fetal tissues (*left*) or various brain regions (*right*) were hybridized with a radiolabeled *RELN*-specific probe. A single hybridizing band of ~12 kb (arrow) was seen in some RNA samples. An actin-specific probe was hybridized to the same membranes, and relatively equal hybridization signals were seen for all samples except for cerebellum RNA, which showed a slightly weaker signal (data not shown).

taken the first step toward investigating the structure and function of the human homolog of the gene responsible for the mouse neurodevelopmental mutation known as reeler.

The isolation of an overlapping set of cDNA clones allowed us to establish the nucleotide sequence of *RELN*. The human and mouse reelin proteins are both large and exhibit 94.2% identity at the amino acid level. The amino terminus of the human reelin protein consists of a signal peptide followed by a consensus cleavage site. The presence of a signal peptide with the lack of any other apparent transmembrane domain strongly suggests that human reelin is a secreted protein. The amino terminus also displays 28% amino acid identity to F-spondin, a protein secreted by the floor plate of the spinal cord that is thought to be involved in regulating adhesion and extension of axons in the developing central nervous system (Klar et al. 1992). Human reelin, like mouse reelin, has eight consecutive repeat sequences comprised of two related subdomains separated by an EGF-like motif. The latter is often associated with receptor–ligand interactions (Reichardt and Tomaselli 1991). The EGF-like motifs in reelin have the highest similarity with the extracellular matrix proteins tenascin, restrictin, and the integrin β-chain family (Reichardt and Tomaselli 1991). The *RELN* transcript is expressed in brain and liver, both at fetal and adult ages. In the adult brain, *RELN* mRNA levels are particularly elevated in the cerebellum. High levels of *Reln* mRNA in adult liver were not observed in the mouse (D'Arcangelo et al. 1995).

Currently, no human disease has been associated with *RELN*. One possibility is that *RELN* mutations are lethal in human embryos. Nevertheless, neuronal migration abnormalities have been observed in a number of human diseases. In one case, Kallmann syndrome, the disease affects migration of neurons secreting the hormone GnRH in the hypothalamus, resulting in hypogonadism and anosmia (Kallman et al. 1944; Rugarli and Ballabio 1993). The gene responsible for the X-linked form of Kallmann syndrome (KAL) has been identified and shown to code for a protein that shares sequence similarities with several neuronal cell adhesion molecules (Franco et al. 1991; Legouis et al. 1991). Interestingly, an autosomal form of Kallmann syndrome has been reported that involves a translocation involving 7q22 (Best et al. 1990), which is the general cytogenetic location of *RELN*. The possible association of *RELN* with this form of Kallmann syndrome is currently being investigated. The reagents and information reported here, particularly the cDNA sequence, the genomic and cDNA clones, and the availability of tightly linked genetic markers, should greatly enhance the study of *RELN*. Undoubtedly, this protein serves an important function in mammalian brain development, and it may be associated with human brain malformations.

METHODS

Southern Hybridization Analysis

Genomic DNA from various vertebrate species was obtained commercially (Clontech). The DNA (10 μg per lane) was digested with *Eco*RI, separated in a 0.8% agarose gel, and transferred to a Hybond nylon membrane (Amersham). The membrane was then hybridized with a mouse *Reln* probe corresponding to nucleotides 3268–3613, which was radiolabeled with [α-^{32}P]dCTP using an oligolabeling kit (Pharmacia). Following hybridization at 37°C in a standard buffer containing 50% formamide, the membrane was washed twice in 2× SSC/0.2% SDS at 37°C and exposed to Kodak X-OMAT film.

Isolation of cDNA Clones

Based on the cDNA sequence of *Reln* (D'Arcangelo et al. 1995), a series of PCR assays were developed that amplified the appropriately sized products from human genomic DNA. In some cases, low-stringency PCR conditions (e.g., low annealing temperature) were required to achieve robust amplification from human DNA. The resulting PCR products were cloned into pCRII (Invitrogen) and sequenced using dye-labeled primers on an Applied Biosystems 373A sequencer.

The resulting human DNA sequence was used to design *RELN*-specific oligonucleotides, which in turn were used to

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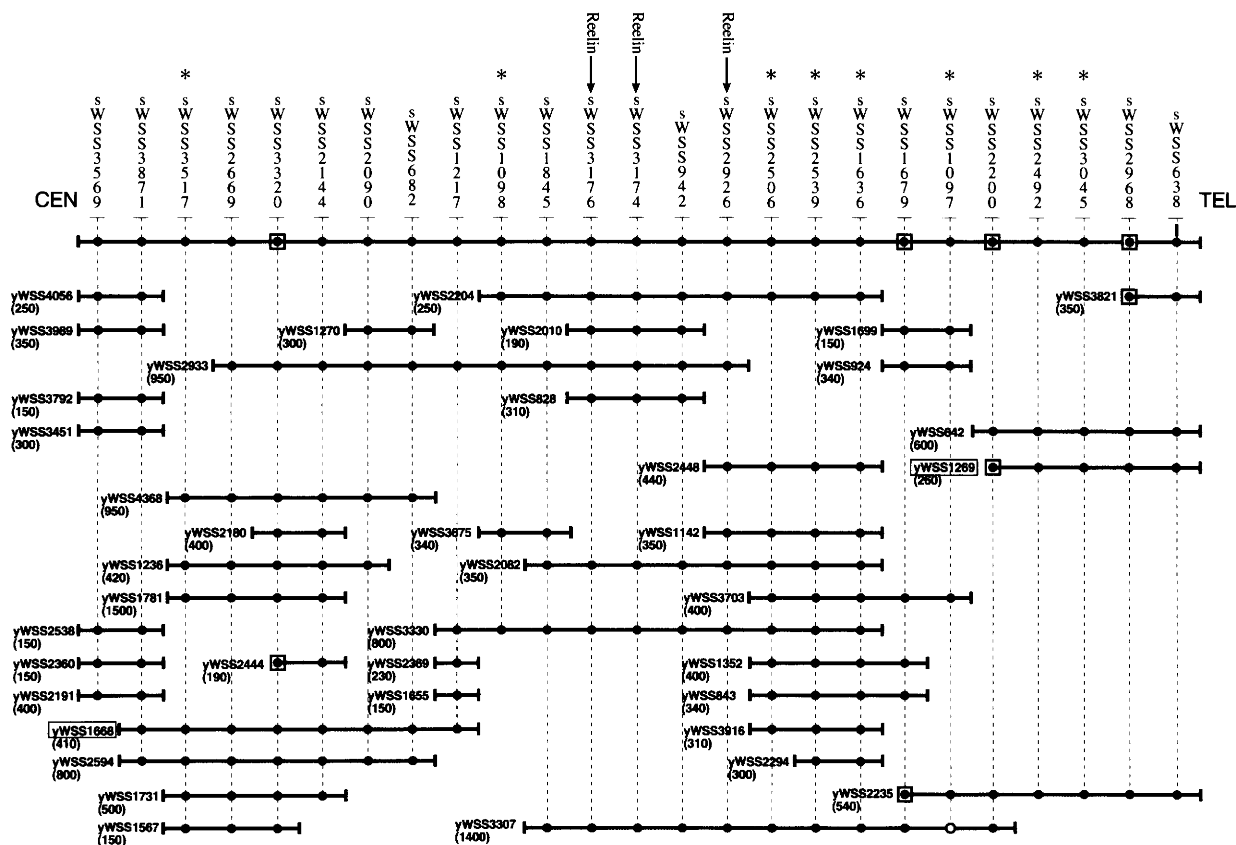

Figure 5 Localization of *RELN* on the YAC-based physical map. Three *RELN*-specific STSs were developed and mapped to a large YAC contig from human chromosome 7q22. A portion of this YAC contig is shown. Relevant information about all of the STSs (listed along the top) is available in GenBank and the Genome Database (GDB). The eight STSs corresponding to genetic markers are indicated with an asterisk (*; see Table 1). A partial set of the YACs from this region is shown, with each estimated YAC size (in kb, measured by pulsed-field gel electrophoresis) provided in parenthesis. The presence of an STS in a YAC is indicated (●) at the appropriate position, with the absence of an STS expected to be present (○). When an STS corresponds to the insert end of a YAC, a square (□) is placed around the corresponding ● [both along the top (near the STS name) and at the end of the YAC from which it was derived]. Additional information about the YACs is available in GDB. Boxes are placed around the names of the two YACs (yWSS1269 and yWSS1668) that were mapped to 7q22 by FISH analysis (Green et al. 1994; data not shown). The relative positions of the centromere (CEN) and the 7q telomere (TEL) are indicated. Note that *RELN* is transcribed in the telomeric to centromeric direction.

isolate clones from a Superscript human brain cDNA library (Life Technologies) using the GeneTrapper cDNA positive selection system (Life Technologies). A total of seven cDNA clones (ranging in size from 2.2 to 4.8 kb) were isolated (see Fig. 2). Sequences of the insert ends of each clone were established and aligned with the mouse sequence to establish the extent of continuity. When gaps in clone coverage were detected and subsequent GeneTrapper cDNA isolation attempts failed to yield a desired clone, hybridization-based screening of an alternate cDNA library was performed. Approximately 10^6 clones from a human cerebellum "5'-Stretch" cDNA library (Clontech) were plated at a density of ~5×10^5 plaques per 24×24-cm screening plate (Nunc). Phage plaques were transferred in duplicate to Nextran Plus nylon membranes (Schleicher & Schuell) and then denatured in 1.5 M NaCl/0.5 N NaOH for 5 min, neutralized in 1.5 M NaCl/0.5 M Tris-HCl for 5 min, briefly washed in 2× SSC, and dried. Appropriate probes derived from existing cDNA clones (see above) were radiolabeled and hybridized to the nylon membranes using standard conditions. Two positive plaques were picked and rescreened. Inserts from these two cDNA clones were amplified by PCR, cloned into pCRII, and used for sequencing.

Sequencing of cDNA Clones

A subset of the isolated cDNA clones, selected to provide two-fold coverage of most of the *RELN* sequence (see Fig. 2), were used to construct a custom subclone library suitable for shotgun sequencing (SeqWright, Houston, TX). Following excision with *Mlu*I, the inserts of the cDNA clones were mixed together, ligated end-to-end, and sheared with a nebulizer. The DNA fragments were end-repaired and subcloned into M13mp19 (Andersson et al. 1996). Random subclones were

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Table 1. Microsatellite Markers Mapping Near *RELN*

Name	Genetic marker name	Accession no. GDB	Accession no. GenBank
sWSS3517	UT997	GDB:312942	L39153, L39154
sWSS1098	AGM273vg5	GDB:612027	Z51237
sWSS2506	AFMb348zb5	GDB:661540	Z53797
sWSS2539	GATA6G06	GDB:228764	G09119
sWSS1636	GATA4E02	GDB:686670	G08623
sWSS1097	AFM269zg1	GDB:199652	Z23925
sWSS2493	AFMa275yc1	GDB:608169	Z52739
sWSS3045	AFMb320ve1	GDB:610899	Z53567

The eight genetic markers mapping in close physical proximity to *RELN* (see Fig. 5) are listed. The sWSS name corresponds to the STS used to localize the corresponding marker on the YAC-based physical map. The genetic marker name reflects the microsatellite marker used for genetic mapping (with the appropriate GDB and GenBank numbers indicated). Note that the actual PCR assays used for YAC-based physical mapping are usually different from those used for genetic mapping, as the former typically employ primers that do not flank the polymorphic tracts (Green et al. 1994).

sequenced to provide roughly sixfold coverage using fluorescent dye-labeled -21M13 universal primer (Applied Biosystems) and an Applied Biosystems 373A sequencer. Sequence assembly was performed with DNASTAR sequence analysis software. Gaps and ambiguities were resolved by targeted sequencing of selected subclones with custom primers and fluorescent dye-labeled terminators (Applied Biosystems). All bases were read on both strands to confirm the final sequence. Subsequent assembly and analysis of the DNA sequence was performed with either the DNASTAR or MacVector (Kodak) sequence analysis software.

Northern Hybridization Analysis

For analyzing *RELN* expression in fetal tissues, a 180-bp fragment corresponding to nucleotides 1114–1294 of the *RELN* cDNA was amplified by PCR (5′-CTCTACCTTCCTGAGGACGCCAA-3′, 5′-AAGCCAGTTGCCTGTGTCCACTG-3′), gel purified, radiolabeled, and hybridized to a fetal tissue RNA blot (Clontech) in ExpressHyb (Clontech) as recommended by the manufacturer. For analyzing *RELN* expression in adult brain, poly(A) RNA from different brain regions (Clontech) was electrophoresed through a 1% agarose/formaldehyde gel (2 μg per lane) and transferred to a Hybond nylon membrane (Amersham). The same probe as above was hybridized to these mRNAs in standard buffer containing 50% formamide at 42°C. The membrane was washed twice in 2× SSC/0.2% SDS, then once in 0.1× SSC/0.1% SDS at 50°C and exposed to Kodak X-OMAT film.

Mapping *RELN* on the YAC-Based Physical Map

Three *RELN*-specific PCR assays (sWSS2926, sWSS3174, and sWSS3176; GenBank accession nos. G30936, G30937, G30938, respectively) were developed and optimized essentially as described (Green et al. 1991; Green 1993). These PCR assays were in turn used to screen the chromosome 7 YAC resource (Green et al. 1995).

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