Corin, a Mosaic Transmembrane Serine Protease Encoded by a Novel cDNA from Human Heart*

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A novel cDNA has been identified from human heart that encodes an unusual mosaic serine protease, designated corin. Corin has a predicted structure of a type II transmembrane protein and contains two frizzled-like cysteine-rich motifs, seven low density lipoprotein receptor repeats, a macrophage scavenger receptor-like domain, and a trypsin-like protease domain in the extracellular region. Northern analysis showed that corin mRNA was highly expressed in the human heart. In mice, corin mRNA was detected by in situ hybridization in the cardiac myocytes of the embryonic heart as early as embryonic day (E) 9.5. By E11.5–13.5, corin mRNA was most abundant in the primary atrial septum and the trabecular ventricular compartment. Expression in the heart was maintained through the adult. In addition, mouse corin mRNA was also detected in the prehypertrophic chondrocytes in developing bones. By fluorescent in situ hybridization analysis, the human corin gene was mapped to 4p12–13 where a congenital heart disease locus, total anomalous pulmonary venous return, had been previously localized. The unique domain structure and specific embryonic expression pattern suggest that corin may have a function in cell differentiation during development. The chromosomal localization of the human corin gene makes it an attractive candidate gene for total anomalous pulmonary venous return.

Serine proteases are essential for a variety of biological processes including food digestion, complement activation, and blood coagulation (1–3). In Drosophila, serine proteases are also involved in developmental pathways. For example, serine proteases encoded by the nudel, gastrulation defective, easter, and snade genes are key components of a proteolytic cascade that is critical for the establishment of the dorsal-ventral pattern in developing embryos (4–6). Genetic defects in these genes often lead to the disruption of the dorsal-ventral axis, resulting in embryonic lethality (7).

Most serine proteases of the trypsin family are secreted proteins. Several members from this family have been identified that contain an integral transmembrane domain. Hepsin, for example, is a serine protease expressed on the surface of hepatocytes. Structurally, hepsin is a type II transmembrane protein with the transmembrane domain at its amino terminus and the protease domain at the carboxyl terminus exposed to the outside of the cell (8). In tissue culture studies, hepsin was shown to contribute to hepatocyte growth (9). However, the physiological significance of the growth stimulating activity of hepsin remains unknown (10). In Drosophila, Stubble-stubbloid protein, another transmembrane serine protease, shares structural similarities with hepsin (11). Genetic studies demonstrated that Stubble-stubbloid is essential for epithelial morphogenesis and development of the fruit fly. Defects in the Stubble-stubbloid gene cause malformation of legs, wings, and bristles. Most recently, other transmembrane serine proteases were isolated and cloned from human tissue. Several members from this family have been identified by searching for ESTs (expressed sequence tag) clones that contained inserts of 3.5 and 3.1 kb, respectively. The DNA sequence of the EST report in this paper was submitted to the GenBank(12). BRL Data Bank with accession number(s) AF139845.

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The abbreviations used are: LDL, low density lipoprotein; EST, expressed sequence tag; FISH, fluorescent in situ hybridization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ORF, open reading frame; RT, reverse transcriptase; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; TAPVR, total anomalous pulmonary venous return; kb, kilobase pair; bp, base pair; E, embryonic day.

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ers were designed to clone further 5' end cDNA sequences by 5' rapid amplification of cDNA ends (RACE) using Marathon-ready human heart cDNA templates (CLONTECH). The PCR products from 5' RACE were cloned into pCRII vector (Invitrogen, San Diego, CA) and sequenced. Oligonucleotide primers used in the 5' RACE experiments were 5'-CAGTTCTTGGTGAACTGTCGAGG-3', 5'-TGCAAGAGGAGGATACGCCTGCGT-3', 5'-AACTCCAGAGAACAAGCTCACGAG-3', 5'-CAGGTCCACAGAGAGCTCACCAC-3', 5'-GGTGCTCTTGTGATGCTGCTTG-3', 5'-CGAGGCCCCATGAAGTTAAAACCA-3', and 5'-AACAAAGGATCTTGGTCTGCGACTGT-3'. The final 5' end sequence of human corin cDNA was derived from at least three independent clones. The full-length cDNA sequence was compiled using the Genetics Computer Group (GCC) software (version 9.1, Madison, WI).

Northern Analysis—Northern blots containing poly(A)⁺ RNA samples (2 µg/lane) from multiple human and mouse tissues were purchased from CLONTECH. Human and mouse corin cDNA probes were labeled with [32P]dCTP using a random primed DNA labeling kit (Roche Molecular Biochemicals). Northern hybridization was performed at 42 °C overnight in a solution containing 40% formamide, 5× Denhardt’s solution, 6× SSC, 100 µg/ml salmon sperm DNA, and 0.1% SDS. Blots were washed with 0.2× SSC, 0.1% SDS at 60 °C and then exposed to Fuji imaging plates. As a control, the blots were reprobed with a human actin cDNA probe provided by CLONTECH.

RT-PCR—mRNA samples were isolated from Hec-1-A, U2-OS, SKLMS-1, and AN3-CA cells using a commercial RNA preparation kit (Oligotex Direct mRNA Mini Kits, Qiagen). First strand cDNAs were synthesized using SuperScript II RNase−reverse transcriptase (Life Technologies Inc.). Human corin-specific oligonucleotide primers (sense primer, 5'-AACAAGAGGATCTTGGTCTGCGACTGT-3', and anti-sense primer, 5'-CAGGTCCACAGAGAGCTCACCAC-3') were used to amplify a 630-bp fragment of corin cDNA between nucleotides 2475 and 3105. Oligonucleotide primers TFR1 (5'-GCTCAATGCTCCCAAAGCTCACCAGA-3') and TFR2 (5'-ATTCGGGGAATGCTGAGAAAACAGA-3'), derived from the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, were used as an internal quantification control. PCR reactions were performed with a thermal cycler (Perkin-Elmer, model 480). PCR products were separated on 1% agarose gels and visualized by ethidium bromide staining.

In Situ Hybridization—Mouse adult heart and embryonic tissue sections were deparaffinized in xylene, rehydrated, and fixed in 4% paraformaldehyde. The tissues were digested with proteinase K (20 µg/ml), then treated with triethanolamine/acetic anhydride, and dehydrated. An 800-bp mouse corin cDNA fragment from the coding region was cloned into pCRII (Invitrogen) in two orientations to yield plasmids pM11 and pM41. The plasmids were linearized by HinIII digestion. Sense and antisense probes were synthesized using T7 RNA polymerase−reverse transcriptase (Life Technologies Inc.). Hybridization signals were detected by fluorescent-labeled antidigoxigenin antibodies and counter-staining with D-19 developer. The slides were stained with hematoxylin/eosin and analyzed using a Zeiss microscope.

Fluorescent In Situ Hybridization (FISH) Analysis—P1 phage clones containing the human corin gene were isolated by filter hybridization using a human corin cDNA as the probe. One clone was confirmed by DNA sequencing using a primer from human corin cDNA. The DNA fragment from this P1 phage was labeled with digoxigenin-dUTP. The labeled probe was combined with sheared human DNA and hybridized to metaphase chromosomes derived from PHA-stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate, and 2× SSC. Hybridization signals were detected by fluorescent-labeled antidigoxigenin antibodies and counter-staining with 4',6-diamidino-2-phenylindole. A total of 80 metaphase cells were analyzed of which 74 cells exhibited specific labeling.

Homology Model of the Protease Domain of Corin—A model of the corin protease domain (amino acids 802–1042) was built based on the structure of bovine chymotrypsinogen A at 1.8 Å resolution (15, 16), using the homology program (Insight II, 1995, MSI, San Diego, CA). Rotamers for the non-identical side chain replacements (18). Coordinates for the loop insertions were extracted from the Brookhaven protein data bank (17). The model was refined by energy minimization using the AMBER force field (Discover 95.0), with a distance-dependent dielectric constant. The minimization used the steepest descents and conjugate gradient methods as follows: first for the loops only where insertions and deletions occurred, then side chains, and a final round of minimization keeping the Ca atoms fixed. The residues of corin (His43, Asp492, and Ser985) corresponding to the catalytic triad of the template structure were also held fixed.

RESULTS

Cloning of the Full-Length Human Corin cDNA—A computer search using the BLAST program identified an EST clone from a human heart library that shared significant homology with serine protease family members, such as trypsin. The EST clone was used to isolate the full-length cDNA of a novel gene, designated corin for its abundant expression in the heart. The sequence of the full-length corin cDNA, 4933 bp in length, is shown in Fig. 1. The size of the cDNA is consistent with the length of corin mRNA (~5 kb) detected by Northern analysis (Fig. 4A). An ATG codon is located at position 95 that may represent the translation initiation site. The open reading frame (ORF) spans 3126 bp with a 5'-untranslated region of 94 nucleotides before the initiation codon. At the 3' end, there is a 1.7-kb 3'-untranslated region after the stop codon at position 3221. A polyadenylation signal of AAATAA is present 12 nucleotides before the poly(A)⁺ tail.

The Domain Structure of Human Corin—The ORF of the human corin cDNA encodes a polypeptide of 1042 amino acids with a calculated mass of 116 kDa. At the amino terminus of the predicted corin protein, there is no discernable signal peptide sequence. Hydropathy plots using the GCG program identified a highly hydrophobic region between amino acids 46 and 66 (Fig. 2B). This hydrophobic sequence could serve as a potential transmembrane domain. There are positively charged amino acid residues immediately preceding the putative transmembrane segment, suggesting that corin is a type II transmembrane protein with the amino terminus present in the cytosol (18). Consistent with this hypothesis, there are 19 predicted N-linked glycosylation sites present in the extracellular domains of corin (Fig. 1).

Analysis of the corin protein sequence showed that in the extracellular region there are two frizzled-like cysteine-rich domains, seven LDL receptor repeats, one macrophage scavenger receptor-like domain, and one trypsin-like serine protease domain (Fig. 2A). As shown in Fig. 2A, two frizzled-like cysteine-rich domains are located at amino acids 134–259 and 450–573, respectively. Amino acid sequences of these two domains share significant similarities with the extracellular cysteine-rich domain of the Drosophila Frizzled protein, a seven-transmembrane receptor essential for polarity determination during the development of the fruit fly (19). The frizzled-like cysteine-rich domains have also been found in other proteins, such as Dfz2 in Drosophila (20), Lin-17 in Caenorhabditis elegans (21), and FZ-1 in human (22). The sequences of the two frizzled-like cysteine-rich domains in corin are closest to those in Lin-17 and FZ-1. As shown in Fig. 2C, all the 10 conserved cysteine residues are present in the frizzled-like cysteine-rich domains of corin.

Between amino acids 268–415 and 579–690 (Fig. 2, A and D), there are seven cysteine-rich repeats homologous to the LDL receptor class A repeats (23). Each repeat is about 36 amino acids long and contains six cysteine residues as well as a highly conserved cluster of negatively charged amino acids. In the LDL receptor, these cysteine-rich repeats bind calcium ions and play an essential role in endocytosis of the extracellular ligands (23). Similar motifs have been found in the extracellular domain of other membrane receptors such as the LDL receptor-related protein (LRP1) (24), megalin (also known as LRPI or gp330) (25), complement proteins (26), enterokinase (27), and Drosophila protease yolkless and nudel (28, 29).

In addition to the frizzled-like cysteine-rich domains and LDL receptor-like repeats, there is another cysteine-rich region between amino acids 713 and 801 in corin (Fig. 2, A and E).
FIG. 1. Nucleotide sequence of human corin cDNA and its deduced amino acid sequence. The potential codon for the initial methionine, the translation stop codon, and the polyadenylation signal were in bold-face type and underlined. The putative transmembrane domain was double underlined. The 19 potential N-linked glycosylation sites are in boldface type and double underlined. A n arrow indicates the putative cleavage site for the activation of the serine protease. The active site residues of the catalytic triad (His843, Asp892, and Ser985) are in boldface type and underlined.
FIG. 2. A, a schematic presentation of the domain structure of corin protein. The transmembrane domain (TM), frizzled-like cysteine-rich domains (CRD), LDL receptor repeats (LDLR), scavenger receptor cysteine-rich domain (SRCR), and serine protease catalytic domain (Catalytic) are indicated. Numbers correspond to the amino acid residues of the ORF shown in Fig. 1. B, hydropathy plots of the deduced amino acid sequence of corin by Goldman and Kyte-Doolittle methods, respectively (36). *Hphobic*, hydrophobic; *Hphilic*, hydrophilic. C, alignment of amino acid sequences of the frizzled-like cysteine-rich domains from corin and other members of the frizzled family, including Frizzled in *Drosophila*, lin-17 in *C. elegans*, and FZ-1 in human. D, alignment of amino acid sequences of the seven LDL receptor repeats of corin with the consensus sequence derived from the human LDL receptor. E, alignment of amino acid sequences of the scavenger receptor-like cysteine-rich domains from corin and human enterokinase (*Enlk*), sea urchin speract receptor (*q17064*) and human scavenger receptor I (*o15393*). Asterisks indicate conserved residues. F, alignment of amino acid sequences of protease domains from human corin, prekallikrein (*KAL*), enterokinase (*ENTK*), trypsin (*TRP1*), and bovine chymotrypsinogen A (*CTRA*).
This region contains 88 amino acids and is homologous to the cysteine-rich motif found in the macrophage scavenger receptor (30). This motif is also present in the sea urchin spermatozoa speract receptor (31, 32) and the vertebrate serine protease, enterokinase (27).

At the carboxyl terminus of corin protein between amino acid residues 802 and 1042, there is a trypsin-like serine protease domain (Fig. 2A). This protease domain is highly homologous to the catalytic domain of members of the trypsin superfamily. For example, amino acid sequence identities between corin and prekallikrein (33), factor XI (34), and hepsin (35) are 40, 40, and 38%, respectively. All essential features of serine protease sequences are well conserved in corin (Figs. 1 and 2F). The active site residues of the catalytic triad are located at His843, Asp892, and Ser985. The amino acid residues forming the substrate specificity pocket are located at Asp979, Gly1007, and Gly1018. These residues are predicted to bind the substrate P1 residues, suggesting that corin would cleave its substrate after basic residues, such as lysine or arginine. In addition, a putative activation cleavage site was found at Arg801, suggesting that corin would be synthesized as an inactivezymogen and that another trypsin-like enzyme was required for its activation.

In the protease domain, there are 12 cysteine residues. Potential pairing of these cysteine residues can be predicted by comparing with other well studied serine proteases, such as trypsin and chymotrypsin. First three pairs of cysteine residues present in essentially all members of the trypsin superfamily are located at Cys828–Cys844, Cys905–Cys970, and Cys981–Cys1010. Two more pairs of cysteine residues are present at the positions Cys790–Cys830 and Cys912–Cys991. These two pairs of cysteine residues are commonly found in a subfamily of two-chain serine proteases, such as chymotrypsin and prekallikrein (33). The presence of Cys790 and Cys912 indicated that, after the activation cleavage at Arg801, the catalytic domain of corin would remain attached to the rest of molecule by a disulfide bond. Interestingly, there is one additional pair of cysteine residues, Cys817 and Cys830, present in corin. Cysteine residues at these two positions were not found in any other serine proteases in vertebrates. A search of databases showed that a chymotrypsinogen-like serine protease from the lugworm, Arenicola marina, had two cysteine residues at the corresponding positions. A model of the corin protease domain was built based on the structure of bovine chymotrypsinogen A (Fig. 3). Based on this corin model, where the Cα atoms of these two cysteine residues were held fixed during energy minimization, the distance between the sulfur atoms of their side chains is about 2.5 Å after rotamer searching. The model indicates that these two cysteines are likely to form a disulfide bond connecting two β-sheets in the core of the protease domain (Fig. 3).

Northern Analysis of Corin mRNA Expression—To determine expression of the corin gene in human tissues, Northern hybridization was performed using human corin cDNA probes. As shown in Fig. 4A, an ~5-kb transcript was detected only in the heart but not in other tissues including brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, colon, and leukocytes. Since the heart is mainly composed of cardiac muscles, Northern analysis was

2 J. Eberhardt, GenBank™ accession number G1160388.
performed to examine the presence of corin mRNA in other human muscle-rich tissues. Again, corin mRNA was detected in the heart but not in uterus, small intestine, bladder, stomach, and prostate (Fig. 4).

To examine corin mRNA expression in mice, the full-length mouse corin cDNA was cloned by a PCR-based strategy. Mouse corin cDNA shared 89% sequence identities with human corin cDNA (data not shown). Northern analysis was performed with RNA samples from mouse tissues. As shown in Fig. 4C, a prominent transcript of ~5 kb was detected in samples derived from the heart. In contrast to Northern analysis with human samples, low levels of corin mRNA were also detected in samples derived from the testes and kidneys.

Mouse Corin mRNA Expression in Adult and Embryonic Hearts—In situ hybridization was performed to determine the temporal and spatial expression of corin mRNA. In adult mice (Fig. 5), corin mRNA was detected in cardiac myocytes of both atrium and ventricle. The level of expression appeared to be higher in the atrium than the ventricle. During embryonic development, corin mRNA was first detected at E9.5 in both
atrium and ventricle of the developing heart (Fig. 6B). Between E11.5 and E13.5, corin mRNA was highly expressed in the thickened atrial wall and in the regions that underwent trabeculation in the ventricle (Fig. 6, D and F). By E15.5, corin mRNA in the heart was more abundant, especially in primary atrial septa (Fig. 6H). Weak signals appeared to be present in developing aorta and vena cava but not in the esophagus and lungs (Fig. 6H). The expression of corin mRNA in the heart was maintained in the subsequent embryonic stages (not shown).

Corin mRNA Expression in Other Tissues—In addition to the heart, corin mRNA was also detected in other mouse tissues by in situ hybridization. For example, corin mRNA was present in the uterus of pregnant mice and in the developing kidneys. In the uterus (Fig. 7L), corin mRNA expression was most abundant in the decidual cells close to the implantation site of the embryo. In the developing kidneys at E15.5, corin mRNA was
highly expressed in the stromal cells in the medulla but not in the cortex of the kidney (Fig. 7J). This finding was consistent with the results of Northern analysis in which a corin transcript was found in RNA samples from mouse kidneys (Fig. 3C).

Interestingly, in situ hybridization also identified corin mRNA in several cartilage-derived structures, such as the vertebra in the tail, the turbinate in the head, and the long bones in the limbs (Fig. 7, B, D, F, and H). Fig. 7B showed the expression of corin mRNA in cartilage primordia of vertebral bodies in the posterior of an E13.5 embryo. By E15.5, the level of corin mRNA expression in the vertebra was much lower as the vertebra became more matured (data not shown), indicating that corin may play a role in the differentiation of chondrocytes. This notion was supported by the expression of corin mRNA in developing limbs. Fig. 7, E and F, showed an early developing digital bone that consisted of three types of cells as follows: hypertrophic chondrocytes at the center, prehypertrophic chondrocytes next to the hypertrophic zone, and proliferating chondrocytes at the both ends. Corin mRNA was found mostly in the prehypertrophic chondrocytes (Fig. 7F). Hybridization signals were also present in perichondrium (Fig. 7F). Fig. 7, G and H, showed a long bone in a hind limb that was at a more advanced developmental stage. The central hypertrophic zone was replaced by vascularized tissues containing bone marrow cells and osteoblasts. Nevertheless, similar expression pattern of corin mRNA was found in the narrow zone of the prehypertrophic chondrocytes and in the perichondrium. These results indicated that corin expression was associated with a specific stage of chondrocyte differentiation.

Corin mRNA Expression in Human Tumor Cell Lines—A number of human cancer cell lines were screened by Northern and RT-PCR analyses for the presence of corin mRNA. In most cell lines, such as HL60, HeLa, K562, MOLT-4, RAJI, SW480, A549, and G36, corin mRNA was undetectable (data not shown). However, corin mRNA was found in several cell lines derived from uterus tumors or osteosarcoma. As shown in Fig. 8, corin mRNA was detected by RT-PCR in endometrium carcinoma cell lines IEC-1-A, AN3 CA, and RL95-2, leiomyosarcoma cell line SK-LMS-1, as well as in osteosarcoma cell line U2-OS. The result is consistent with the finding by in situ hybridization in which corin mRNA was highly expressed in the developing bones in embryos as well as in the maternal uterus.

Chromosomal Localization of the Human Corin Gene—FISH analysis was performed to determine the chromosomal locus of the human corin gene. Specific fluorescent spots were found at 4p12-13, a region adjacent to the centromere on the short arm of chromosome 4 (Fig. 9). The result was confirmed in a subsequent experiment in which a genomic probe previously mapped to 4p15.3 was co-localized with the corin gene probe (data not shown). A search of the OMNI human genetic data base indicated that a congenital heart disease locus, total anomalous pulmonary venous return (TAPVR), was previously mapped to this region at 4p13-q12 (37).

**DISCUSSION**

In this study, we describe the cloning and initial characterization of a novel cDNA from the human heart that encodes a putative transmembrane serine protease, which we have designated as corin. The presence of a hydrophobic transmembrane domain at its amino terminus and the absence of a signal peptide suggest that corin is a type II transmembrane protein. In the extracellular region of corin, there is a trypsin-like catalytic domain that contains all conserved structural features of serine proteases, such as the catalytic triad, the activation cleavage site, the substrate specificity pocket, and the essential cysteine residues. Interestingly, the protease domain of corin contains two unique cysteine residues, Cys<sup>817</sup> and Cys<sup>830</sup>, that are not present in other trypsin-like serine proteases in vertebrates. Molecular modeling showed that these two cysteine residues are likely to form a disulfide bond connecting two β-sheets in the core of the protease domain (Fig. 3). A search of genomic data bases showed that a chymotrypsin-like protease found in the lugworm, <i>A. marina</i>, also has two cysteine residues at the corresponding positions. It is not clear whether these two cysteine residues are maintained through a convergent or divergent evolution. Nevertheless, the presence of such an unusual pair of cysteine residues in both corin and the lugworm protease suggests an important biological function of the disulfide bond. One potential possibility is that the disulfide bond may contribute to stability of the proteases.

Although members of the trypsin superfamily are known to contain a variety of domain structures such as kringle and epidermal growth factor-like domains that are important for protein-protein interactions, this is the first report of the presence of a frizzled-like cysteine-rich domain in this extended family. Originally, the frizzled gene was identified in Drosophila (38). The gene encodes a seven-transmembrane receptor that is required for proper development of hairs, bristles, and ommatidia of the fruit fly (19, 39). Later, other Frizzled proteins have been identified in many other species. They all contain a well conserved extracellular cysteine-rich domain and a seven-transmembrane domain and act as receptors for secreted Wnt glycoproteins (for review see Refs. 40 and 41). The cysteine-rich domain, which is about 120 amino acids in length and contains a motif of 10 invariantly spaced cysteine residues, has been shown to be necessary and sufficient for the binding of the Wnt ligands (20, 42). Recent studies demonstrated that Fzrb, a secreted frizzled-like protein without the seven-transmembrane domain, is expressed in the Spermann organizer of frog embryos and can bind and inhibit Wnt-8 (43, 44). In addition, similar frizzled-like cysteine-rich domains have also been found in several other proteins, including mouse collagen (XVIII) α1 chain (45), human carboxypeptidase Z (46), and several receptor tyrosine kinases (47–49). The function of the cysteine-rich domain in these proteins has not been determined. Corin is unique in that it contains the frizzled-like cysteine-rich domains and a serine protease domain. The presence of frizzled-like domains in corin implies that corin may play an important role in development by directly interacting with Wnt proteins.

The temporal and spatial pattern of corin gene expression further supported a potential developmental function of corin. In mice, corin mRNA was detected in the cardiac myocytes of the embryonic heart as early as E9.5 (Fig. 6D). The expression was most prominent in the primary atrial septum and the trabecular ventricular compartment by E11.5–13.5 (Fig. 6, D, E, and F).
and F). During this period, an active process of looping and remodeling takes place in the embryonic heart. As a result, outflow tracts are formed, and the original single tube-like heart is reorganized into a four-chambered structure. Growth factors, such as bone morphogenic proteins and the transforming growth factor-β family members, are known to play a critical role during the embryonic heart development (50). Recent studies in Drosophila showed that the wingless (wg) gene, a homologue of the wnt oncogene in mammals, is directly involved in heart formation (51). It has been suggested that similar signaling pathways also contributed to the heart development in vertebrate (52). It is possible that corin could participate in such developmental pathways by interacting directly with Wnt proteins or other growth factors.

In addition to the heart, corin mRNA was identified in other tissues, such as the pregnant uterus and developing kidneys and bones. The expression of corin mRNA in these tissues appeared to be cell type-specific. For example, in developing long bones corin mRNA was specifically expressed in the prehypertrophic chondrocytes. It is known that skeletal bones are derived from two different processes, intramembranous and endochondral ossification. In the former case, mesenchymal cells first become chondrocytes that in turn differentiate from proliferating chondrocytes to prehypertrophic chondrocytes and finally to hypertrophic chondrocytes. The hypertrophic chondrocytes eventually undergo apoptosis followed by vascularization and ossification. This process of chondrocyte differentiation has been shown to be tightly regulated by hedgehog proteins, bone morphogenic proteins, and parathyroid hormone-related protein (54–57). The expression of corin mRNA in these tissues, such as the pregnant uterus and developing kidneys and bones, is tightly regulated by hedgehog proteins, bone morphogenic proteins, and parathyroid hormone-related protein (54–57). The expression of corin mRNA in these tissues is directly with Wnt proteins or other growth factors.

Finally, by FISH analysis the human corin gene was located on the short arm of chromosome 4 (4p12-13) (Fig. 9). A search of the OMNI human genetic data base showed that a disease locus, total anomalous pulmonary venous return (TAPVR), had been previously mapped to this region. TAPVR is a rare cyanotic form of congenital heart defects in which the pulmonary vein connected abnormally to the right atrium or one of the venous tributaries instead of the left atrium. The molecular mechanism responsible for this developmental defect in the heart is unknown. A linkage study of a large Utah-Idaho family that included 14 affected individuals localized the TAPVR locus to a 30-centimorgan interval on 4p13-q12 (37). The findings that the corin gene and the TAPVR locus are co-localized on chromosome 4 and that corin mRNA is highly expressed in the embryonic heart, particularly in the region where outflow tracts were formed, suggest that corin is an attractive candidate for the TAPVR gene. The isolation of the corin cDNA provided a useful tool to study further this intriguing possibility.

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