A Novel Member of the LDL Receptor Gene Family with Eleven Binding Repeats is Structurally Related to Neural Adhesion Molecules and a Yeast Vacuolar Protein Sorting Receptor

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We now have discovered and characterized a novel multi-domain protein and classified it as a member of the LDL receptor gene family. The ~250 kDa membrane protein, termed LR11, highly conserved in man, rabbit and chicken, contains a cluster of 11 LDL receptor ligand binding repeats, a group of 5 LDL receptor "YWTD" repeats, a large hexarepeat domain of structural elements found in neural cell adhesion molecules, and a domain with similarity to a yeast receptor for vacuolar protein sorting, VPS10. The cytoplasmic domain exhibits features typical of endocytosis-competent coated-pit receptors. The mosaic, and presumably multifunctional, receptor is expressed abundantly in brain, liver and adrenal glands. Ligand blotting of LR11-transfected cells demonstrated that LR11 binds apolipoprotein E-containing lipoproteins, as well as other members of LDL receptor gene family. In contrast to the LDL receptor, the mRNA levels in rabbit liver is unaffected by hyperlipidemia. The features of this highly conserved and complex mosaic protein suggest the importance of the ever expanding LDL receptor gene family in the evolution and proposed multifunctionality. *J Atheroscler Thromb*, 1997; 4: 20-26.

Key words : Gene family, Low density lipoprotein, Adhesion molecule, Vacuolar sorting

In 1984, the primary structure of the low density lipoprotein (LDL) receptor was elucidated by cDNA cloning (1). This was a hallmark in lipoprotein and receptor research and was the molecular basis of familial hypercholesterolemia (FH), one of the most common genetic disease in man (2). As compared with the LDL receptor (LDLR), the physiological roles of newly identified in the past decade and ever expanding LDLR gene family (Fig. 1), are not yet elucidated. These identified common structural modules are (i) the so-called "LDLR ligand binding repeats", complement-type domains consisting of ~40 residues displaying a triple-disulfide-bond-stabilized negatively charged

surface; (ii) epidermal growth factor precursor-type repeats, also containing six cysteines each; (iii) modules of ~50 residues each, most often in groups of five, with a consensus tetrapeptide, Tyr-Trp-Thr-Asp (LDLR "YWTD" repeats); and (iv), in the cytoplasmic region, signals for receptor internalization via coated pits, containing the consensus tetrapeptide Asn-Pro-Xaa-Tyr (NPXY) (3).

The best characterized binding domain is that of the LDLR, which consists of seven complement-type repeats and recognizes apolipoprotein (apo) B and apoE (4). LDLR family members harbor from 1 to 4 clusters with varying numbers of ligand binding repeats. To date, the family includes in addition to the LDLR, the very low density lipoprotein (VLDL) receptor (5, 6), *Drosophila* yolkless (YI) (7), LDLR related protein/ α 2-macroglobulin receptor (LRP) (8), the *C. elegans* LRP-like gene, (9), gp 330/megalin (10), and the most recent additions, the apoE receptor 2 (11) and brain-specific receptor termed LR8B

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Fig. 1. The LDL receptor gene family. The diagram compares the functional domains in the LDL receptor, VLDL receptor, *Drosophila* yolkless, LDL receptor-related protein/ α_2 -macroglobulin receptor (LRP/ α_2 MR), *C. elegans* LRP-like protein and gp 330/megalin.

(12). Extensive studies on the ligand specificities of these newly identified proteins have shown that most of them play roles as multifunctional receptors rather than the specific receptors for cellular uptake of plasma lipoproteins. One of the functionally best documented receptor, the VLDL receptor (VLDLR) in chicken oocytes, termed LR8 (13), takes up the yolk precursors, riboflavin binding protein (14), α_2 -macroglobulin (α_2 M) (15), VLDL, and vitellogenin (6, 16). Chicken LR8 also recognizes the iron-binding protein, lactoferrin (17). YI is a vitellogenin receptor in *Drosophila*, although its ligand property has not been elucidated (7). LRP and gp 330/megalin have overlapping broad ligand specificities, such as tissue-type plasminogen activator (tPA), urinary-type plasminogen activator, apoE-rich lipoproteins, lactoferrin and lipoprotein lipase. In addition, LRP binds activated α_2 M and *Pseudomonas* exotoxin A. Recently, LRP and gp 330/megalin have been proposed to be the responsible receptors for multifunctional glycoproteins, thrombospondin and apoprotein J/clusterin, respectively (18–20).

We have recently discovered a novel and unusually complex member of the LDLR gene family in man, rabbit and chicken (21, 22), and found that the gene products are highly conserved in such diverse species. The predominant domain of the type I membrane protein consists of a cluster of eleven LDLR ligand binding repeats; according to our preferred nomenclature (13), the new receptor is termed LR11. It also contains sequences highly homologous to a yeast receptor for vacuolar protein

sorting, and to cellular adhesion molecules. Highly expressed in brain, LR11 is proposed to be a member of a hitherto unknown branch of the LDLR gene family which may participate in brain-specific physiological processes.

Identification of a new member of the LDLR gene family, LR11

cDNA encoding a novel LDLR gene family member, designated LR11 (the LDLR relative with 11 binding repeats) was first identified from a rabbit liver cDNA library using as a probe cDNA encoding the ligand binding domain of the rabbit LDLR (21). Sequencing analysis defined a long open reading frame of 6639 bp which codes for 2213 amino acids. Subsequently, using as a probe rabbit LR11 cDNA clone, human and chicken (partial) LR11 cDNAs were identified from brain cDNA libraries from respective species. (Fig. 2). Despite their considerable size and evolutionary distance, the proteins possess 94% (man vs. rabbit) and 84% (man vs. chicken) identical residues, respectively (Figs. 2 and 4). Thus, we now have obtained LR11 cDNAs from three species.

Homology searches of data banks together with a systemic analysis for internal repeats of the predicted amino acid sequence of LR11 demonstrated that the polypeptide has seven distinct domains (Fig. 3): domain I, N-terminal ~ 350 amino acids following the signal sequence; domain II, a segment related to a yeast receptor for vacuolar protein sorting; domain III, an EGF precursor

human	MATRSSRRSRSLPFLTLVALLPPGALCEVWTF	LHGGSAFLPQGRGTVVQGDPRELRLW	RGDARGASRADEKPLRR	RSAAALOPEPKVYGOVSLND	100			
rabbit	MATRSSRRSRSLPFLTLVALLPPGALCEVWTF	LHGGSAFLPQGRGTVVQGDPRELRLW	RGDARGASRADEKPLRR	RSAAALOPEPKVYGOVSLND	100			
chicken	MATRSSRRSRSLPFLTLVALLPPGALCEVWTF	LHGGSAFLPQGRGTVVQGDPRELRLW	RGDARGASRADEKPLRR	RSAAALOPEPKVYGOVSLND	7			
human	SHNQMVVHWAGEKSNVIVALARDSLALARPSSDVVSYDYGKSPFKKIS	KLNFGLGNHSEAVIAQFYHSPADNKRIYFADAYAQYLWITP	DFCNTLGGF		200			
rabbit	SHNQMVVHWAGEKSNVIVALARDSLALARPSSDVVSYDYGKSPFKKIS	KLNFGLGNHSEAVIAQFYHSPADNKRIYFADAYAQYLWITP	DFCNTLGGF		200			
chicken	SHNQMVVHWAGEKSNVIVALARDSLALARPSSDVVSYDYGKSPFKKIS	KLNFGLGNHSEAVIAQFYHSPADNKRIYFADAYAQYLWITP	DFCNTLGGF		107			
human	SIPFRAADLLLSKASNLLGDFDRSHPNKQLWKSDDFGQTWIMIQEHVKS	FSWGIDDPYDKPNTIYIERHEPSGYSTVFRSTDDFQSRNQEVILEEVRDF			300			
rabbit	SIPFRAADLLLSKASNLLGDFDRSHPNKQLWKSDDFGQTWIMIQEHVKS	FSWGIDDPYDKPNTIYIERHEPSGYSTVFRSTDDFQSRNQEVILEEVRDF			300			
chicken	SIPFRAADLLLSKASNLLGDFDRSHPNKQLWKSDDFGQTWIMIQEHVKS	FSWGIDDPYDKPNTIYIERHEPSGYSTVFRSTDDFQSRNQEVILEEVRDF			207			
human	QLRDKYMFATKVHLLGSP	QSSSVQLWVWSFGRRKPMRAAQFVTRHPINEYY	IADASEDVQVFCVSHSNRNNTNLYISEAEGKFLSLLENVLYSPGGAGSD		400			
rabbit	QLRDKYMFATKVHLLGSP	QSSSVQLWVWSFGRRKPMRAAQFVTRHPINEYY	IADASEDVQVFCVSHSNRNNTNLYISEAEGKFLSLLENVLYSPGGAGSD		400			
chicken	QLRDKYMFATKVHLLGSP	QSSSVQLWVWSFGRRKPMRAAQFVTRHPINEYY	IADASEDVQVFCVSHSNRNNTNLYISEAEGKFLSLLENVLYSPGGAGSD		307			
human	TLVRYFANEFFADFHRVEGLQGVYIATLINGSMNEENMRASVITFDKGGTWEFLQAPFTGYGEKINCELSQ	GCSSLHLAQLRSLQLNLQLRRMPILSKESA			500			
rabbit	TLVRYFANEFFADFHRVEGLQGVYIATLINGSMNEENMRASVITFDKGGTWEFLQAPFTGYGEKINCELSQ	GCSSLHLAQLRSLQLNLQLRRMPILSKESA			500			
chicken	TLVRYFANEFFADFHRVEGLQGVYIATLINGSMNEENMRASVITFDKGGTWEFLQAPFTGYGEKINCELSQ	GCSSLHLAQLRSLQLNLQLRRMPILSKESA			407			
human	PGLIATGSGVGNLASKTNVYISSAGARWREALPGHYTTWGDHGGIT	ATAOQMETNELKYSTNEGETWRT	FLFSEKPVFVYGLLTEPEGEKSTVFTIF		600			
rabbit	PGLIATGSGVGNLASKTNVYISSAGARWREALPGHYTTWGDHGGIT	ATAOQMETNELKYSTNEGETWRT	FLFSEKPVFVYGLLTEPEGEKSTVFTIF		600			
chicken	PGLIATGSGVGNLASKTNVYISSAGARWREALPGHYTTWGDHGGIT	ATAOQMETNELKYSTNEGETWRT	FLFSEKPVFVYGLLTEPEGEKSTVFTIF		507			
human	GSNKENVHSLILQVNTDALGVPCETENDYKLWSPSDERGNELGHKT	VFKRRTPHATCFNGEDFDRPVVSNCSCTREDYCDFGFKMSDEL	SLVVCV		700			
rabbit	GSNKENVHSLILQVNTDALGVPCETENDYKLWSPSDERGNELGHKT	VFKRRTPHATCFNGEDFDRPVVSNCSCTREDYCDFGFKMSDEL	SLVVCV		700			
chicken	GSNKENVHSLILQVNTDALGVPCETENDYKLWSPSDERGNELGHKT	VFKRRTPHATCFNGEDFDRPVVSNCSCTREDYCDFGFKMSDEL	SLVVCV		607			
human	PDPGFSKGSYSPVPVPCVGSYTYRRTRGYRKISGDTCSGGDVEARLEL	GPLAEENEFILYATRKSHRYDLASG	SQELPLAGRAAVALDFDYEH		800			
rabbit	PDPGFSKGSYSPVPVPCVGSYTYRRTRGYRKISGDTCSGGDVEARLEL	GPLAEENEFILYATRKSHRYDLASG	SQELPLAGRAAVALDFDYEH		799			
chicken	PDPGFSKGSYSPVPVPCVGSYTYRRTRGYRKISGDTCSGGDVEARLEL	GPLAEENEFILYATRKSHRYDLASG	SQELPLAGRAAVALDFDYEH		707			
human	CLVWSDIALDVIQRLCLNGSTGOEVIINS	GLETVEALAEPLSQLLYWDA	GFKKEVANPDGDFRLTLVNSVLDPRALVLPQEG	VMFWTWDGDLKP	900			
rabbit	CLVWSDIALDVIQRLCLNGSTGOEVIINS	GLETVEALAEPLSQLLYWDA	GFKKEVANPDGDFRLTLVNSVLDPRALVLPQEG	VMFWTWDGDLKP	899			
chicken	CLVWSDIALDVIQRLCLNGSTGOEVIINS	GLETVEALAEPLSQLLYWDA	GFKKEVANPDGDFRLTLVNSVLDPRALVLPQEG	VMFWTWDGDLKP	807			
human	GIYSNMDGSAAYLVSEDKWPNGISVDDOWIYWDAYLDCIERIT	TFSSGO	ORSVILDLRPHYPATAVFKNEIYDDWSQSLIFRASKYSGSOMEILANO		1000			
rabbit	GIYSNMDGSAAYLVSEDKWPNGISVDDOWIYWDAYLDCIERIT	TFSSGO	ORSVILDLRPHYPATAVFKNEIYDDWSQSLIFRASKYSGSOMEILANO		999			
chicken	GIYSNMDGSAAYLVSEDKWPNGISVDDOWIYWDAYLDCIERIT	TFSSGO	ORSVILDLRPHYPATAVFKNEIYDDWSQSLIFRASKYSGSOMEILANO		907			
human	LTGLMDMKIFYKGNKTSNACVPRPCSLCLCPKANNRSCRCEP	EVSSSVLPSGDLMDCCP	GVOLKNNTCVKEENTCLRNQYRCSNGNCINSIWWCDF		1100			
rabbit	LTGLMDMKIFYKGNKTSNACVPRPCSLCLCPKANNRSCRCEP	EVSSSVLPSGDLMDCCP	GVOLKNNTCVKEENTCLRNQYRCSNGNCINSIWWCDF		1099			
chicken	LTGLMDMKIFYKGNKTSNACVPRPCSLCLCPKANNRSCRCEP	EVSSSVLPSGDLMDCCP	GVOLKNNTCVKEENTCLRNQYRCSNGNCINSIWWCDF		1007			
human	NDCGDMDEKNCPTTICDLDTFRCE	SGTCIPLSYKCDLEDDCGNSDES	HECEMHCQRSDYNCSSGMC	IRSSWVCDGNDNCRDWSDEANCAIYHTC	1199			
rabbit	NDCGDMDEKNCPTTICDLDTFRCE	SGTCIPLSYKCDLEDDCGNSDES	HECEMHCQRSDYNCSSGMC	IRSSWVCDGNDNCRDWSDEANCAIYHTC	1198			
chicken	NDCGDMDEKNCPTTICDLDTFRCE	SGTCIPLSYKCDLEDDCGNSDES	HECEMHCQRSDYNCSSGMC	IRSSWVCDGNDNCRDWSDEANCAIYHTC	1106			
human	EASNFQCRNGHCIPQWACDGD	ADCDGSDSEDT	CEKKCNCRGFCNPGTCIP	SSKHCDGLD	DCSDGSDDEHCE	PLCTR	FMDFVCKNRQOCLFHSVMVCDG	1298
rabbit	EASNFQCRNGHCIPQWACDGD	ADCDGSDSEDT	CEKKCNCRGFCNPGTCIP	SSKHCDGLD	DCSDGSDDEHCE	PLCTR	FMDFVCKNRQOCLFHSVMVCDG	1297
chicken	EASNFQCRNGHCIPQWACDGD	ADCDGSDSEDT	CEKKCNCRGFCNPGTCIP	SSKHCDGLD	DCSDGSDDEHCE	PLCTR	FMDFVCKNRQOCLFHSVMVCDG	1206
human	IIQCRDGSDEDAFAGCSQDPEFHVKVDEFGFCQ	QNGVCSILWKC	DMDCDGDYSD	DEANCPTEAPNCSRYFQFRCE	NGHCIPNWRKCDRENDCGDWS			1398
rabbit	IIQCRDGSDEDAFAGCSQDPEFHVKVDEFGFCQ	QNGVCSILWKC	DMDCDGDYSD	DEANCPTEAPNCSRYFQFRCE	NGHCIPNWRKCDRENDCGDWS			1397
chicken	IIQCRDGSDEDAFAGCSQDPEFHVKVDEFGFCQ	QNGVCSILWKC	DMDCDGDYSD	DEANCPTEAPNCSRYFQFRCE	NGHCIPNWRKCDRENDCGDWS			1306
human	DEKDCGSHLPLPSTPSTCLPNYRC	SGTFTVMDTWVCDGYRDCADGSD	DEACHTANVTASHT	PLQGRCDRFEFECHQPKC	IPNWRKCDGHODCO			1498
rabbit	DEKDCGSHLPLPSTPSTCLPNYRC	SGTFTVMDTWVCDGYRDCADGSD	DEACHTANVTASHT	PLQGRCDRFEFECHQPKC	IPNWRKCDGHODCO			1497
chicken	DEKDCGSHLPLPSTPSTCLPNYRC	SGTFTVMDTWVCDGYRDCADGSD	DEACHTANVTASHT	PLQGRCDRFEFECHQPKC	IPNWRKCDGHODCO			1406
human	DGRDEANCPHSTLTCSR	EFQCEDEGEACIVLSERCDGLDCSD	DESEKACSD	DELTVYKVNQLQWTADEFS	QNTLTWMPKPKMPSASCVNVVYRVVGS			1598
rabbit	DGRDEANCPHSTLTCSR	EFQCEDEGEACIVLSERCDGLDCSD	DESEKACSD	DELTVYKVNQLQWTADEFS	QNTLTWMPKPKMPSASCVNVVYRVVGS			1597
chicken	DGRDEANCPHSTLTCSR	EFQCEDEGEACIVLSERCDGLDCSD	DESEKACSD	DELTVYKVNQLQWTADEFS	QNTLTWMPKPKMPSASCVNVVYRVVGS			1505
human	IWKTLETHSNKTNVFLVKLPD	TTYQVKVQVQCLSKV	HNNTNDFVTLRTEP	GLEPDAF	PNQLSLHCE	EGVIVGHSPPH	HTHGLIREYIVEYRSRGS	1698
rabbit	IWKTLETHSNKTNVFLVKLPD	TTYQVKVQVQCLSKV	HNNTNDFVTLRTEP	GLEPDAF	PNQLSLHCE	EGVIVGHSPPH	HTHGLIREYIVEYRSRGS	1697
chicken	IWKTLETHSNKTNVFLVKLPD	TTYQVKVQVQCLSKV	HNNTNDFVTLRTEP	GLEPDAF	PNQLSLHCE	EGVIVGHSPPH	HTHGLIREYIVEYRSRGS	1592
human	ASDRAASNFTEIKNLLVNTLYTVRAAVTSRGIGNWS	DSKSITTVK	GKALPPEN	HIIDNMDENS	LSFTLTV	DGNIKVNGYVNVLF	WAFDTHKQEK	1798
rabbit	ASDRAASNFTEIKNLLVNTLYTVRAAVTSRGIGNWS	DSKSITTVK	GKALPPEN	HIIDNMDENS	LSFTLTV	DGNIKVNGYVNVLF	WAFDTHKQEK	1797
chicken	ASDRAASNFTEIKNLLVNTLYTVRAAVTSRGIGNWS	DSKSITTVK	GKALPPEN	HIIDNMDENS	LSFTLTV	DGNIKVNGYVNVLF	WAFDTHKQEK	1592
human	RSSILSHKVGNTLTA	HTVEYISAWAKTDLGDSPT	AFHVMT	TRGVRPPAPSLK	AINQTA	VECTWTGPRNVVYGIFYATS	FLDLYRNP	1898
rabbit	RSSILSHKVGNTLTA	HTVEYISAWAKTDLGDSPT	AFHVMT	TRGVRPPAPSLK	AINQTA	VECTWTGPRNVVYGIFYATS	FLDLYRNP	1897
chicken	RSSILSHKVGNTLTA	HTVEYISAWAKTDLGDSPT	AFHVMT	TRGVRPPAPSLK	AINQTA	VECTWTGPRNVVYGIFYATS	FLDLYRNP	1592
human	VSKDEQYLFLVRVVM	PYQGPSSDYVVVKMIPDSRLPPRHLM	VHTGKTS	V	VIKWESPYDSPDQDL	FAIAVKDLIRKTRDSYKVKSRNSTVEYITL	KLEP	1998
rabbit	VSKDEQYLFLVRVVM	PYQGPSSDYVVVKMIPDSRLPPRHLM	VHTGKTS	V	VIKWESPYDSPDQDL	FAIAVKDLIRKTRDSYKVKSRNSTVEYITL	KLEP	1997
chicken	VSKDEQYLFLVRVVM	PYQGPSSDYVVVKMIPDSRLPPRHLM	VHTGKTS	V	VIKWESPYDSPDQDL	FAIAVKDLIRKTRDSYKVKSRNSTVEYITL	KLEP	1592
human	GGKYHILVOLGNMSKD	SIKITTVSL	SAPDALKIITENDHVLFWKSLAKEN	H	FNESRGYEIHMFD	SAMNITAYLGN	TDFNFKISNLKMGHNYTFTVQ	2098
rabbit	GGKYHILVOLGNMSKD	SIKITTVSL	SAPDALKIITENDHVLFWKSLAKEN	H	FNESRGYEIHMFD	SAMNITAYLGN	TDFNFKISNLKMGHNYTFTVQ	2097
chicken	GGKYHILVOLGNMSKD	SIKITTVSL	SAPDALKIITENDHVLFWKSLAKEN	H	FNESRGYEIHMFD	SAMNITAYLGN	TDFNFKISNLKMGHNYTFTVQ	1592
human	ARCLFQDICEGEP	ALLYLDELGSG	ASASAMCAARST	DVAAVVVPILFLILL	SLGVG	FAILYTKHRLQSSFTAFANS	SHYSSRLGSAIFSSGDDL	2198
rabbit	ARCLFQDICEGEP	ALLYLDELGSG	ASASAMCAARST	DVAAVVVPILFLILL	SLGVG	FAILYTKHRLQSSFTAFANS	SHYSSRLGSAIFSSGDDL	2197
chicken	ARCLFQDICEGEP	ALLYLDELGSG	ASASAMCAARST	DVAAVVVPILFLILL	SLGVG	FAILYTKHRLQSSFTAFANS	SHYSSRLGSAIFSSGDDL	1592
human	AFMITGSDSDVPMVIA							2214
rabbit	AFMITGSDSDVPMVIA							2213
chicken	AFMITGSDSDVPMVIA							1592

Fig. 2. Comparison of amino acid sequences of human, rabbit and chicken LR11s. The amino acids are numbered from the N-terminus of the human and rabbit LR11 and from the first identified amino acid of chicken LR11, respectively. Gaps (-) have been introduced to optimize the alignment. Identical residues in the three proteins are boxed.

sor homology region; domain IV, a cysteine-rich segment containing 11 copies of the sequence similar to the LDL-receptor ligand binding repeats; domain V, 6 motifs related to the fibronectin type (FN) III repeat; domain VI, a putative membrane spanning region; and domain VII, at the C-terminus 54 amino acids predicted to be a cytoplasmic region.

Domain I is, to our knowledge, the only region of LR11

without significant similarities to other proteins, yet highly conserved in the regions known from all three LR11s (over 80% identical residues) (Figs. 2 and 4).

Domain II shows high homology to a yeast gene, VPS10. The VPS10 has been reported to be a yeast sorting receptor for vacuolar carboxypeptidase Y (23) and a type I transmembrane protein with a large luminal domain containing two homologous repeats of approximately 650

Fig. 3. Schematic diagram of the proposed multidomain structure of the LR11 protein. Seven domains are defined, characterized by: domain I, no particular similarity to other proteins; domain II, homology to a yeast receptor for vacuolar protein sorting, VPS10; domain III, five repeats with F/YWTD consensus sequences found in the EGF-precursor; domain IV, 11 tandemly arranged complement-type ligand binding repeats highly homologous to those constituting the ligand binding domain of the LDLR; domain V, 6 copies of FNIII repeats; domain VI, a putative membrane spanning region; and domain VII, the cytoplasmic domain. The signal sequence (SS) and the transmembrane domain (TM) are indicated by black boxes.

Fig. 4. Amino acid conservation of man, rabbit and chicken LR11s. Seven functional domains (I to VII) in the rabbit and human LR11s are shown. The extents of homology (% identity) between the domains of rabbit and human LR11s, and of chicken and human LR11s are indicated. Open boxes represent the known portions of domains I and V of the chicken protein.

amino acids each. The LR11 protein has a single repeat in this domain.

Domain III has the five repeats with characteristic tetrapeptides, F/YWTD. This region, in all three species, is the most highly conserved feature of LR11.

Domain IV is characterized by the 11 tandemly arranged 6-cysteine repeats with remarkable homology to the LDLR ligand binding domain. The LDLR has seven repeats in the binding domain and recognizes apoB and apoE as receptor ligands (2). Other family members have more binding repeats compared to the LDLR: the VLDLR (5, 8; YI (7), 13; LRP (8), 31; the *C. elegans* LRP-like protein (9), 35; and gp 330/megalin (10), 36. Recent studies on the ligand specificities for these receptors showed a wide range of ligands bind to most family members *in vitro* in addition to lipoproteins (17, 24–26).

The different ligand specificities are most likely to be caused by the different repeated numbers or combinations of the complement-type domains consisting of ~40 amino acid residues. The eleven binding repeats exist as a cluster in LRP (8), the *C. elegans* LRP-like protein (9), and gp 330/megalin (10) as well as LR11. These clusters of eleven repeats might be derived from the same ancestral gene because of the popular use in the LDLR gene family members.

Domain V, the ~600 amino acids stretch following the LDLR binding domain homology region is composed of six putative FNIII repeats. The FNIII repeats are shared with several adhesion molecules and a variety of other proteins (27). Part of domain V is most homologous to the FNIII repeat regions in neural cell adhesion molecules.

Domain VI, the putative membrane spanning region is followed by the carboxy terminal domain VII, which are presumed to be a putative cytoplasmic region. In the short cytoplasmic tail, the sequence Phe-Ala-Asn-Ser-His-Tyr (FANSHY) is similar to the internalization signal of coated pit receptors (3, 28); there are also positively and negatively charged residues near the transmembrane and the C-terminus, respectively, equivalent to those in the similarly short cytoplasmic domains in LDLRs and VLDLRs. Although further studies are needed to determine the role of the consensus motif in LR11, the characteristic signature sequence, together with clusters of charged residues in the cytoplasmic domain also found in other LDLR gene family members, strongly suggest that the LR11 protein is an endocytosis-competent receptor.

Genetic and structural elements defining the LDLR gene family are widespread. Family members identified so far are products of genes that are entirely made up of elements found in the LDLR itself. It is believed that the wide variety of ligands that interact with LDLR family members do so via these complement-type repeats; information on the functional contribution of additional protein domains may be obtained from genes in which LDLR- and non-LDLR-elements are present in novel combinations. We have identified the product of exactly such a gene in man, LR11, which is a striking mosaic protein containing several structural elements so far not found in LDLR gene family members. The elements that put LR11 in the LDLR gene family member, domains III and IV, are located in the center of the molecule, domains VI and VII, are located at the carboxy terminal region of the molecule (Fig. 3).

Chromosomal localization and tissue-specific expression of LR11

The human LR11 gene locus was mapped to chromosome 11q23.3–24 by fluorescence *in situ* hybridization (22). The human gene loci of some LDLR gene family members have been identified (Table 1). Each LDLR gene family member is located on the different chromo-

Table 1

Receptor	Locus	Reference
LDL receptor	19p13.1-13.3	29
VLDL receptor	9p24	30
LRP	12q13-14	31
LRP2 (gp330)	2q24-31	32
LR11	11q23.3-24	22

some (29-32). The LR11 gene is also on different chromosome from those other members located on.

The rabbit LR11 transcripts are abundantly expressed in brain, liver and adrenal glands by Northern blot analysis. Furthermore, the LR11 transcripts are also present in pancreas, testis, small intestine, lung and thymus, and barely detected in other tissues, such as skeletal muscle, heart, spleen and kidney. In man, the transcripts are mainly observed in brain, liver, kidney, and pancreas (21, 22). LR11 is expressed in brain of all three species; particularly in man, brain is the predominant site of expression.

Immunoblot analysis with anti-LR11 monoclonal antibodies revealed that approximately 250 kDa proteins were present in rabbit brain and liver extracts. These monoclonal antibodies showed no crossreactivity with any other protein in these tissues. Detailed immunoblot analysis of LR11 expression in rabbit brain, using extracts from different portions of brain, showed that the LR11 protein was present predominantly in the cerebrum, but also in the cerebellum and brain stem. Moreover, immunohistochemical analysis to determine the localization of LR11 in rabbit cerebrum revealed the presence of LR11 mainly in the hippocampal CA regions and dentate gyrus, with low level signals in the thalamus, as well as cerebral cortex (21).

Functional analysis of LR11

ApoE-rich lipoprotein, β -migrating VLDL (β -VLDL) is known to be a ligand to all members of the LDLR gene family reported to date (2, 5, 33-36). Therefore, we examined the binding of β -VLDL to the LR11-transfected cells by ligand blot analysis (21). A clear signal was obtained in the same position (~250 kDa) as that revealed by immunoblot analysis. These results showed that the LR11 has retained the common binding property of the LDLR gene family.

The expression of the LDLR is tightly regulated by cholesterol (2). To determine the effects of cholesterol on LR11 expression, we analyzed the transcript levels in the liver of a genetically hypercholesterolemic animal, WHHL rabbit (37), by Northern blot analysis. The LR11 expression in the liver of WHHL rabbits was similar to that of normal rabbits (21), clearly indicating that LR11 expression is not affected by cholesterol levels *in vivo*.

The expression of the LDLR and VLDLR have been

known to be stimulated by estrogen (38). We next tested the effects of estrogen on the expression of the LR11 mRNA. For this aim, a chicken estrogen receptor-transfected hepatic cell line, LMH/2A cells were used because of the well responsibility to estrogen *in vitro* (39). PCR amplification indicated that the amount of LR11 transcripts are not changed after the incubation with estrogen. In addition, the LR11 mRNA levels were not affected by the addition of fetal bovine serum (22). These results are rather different from the LDLR, suggesting the LR11 is possibly playing roles in some other function in addition to lipoprotein metabolism.

The most characteristic structural module of the LDLR gene family, LDLR ligand binding repeats are also found in a wide variety of proteins whose functions seem to be unrelated to lipoprotein metabolism. Among these are several proteins of the blood complement system (40, 41); a basement membrane heparan sulfate proteoglycan, perlecan (42, 43); a rat apical early endosomal glycoprotein (44); a cortical granule protein in sea urchin (45); a linker chain of earthworm hemoglobin (46); a G-protein coupled receptor in the ganglion of *Lymnaea* (47); and a chicken rous sarcoma virus receptor (48). Thus, the complement-type repeats in the LDLR are shared by a number of proteins that participate in diverse biological processes. For instance, the occurrence of complement-type repeats together with EGF precursor homology domain(s) in some extracellular matrix proteins, such as perlecan, suggests that certain family members might be involved in cell growth and cellular attachment.

Conclusions

Receptors belonging to the LDLR gene family are playing key roles for wide varied species in cellular endocytosis of a broad spectrum of ligands including spent, biologically inactive and/or unwanted plasmatic carrier complexes, certain toxins, yolk precursors, as well as circulating plasma lipoproteins. Recently, Kounnas *et al.* reported that LRP binds, in addition to many other possible ligands, β -amyloid precursor protein and mediates its degradation *in vitro* (49). At present, we have no idea which ligand(s) may be the true physiological partner(s) of LR11. The localization of rabbit LR11 in the hippocampus, dentate gyrus and cerebral cortex suggests functional significance of LR11 in metabolically active regions of brain. For instance, glutamate receptors, thought to play key roles in active brain functions such as learning (50), are highly expressed in the same brain structures (51).

Here we described a novel member of the LDLR gene family discovered in man, rabbit, and chicken. The LR11, as well as the recently identified apoE receptor 2 (11) and LR8B (12) appear to define a new group of LDLR family members with possible brain-specific functions (52). The novel complex mosaic structure of LR11 sheds new

lights on the evolution and proposed multifunctionality of the LDLR gene family.

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