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(54) **LSR RECEPTOR, ITS ACTIVITY, ITS CLONING, AND ITS APPLICATIONS TO THE DIAGNOSIS, PREVENTION AND/OR TREATMENT OF OBESITY AND RELATED RISKS OR COMPLICATIONS**

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C07K 7/06	(2006.01)
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A61K 35/00	(2006.01)
C07K 16/28	(2006.01)
A61K 39/395	(2006.01)
A61P 3/04	(2006.01)
A61P 3/06	(2006.01)
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C12N 5/10	(2006.01)
C12N 1/00	(2006.01)
C12Q 1/02	(2006.01)
C12P 21/02	(2006.01)

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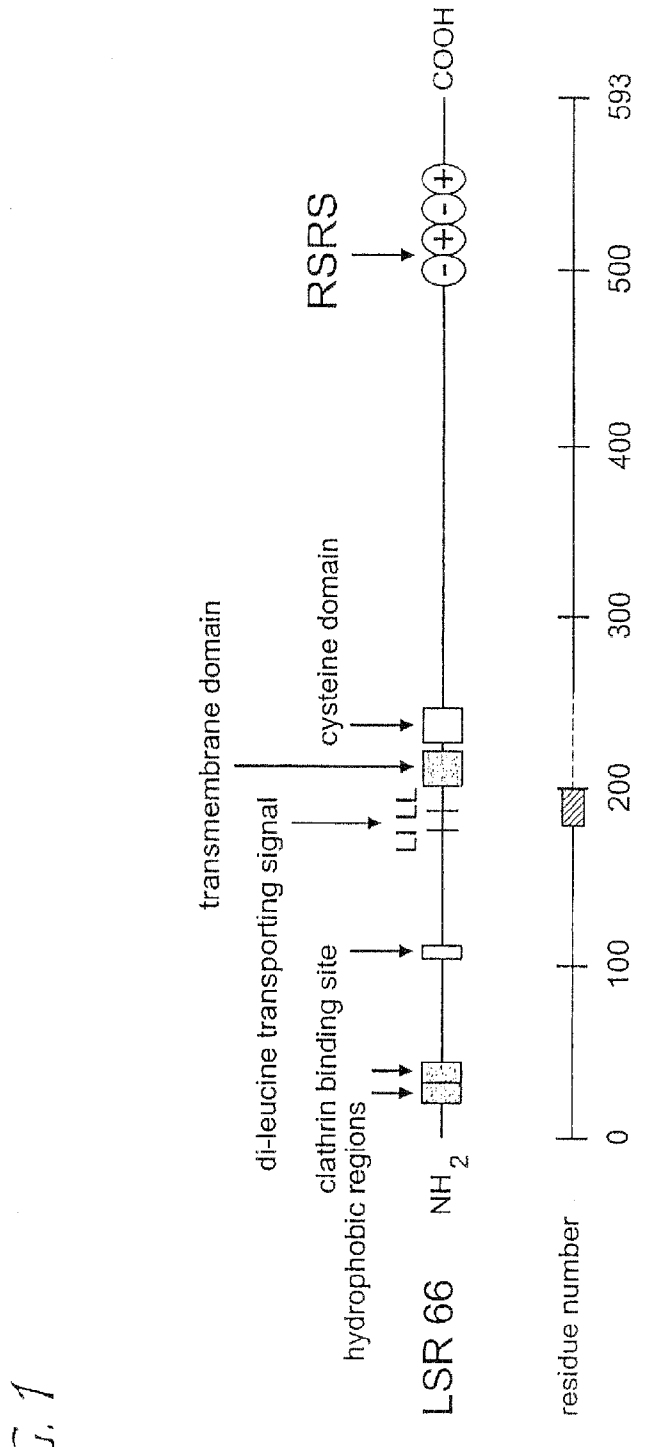
(57) **ABSTRACT**

The present invention relates to a new complex receptor polypeptide LSR (Lipolysis Stimulated Receptor), characterized by its functional activities, the cloning of the cDNAs complementary to the messenger RNAs encoding each of the subunits of the multimeric complex, vectors and transformed cells, methods of diagnosis and of selection of compounds which can be used as medicament for the prevention and/or treatment of pathologies and/or of pathogeneses such as obesity and anorexia, hyperlipidemias, atherosclerosis, diabetes, hypertension, and more generally the various pathologies associated with abnormalities in the metabolism of cytokines.

FIG. 1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clathrin binding site
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cysteine domain
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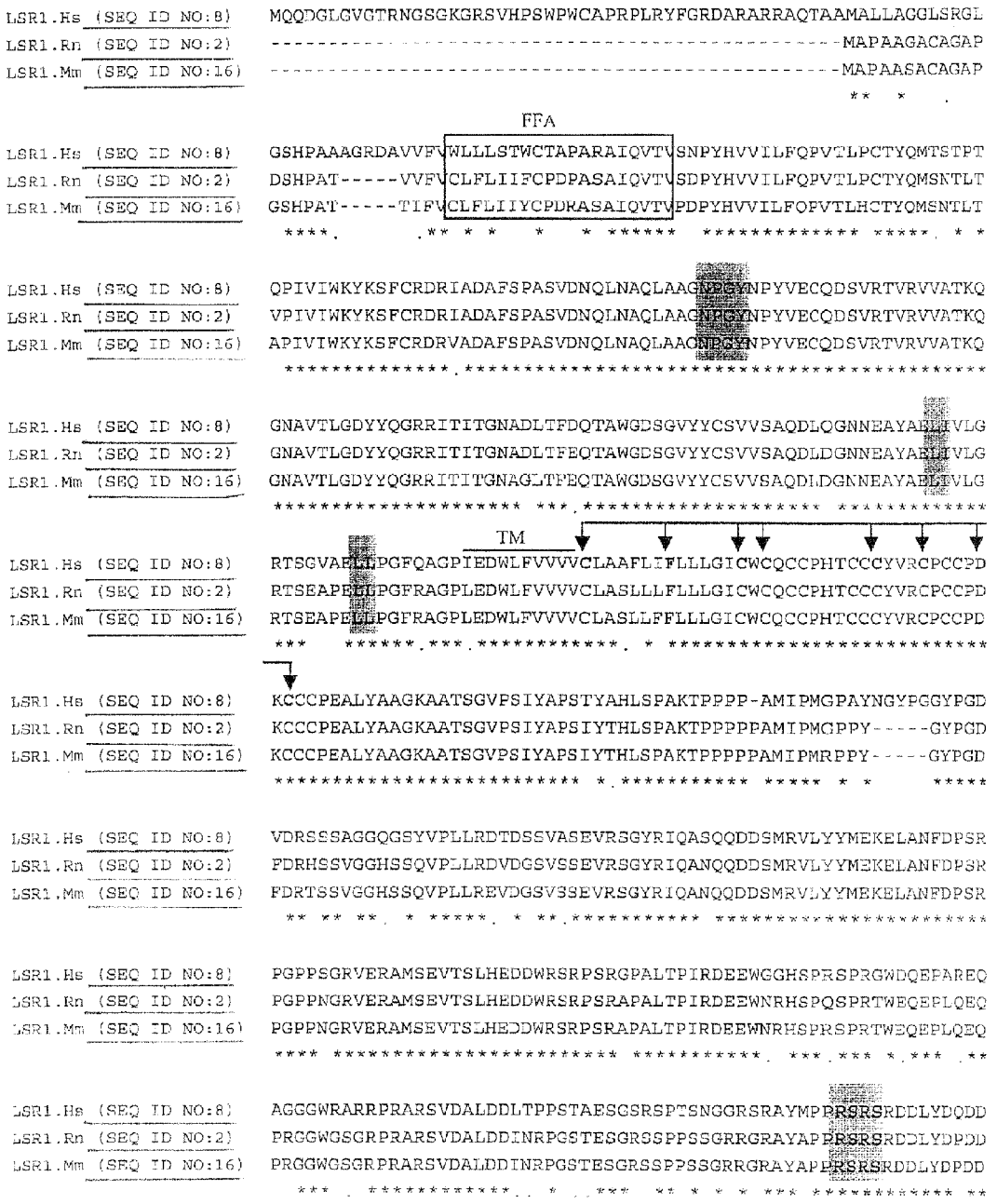


FIG. 2A

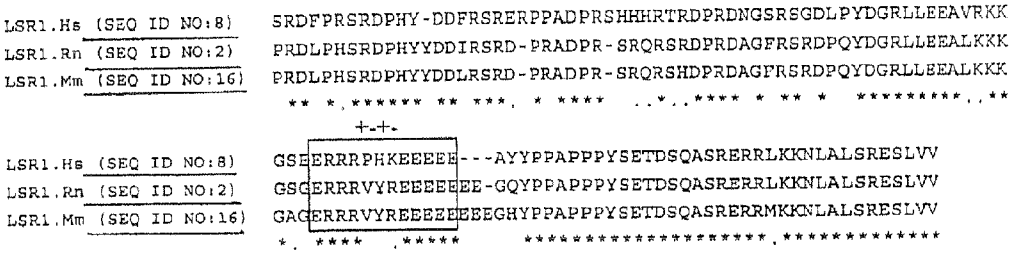

LSR1.Hs (SEQ ID NO:8) SRDFPRSRDPHY-DDFRSRERPPADPRSHHHRTRDPRDNGSRSGDLPYDGRLLEEAVRKK
LSR1.Rn (SEQ ID NO:2) PRDLPHSRDPHYYDDIRSRD-PRADPR-SRQRSRDPRDAGFRSRDPQYDGRLLEEALKKK
LSR1.Mm (SEQ ID NO:16) PRDLPHSRDPHYYDDLRSRD-PRADPR-SRQRSHDPRDAGFRSRDPQYDGRLLEEALKKK
+-+-
LSR1.Hs (SEQ ID NO:8) GSEERRRPHKEEEEE---AYYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR1.Rn (SEQ ID NO:2) GSGERRRVYREEEEEEE-GQYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR1.Mm (SEQ ID NO:16) GAGERRRVYREEEEEEEEGHYPPAPPPYSETDSQASRERRMKKNLALSRESLVV

FIG. 2B

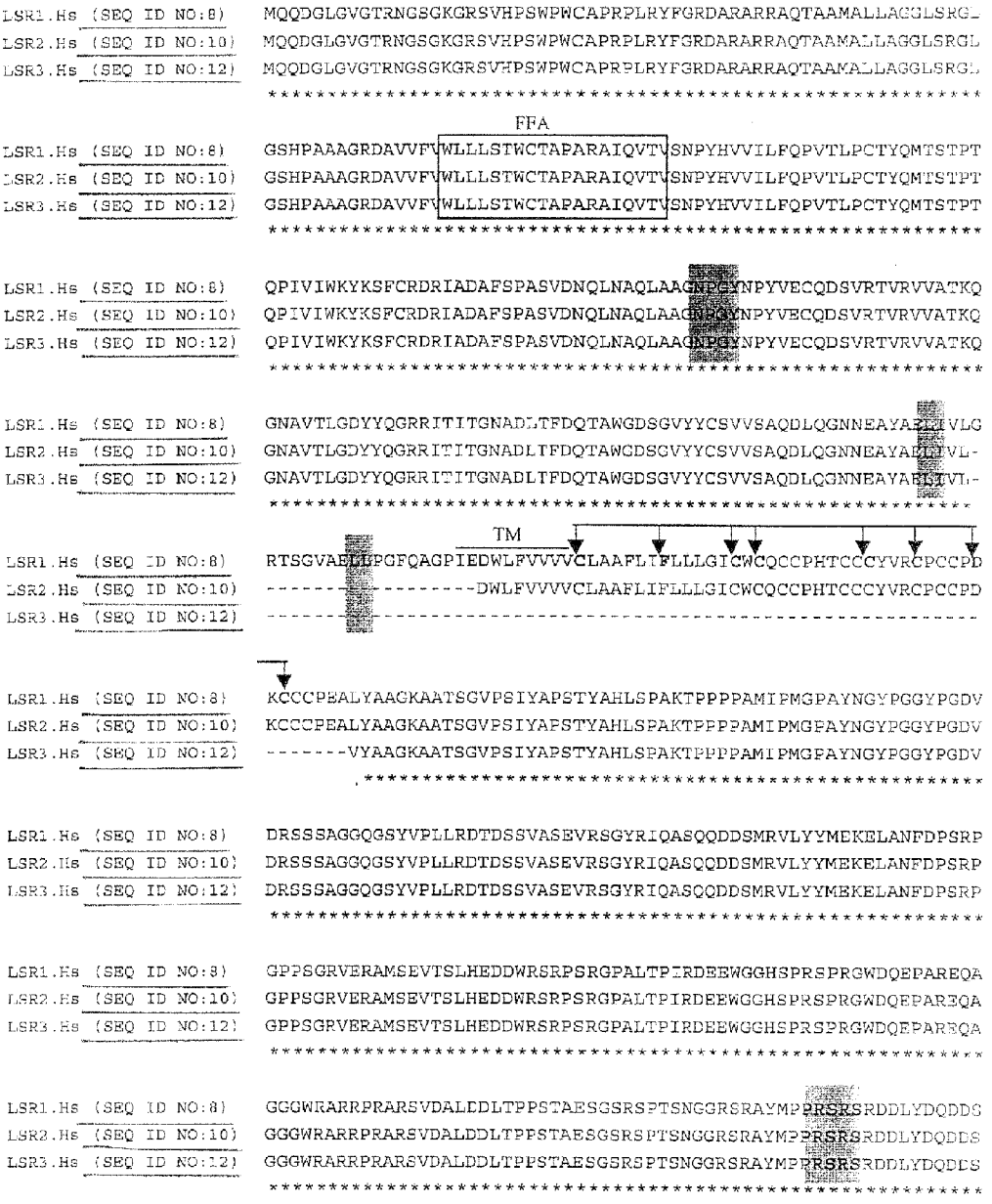

LSR1.Hs (SEQ ID NO:8) MQQDGLGVGTRNGSGKGRSVHPSWPWCAPRPLRYFGRDARARRAQTAAMALLAGGLSRGL
LSR2.Hs (SEQ ID NO:10) MQQDGLGVGTRNGSGKGRSVHPSWPWCAPRPLRYFGRDARARRAQTAAMALLAGGLSRGL
LSR3.Hs (SEQ ID NO:12) MQQDGLGVGTRNGSGKGRSVHPSWPWCAPRPLRYFGRDARARRAQTAAMALLAGGLSRGL
FFA
LSR1.Hs (SEQ ID NO:8) GSHPAAAGRDAVVFVWLLLSTWCTAPARAIQVTVSNPYHVVILFQPVTLPCTYQMTSTPT
LSR2.Hs (SEQ ID NO:10) GSHPAAAGRDAVVFVWLLLSTWCTAPARAIQVTVSNPYHVVILFQPVTLPCTYQMTSTPT
LSR3.Hs (SEQ ID NO:12) GSHPAAAGRDAVVFVWLLLSTWCTAPARAIQVTVSNPYHVVILFQPVTLPCTYQMTSTPT
LSR1.Hs (SEQ ID NO:8) QPIVIWKYKSFCRDRIADAFSPASVDNQLNAQLAAGNPGYNPYVECQDSVRTVRVVATKQ
LSR2.Hs (SEQ ID NO:10) QPIVIWKYKSFCRDRIADAFSPASVDNQLNAQLAAGNPGYNPYVECQDSVRTVRVVATKQ
LSR3.Hs (SEQ ID NO:12) QPIVIWKYKSFCRDRIADAFSPASVDNQLNAQLAAGNPGYNPYVECQDSVRTVRVVATKQ
LSR1.Hs (SEQ ID NO:8) GNAVTLGDYYQGRRITITGNADLTFDQTAWGDSGVYYCSVVSAQDLQGNNEAYAELIVLG
LSR2.Hs (SEQ ID NO:10) GNAVTLGDYYQGRRITITGNADLTFDQTAWGDSGVYYCSVVSAQDLQGNNEAYAELIVL-
LSR3.Hs (SEQ ID NO:12) GNAVTLGDYYQGRRITITGNADLTFDQTAWGDSGVYYCSVVSAQDLQGNNEAYAELIVL-
TM
LSR1.Hs (SEQ ID NO:8) RTSGVAELLPGFQAGPIEDWLFVVVVCLAAFLTFLLLGICWCQCCPHTCCCYVRCPCCPD
LSR2.Hs (SEQ ID NO:10) ----------DWLFVVVVCLAAFLIFLLLGICWCQCCPHTCCCYVRCPCCPD
LSR3.Hs (SEQ ID NO:12) ----------
LSR1.Hs (SEQ ID NO:8) KCCCPEALYAAGKAATSGVPSIYAPSTYAHLSPAKTPPPPAMIPMGPAYNGYPGGYPGDV
LSR2.Hs (SEQ ID NO:10) KCCCPEALYAAGKAATSGVPSIYAPSTYAHLSPAKTPPPPAMIPMGPAYNGYPGGYPGDV
LSR3.Hs (SEQ ID NO:12) -------VYAAGKAATSGVPSIYAPSTYAHLSPAKTPPPPAMIPMGPAYNGYPGGYPGDV
LSR1.Hs (SEQ ID NO:8) DRSSSAGGQGSYVPLLRDTDSSVASEVRSGYRIQASQQDDSMRVLYYMEKELANFDPSRP
LSR2.Hs (SEQ ID NO:10) DRSSSAGGQGSYVPLLRDTDSSVASEVRSGYRIQASQQDDSMRVLYYMEKELANFDPSRP
LSR3.Hs (SEQ ID NO:12) DRSSSAGGQGSYVPLLRDTDSSVASEVRSGYRIQASQQDDSMRVLYYMEKELANFDPSRP
LSR1.Hs (SEQ ID NO:8) GPPSGRVERAMSEVTSLHEDDWRSRPSRGPALTPIRDEEWGGHSPRSPRGWDQEPAREQA
LSR2.Hs (SEQ ID NO:10) GPPSGRVERAMSEVTSLHEDDWRSRPSRGPALTPIRDEEWGGHSPRSPRGWDQEPAREQA
LSR3.Hs (SEQ ID NO:12) GPPSGRVERAMSEVTSLHEDDWRSRPSRGPALTPIRDEEWGGHSPRSPRGWDQEPAREQA
LSR1.Hs (SEQ ID NO:8) GGGWRARRPRARSVDALDDLTPPSTAESGSRSPTSNGGRSRAYMPPRSRSRDDLYDQDDS
LSR2.Hs (SEQ ID NO:10) GGGWRARRPRARSVDALDDLTPPSTAESGSRSPTSNGGRSRAYMPPRSRSRDDLYDQDDS
LSR3.Hs (SEQ ID NO:12) GGGWRARRPRARSVDALDDLTPPSTAESGSRSPTSNGGRSRAYMPPRSRSRDDLYDQDDS

FIG. 3A

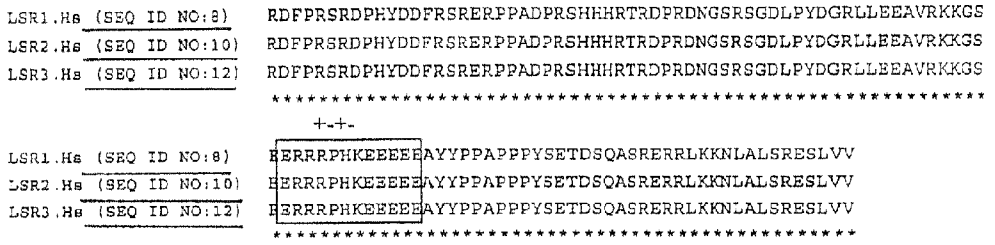

LSR1.Hs (SEQ ID NO:8) RDFPRSRDPHYDDFRSRERPPADPRSHHHRTRDPRDNGSRSGDLPYDGRLLEEAVRKKGS
LSR2.Hs (SEQ ID NO:10) RDFPRSRDPHYDDFRSRERPPADPRSHHHRTRDPRDNGSRSGDLPYDGRLLEEAVRKKGS
LSR3.Hs (SEQ ID NO:12) RDFPRSRDPHYDDFRSRERPPADPRSHHHRTRDPRDNGSRSGDLPYDGRLLEEAVRKKGS
+-+-
LSR1.Hs (SEQ ID NO:8) HERRRPHKEEEEEAYYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR2.Hs (SEQ ID NO:10) HERRRPHKEEEEEAYYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR3.Hs (SEQ ID NO:12) HERRRPHKEEEEEAYYPPAPPPYSETDSQASRERRLKKNLALSRESLVV

FIG. 3B

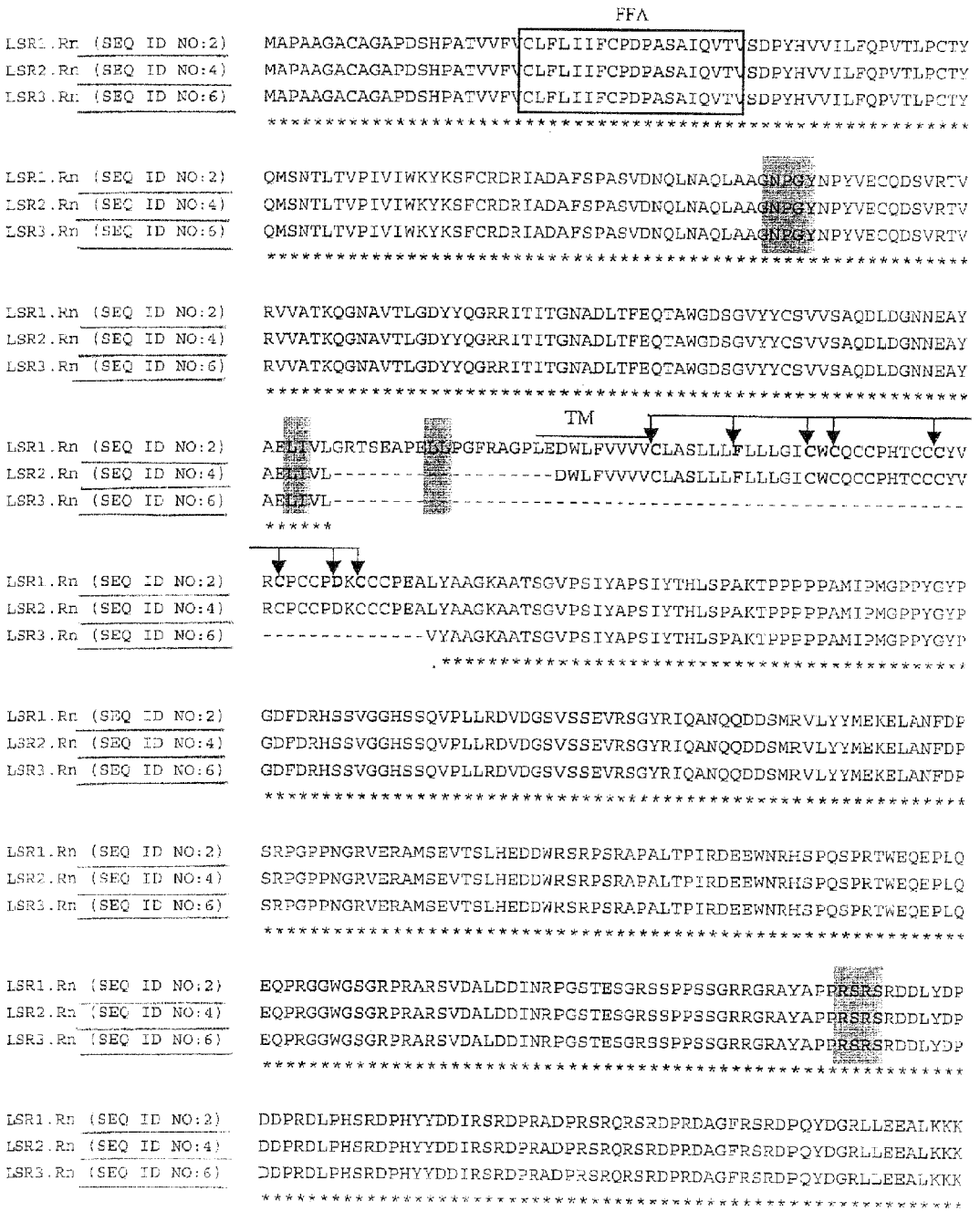


FIG. 4A

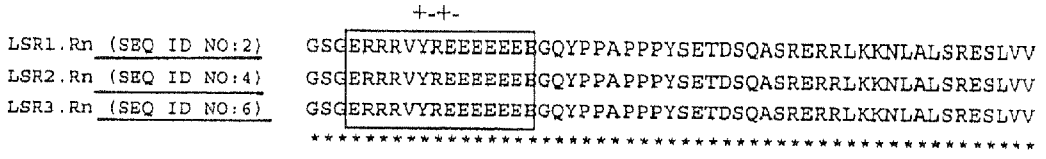

+-+-
LSR1.Rn (SEQ ID NO:2) GSGERRRVYREEEEEEEGQYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR2.Rn (SEQ ID NO:4) GSGERRRVYREEEEEEEGQYPPAPPPYSETDSQASRERRLKKNLALSRESLVV
LSR3.Rn (SEQ ID NO:6) GSGERRRVYREEEEEEEGQYPPAPPPYSETDSQASRERRLKKNLALSRESLVV

FIG. 4B

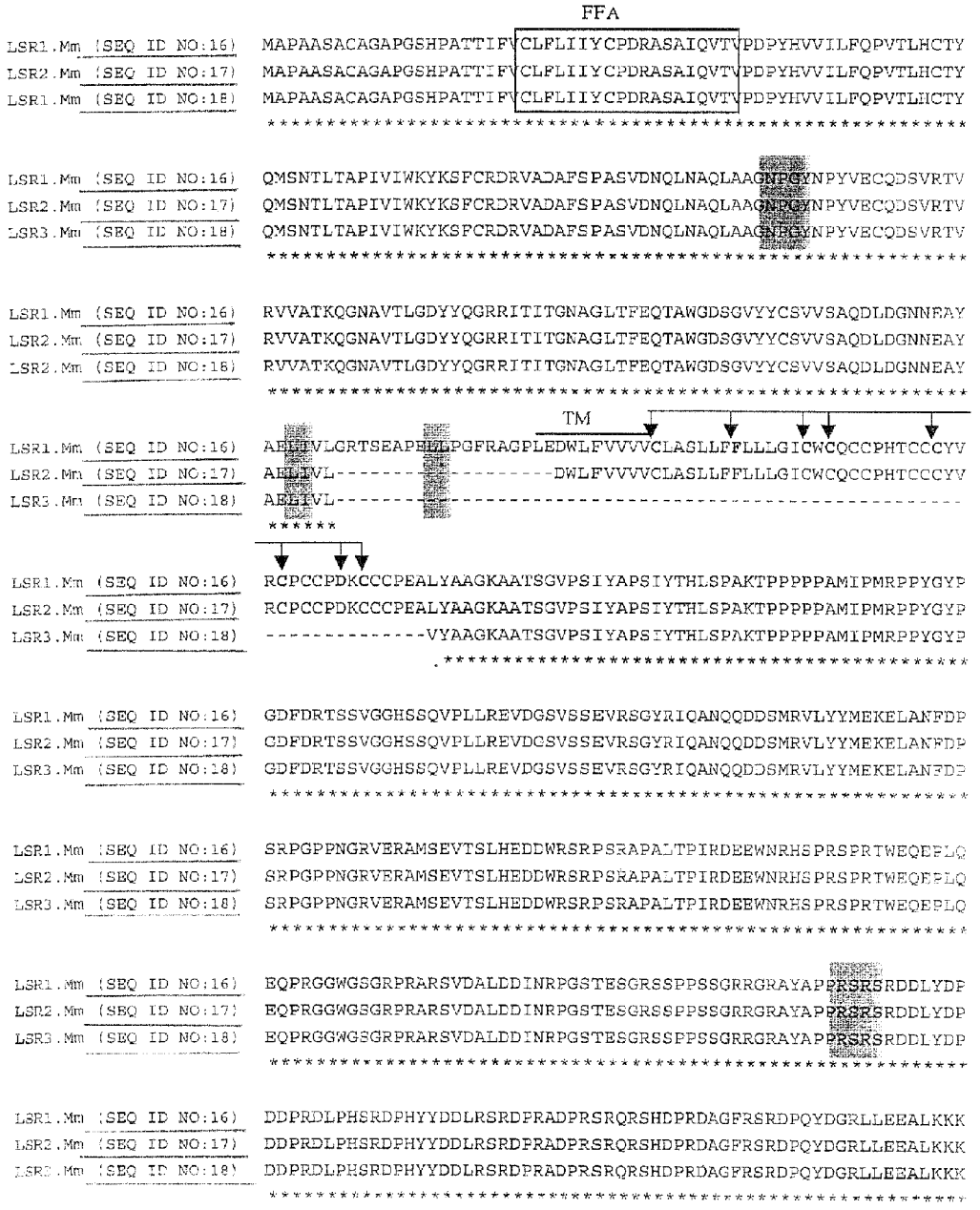

FFA
TM
LSR1.Mm (SEQ ID NO:16)
LSR2.Mm (SEQ ID NO:17)
LSR3.Mm (SEQ ID NO:18)

FIG. 5A

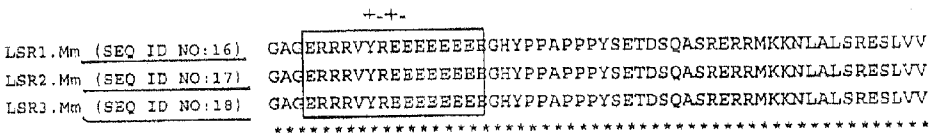

+-+-
LSR1.Mm (SEQ ID NO:16) GACERRRVYREEEEEEEEGHYPPAPPPYSETDSQASRERRMKKNLALSRESLVV
LSR2.Mm (SEQ ID NO:17) GACERRRVYREEEEEEEEGHYPPAPPPYSETDSQASRERRMKKNLALSRESLVV
LSR3.Mm (SEQ ID NO:18) GACERRRVYREEEEEEEEGHYPPAPPPYSETDSQASRERRMKKNLALSRESLVV

FIG. 5B

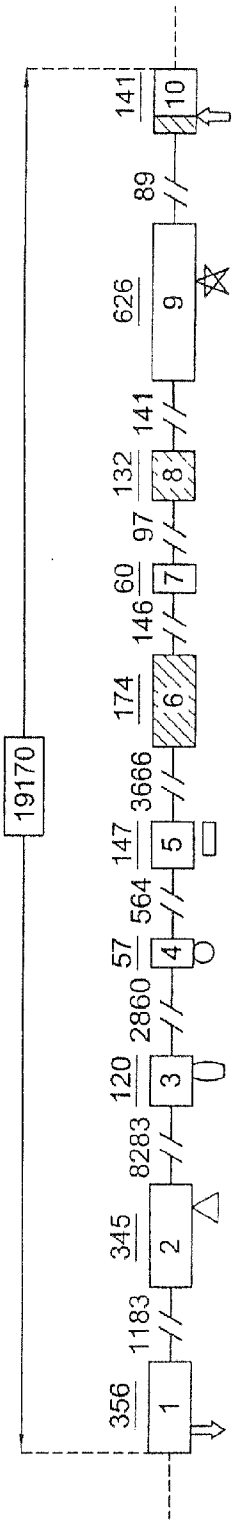
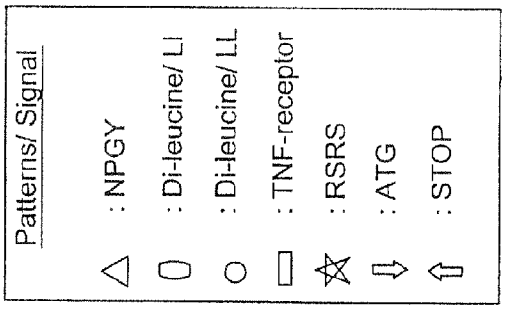
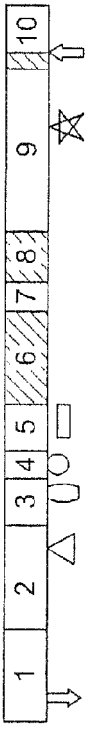


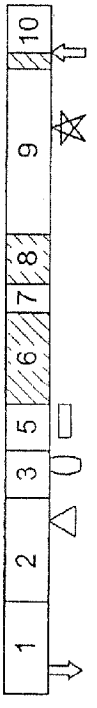
FIG. 6A





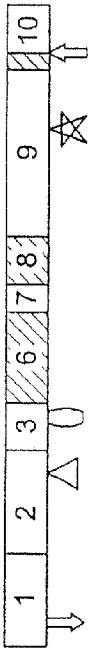
LSR_Hs_2062 (649 aa)

FIG. 6B



LSR_Hs_2005 (630 aa)

FIG. 6C



LSR_Hs_1858 (581 aa)

FIG. 6D

```
lsr1.Hs (SEQ ID NO:7)    TGGAGTGTGGCTCGGAGGACCGCGGCGGGTCAAGCACCTTTCTCCCCCATATCTGAAAGC
lsr1.Rn (SEQ ID NO:1)    ----------ACCGCTCACCAGGTCAGTTGTCCCCGGAAAGCCGAAGGC
lsr1.Mm (SEQ ID NO:13)   ------------------------------------------------------------

lsr1.Hs (SEQ ID NO:7)    ATGCCCTTTGTCCACGTCGTTTACGCTCATTAAAACT--TCCAGAATGCAACAGGACGGA
lsr1.Rn (SEQ ID NO:1)    ATGAGCTTCGCCCAAGTTCTTTTTATGGGTTAGAACTCCTCCAGAGCGGGGGAAAAAGGA
lsr1.Mm (SEQ ID NO:13)   ------------------------------------------------------------

lsr1.Hs (SEQ ID NO:7)    CTTGGAGTAGGGACAAGGAACGGAAGTGGGAAGGGGAGGAGCGTGCACCCCTCCTGGCCT
lsr1.Rn (SEQ ID NO:1)    CTTGGAATAGGGGC----------GGGACGGAGCACGCACCCTTCTCCGCCT
lsr1.Mm (SEQ ID NO:13)   ------------------------------------------------------------

lsr1.Hs (SEQ ID NO:7)    TGGTGCGCGCCGCGCCCCCTA------AGGTACTTTGGAAGGGACGCGCGGGC-CAGACG
lsr1.Rn (SEQ ID NO:1)    TGGTTCTCGCCGCGCCCCCTACTCTCGGGATACTTGGGAGGGGACGCGCGGGCACCGTCG
lsr1.Mm (SEQ ID NO:13)   ----------------------------------------------------GCACCGTCG
                                                                              ** * * **

lsr1.Hs (SEQ ID NO:7)    CGCCCAGACGGCCGCGATGGCGCTGTTGGCCGGCGGGCTCTCCAGAGGGCTGGGCTCCCA
lsr1.Rn (SEQ ID NO:1)    CTGCTAGACGGCCGCGATGGCGCCGGCGGGCCGGCGCGTGTGCTGGGGCGCCTGACTCCCA
lsr1.Mm (SEQ ID NO:13)   CTGCTAGACGGCCGCGATGGCGCCGGCGGCCAGCGCGTGTGCTGGGGCGCCTGGCTCCCA
                         *  * ******************* *  **** *** *    *  * * **  * ******

lsr1.Hs (SEQ ID NO:7)    CCCGGCCGCCGCAGGCCGGGACGCGGTCGTCTTCGTGTGGCTTCTGCTTAGCACCTGGTG
lsr1.Rn (SEQ ID NO:1)    CCCAGCTACCGTGG--------------TCTTCGTGTGTCTCTTTCTCATCATTTTCTG
lsr1.Mm (SEQ ID NO:13)   CCCGGCCACCACGA--------------TCTTCGTGTGTCTTTTTCTCATCATTTACTG
                         *** **  **                  ********** **  * ** * **  *  **

lsr1.Hs (SEQ ID NO:7)    CACAGCTCCTGCCAGGGCCATCCAGGTGACCGTGTCCAACCCCTACCACGTGGTGATCCT
lsr1.Rn (SEQ ID NO:1)    CCCAGACCCTGCCAGTGCCATCCAGGTGACTGTGTCTGACCCCTACCACGTAGTGATCCT
lsr1.Mm (SEQ ID NO:13)   CCCAGACCGTGCCAGTGCCATCCAGGTGACCGTGCCTGACCCCTACCACGTAGTGATCCT
                         * ***  * ****** *************** *** *  ************ ********

lsr1.Hs (SEQ ID NO:7)    CTTCCAGCCTGTGACCCTGCCCTGTACCTACCAGATGACCTCGACCCCCACGCAACCCAT
lsr1.Rn (SEQ ID NO:1)    GTTCCAGCCAGTGACCCTGCCCTGCACCTATCAGATGAGCAACACTCTCACAGTCCCCAT
lsr1.Mm (SEQ ID NO:13)   GTTCCAGCCAGTGACACTACACTGCACCTACCAGATGAGCAATACCCTCACAGCCCCTAT
                          ******** ***** ** * *** ***** ******* *   ** * ***    ** **

lsr1.Hs (SEQ ID NO:7)    CGTCATCTGGAAGTACAAGTCTTTCTGCCGGGACCGCATCGCCGATGCCTTCTCCCCGGC
lsr1.Rn (SEQ ID NO:1)    CGTGATCTGGAAGTACAAGTCATTCTGCCGGGACCGTATTGCCGATGCCTTCTCTCCTGC
lsr1.Mm (SEQ ID NO:13)   CGTGATCTGGAAGTATAAGTCGTTCTGTCGGGACCGTGTTGCCGACGCCTTCTCCCCTGC
                         *** *********** ***** ***** ********  * ***** ******** ** **
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FIG. 7A

```
lsr1.Hs (SEQ ID NO:7)    CAGCGTCGACAACCAGCTCAATGCCCAGCTGGCAGCCGGGAACCCAGGCTACAACCCCTA
lsr1.Rn (SEQ ID NO:1)    CAGTGTGGACAACCAGCTAAATGCCCAGTTGGCAGCTGGCAACCCCGGCTACAACCCCTA
lsr1.Mm (SEQ ID NO:13)   CAGCGTGGACAACCAGCTCAACGCCCAGCTGGCGGCTGGCAACCCCGGCTACAACCCCTA
                         *** ** *********** ** ****** **** ** ** ***** **************

lsr1.Hs (SEQ ID NO:7)    CGTTGAGTGCCAGGACAGCGTGCGCACCGTCAGGGTCGTGGCCACCAAGCAGGGCAACGC
lsr1.Rn (SEQ ID NO:1)    TGTGGAGTGCCAGGACAGTGTACGCACTGTCAGGGTGGTGGCCACCAAACAGGGCAATGC
lsr1.Mm (SEQ ID NO:13)   TGTGGAGTGCCAGGACAGCGTACGCACTGTCAGGGTGGTGGCCACCAAACAGGGCAATGC
                          ** *************** ** ***** ******** ********** ******** **

lsr1.Hs (SEQ ID NO:7)    TGTGACCCTGGGAGATTACTACCAGGGCCGGAGGATTACCATCACCGGAAATGCTGACCT
lsr1.Rn (SEQ ID NO:1)    GGTGACCCTGGGAGACTACTACCAAGGCAGGAGGATCACCATAACAGGAAATGCTGACCT
lsr1.Mm (SEQ ID NO:13)   TGTGACCCTGGGAGACTACTACCAGGGCAGGAGAATCACCATCACAGGAAATGCTGGCCT
                          ************** ******** *** **** ** ***** ** ********** ***

lsr1.Hs (SEQ ID NO:7)    GACCTTTGACCAGACGGCGTGGGGGGACAGTGGTGTGTATTACTGCTCCGTGGTCTCAGC
lsr1.Rn (SEQ ID NO:1)    GACCTTCGAGCAGACAGCCTGGGGAGACAGTGGAGTGTATTACTGCTCTGTGGTCTCGGC
lsr1.Mm (SEQ ID NO:13)   GACCTTCGAGCAGACGGCCTGGGGAGACAGTGGAGTGTATTACTGCTCCGTGGTCTCAGC
                         ******* ** ***** ** ***** ******** ************** ******** **

lsr1.Hs (SEQ ID NO:7)    CCAGGACCTCCAGGGGAACAATGAGGCCTACGCAGAGCTCATCGTCCTTGGGAGGACCTC
lsr1.Rn (SEQ ID NO:1)    CCAAGATCTGGATGGAAACAACGAGGCGTACGCAGAGCTCATCGTCCTTGGCAGGACCTC
lsr1.Mm (SEQ ID NO:13)   CCAAGATCTGGATGGGAACAACGAGGCGTACGCAGAGCTCATTGTCCTTGGCAGGACCTC
                         *** ** **  * ** ***** ***** ************** ******** ********

lsr1.Hs (SEQ ID NO:7)    AGGGGTGGCTGAGCTCTTACCTGGTTTTCAGGCGGGGCCCATAGAAGACTGGCTCTTCGT
lsr1.Rn (SEQ ID NO:1)    AGAGGCCCCTGAGCTCCTACCTGGTTTTCGGGCGGGGCCCTTGGAAGATTGGCTCTTTGT
lsr1.Mm (SEQ ID NO:13)   AGAAGCCCCTGAGCTCCTACCTGGTTTTCGGGCGGGGCCCTTGGAAGATTGGCTCTTTGT
                         **  *   ******** ************ ********** * ***** ******** **

lsr1.Hs (SEQ ID NO:7)    GGTTGTGGTATGCCTGGCTGCCTTCCTCATCTTCCTCCTCCTGGGCATCTGCTGGTGCCA
lsr1.Rn (SEQ ID NO:1)    GGTCGTGGTCTGCCTGGCGAGCCTCCTCCTCTTCCTCCTCCTGGGCATCTGCTGGTGCCA
lsr1.Mm (SEQ ID NO:13)   GGTCGTGGTCTGCCTGGCAAGCCTCCTCTTCTTCCTCCTCCTGGGCATCTGCTGGTGCCA
                         *** ***** ********   * ***** **********************************

lsr1.Hs (SEQ ID NO:7)    GTGCTGCCCGCACACTTGCTGCTGCTACGTCAGGTGCCCCTGCTGCCCAGACAAGTGCTG
lsr1.Rn (SEQ ID NO:1)    GTGCTGTCCTCACACCTGCTGCTGCTATGTCCGATGTCCCTGCTGCCCAGACAAGTGCTG
lsr1.Mm (SEQ ID NO:13)   GTGCTGTCCCCACACCTGCTGCTGCTATGTCAGATGTCCCTGCTGCCCAGACAAGTGCTG
                         ****** ** ***** ************ *** * ** ************************

lsr1.Hs (SEQ ID NO:7)    CTGCCCCGAGGCCCTGTATGCCGCCGGCAAAGCAGCCACCTCAGGTGTTCCCAGCATTTA
lsr1.Rn (SEQ ID NO:1)    TTGCCCTGAGGCTCTTTATGCTGCTGGCAAAGCAGCCACCTCAGGTGTCCCGAGCATCTA
lsr1.Mm (SEQ ID NO:13)   TTGCCCTGAGGCCCTTTATGCTGCTGGCAAAGCAGCCACCTCAGGTGTGCCAAGCATCTA
                          ***** ***** ** ***** ** ************************ ** ***** **
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FIG. 7B

```
lsr1.Hs (SEQ ID NO:7)   TGCCCCCAGCACCTATGCCCACCTGTCTCCCGCCAAGACCCCACC---CCCACCAGCTAT
lsr1.Rn (SEQ ID NO:1)   TGCCCCCAGCATCTATACCCACCTCTCACCTGCCAAGACCCCACCACCTCCGCCTGCCAT
lsr1.Mm (SEQ ID NO:13)  TGCCCCCAGCATCTATACCCACCTCTCTCCTGCCAAGACTCCGCCACCTCCGCCTGCCAT
                        *********** **** ******* ** ** ******** ** **    ** ** ** **

lsr1.Hs (SEQ ID NO:7)   GATTCCCATGGGCCCTGCCTACAACGGGTACCCTGGAGGATACCCTGGAGACGTTGACAG
lsr1.Rn (SEQ ID NO:1)   GATTCCCATGGGCCCTCCCTAT--------------GGGTACCCTGGAGACTTTGACAG
lsr1.Mm (SEQ ID NO:13)  GATTCCCATGCGTCCTCCCTAT--------------GGGTACCCTGGAGACTTTGACAG
                        ********** * *** ****              ** ************ *******

lsr1.Hs (SEQ ID NO:7)   GAGTAGCTCAGCTGGTGGCCAAGGCTCCTATGTACCCCTGCTTCGGGACACGGACAGCAG
lsr1.Rn (SEQ ID NO:1)   ACATAGCTCAGTTGGTGGCCACAGCTCCCAAGTACCCCTGCTGCGTGACGTGGATGGCAG
lsr1.Mm (SEQ ID NO:13)  GACCAGCTCAGTTGGTGGCCACAGCTCCCAGGTGCCCCTGCTGCGTGAAGTGGATGGGAG
                        ******* *********  ***** * ** ******** ** **   *** * **

lsr1.Hs (SEQ ID NO:7)   TGTGGCCTCTGAAGTCCGCAGTGGCTACAGGATTCAGGCCAGCCAGCAGGACGACTCCAT
lsr1.Rn (SEQ ID NO:1)   TGTATCTTCAGAAGTACGAAGTGGCTACAGGATCCAGGCTAACCAGCAAGATGACTCCAT
lsr1.Mm (SEQ ID NO:13)  CGTATCTTCAGAAGTACGAAGTGGCTACAGGATCCAGGCTAACCAGCAAGATGACTCCAT
                        **  * ** ***** ** ************** ***** * ****** ** ********

lsr1.Hs (SEQ ID NO:7)   GCGGGTCCTGTACTACATGGAGAAGGAGCTGGCCAACTTCGACCCTTCTCGACCTGGCCC
lsr1.Rn (SEQ ID NO:1)   GAGGGTCCTATACTATATGGAGAAAGAGCTAGCCAACTTTGACCCTTCCCGACCTGGCCC
lsr1.Mm (SEQ ID NO:13)  GAGGGTCCTATACTATATGGAGAAGGAGCTAGCCAACTTCGATCCTTCCCGGCCTGGCCC
                        * ******** ***** ******** ***** ******** ** ***** ** ********

lsr1.Hs (SEQ ID NO:7)   CCCCAGTGGCCGTGTGGAGCGGGCCATGAGTGAAGTCACCTCCCTCCACGAGGACGACTG
lsr1.Rn (SEQ ID NO:1)   TCCCAATGGCAGAGTGGAACGGGCCATGAGTGAAGTAACCTCCCTCCATGAAGATGACTG
lsr1.Mm (SEQ ID NO:13)  TCCCAATGGCCGAGTGGAACGGGCCATGAGTGAAGTAACCTCCCTCCATGAAGATGACTG
                        **** **** * ***** ****************** *********** ** ** *****

lsr1.Hs (SEQ ID NO:7)   GCGATCTCGGCCTTCCCGGGGCCCTGCCCTCACCCCGATCCGGGATGAGGAGTGGGGTGG
lsr1.Rn (SEQ ID NO:1)   GCGATCGAGGCCTTCCAGGGCTCCTGCCCTCACCCCCATCAGGGATGAGGAGTGGAATCG
lsr1.Mm (SEQ ID NO:13)  GCGATCTCGGCCTTCCAGGGCTCCTGCCCTCACACCCATCAGGGATGAGGAGTGGAATCG
                        ******  ******** ***  *********** ** *** ************** * *

lsr1.Hs (SEQ ID NO:7)   CCACTCCCCCCGGAGTCCCAGGGGATGGGACCAGGAGCCCGCCAGGGAGCAGGCAGGCGG
lsr1.Rn (SEQ ID NO:1)   CCACTCCCCACAGAGTCCCAGAACATGGGAGCAGGAACCCCTTCAAGAACAACCAAGGGG
lsr1.Mm (SEQ ID NO:13)  CCACTCCCCTCGGAGTCCCAGAACATGGGAGCAGGAACCCCTTCAAGAACAGCCAAGGGG
                        ********* * *********   ****** ***** ***      ** **  ** * **

lsr1.Hs (SEQ ID NO:7)   GGGCTGGCGGGCCAGGCGGCCCCGGGCCCGCTCCGTGGACGCCCTGGACGACCTCACCCC
lsr1.Rn (SEQ ID NO:1)   TGGTTGGGGGTCTGGACGCCCTCGGGCCCGCTCTGTGGATGCTCTAGATGATATCAACCG
lsr1.Mm (SEQ ID NO:13)  TGGTTGGGGGTCTGGGCGGCCTCGGGCCCGCTCTGTGGATGCTCTAGATGACATCAACCG
                        ** *** ** *  * ** ** ********** ***** ** ** ** **  *** **
```

FIG. 7C

```
lsr1.Hs (SEQ ID NO:7)    GCCGAGCACCGCCGAGTCAGGGAGCAGGTCTCCCACGAGTAATGGTGGGAGAAGCCGGGC
lsr1.Rn (SEQ ID NO:1)    GCCTGGCTCCACTGAATCAGGACGGTCTTCTCCCCCAAGTAGTGGACGGAGAGGACGGGC
lsr1.Mm (SEQ ID NO:13)   GCCTGGCTCCACTGAATCAGGAAGGTCTTCTCCCCCAAGTAGTGGACGGAGAGGGCGGGC
                         ***  ** ** * ** ***** *    ****** * **** ***  ***** * *****

lsr1.Hs (SEQ ID NO:7)    CTACATGCCCCCGCGGAGCCGCAGCCGGGACGACCTCTATGACCAAGACGACTCGAGGGA
lsr1.Rn (SEQ ID NO:1)    CTATGCACCTCCAAGAAGTCGCAGCCGGGATGACCTCTATGACCCGGACGATCCTAGGGA
lsr1.Mm (SEQ ID NO:13)   CTATGCACCTCCGAGAAGTCGCAGCCGGGATGACCTCTATGACCCCGACGATCCTAGAGA
                         ***    ** **  * ** ********** ********** *****  * ** **

lsr1.Hs (SEQ ID NO:7)    CTTCCCACGCTCCCGGGACCCCCACTAC---GACGACTTCAGGTCTCGGGAGCGCCCTCC
lsr1.Rn (SEQ ID NO:1)    CTTGCCACATTCCCGAGATCCCCACTATTATGACGACATCAGGTCTAGAGA---TCCACG
lsr1.Mm (SEQ ID NO:13)   CTTGCCACATTCCCGAGATCCCCACTATTATGATGATTTGAGGTCTAGGGA---TCCACG
                         *** ****  ***** ** ********    ** **  * ****** * **    ** *

lsr1.Hs (SEQ ID NO:7)    TGCCGACCCCAGGTCCCACCACCACCGTACCCGGGACCCTCGGGACAACGGCTCCAGGTC
lsr1.Rn (SEQ ID NO:1)    TGCTGACCCCAGATCCCGTCAGC---GATCCCGAGATCCTCGGGATGCTGGCTTCAGGTC
lsr1.Mm (SEQ ID NO:13)   TGCTGACCCCAGATCCCGTCAGC---GATCCCACGATCCTCGGGATGCTGGCTTCAGGTC
                         *** ******** ****  ** *   *  ***  ** ********    **** ******

lsr1.Hs (SEQ ID NO:7)    CGGGGACCTCCCCTATGATGGGCGGCTACTGGAGGAGGCTGTGAGGAAGAAGGGGTCGGA
lsr1.Rn (SEQ ID NO:1)    AAGGGACCCTCAGTATGATGGGCGACTATTAGAAGAGGCTTTAAAGAAAAAGGGGTCGGG
lsr1.Mm (SEQ ID NO:13)   ACGGGACCCTCAGTATGATGGGCGACTCTTAGAAGAGGCTTTAAAGAAAAAAGGGGCTGG
                           ******  *  ********** **  * ** ****** * * *** ** *** * *

lsr1.Hs (SEQ ID NO:7)    GGAGAGGAGGAGACCCCACAAGGAGGA---------GGAGGAAGAGGCCTACTACCCGCC
lsr1.Rn (SEQ ID NO:1)    CGAGAGAAGGAGGCTTTACAGGGAGGAAGAAGA---GGAAGAGGAGGGCCAATACCCCCC
lsr1.Mm (SEQ ID NO:13)   GGAGAGAAGACGCGTTTACAGGGAGGAAGAAGAAGAAGAAGAGGAGGGCCACTATCCCCC
                          ***** **  *     *** ******          ** ** **** * * ** ** **

lsr1.Hs (SEQ ID NO:7)    CGCGCCGCCCCCGTACTCGGAGACCGACTCGCAGGCGTCCCGAGAGCGCAGGCTCAAGAA
lsr1.Rn (SEQ ID NO:1)    AGCACCTCCACCTTACTCAGAGACTGACTCGCAGGCCTCACGGGAGAGGAGGCTGAAAAA
lsr1.Mm (SEQ ID NO:13)   AGCACCTCCGCCTTACTCTGAGACTGACTCGCAGGCCTCGAGGGAGCGGAGGATGAAAAA
                          ** ** ** ** ***** ***** ********** **  * *** * *** * ** **

lsr1.Hs (SEQ ID NO:7)    GAACTTGGCCCTGAGTCGGGAAAGTTTAGTCGTCTGATCTGACGTT: ------------
lsr1.Rn (SEQ ID NO:1)    GAATTTGGCCCTGAGTCGGGAAAGTTTAGTCGTCTGATCC-ACGTTTTGT-ATGTAGCTT
lsr1.Mm (SEQ ID NO:13)   GAATTTGGCCCTGAGTCGGGAAAGTTTAGTCGTCTGATCCCACGTTTTGTTATGTAGCTT
                         *** ************************************  *****

lsr1.Hs (SEQ ID NO:7)    ------------------------------------------------------------
lsr1.Rn (SEQ ID NO:1)    TTGTACTTTTTTTTTAATTGGAATCAATATTGATGAAACTTCAAGCCTAATAAAATGTCT
lsr1.Mm (SEQ ID NO:13)   TTATACTTTTTTAATTGGAATATTGATGA--AACTCTTCACCAAGCCTAATAAAA-----
```

FIG. 7D

```
lsr1.Hs (SEQ ID NO:7)    ------------------
lsr1.Rn (SEQ ID NO:1)    AATCACAAAAAAAAAAA
lsr1.Mm (SEQ ID NO:13)   ------------------
```

FIG. 7E

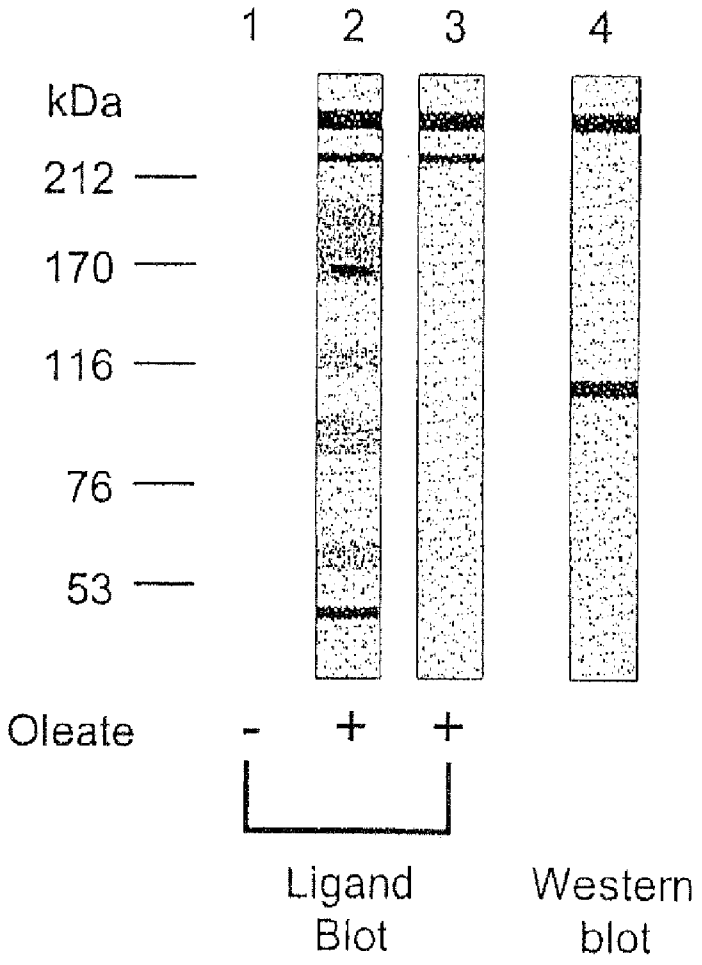


FIG. 8

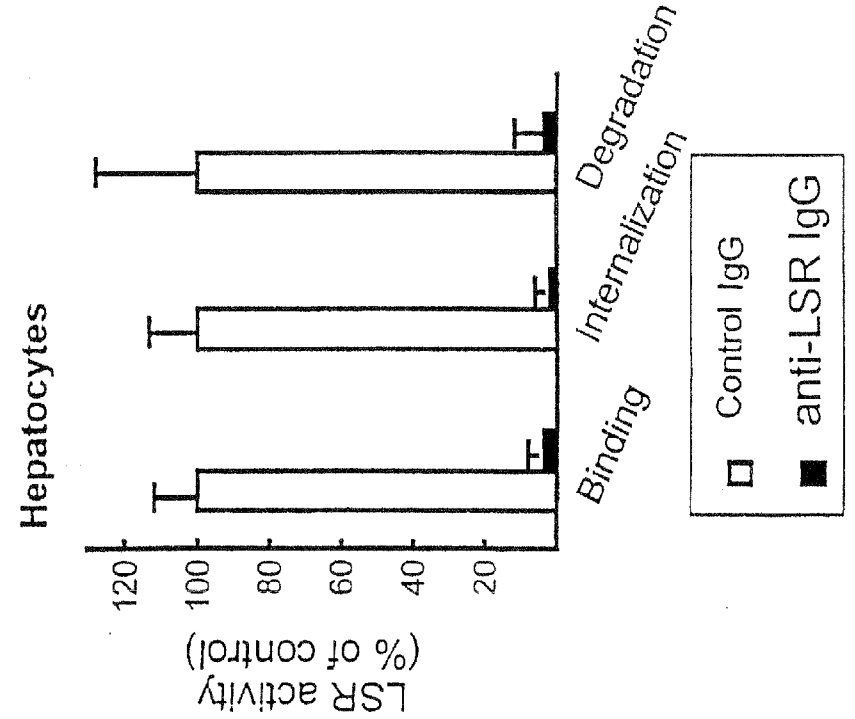

Hepatocytes
LSR activity
(% of control)
120
100
80
60
40
20
Binding
Internalization
Degradation
Control IgG
anti-LSR IgG

FIG. 9B

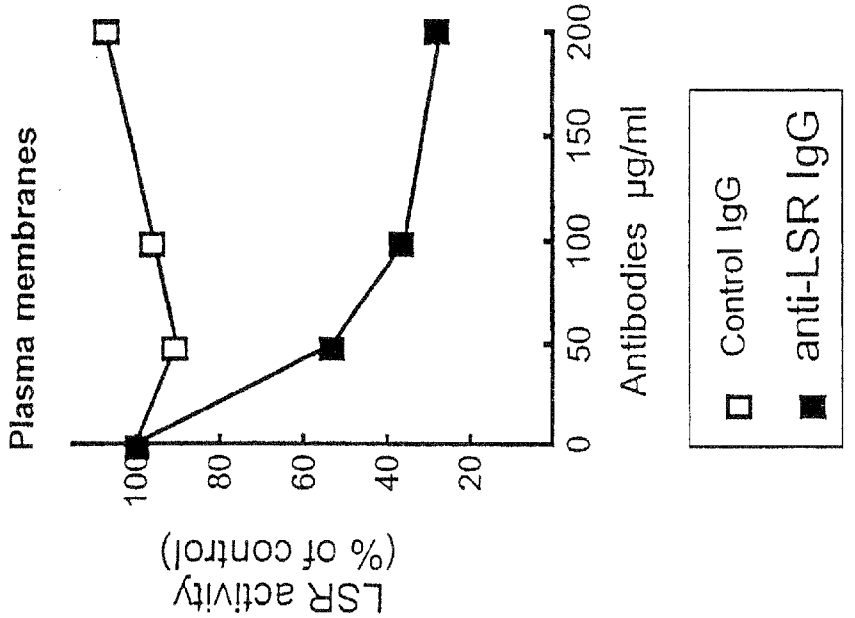

Plasma membranes
LSR activity
(% of control)
100
80
60
40
20
0
50
100
150
200
Antibodies µg/ml
Control IgG
anti-LSR IgG

FIG. 9A

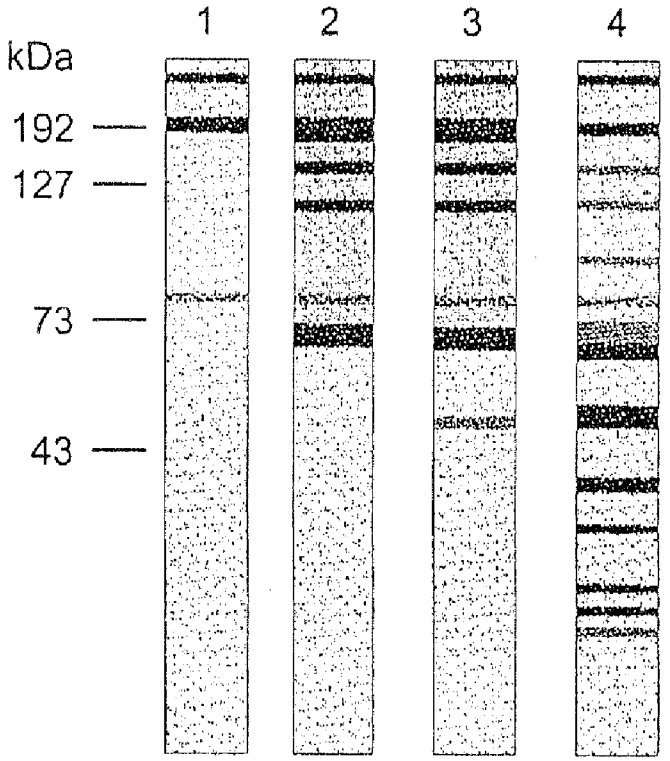


FIG. 10

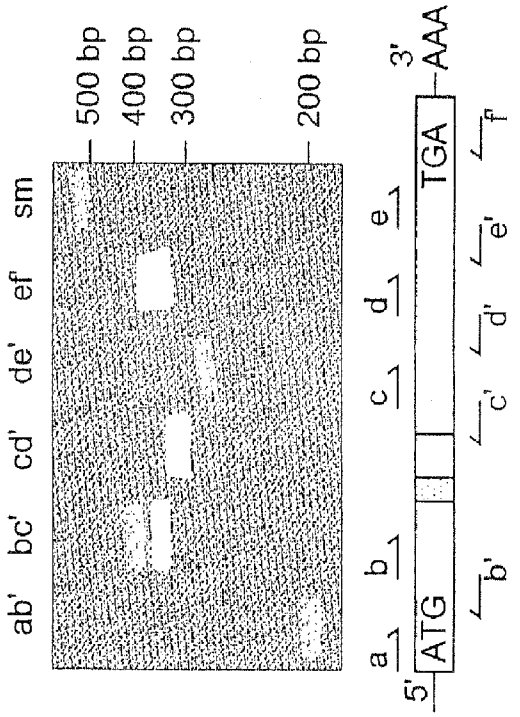


FIG. 11C

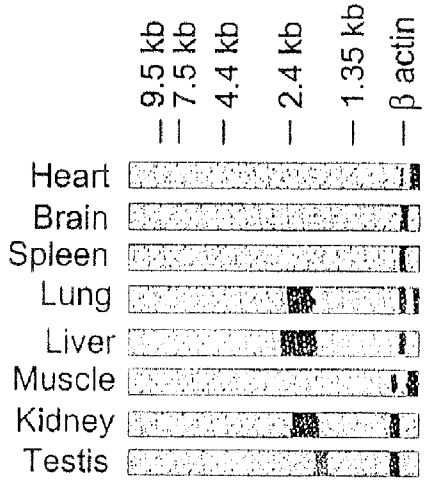


FIG. 11B

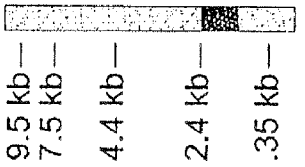


FIG. 11A

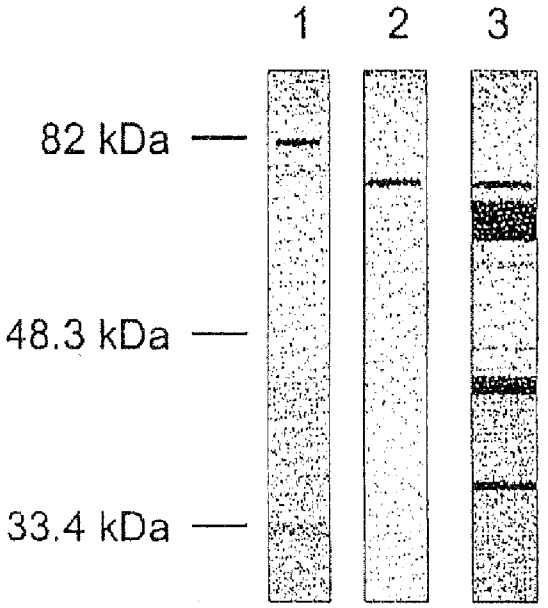


FIG. 12

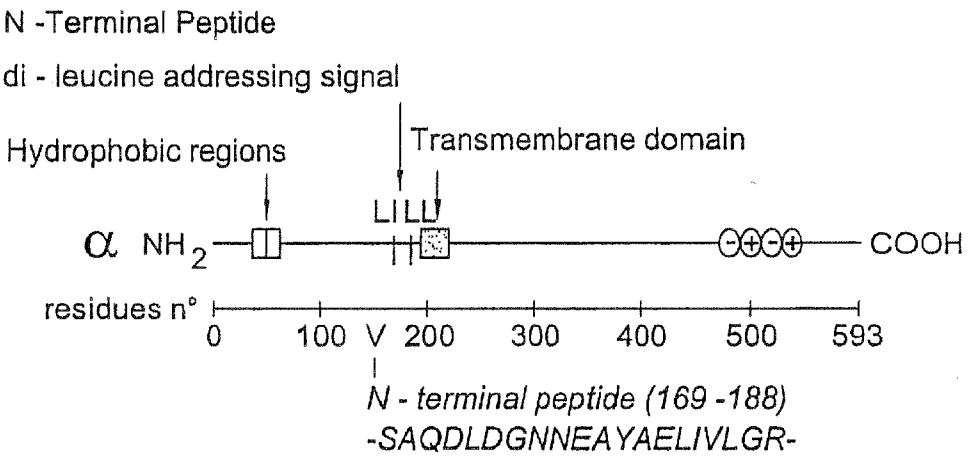


FIG. 13A

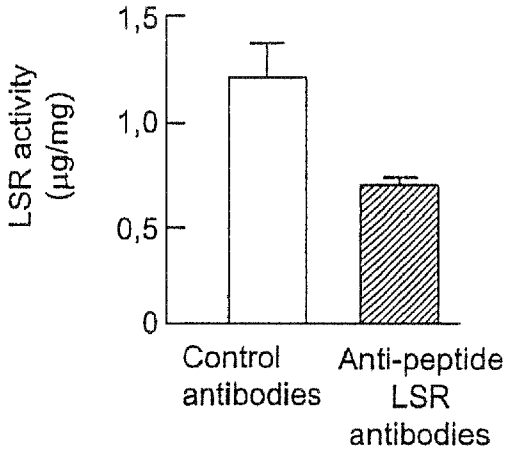


FIG. 13B

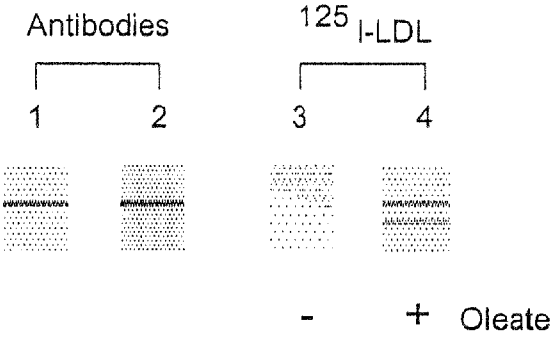


FIG. 13C

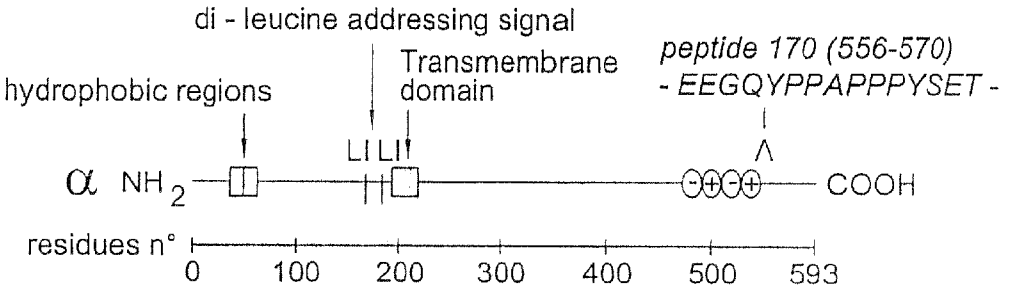


FIG. 14A Peptide 170

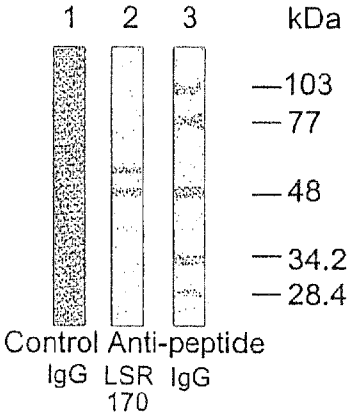


FIG. 14B Western

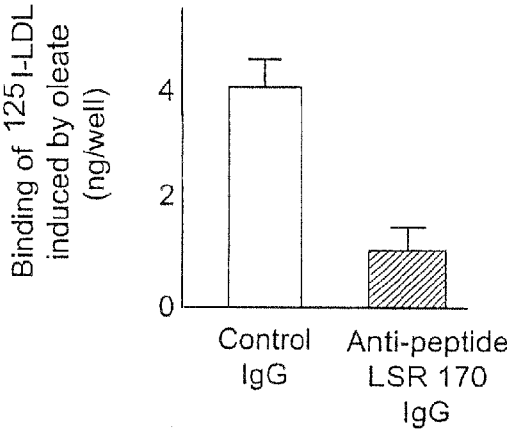


FIG. 14C Immunionhibition

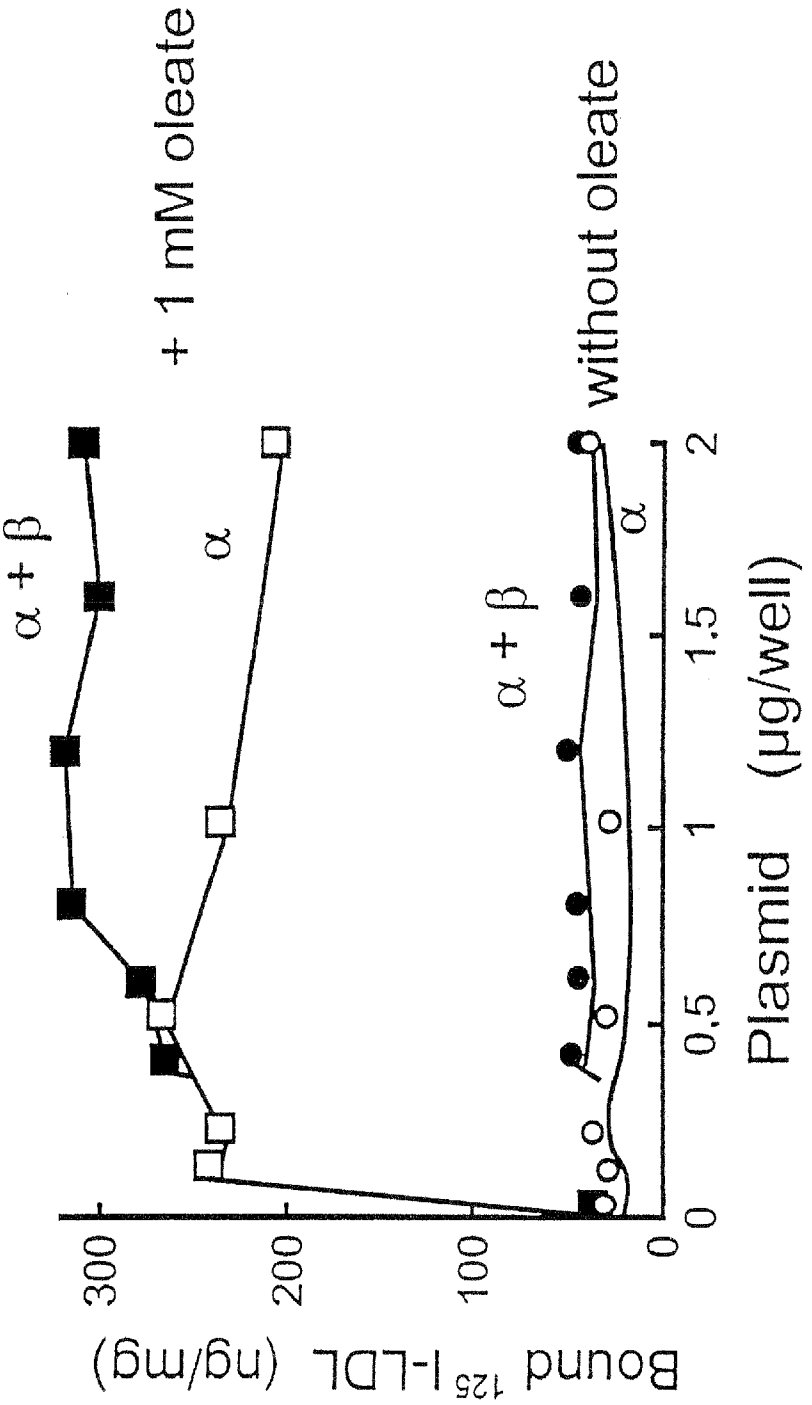

+ 1 mM oleate
without oleate
α + β
α
α + β
α
Bound 125I-LDL (ng/mg)
300
200
100
0
0
0,5
1
1,5
2
Plasmid (µg/well)

FIG. 15

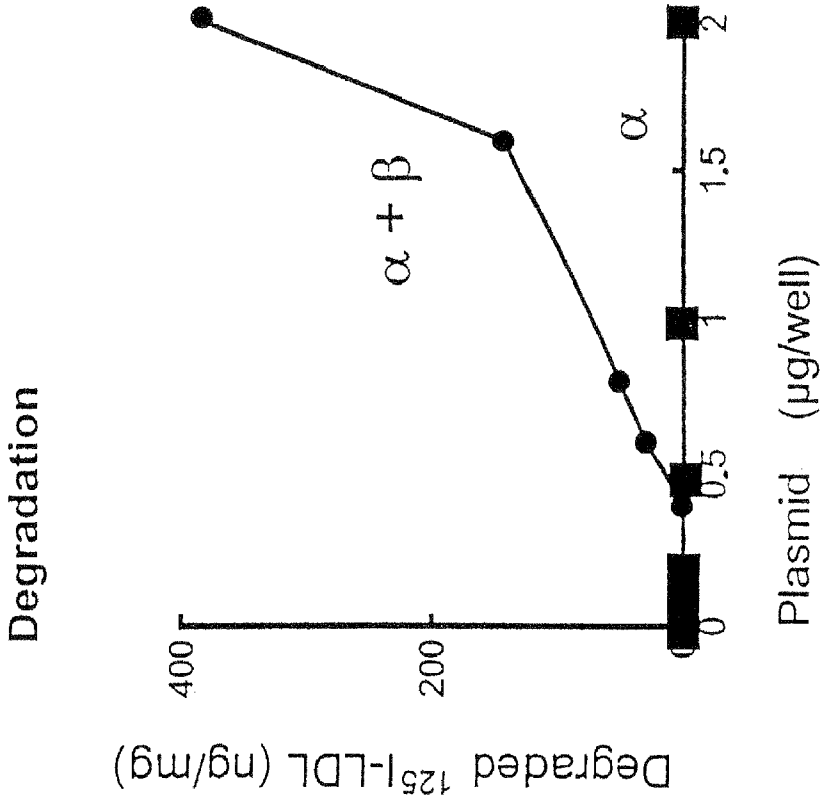


FIG. 16B

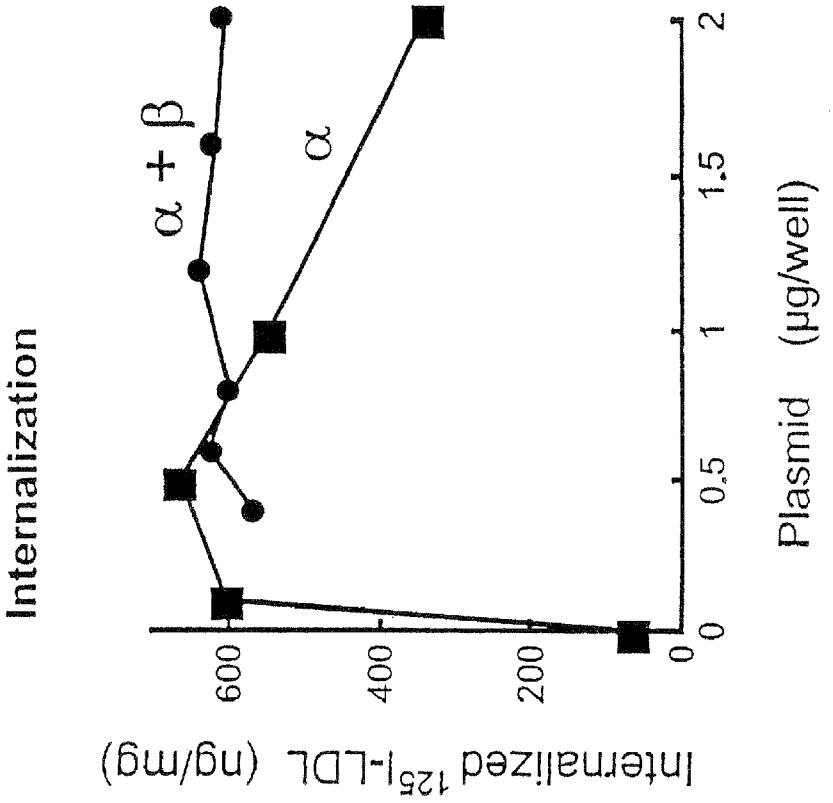


FIG. 16A

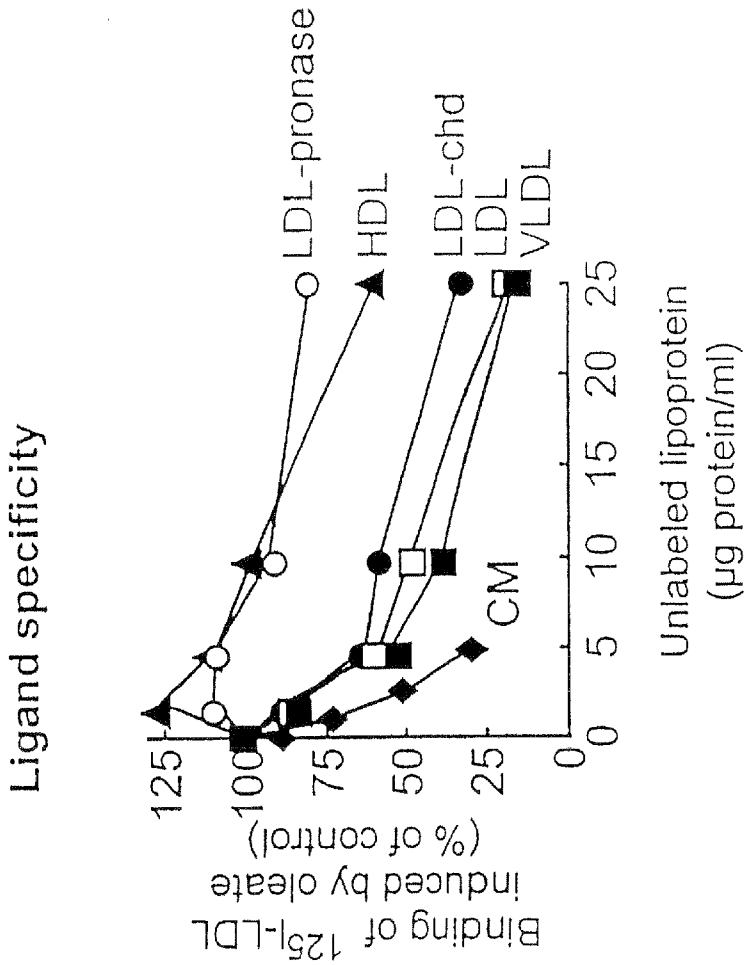


FIG. 17B

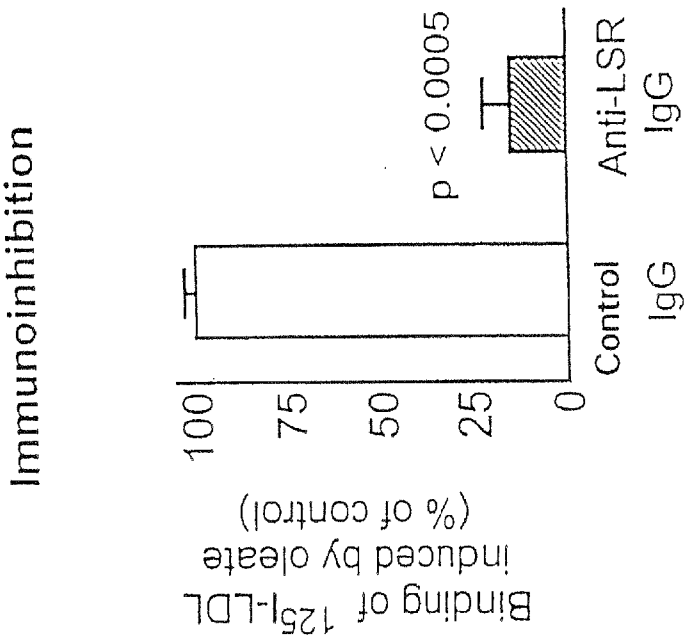
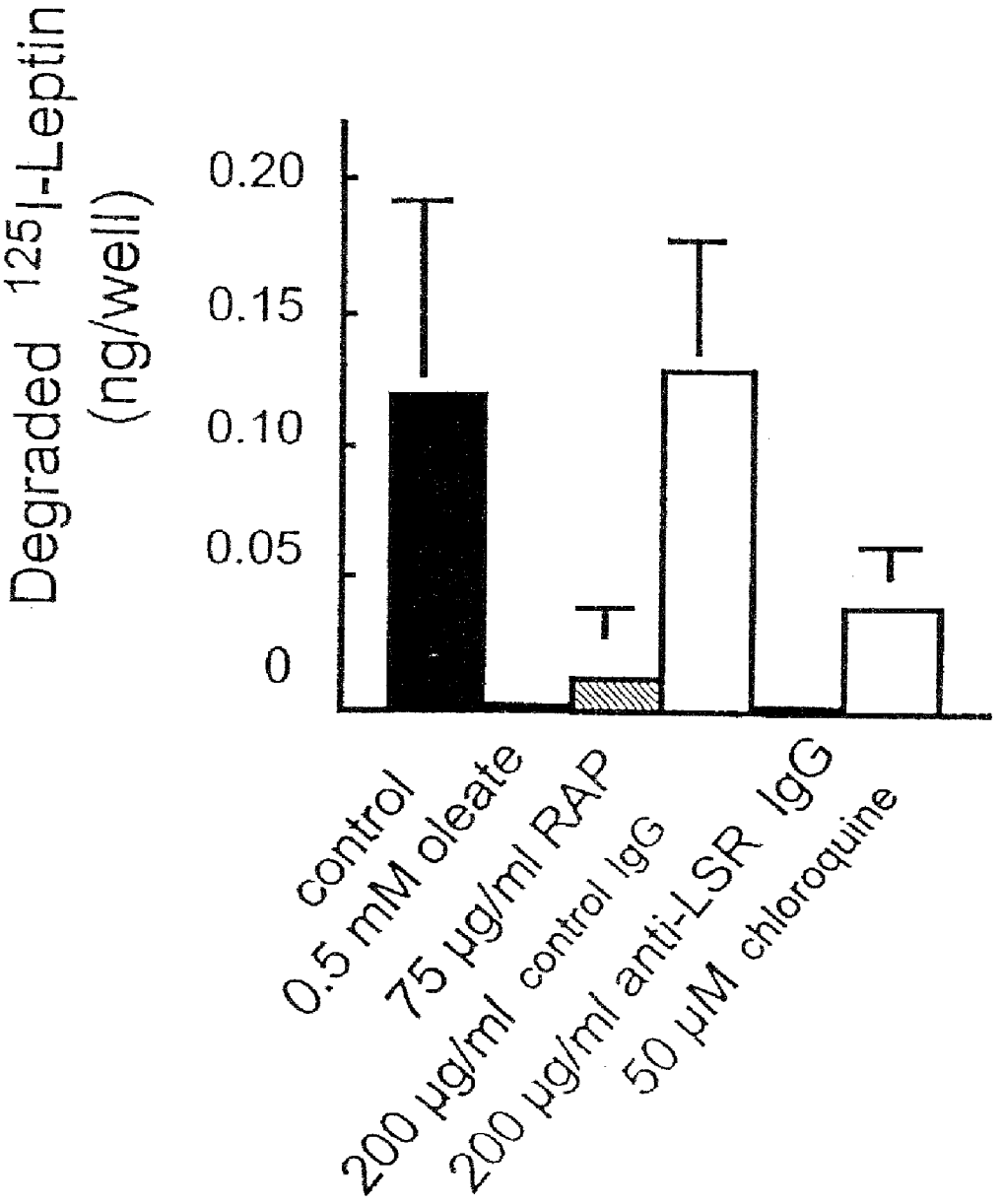


FIG. 17A

FIG. 18


Degraded ^{125}I-Leptin (ng/well)
0.20
0.15
0.10
0.05
0
control
0.5 mM oleate
75 µg/ml RAP
200 µg/ml control IgG
200 µg/ml anti-LSR IgG
50 µM chloroquine

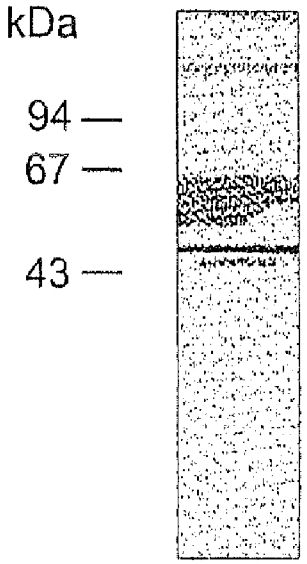


FIG. 19

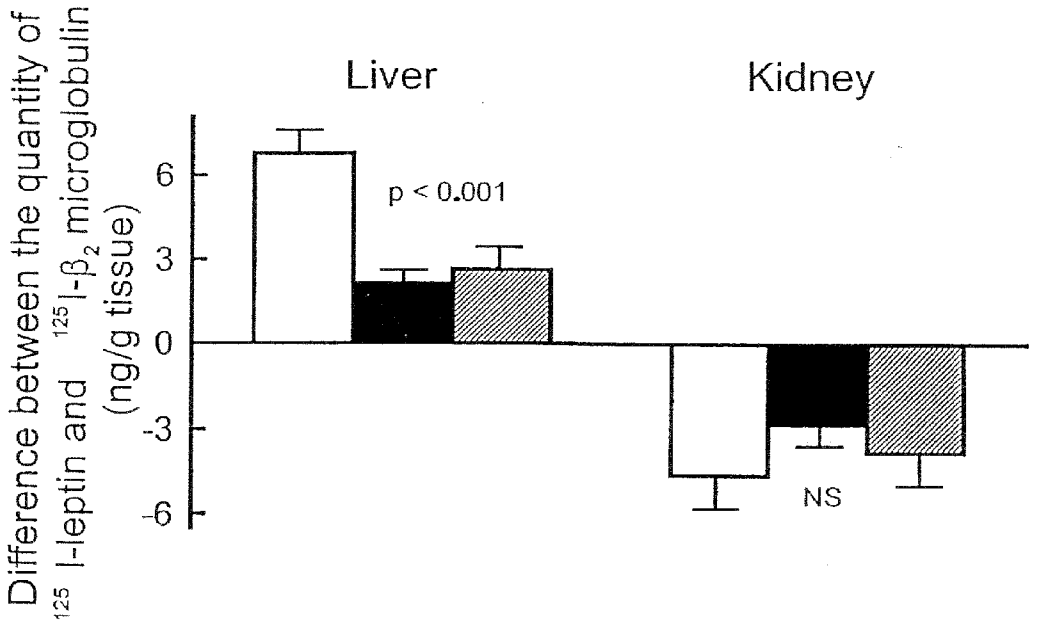

Difference between the quantity of
125 I-leptin and 125 I-β2 microglobulin
(ng/g tissue)
Liver
Kidney
6
3
0
-3
-6
p < 0.001
NS

FIG. 20

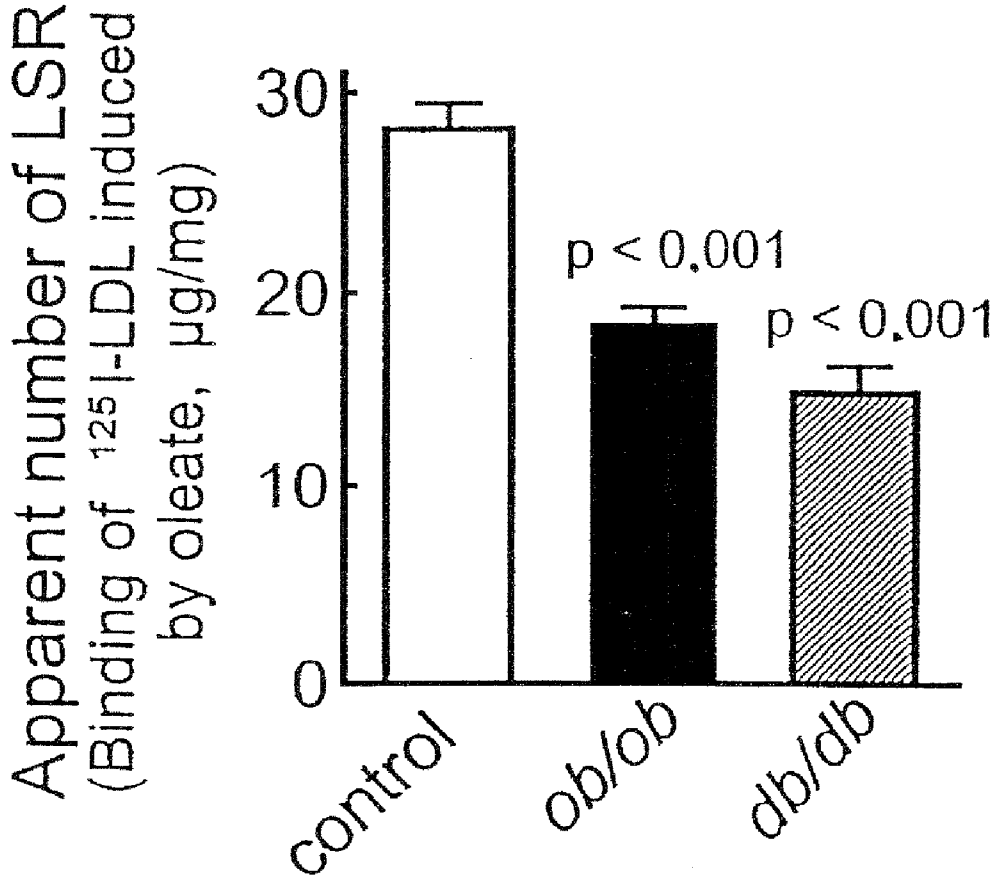

Apparent number of LSR
(Binding of ^{125}I-LDL induced by oleate, μg/mg)
30
20
10
0
p < 0.001
p < 0.001
control
ob/ob
db/db

FIG. 21

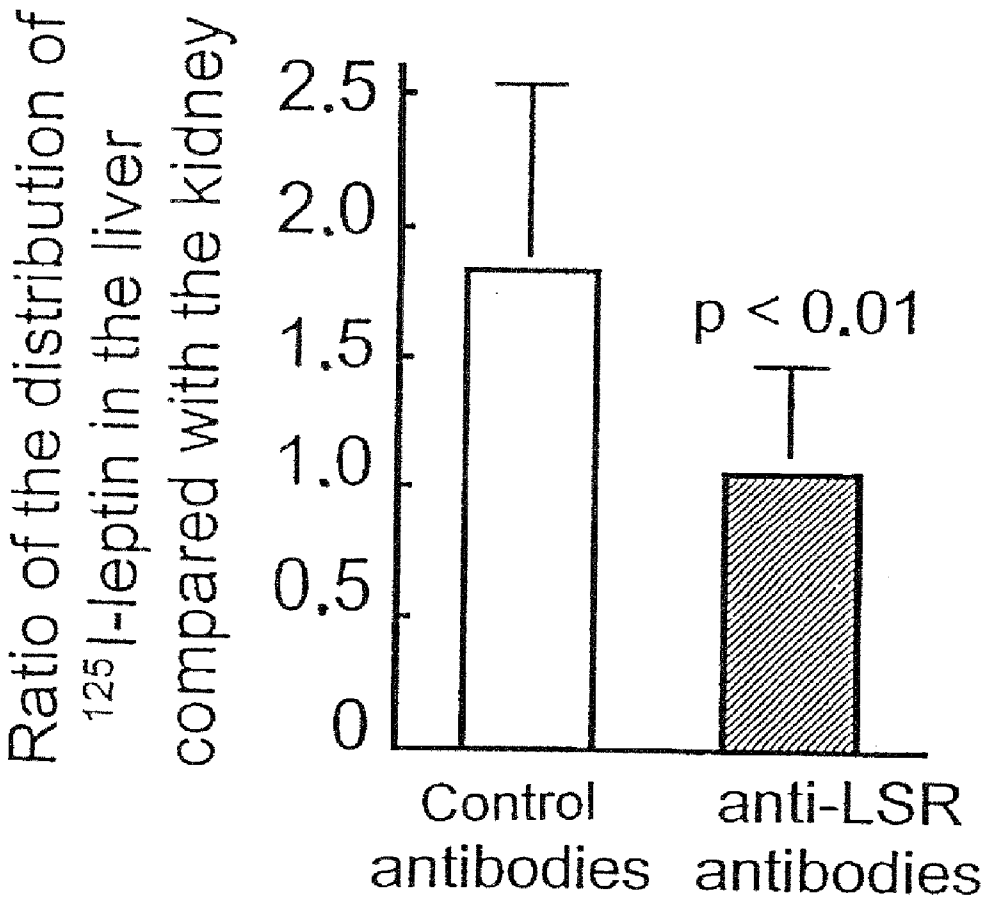

Ratio of the distribution of
125I-leptin in the liver
compared with the kidney
2.5
2.0
1.5
1.0
0.5
0
p < 0.01
Control
antibodies
anti-LSR
antibodies

FIG. 22

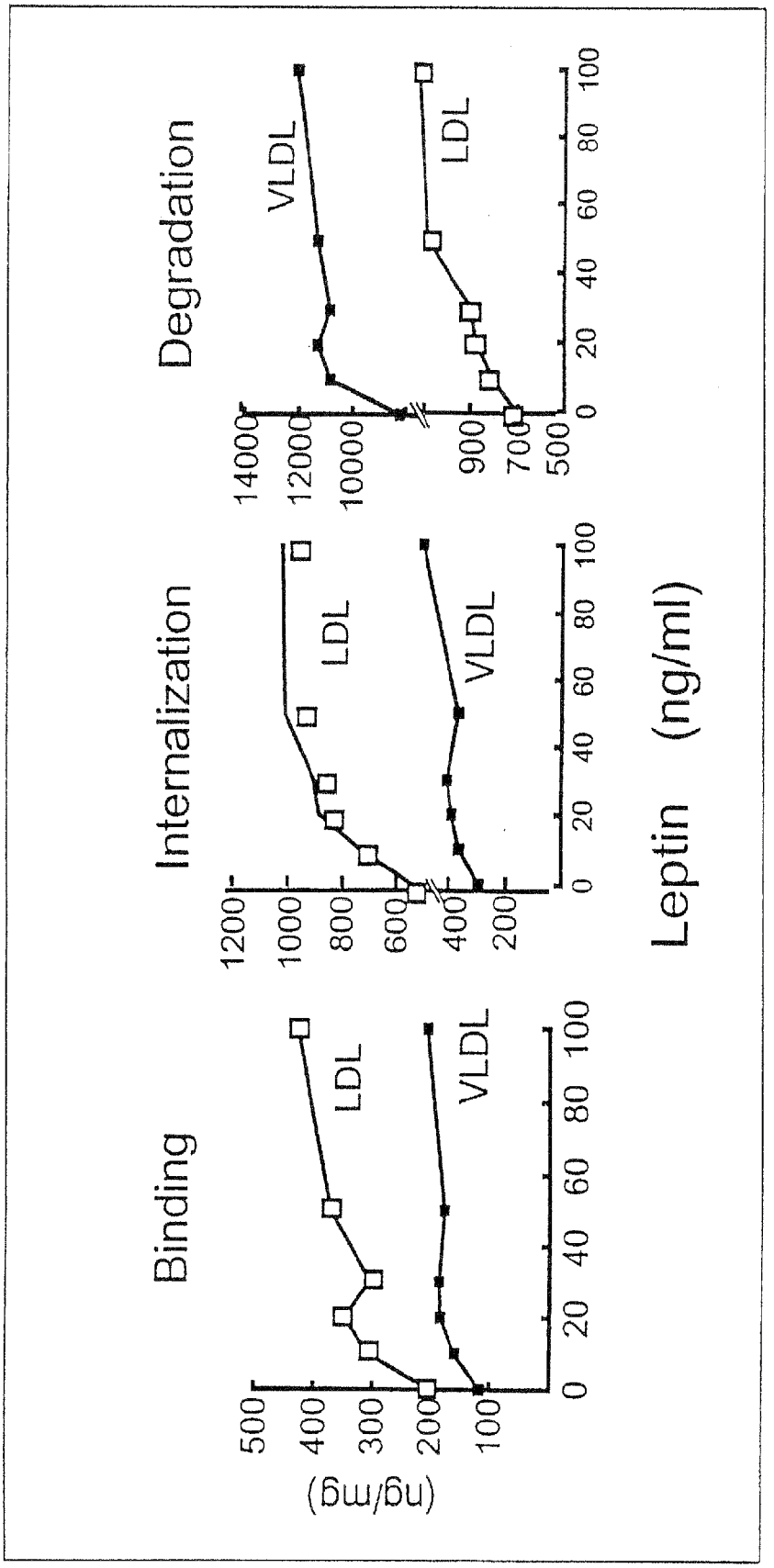

Binding
Internalization
Degradation
LDL
VLDL
(ng/mg)
Leptin (ng/ml)

FIG. 23

FIG. 24A

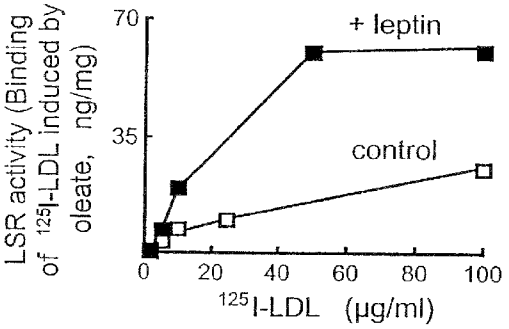
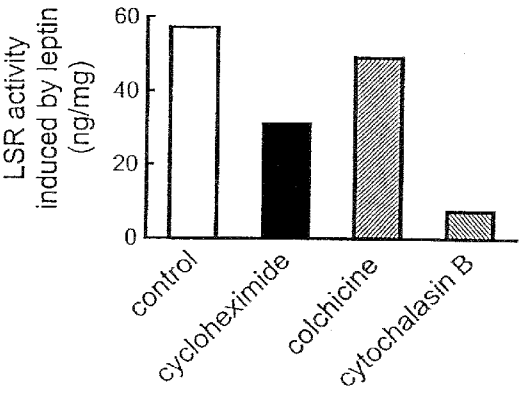


FIG. 24B



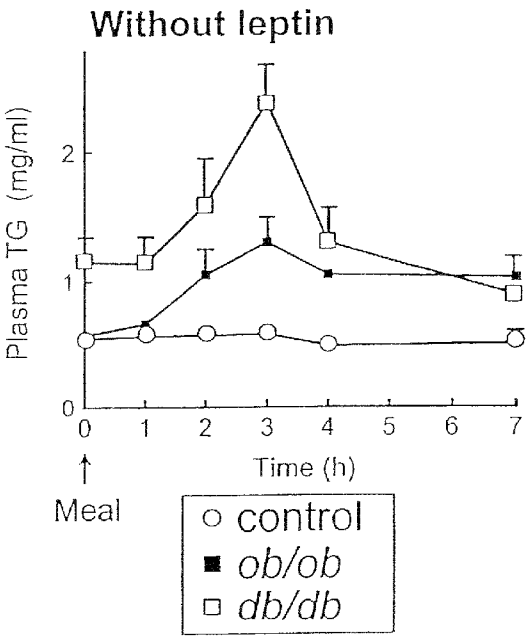


FIG. 25A

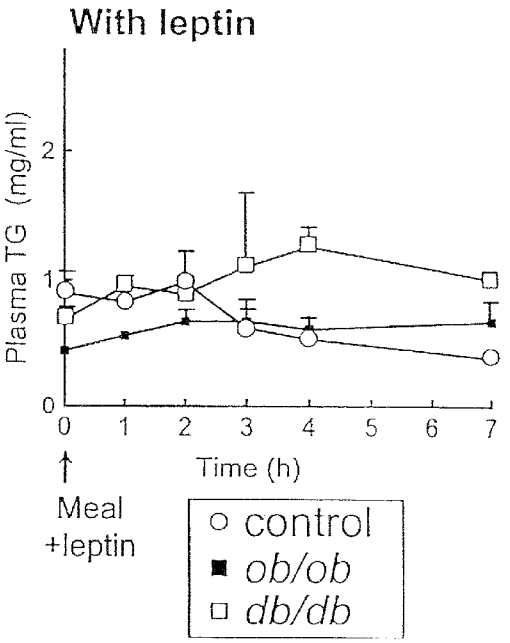


FIG. 25B

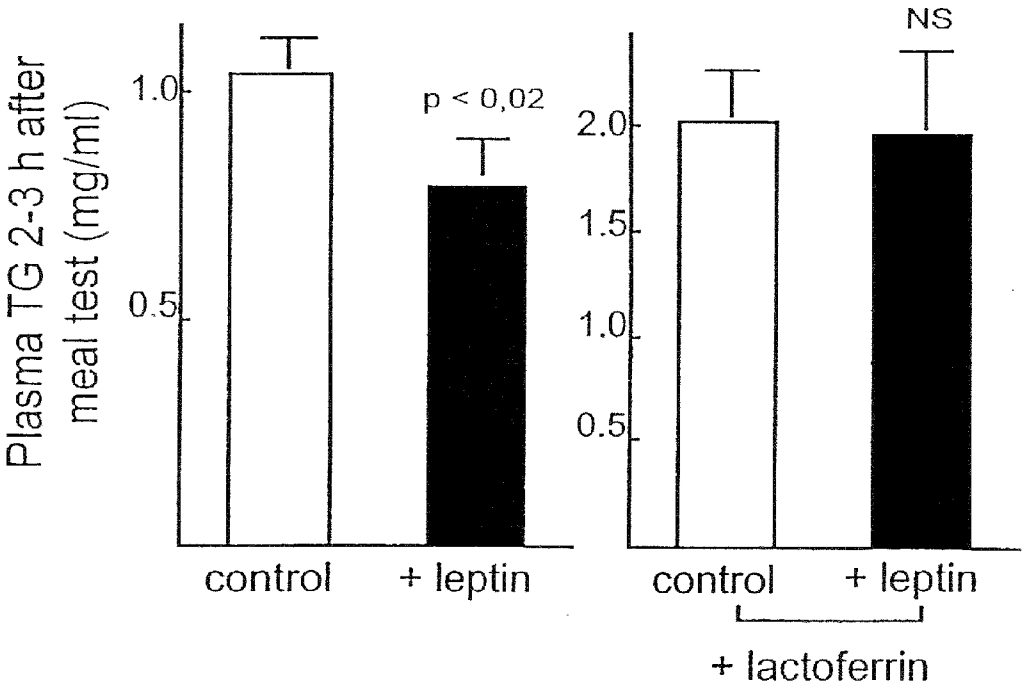

Plasma TG 2-3 h after meal test (mg/ml)
1.0
0.5
p < 0,02
control
+ leptin
2.0
1.5
1.0
0.5
NS
control
+ leptin
+ lactoferrin

FIG. 26

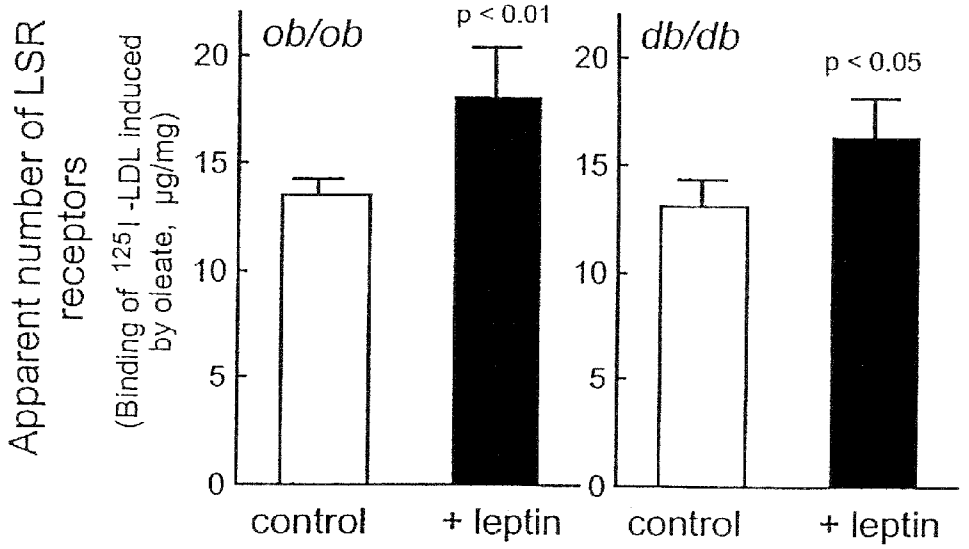

Apparent number of LSR receptors
(Binding of ^{125}I -LDL induced by oleate, µg/mg)
ob/ob
p < 0.01
db/db
p < 0.05
20
15
10
5
0
control
+ leptin
control
+ leptin

FIG. 27

FIG. 28A Weight

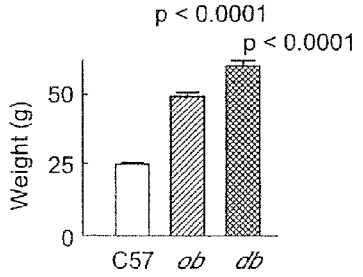


FIG. 28B Postrandial lipemic response

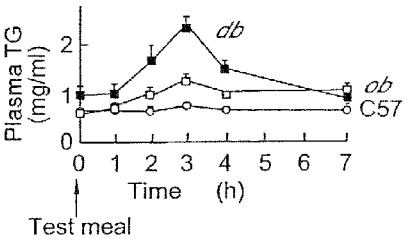


FIG. 28C LSR activity

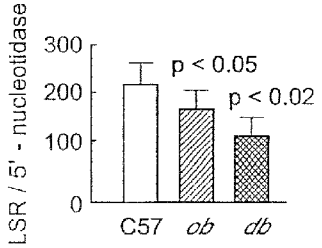


FIG. 28D Northern blot

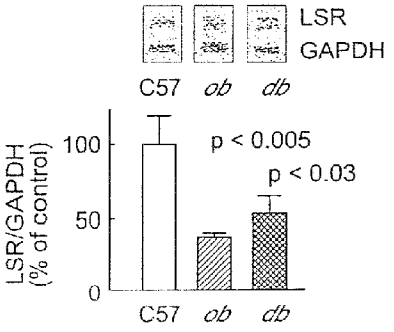


FIG. 29A Weight

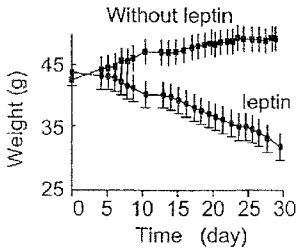


FIG. 29B Postrandial lipemic response

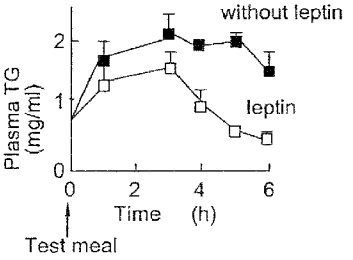


FIG. 29C LSR activity

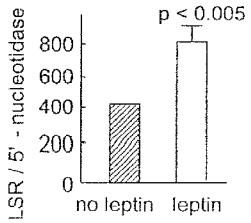
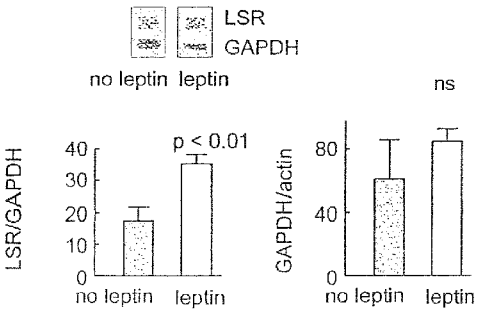


FIG. 29D Northern blot



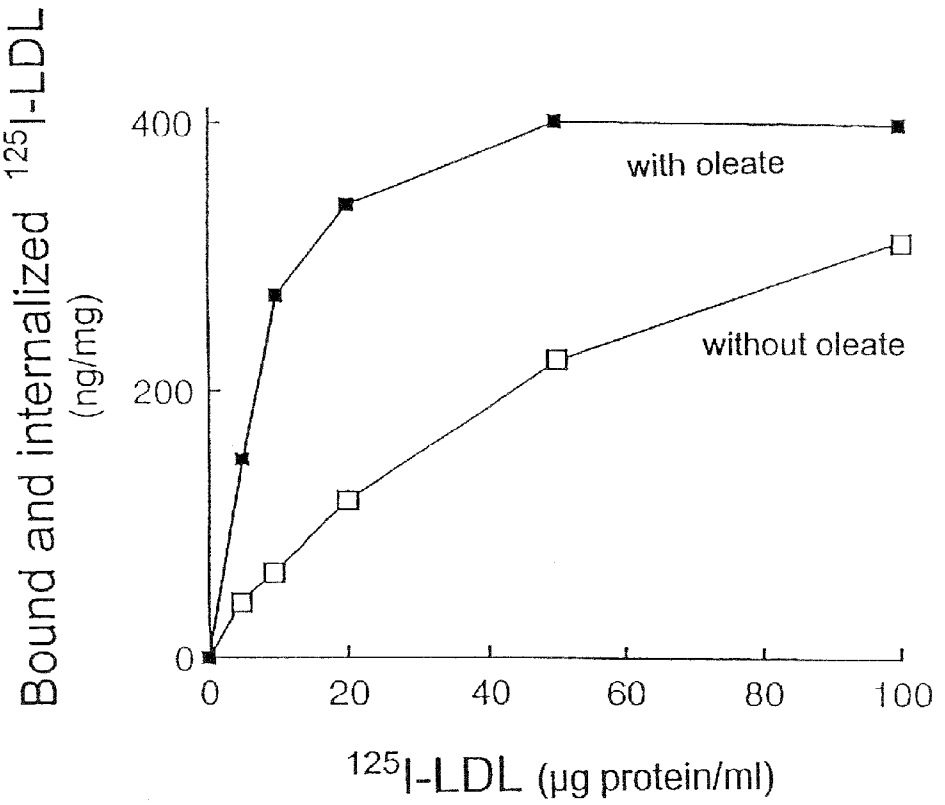

Bound and internalized ^{125}I-LDL (ng/mg)
400
200
0
0
20
40
60
80
100
with oleate
without oleate
^{125}I-LDL (µg protein/ml)

FIG. 30

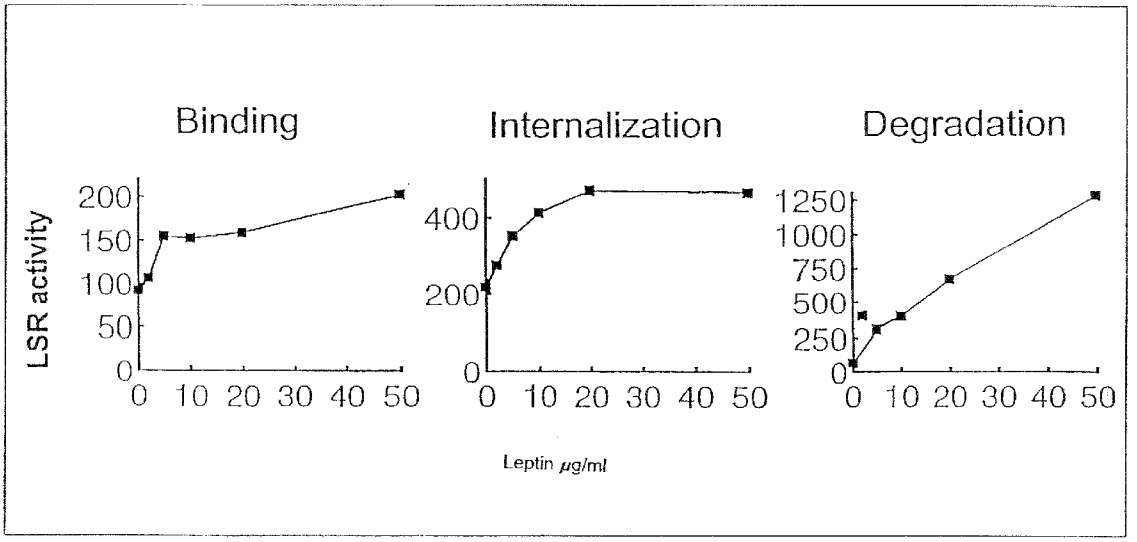

Binding
Internalization
Degradation
LSR activity
200
150
100
50
0
400
200
0
1250
1000
750
500
250
0 10 20 30 40 50
Leptin μg/ml

FIG. 31

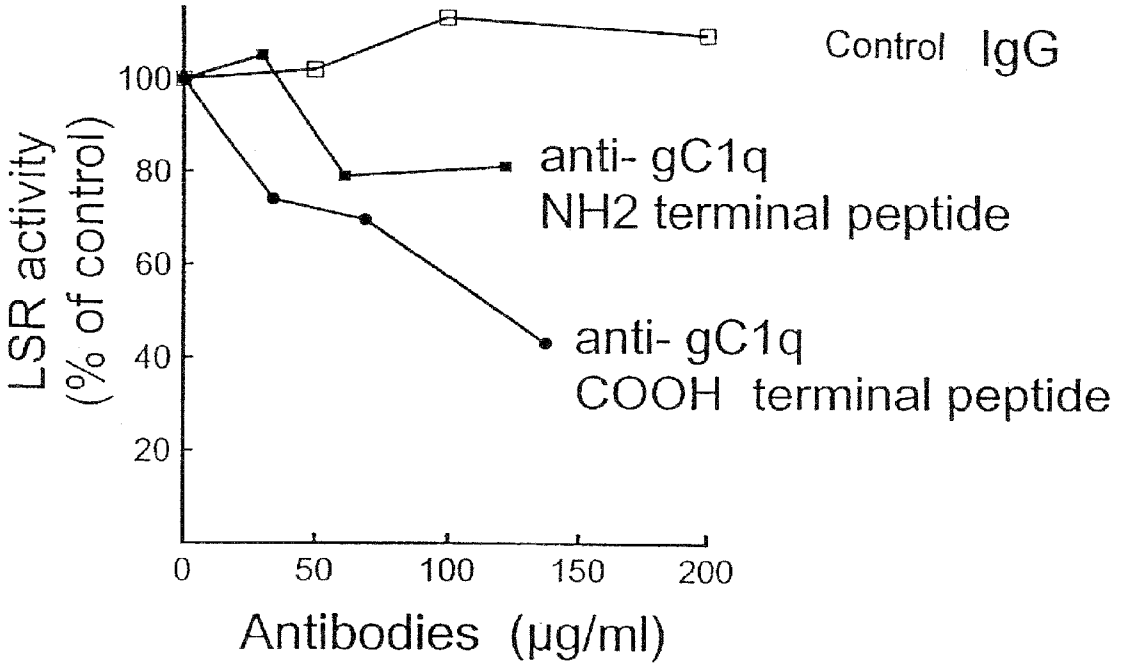

Control IgG
anti- gC1q
NH2 terminal peptide
anti- gC1q
COOH terminal peptide
LSR activity
(% of control)
100
80
60
40
20
0
50
100
150
200
Antibodies (µg/ml)

FIG. 32

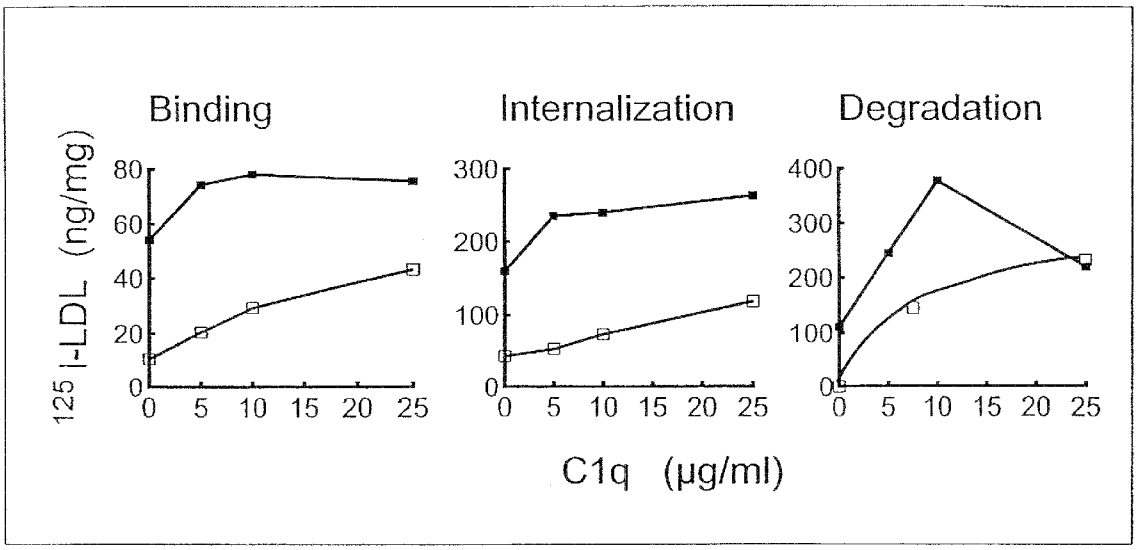

Binding
Internalization
Degradation
125 I-LDL (ng/mg)
80
60
40
20
0
300
200
100
400
0 5 10 15 20 25
C1q (µg/ml)
oleate
without oleate

FIG. 33

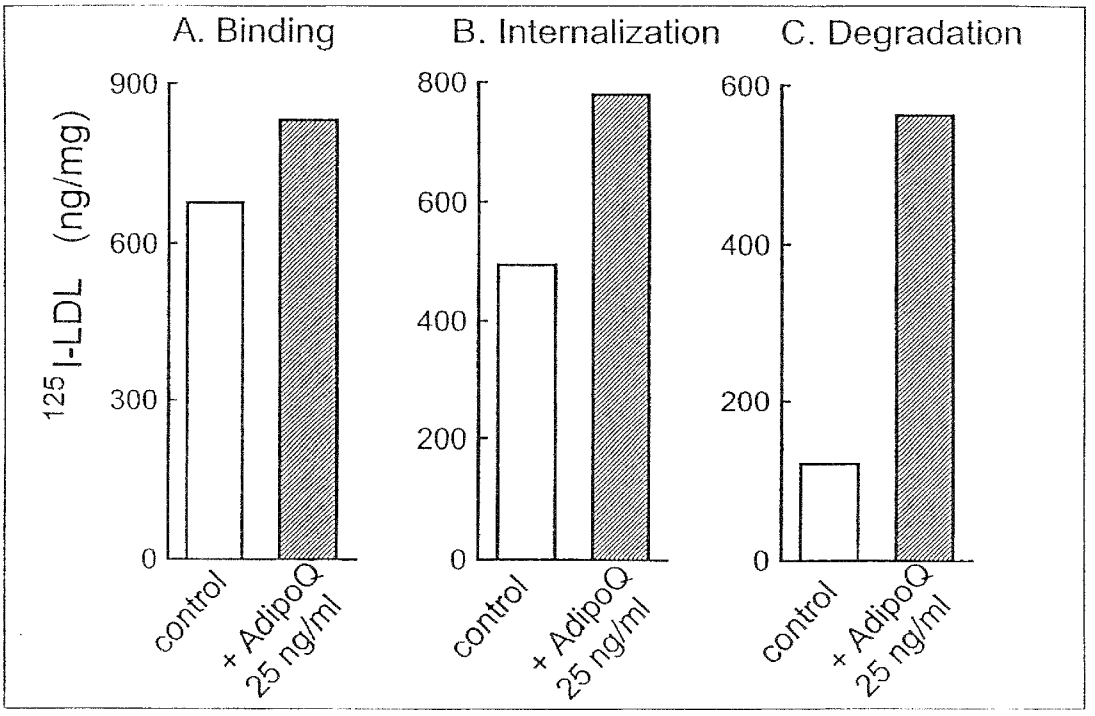

A. Binding
B. Internalization
C. Degradation
^{125}I-LDL (ng/mg)
900
600
300
0
800
600
400
200
0
600
400
200
0
control
+ AdipoQ 25 ng/ml
control
+ AdipoQ 25 ng/ml
control
+ AdipoQ 25 ng/ml

FIG. 34

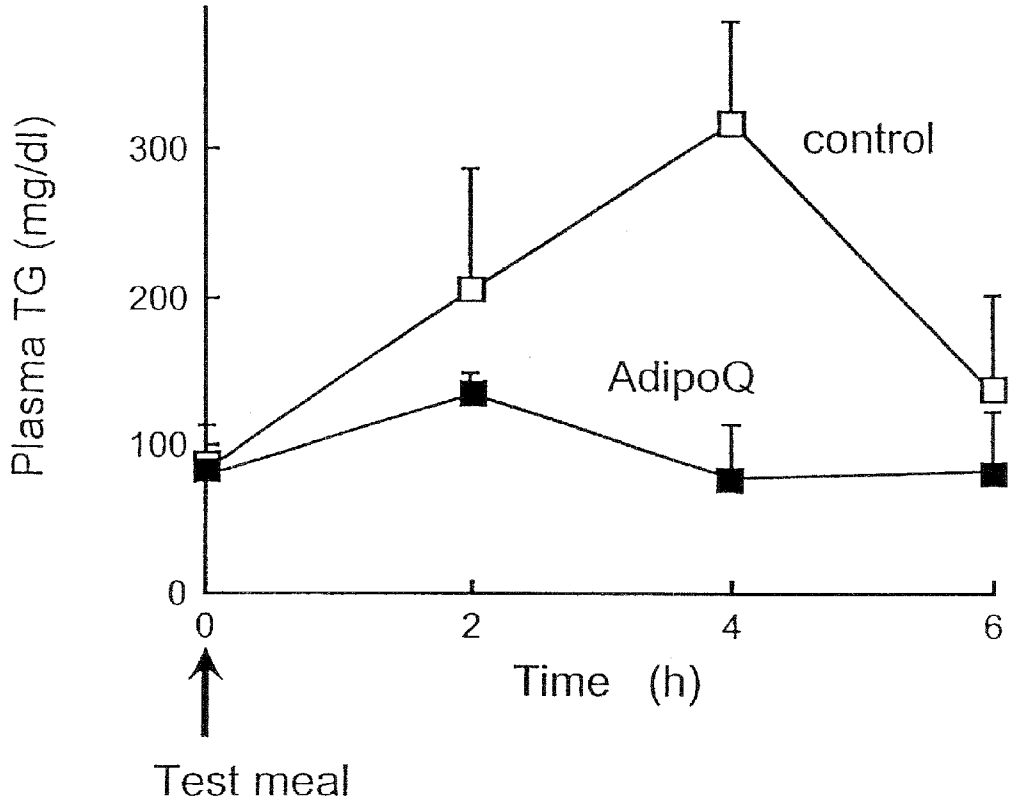

Plasma TG (mg/dl)
300
200
100
0
control
AdipoQ
0
2
4
6
Time (h)
Test meal

FIG. 35

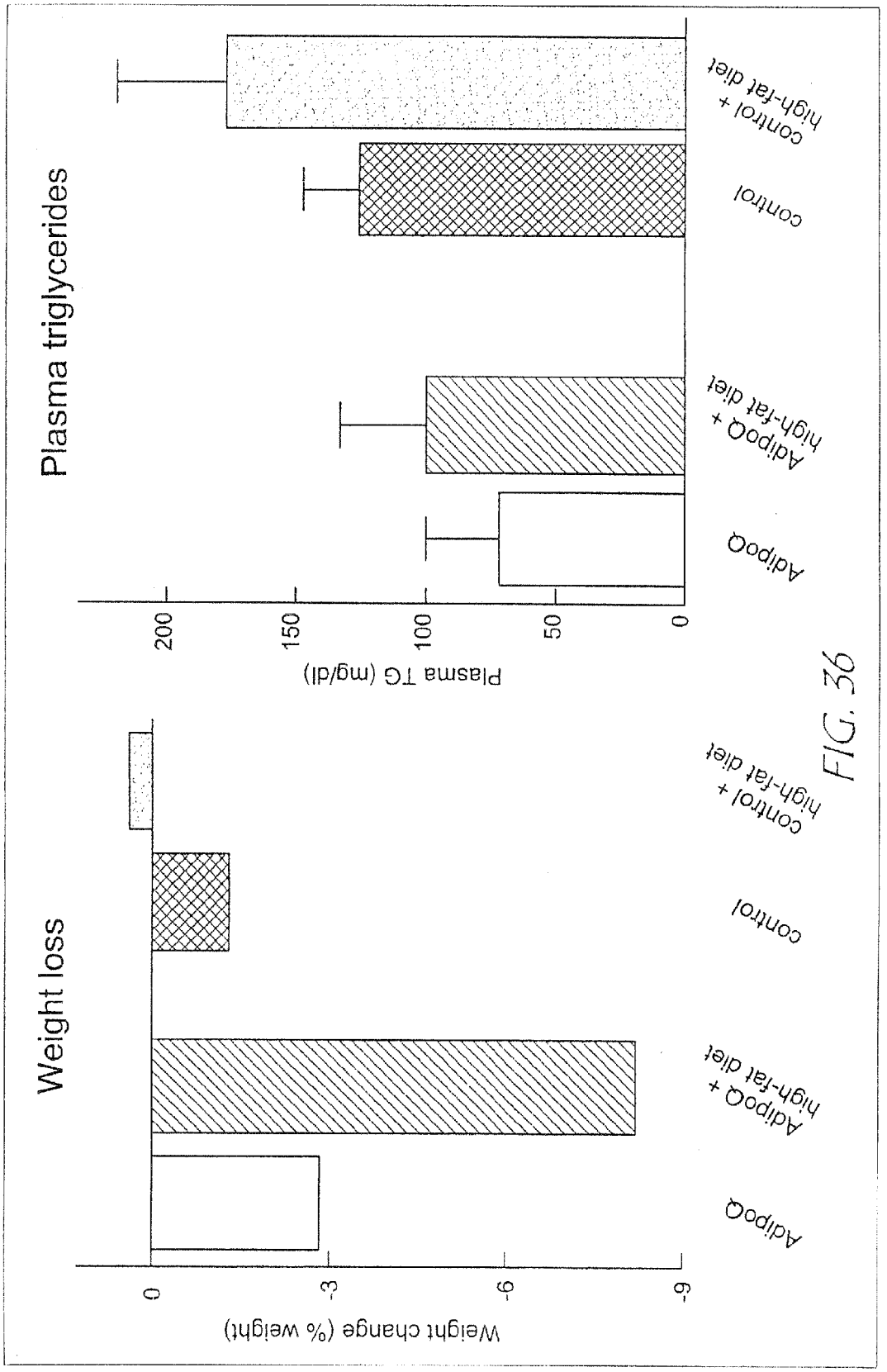

Plasma triglycerides
Plasma TG (mg/dl)
0
50
100
150
200
AdipoQ
AdipoQ + high-fat diet
control
control + high-fat diet
Weight loss
Weight change (% weight)
0
-3
-6
-9
AdipoQ
AdipoQ + high-fat diet
control
control + high-fat diet

FIG. 36

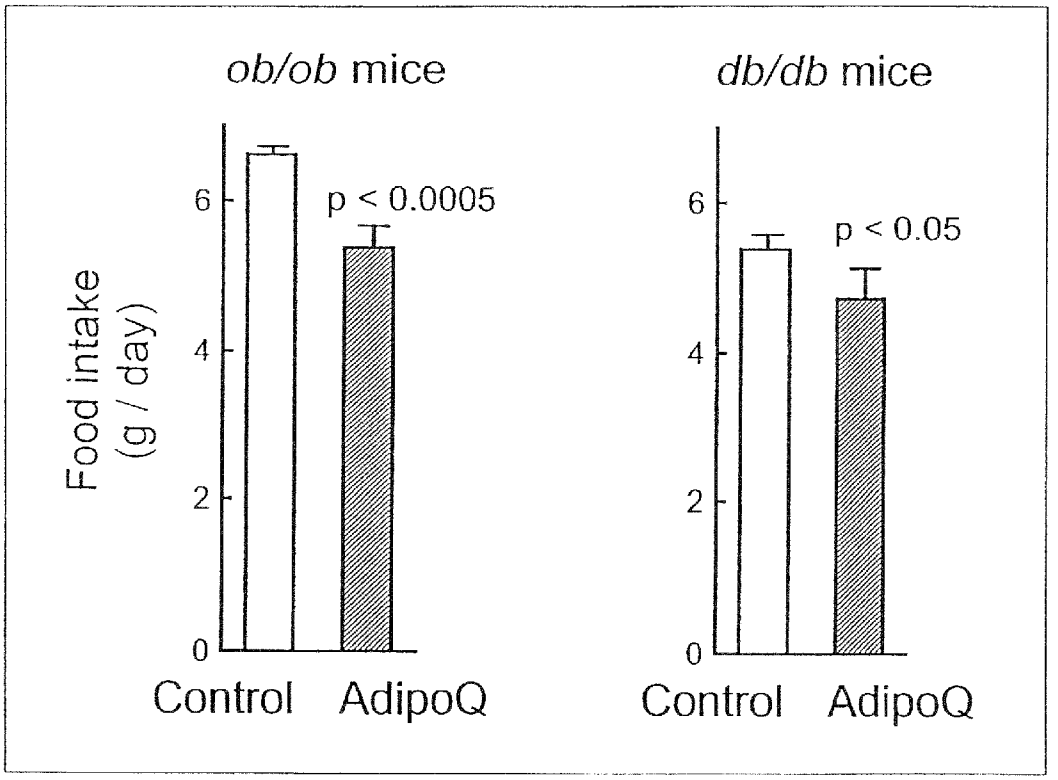

ob/ob mice
db/db mice
Food intake
(g / day)
6
4
2
0
p < 0.0005
p < 0.05
Control
AdipoQ
Control
AdipoQ

FIG. 37

LSR RECEPTOR, ITS ACTIVITY, ITS CLONING, AND ITS APPLICATIONS TO THE DIAGNOSIS, PREVENTION AND/OR TREATMENT OF OBESITY AND RELATED RISKS OR COMPLICATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 11/862,842, filed Sep. 27, 2007, which is a continuation of U.S. application Ser. No. 10/650,507, filed Aug. 27, 2003, now U.S. Pat. No. 7,291,709, which is a divisional of U.S. application Ser. No. 09/269,939, filed May 28, 1999, now U.S. Pat. No. 6,635,431, which is the national stage of international application No. PCT/IB98/01257, filed Aug. 6, 1998.

INTRODUCTION

[0002] The present invention relates to a new complex receptor polypeptide LSR (Lipolysis Stimulated Receptor), characterized by its functional activities, the cloning of the cDNAs complementary to the messenger RNAs encoding each of the subunits of the multimeric complex, vectors and transformed cells, methods of diagnosis and of selection of compounds which can be used as medicament for the prevention and/or treatment of pathologies and/or of pathogeneses such as obesity and anorexia, hyperlipidemias, atherosclerosis, diabetes, hypertension, and more generally the various pathologies associated with abnormalities in the metabolism of cytokines.

[0003] Obesity is a public health problem which is both serious and widespread: in industrialized countries, a third of the population has an excess weight of at least 20% relative to the ideal weight. The phenomenon continues to worsen, in regions of the globe whose economies are being modernized, such as the Pacific islands, and in general. In the United States, the number of obese people has passed from 25% at the end of the 70s to 33% at the beginning of the 90s.

[0004] Obesity considerably increases the risk of developing cardiovascular or metabolic diseases. It is estimated that if the entire population had an ideal weight, the risk of coronary insufficiency would decrease by 25% and that of cardiac insufficiency and of cerebral vascular accidents by 35%. Coronary insufficiency, atheromatous disease and cardiac insufficiency are at the forefront of the cardiovascular complications induced by obesity. For an excess weight greater than 30%, the incidence of coronary diseases is doubled in subjects under 50 years. Studies carried out for other diseases are equally eloquent. For an excess weight of 20%, the risk of high blood pressure is doubled. For an excess weight of 30%, the risk of developing a non-insulin-dependent diabetes is tripled. That of hyperlipidemias is multiplied by 6.

[0005] The list of diseases whose onset is promoted by obesity is long: hyperuricemia (11.4% in obese subjects, against 3.4% in the general population), digestive pathologies, abnormalities in hepatic functions, and even certain cancers.

[0006] Whether the physiological changes in obesity are characterized by an increase in the number of adipose cells, or by an increase in the quantity of triglycerides stored in each adipose cell, or by both, this excess weight results mainly from an imbalance between the quantities of calories consumed and those of the calories used by the body. Studies on the causes of this imbalance have been in several directions. Some have focused on studying the mechanism of absorption of foods, and therefore the molecules which control food intake and the feeling of satiety. Other studies have been related to the basal metabolism, that is to say the manner in which the body uses the calories consumed.

[0007] The treatments for obesity which have been proposed are of four types. Food restriction is the most frequently used. The obese individuals are advised to change their dietary habits so as to consume fewer calories. This type of treatment is effective in the short-term. However, the recidivation rate is very high. The increase in calorie use through physical exercise is also proposed. This treatment is ineffective when applied alone, but it improves, however, weight loss in subjects on a low-calorie diet. Gastrointestinal surgery, which reduces the absorption of the calories ingested, is effective but has been virtually abandoned because of the side effects which it causes. The medicinal approach uses either the anorexigenic action of molecules involved at the level of the central nervous system, or the effect of molecules which increase energy use by increasing the production of heat. The prototypes of this type of molecule are the thyroid hormones which uncouple oxidative phosphorylations of the mitochondrial respiratory chain. The side effects and the toxicity of this type of treatment make their use dangerous. An approach which aims to reduce the absorption of dietary lipids by sequestering them in the lumen of the digestive tube is also in place. However, it induces physiological imbalances which are difficult to tolerate: deficiency in the absorption of fat-soluble vitamins, flatulence and steatorrhoea. Whatever the envisaged therapeutic approach, the treatments of obesity are all characterized by an extremely high recidivation rate.

[0008] The molecular mechanisms responsible for obesity in humans are complex and involve genetic and environmental factors. Because of the low efficiency of the treatments known up until now, it is urgent to define the genetic mechanisms which determine obesity, so as to be able to develop better targeted medicaments.

[0009] More than 20 genes have been studied as possible candidates, either because they have been implicated in diseases of which obesity is one of the clinical manifestations, or because they are homologues of genes involved in obesity in animal models. Situated in the 7q31 chromosomal region, the OB gene is one of the most widely studied. Its product, leptin, is involved in the mechanisms of satiety. Leptin is a plasma protein of 16 kDa produced by the adipocytes under the action of various stimuli. Obese mice of the ob/ob type exhibit a deficiency in the leptin gene; this protein is undetectable in the plasma of these animals. The administration of leptin obtained by genetic engineering to ob/ob mice corrects their relative hyperphagia and allows normalization of their weight. This anorexigenic effect of leptin calls into play a receptor of the central nervous system: the ob receptor which belongs to the family of class 1 cytokine receptors. The ob receptor is deficient in obese mice of the db/db strain. The administration of leptin to these mice has no effect on their food intake and does not allow substantial reduction in their weight. The mechanisms by which the ob receptors transmit the signal for satiety are not precisely known. It is possible that neuropeptide Y is involved in this signalling pathway. It is important to specify at this stage that the ob receptors are not the only regulators of appetite. The Melanocortin 4 receptor is also involved since mice made deficient in this receptor are obese (Gura, 1997).

[0010] The discovery of leptin and the characterization of the leptin receptor at the level of the central nervous system have opened a new route for the search for medicaments against obesity. This model, however, rapidly proved disappointing. Indeed, with only one exception (Montague et al., 1997), the genes encoding leptin or its ob receptor have proved to be normal in obese human subjects. Furthermore and paradoxically, the plasma concentrations of leptin, the satiety hormone, are abnormally high in most obese human subjects. Most of the therapeutic research efforts in this direction have centered on the characterization of the effect of leptin at the level of the central nervous system.

SUMMARY OF THE INVENTION

[0011] The present invention results from a focusing of the research effort on the discovery of the mechanisms of leptin elimination. The most widely accepted working hypothesis is that the plasma levels of leptin are high in obese subjects because this hormone is produced by the adipose tissue and that the fatty mass is increased in obese subjects. The inventors have formulated a different hypothesis and have postulated that the concentrations of leptin are increased in obese individuals because the clearance of this hormone is reduced. This deficiency causes a leptin resistance syndrome and the obese individual develops a suitable response to the high concentrations of leptin. In this perspective, the treatment of obese subjects ought to consist not in an increase in the leptin levels but in a normalization thereof. At this stage, it is essential to recall that the ob type receptors are signalling type receptors. These receptors can bind leptin at the level of the plasma membrane but cannot cause the protein to enter inside the cell for it to be degraded therein. The ob receptors are not endocytosis receptors.

LSR Receptor

[0012] The inventors have characterized a receptor, in particular hepatic, called LSR receptor, whose activity is dual. The LSR receptor allows, on the one hand, endocytosis of lipoproteins, when it is activated by the free fatty acids, thus serving as a pathway for the clearance of lipoproteins. This pathway serves mainly, but not exclusively, for the clearance of particles high in triglycerides of intestinal origin (Mann et al., 1995). This activity, expressed most particularly at the hepatic level, is dependent on the presence of free fatty acids which, by binding to the receptor, induce a reversible change in the conformation of this complex and allow it to bind, with a high affinity, various classes of lipoproteins such as those containing apoprotein B or apoprotein E.

[0013] On the other hand, under normal conditions, in the absence of free fatty acids, the complex receptor LSR does not bind lipoproteins, but is capable of binding a cytokine, in particular leptin, and then of internalizing it and of degrading it.

[0014] The present invention therefore relates to a purified LSR receptor, in particular of hepatic cells, characterized in that it is capable, in the presence of free fatty acids, of binding lipoproteins, and in the absence of free fatty acids, of binding a cytokine, preferably leptin.

[0015] According to the invention, this LSR receptor is, in addition, characterized in that the bound lipoproteins or the bound cytokine are incorporated into the cell and then degraded, the bound lipoproteins containing in particular apoprotein B or E.

[0016] It should be understood that the invention does not relate to the LSR receptors in a natural form, that is to say that they are not taken in their natural environment but obtained by purification from natural sources, or alternatively obtained by genetic recombination, or alternatively by chemical synthesis and capable, in this case, of containing non-natural amino acids, as will be described below. The production of a recombinant LSR receptor, which may be carried out using one of the nucleotide sequences according to the invention, is particularly advantageous because it makes it possible to obtain an increased level of purity of the receptor.

[0017] More particularly, the invention relates to a purified rat LSR receptor, characterized in that it comprises at least one subunit having a molecular weight of about 66 kDa and a subunit having a molecular weight of about 58 kDa.

[0018] Preferably, the purified rat LSR receptor of the present invention is characterized in that it contains an α subunit comprising the amino acid sequence of SEQ ID NO:2 or a sequence homologous thereto, or an α' subunit comprising the amino acid sequence of SEQ ID NO:4 or a sequence homologous thereto, and one, preferably three, β subunits comprising the amino acid sequence of SEQ ID NO:6 or a sequence homologous thereto.

[0019] The invention also relates to a purified mouse LSR receptor, characterized in that it comprises at least one subunit having a molecular weight of about 66 kDa and a subunit having a molecular weight of about 58 kDa.

[0020] Preferably, the purified mouse LSR receptor of the present invention is characterized in that it contains an α subunit comprising the amino acid sequence of SEQ ID NO:16 or a sequence homologous thereto, or an α' subunit comprising the amino acid sequence of SEQ ID NO:17 or a sequence homologous thereto, and one, preferably three, 3 subunits comprising the amino acid sequence of SEQ ID NO:18 or a sequence homologous thereto.

[0021] The invention also relates to a purified human LSR receptor, characterized in that it comprises at least one subunit having a molecular weight of about 72 kDa and a subunit having a molecular weight of about 64 kDa.

[0022] Preferably, the purified human LSR receptor of the present invention is characterized in that it contains an α subunit comprising the amino acid sequence of SEQ ID NO:8 or a sequence homologous thereto, or an α' subunit comprising the amino acid sequence of SEQ ID NO:10 or a sequence homologous thereto, and one, preferably three, 3 subunits comprising the amino acid sequence of SEQ ID NO:12 or a sequence homologous thereto.

[0023] A particularly preferred embodiment of the LSR receptors of the present invention is a recombinant LSR receptor obtained by expressing, in a recombinant host, one or more nucleotide sequences according to the invention. This preferred recombinant receptor consists of an α or α' subunit and one, preferably three, β subunits, in particular an α or α' subunit and three β subunits of a human LSR receptor.

Polypeptide Sequences of LSR

[0024] The invention relates to polypeptides, characterized in that they are a constituent of an LSR receptor according to the invention.

[0025] It should be understood that the invention does not relate to the polypeptides in a natural form, that is to say that they are not taken in their natural environment. Indeed, the invention relates to the peptides obtained by purification from natural sources, or alternatively obtained by genetic recom-

bination, or alternatively by chemical synthesis, and capable, in this case, of containing non-natural amino acids, as will be described below. The production of a recombinant polypeptide, which may be carried out using one of the nucleotide sequences according to the invention or a fragment of one of these sequences, is particularly advantageous because it makes it possible to obtain an increased level of purity of the desired polypeptide.

[0026] The invention therefore relates to a purified, isolated or recombinant polypeptide comprising a sequence of at least 5, preferably at least 10 to 15, consecutive amino acids of an LSR receptor, as well as the homologues, equivalents or variants of the said polypeptide, or one of their fragments. Preferably, the sequence of at least 10 to 15 amino acids of the LSR receptor is a biologically active fragment of an LSR receptor.

[0027] More particularly, the invention relates to purified, isolated or recombinant polypeptides comprising a sequence of at least 10 to 15 amino acids of a rat LSR receptor, of a mouse LSR receptor or of a human LSR receptor.

[0028] In the present description, the term polypeptide will be used to also designate a protein or a peptide.

Nucleotide Sequences of LSR

[0029] The subject of the present invention is also purified nucleic acid sequences, characterized in that they encode an LSR receptor or a polypeptide according to the invention.

[0030] The invention relates to a purified nucleic acid, characterized in that it comprises at least 8, preferably at least 10 and more particularly at least 15 consecutive nucleotides of the polynucleotide of a genomic, cDNA or RNA sequence of the LSR receptor, as well as the nucleic acid sequences complementary to this nucleic acid.

[0031] More particularly, the invention relates to the purified, isolated or recombinant nucleic acids comprising a sequence of at least 8, preferably at least 10 and more particularly at least 15 consecutive nucleotides of the polynucleotide of a nucleic sequence of a mouse LSR receptor or of a human LSR receptor.

[0032] The invention also relates to the variant, mutated, equivalent or homologous nucleic sequences of the nucleic sequences according to the invention, or one of their fragments. It finally relates to the sequences capable of hybridizing specifically with the nucleic sequences according to the invention.

[0033] The invention therefore also relates to the nucleic acid sequences contained in the gene encoding the LSR receptor, in particular each of the exons of the said gene or a combination of exons of the said gene, or alternatively a polynucleotide extending over a portion of one or more exons. Preferably, these nucleic acids encode one or more biologically active fragments of the human LSR receptor.

[0034] The present invention also relates to the purified nucleic acid sequences encoding one or more elements for regulating the expression of the LSR gene. Also included in the invention are the nucleic acid sequences of the promoter and/or regulator of the gene encoding the receptor according to the invention, or one of their allelic variants, the mutated, equivalent or homologous sequences, or one of their fragments.

[0035] The invention also relates to the purified nucleic sequences for hybridization comprising at least 8 nucleotides, characterized in that they can hybridize specifically with a nucleic sequence according to the invention.

[0036] Preferably, nucleic acid fragments or oligonucleotides, having as sequences the nucleotide sequences according to the invention can be used as probes or primers.

[0037] The invention also comprises methods for screening cDNA and genomic DNA libraries, for the cloning of the isolated cDNAs and/or the genes coding for the receptor according to the invention, and for their promoters and/or regulators, characterized in that they use a nucleic sequence according to the invention.

[0038] The nucleic sequences, characterized in that they are capable of being obtained by one of the preceding methods according to the invention or the sequences capable of hybridizing with the said sequences, form part of the invention.

Vectors, Host Cells and Transgenic Animals

[0039] The invention also comprises the cloning and/or expression vectors containing a nucleic acid sequence according to the invention.

[0040] The vectors according to the invention, characterized in that they comprise elements allowing the expression and/or the secretion of the said sequences in a host cell, also form part of the invention.

[0041] The invention comprises, in addition, the host cells, in particular the eukaryotic and prokaryotic cells, transformed with the vectors according to the invention, as well as the mammals, except man, comprising one of the said transformed cells according to the invention.

[0042] Among the mammals according to the invention, there will be preferred animals such as mice, rats or rabbits, expressing a polypeptide according to the invention, the phenotype corresponding to the normal or variant LSR receptor, in particular mutated of human origin.

[0043] These cells and animals can be used in a method of producing a recombinant polypeptide according to the invention and can also serve as a model for analysis and screening.

[0044] The invention also relates to the use of a cell, of a mammal or of a polypeptide according to the invention for studying the expression and the activity of the receptor according to the invention, and the direct or indirect interactions between the said receptor and chemical or biochemical compounds which may be involved in the activity of the said receptor.

[0045] The invention also relates to the use of a cell, of a mammal or of a polypeptide according to the invention for screening a chemical or biochemical compound capable of interacting directly or indirectly with the receptor according to the invention, and/or capable of modulating the expression or the activity of the said receptor.

Production of Polypeptides Derived from the LSR Receptor

[0046] The invention also relates to the synthesis of synthetic or recombinant polypeptides of the invention, in particular by chemical synthesis or using a nucleic acid sequence according to the invention.

[0047] The polypeptides obtained by chemical synthesis and capable of comprising non-natural amino acids corresponding to the said recombinant polypeptides are also included in the invention.

[0048] The method of producing a polypeptide of the invention in recombinant form is itself included in the present invention, and is characterized in that the transformed cells are cultured under conditions allowing the expression of a recombinant polypeptide having a polypeptide sequence according to the invention, and in that the said recombinant polypeptide is recovered.

[0049] The recombinant polypeptides, characterized in that they are capable of being obtained by the said method of production, also form part of the invention.

Antibodies

[0050] The mono- or polyclonal antibodies or fragments thereof, chimeric or immunoconjugated antibodies, characterized in that they are capable of specifically recognizing a polypeptide or a receptor according to the invention, form part of the invention.

[0051] There may be noted in particular the advantage of antibodies specifically recognizing certain polypeptides, variants or fragments, which are in particular biologically active, according to the invention.

[0052] The invention also relates to methods for the detection and/or purification of a polypeptide according to the invention, characterized in that they use an antibody according to the invention.

[0053] The invention comprises, in addition, purified polypeptides, characterized in that they are obtained by a method according to the invention.

[0054] Moreover, in addition to their use for the purification of polypeptides, the antibodies of the invention, in particular the monoclonal antibodies, may also be used for the detection of these polypeptides in a biological sample.

[0055] More generally, the antibodies of the invention may be advantageously used in any situation where the expression, normal or abnormal, of a polypeptide of the LSR receptor, normal or mutated, needs to be observed.

Detection of Allelic Variability and Diagnosis

[0056] Also forming part of the invention are the methods for the determination of an allelic variability, a mutation, a deletion, a loss of heterozygosity or a genetic abnormality, characterized in that they use a nucleic acid sequence or an antibody according to the invention.

[0057] These methods relate to, for example, the methods for the diagnosis of the predisposition to obesity, to the associated risks, or to pathologies associated with abnormalities in the metabolism of cytokines, by determining, in a biological sample from the patient, the presence of mutations in at least one of the sequences described above. The nucleic acid sequences analysed may be either the genomic DNA, the cDNA or the mRNA.

[0058] Nucleic acids or antibodies based on the present invention can also be used to allow a positive and differential diagnosis in a patient taken in isolation, or a pre-symptomatic diagnosis in an at risk subject, in particular with a familial history.

[0059] In addition, the detection of a specific mutation may allow an evolutive diagnosis, in particular as regards the intensity of the pathology or the probable period of its appearance.

Screening of Compounds of Interest

[0060] Also included in the invention are the methods for selecting chemical or biochemical compounds capable of interacting, directly or indirectly, with the receptor or the polypeptide or nucleotide sequences according to the invention, and/or allowing the expression or the activity of the LSR receptor to be modulated.

[0061] The invention relates in particular to a method for selecting chemical or biochemical compounds capable of interacting with a nucleic acid sequence contained in a gene encoding an LSR receptor, the said method being characterized in that it comprises bringing a host cell expressing an LSR receptor or a fragment of the said receptor into contact with a candidate compound capable of modifying the expression or the regulation of the expression of the said nucleic sequence, and detecting, directly or indirectly, a modification of the expression or of the activity of the LSR receptor.

[0062] The invention also relates to a method for selecting chemical or biochemical compounds capable of interacting with the LSR receptor, the said method being characterized in that it comprises bringing an LSR receptor or a fragment of the said receptor, or a host cell expressing an LSR receptor or a fragment of the said receptor, into contact with a candidate compound capable of modifying the LSR activity, and detecting, directly or indirectly, a modification of the activity of the LSR receptor or the formation of a complex between the candidate compound and the said LSR receptor or the said polypeptide.

[0063] The invention comprises the compounds capable of interacting directly or indirectly with an LSR receptor as well as the compounds capable of interacting with one or more nucleic sequences of the LSR receptor. It also comprises the chemical or biochemical compounds allowing the expression or the activity of the receptor according to the invention to be modulated. The compounds, characterized in that they were selected by one of the methods according to the present invention, also form part of the invention.

[0064] In particular, among these compounds according to the invention, there are preferred the antibodies according to the invention, the polypeptides according to the invention, the nucleic acids, oligonucleotides and vectors according to the invention, or a leptin or one of its derived compounds, preferably one of its protein variants, or leptins which are chemically modified or are obtained by genetic recombination, or the protein gC1qR or one of its analogues, or one of their fragments.

[0065] The invention comprises, finally, compounds capable of modulating the expression or the activity of the receptor according to the invention, as medicament for the prevention of pathologies and/or of pathogeneses such as obesity and anorexia, hyperlipidemias, atherosclerosis, diabetes, hypertension, and more generally the various pathologies associated with abnormalities in the metabolism of cytokines.

DETAILED DESCRIPTION

The LSR Receptor

[0066] The invention relates to a purified LSR receptor ("Lipolysis Stimulated Receptor"), preferably hepatic, consisting of at least one α or α' subunit and at least one β subunit. The α subunit has a molecular weight of about 66 kDa in rats and in mice and of about 72 kDa in humans. The α' subunit has a molecular weight of about 64 kDa in rats and in mice and of about 70 kDa in humans. The β subunit has a molecular weight of about 58 kDa in rats and in mice and of about 64 kDa in humans.

[0067] The inventors have formulated the hypothesis according to which the most abundant, and probably the most active, form of the LSR receptor is that in which an α or α' subunit and three β subunits exist. It appears, however, possible that the α and α' subunits, on the one hand, and the β subunit, on the other, have distinct biological functions and

that these functions can be performed in a cell independently of their assembly in the form of a receptor.

[0068] The inventors have also observed that a complex can form between the LSR receptor and the gC1qR receptor having a molecular weight of about 33 kDa, or a homologous protein. It appears that the gC1qR receptor is transiently combined with the LSR receptor and that the presence of a C1q protein or of homologous proteins makes it possible not only to dissociate gC1qR from the LSR receptor but also to activate the LSR receptor, including in the absence of fatty acids.

Activity of the LSR Receptor and Applications

[0069] The present invention therefore relates to a receptor, in particular of hepatic cells, characterized in that it is capable, in the presence of free fatty acids, of binding lipoproteins, and in the absence of free fatty acids, of binding a cytokine, preferably the bound leptin, lipoproteins and cytokine being incorporated and then degraded by the cell, it being possible for the said receptor, in addition, to bind the gC1qR protein or one of its analogous proteins.

Clearance of Lipoproteins

[0070] The LSR receptor represents the principal pathway for the elimination of lipoproteins of intestinal origin and of particles high in triglycerides, in particular VLDLs and chylomicrons. The LSR receptor can also serve as a pathway for the elimination of LDLs, particles high in cholesterol, which are for the most part removed by the LDL receptor pathway, but of which about 30% are eliminated at the hepatic level by pathways different from the LDL receptor.

[0071] The inventors have in fact demonstrated that the LSR receptor is capable of binding lipoproteins, in particular the lipoproteins high in triglycerides, and then of internalizing and degrading them. This lipoprotein clearance activity by the receptor requires the presence of free fatty acids, for example oleate, and is inhibited in the presence of antibodies directed against LSR or against peptides derived from LSR.

Clearance of Cytokines

[0072] The inventors have also demonstrated that in the absence of free fatty acids, for example oleate, the LSR receptor is capable of binding cytokines, preferably leptin. The leptin clearance function is, however, only possible if the receptor has not bound fatty acids produced by the hepatic lipase or by the hormone-sensitive lipase of the adipose tissue. Once the cytokines have been bound, the LSR receptor internalizes them and degrades them. This cytokine, preferably leptin, degradation activity is inhibited by antibodies directed against LSR or against peptides derived from LSR.

[0073] The inventors have shown that it is the α subunit of the LSR receptor which is most particularly involved in the binding of cytokines, and preferably of leptin.

[0074] Furthermore, the inventors have shown, with the aid of mice, that, in vivo, the LSR receptors carry out the hepatic capturing of cytokines, preferably of leptin.

[0075] The high levels of leptin in all obese human subjects can be explained by several molecular mechanisms which are capable of reducing the hepatic clearance of leptin, including in particular:

a) alteration of one or more genes for LSR, and/or of their promoters;

b) facilitation, by post-transcriptional modifications, of the allosteric rearrangement allowing the passage from the cytokine-competent conformation to the lipoprotein receptor conformation;

c) deficiency in the transport of vesicles containing LSR from, or towards, the plasma membrane (this function depends on the integrity of the cytoskeleton);

d) increase in the degradation of LSR;

e) increase in the lipid calorie ration which, by diverting the receptor towards the clearance of lipoproteins, reduces in part its capacity to degrade leptin.

Control of LSR Activity by the Cytokines

[0076] Finally, the inventors have demonstrated that cytokines, preferably leptin, modulate the activity of the LSR receptor in the presence of free fatty acids. More particularly, the cytokines increase the lipoprotein clearance activity of the LSR receptor and more precisely, the binding, internalization and degradation of the VLDLs and LDLs. This increase in the LSR activity could be the result of the increase in the apparent number of LSR receptor at the surface of the cells following an increase in protein synthesis and following a mobilization of endocytosis vesicles. In addition, the inventors have shown, with the aid of mice, that, in vivo, cytokines, preferably leptin, are capable of reducing postprandial lipaemic response.

[0077] Leptin, and probably other cytokines, are therefore regulators of the activity of LSR. A syndrome of resistance to leptin, or to other cytokines, can lead to a hypertriglyceridemia, which is either permanent or limited to the postprandial phase.

Treatment of Obesity

[0078] The role played by LSR in the clearance of leptin makes it possible to formulate a physiopathological model which requires a revision of the strategies used for treating obesity. It is indeed essential to reduce the concentrations of leptin in obese human subjects in order to restore the physiological fluctuations of this hormone.

[0079] Accordingly, it is possible to envisage using compounds for the treatment of obesity allowing modulation of the number of LSR receptors, of their recycling rate, or of the change in their conformation, and/or allowing in particular:

1. leptinemia, and therefore the sensations of satiety and of hunger, to be controlled;

2. normal leptin concentrations to be restored and normal regulation of dietary habit by the normal perception of the sensations of hunger and of satiety

3. triglyceridemia to be controlled;

4. the plasma concentrations of residues of chylomicrons, highly atherogenic particles, to be regulated.

[0080] The role played by the LSR receptor in the hepatic clearance of lipoproteins of intestinal region makes it possible to envisage using compounds capable of modulating the expression and/or the activity of LSR in order to modulate the distribution of lipids of dietary origin between the peripheral tissues, in particular the adipose tissues, and the liver. A treatment of obesity will consist in promoting the hepatic degradation of lipoproteins, and thereby reducing their storage in the adipose tissue, and regulating their plasma concentrations. The latter effect makes it possible to envisage the use

of such compounds to reduce the risks associated with obesity, in particular the atherogenic risks.

Treatments of Anorexia and of Cachexia

[0081] It is possible to envisage using methods of regulating the activities of LSR to introduce treatments which make it possible to overcome the vicious circle which characterizes anorexia nervosa. By reducing the number of receptors, it should be possible to promote weight gain in anorexic or undernourished subjects.

[0082] Under these conditions, it is advantageous to selectively inhibit the clearance of leptin by using synthetic peptides or pharmacological molecules which either reduce the synthesis of LSR or block its capacity to bind leptin and/or lipoproteins, or alternatively increase the catabolism of the receptor.

Treatment of Abnormalities in the Metabolism of Cytokines

[0083] Analysis of the primary structure of the α subunit of LSR, as described below, shows a site homologous to the cytokine binding sites present on their receptors, as well as two routing signals which allow endocytosis and rapid degradation of ligands in the lysozomes. This observation is new in the sense that the cytokine receptors do not allow the internalization and the degradation of ligands. These receptors have been characterized on the basis of their intracellular signalling properties.

[0084] Thus, in addition to it having the property of allowing the proteolytic degradation of lipoproteins and of leptin, it is highly probable that the LSR receptor also carries out the degradation of other cytokines. This function can be studied by virtue of the anti-LSR antibodies and of transfected CHO cells expressing the α subunit of LSR as described in Example 4. The involvement of LSR in the clearance of cytokines is essential because these molecules play an important role in the regulation of the metabolism of lipids, of the metabolism of glucose, and in the regulation of food intake and of weight gain.

[0085] The molecular mechanisms by which the cytokines modulate the physiological functions involved in obesity and its complications are numerous and complex. It is worth noting, however, the fact that abnormalities in the metabolism of cytokines are associated with hypertriglyceridemia which frequently accompanies viral, bacterial or protozoal infections. Moreover, cytokines, and more particularly Tumor Necrosis Factor (TNF), induce a transient hypertriglyceridemia similar to that observed in certain forms of obesity-related diabetes.

[0086] The reduction in the number of LSR receptors expressed in the liver of obese mice could explain a deficiency in the elimination of some cytokines, this deficiency causing metabolic disruptions such as those found in obesity. The use of hepatic cells in culture, and of the various models of obese animals cited below, will make it possible to determine, among all the cytokines and more particularly those which induce weight loss (IL-6, LIF, OSM, CNTF, IL-11, IL-12α, as well as TNFα and TNFβ), those which modulate the expression and/or the activity of LSR. The determination of such cytokines can, for example, be carried out using methods such as those presented in Examples 4 to 6.

[0087] Finally, analysis of the primary structure of the α LSR reveals potential phosphorylation sites. This opens the perspective of a regulation of cellular activity by the LSR receptor. A particularly important example would be the involvement of LSR in the regulation of the production of "Acute Phase Proteins" under the impetus of various stimuli, including cytokines.

[0088] The involvement of LSR in the clearance and the degradation of cytokines may, in addition, not be limited to the liver. Indeed, while it has been demonstrated that the expression of LSR is predominantly hepatic, it is also certain that the expression of this receptor is not limited to this organ. Preliminary Northern-blot analysis on various human tissues has been able to reveal, in addition to the hepatic products, expression products in the kidney and in the testicle. A more thorough analysis will make it possible to show the different tissues expressing LSR in humans. In this perspective, LSR could be involved in the degradation of cytokines not only at the hepatic level, but also at the level of the peripheral tissues. A deficiency in this activity could be involved in the pathogenesis of autoimmune diseases, of multiple sclerosis and of rheumatoid arthritis. Accumulation of cytokines is frequently found in the pathogenesis of these diseases.

Polypeptide Sequences of the LSR Receptor

[0089] The invention relates to polypeptides, characterized in that they are a constituent of an LSR receptor according to the invention. The invention relates more particularly to the polypeptides characterized in that they constitute the α, α' or β subunits of the LSR receptor.

[0090] The invention relates more particularly to a purified, isolated or recombinant polypeptide comprising a sequence of at least 5, preferably of at least 10 to 15 consecutive amino acids of an LSR receptor, as well as the homologues, equivalents or variants of the said polypeptide, or one of their fragments. Preferably, the sequence of at least 10 to 15 amino acids of the LSR receptor is a biologically active fragment of an LSR receptor.

[0091] Preferably, the invention relates to purified, isolated or recombinant polypeptides comprising a sequence of at least 10 to 15 amino acids of a rat LSR receptor, of a mouse LSR receptor or of a human LSR receptor.

[0092] In a first preferred embodiment of the invention, the polypeptide is characterized in that it comprises a sequence of at least 10 to 15 consecutive amino acids of a sequence chosen from the group comprising the sequences of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, as well as the variants, equivalents or homologues of this polypeptide, or one of their fragments. Preferably, the polypeptide is a homologue or a biologically active fragment of one of the abovementioned sequences.

[0093] In a second preferred embodiment of the invention, the polypeptide is characterized in that it comprises a sequence of at least 10 to 15 consecutive amino acids of a sequence chosen from the group comprising the sequences of SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18, as well as the variants, equivalents or homologues of this polypeptide, or one of their fragments. Preferably, the polypeptide is a homologue or a biologically active fragment of one of the abovementioned sequences.

[0094] In a third preferred embodiment of the invention, the polypeptide is characterized in that it comprises a sequence of at least 10 to 15 consecutive amino acids of a sequence chosen from the group comprising the sequences of SEQ ID NO:8, SEQ ID NO:10 and SEQ ID NO:12, as well as the variants, equivalents or homologues of this polypeptide, or one of their

fragments. Preferably, the polypeptide is a homologue or a biologically active fragment of one of the abovementioned sequences.

[0095] Among the preferred polypeptides of the invention, there will be noted particularly the polypeptides having the human sequence SEQ ID NO:8, SEQ ID NO:10 or SEQ ID NO:12, as well as those having the rat sequence SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or those having the mouse sequence SEQ ID NO:16, SEQ ID NO:17 or SEQ ID NO:18. The fragments corresponding to the domains represented in FIGS. **1** to **6**, whose positions on the sequences corresponding to SEQ ID NO:2, 8 or 16, are indicated in Tables 1, 3 and 4.

[0096] Finally, the invention also relates to the polypeptides of SEQ ID NO:29 and SEQ ID NO:30.

[0097] The present invention also relates to polypeptides comprising the polypeptides described above, as well as their homologous, equivalent or variant polypeptides, as well as the fragments, preferably biologically active, of the said polypeptides.

[0098] Among the polypeptides according to the invention, also preferred are the polypeptides comprising or consisting of an amino acid sequence chosen from the amino acid sequences as described above, characterized in that the said polypeptides are a constituent of the receptor according to the invention.

Analysis of the Polypeptide Sequences of the α, α' and β Subunits of the LSR Receptor

[0099] The systematic analysis of the products of the 3 rat cDNAs described in the present application is schematically represented in FIG. **1**. The α subunit of the rat LSR receptor, a protein encoded by the longer cDNA (LSR-Rn-2097), has the following characteristics.

[0100] Potential glycosylation sites are found at positions 12-14 and 577-579. A potential site of attachment of glycosaminoglycans is found at position 14-17.

[0101] Several phosphorylation sites are located at the level of the NH_2-terminal end (positions 193-196, 597-600, 169-171, 172-174, 401-403, 424-426, 464-466, 467-469, 185-188, 222-225, 436-439, 396-399, 504-507, 530-533, 624-627, 608-615), suggesting that the latter is oriented towards the intracellular region.

[0102] Moreover, the protein has, on the NH_2-terminal side, a hydrophobic amino acid sequence separated into two parts by 2 amino acids inducing a hairpin structure in which the two arms would consist of hydrophobic amino acids. It is reasonable to assume that this region represents the fatty acid binding site of LSR. The glove-finger structure thus produced can accommodate an aliphatic hydrocarbon chain. The two amino acids are, more precisely in the case of rat LSR, two Prolines situated at positions 31 and 33 of the polypeptide sequence of the α subunit.

[0103] Still on the NH_2-terminal side is a consensus sequence for binding to clathrin, a protein which lawns the inner surface of the "coated pits" (Chen et al., 1990). These specific regions of the plasma membrane allow rapid endocytosis of membrane proteins. Such a consensus sequence is found at the level of the LRP-α_2-macroglobulin receptor, of CRAM and of the LDL receptor (Herz et al., 1988; Lee et al., 1990; Goldstein et al., 1995). The consequence of a mutation at this level is a substantial delay in the internalization of the LDLs and induces familial hypercholesterolemia (Davis et al., 1986).

[0104] The receptor then possesses a hydrophobic amino acid sequence which constitutes a potential transmembrane domain. The length of this segment allows only one passage across the phospholipid bilayer (Brendel et al., 1992).

[0105] Between this clathrin binding signal and the hydrophobic chain corresponding to the single transmembrane segment are 2 motifs LI et LL (Letourneur et al., 1992). These two motifs are found in the following proteins: glut 4 glucose carrier (Verhey et al., 1994); the nonvariant chain and the histocompatibility complex class II (Zhong et al., 1997; Parra-Lopez et al., 1997). These signals control endocytosis and the intracellular addressing of proteins in the peripheral membrane system.

[0106] On the C-terminal side, there is then a cysteine-rich region which exhibits homology with the cytokine receptors and more particularly: the TNF 1 and 2 (Tumor Necrosis Factor 1 and 2) receptors; the low-affinity NGF (Nerve Growth factor) receptor; the Shope fibroma virus TNF soluble receptor; CD40, CD27 and CD30, receptors for the cytokines CD40L, CD27L and CD30L; the T cell protein 4-1 BB, receptor for the putative cytokine 4-1BBL, the FAS antigen (APO 1), receptor for the FASL protein involved in apoptosis, the T cell 0X40 antigen, receptor for the cytokine 0X40L, and the vaccinia virus A53 protein (Cytokines and their receptors, 1996; Banner et al., 1993).

[0107] In addition to this cysteine-rich segment, there is a region of amino acids which are alternately charged + and – (Brendel et al., 1992). This region provides a potential binding site for the apoprotein ligands Apo B and Apo E.

[0108] This region contains, in addition, an RSRS motif found in lamin (Simos et al., 1994) and in SF2' (Krainer et al., 1991).

[0109] The LSR α' form encoded by the LSR-Rn-2040 cDNA possesses all the domains described above based on the LSR α sequence encoded by the LSR-Rn-2097 cDNA, with the exception of the LI/LL element, whose Leucine doublet is removed by alternative splicing. Although possessing sequences which are very similar, the subunits α encoded by LSR-Rn-2097 and α' encoded by LSR-Rn-2040 could therefore differ in their recycling rate and their addressing. The β form encoded by LSR-Rn-1893 does not possess a transmembrane domain or a region rich in cysteines and homologous to the cytokine receptors. However, it possesses at the NH_2-terminal level the hydrophobic region separated by a repetition of prolines, the region rich in charged amino acids and the RSRS motif. This constituent is probably positioned entirely outside the cell where it is bound via disulphide bridges either to the product of LSR-Rn-2040, or to that of LSR-Rn-2097.

[0110] Table 1 below lists the different domains or motifs described above, indicates whether or not they belong to each of the subunits of the LSR receptor, as well as the positions of the start and end of the said domains or motifs, or of regions carrying the said domains or motifs, as indicated in the sequence of SEQ ID NO:2.

TABLE 1

	Position on SEQ ID NO: 2		Presence on:		
Domain or motif	Start	End	α	α'	β
Potential fatty acid binding site	23	41	X	X	X
Potential clathrin binding site	104	107	X	X	X

TABLE 1-continued

Domain or motif	Position on SEQ ID NO: 2 Start	End	Presence on: α	α'	β
Signal for transport:					
LI	183	184	X	X	X
LL	195	196	X		
Transmembrane domain	204	213	X	X	
Potential cytokine receptor site	214	249	X	X	
RSRS motif	470	473	X	X	X
Potential lipoprotein ligand binding site	544	557	X	X	X

Comparison of the Polypeptide Sequences of the LSR Receptors in Rats, Mice and Humans

[0111] The lengths of the polypeptide sequences, as well as the sequence identifier numbers of their respective sequences in the listing included, of the three types of subunit of the LSR receptors according to the invention, in rats, mice and humans, are indicated in Table 2a below.

TABLE 2a

Polypeptide	Rat	Mouse	Human
α subunit	593 aa (SEQ ID NO: 2)	594 aa (SEQ ID NO: 16)	649 aa (SEQ ID NO: 8)
α' subunit	574 aa (SEQ ID NO: 4)	575 aa (SEQ ID NO: 17)	630 aa (SEQ ID NO: 10)
β subunit	525 aa (SEQ ID NO: 6)	526 aa (SEQ ID NO: 18)	581 aa (SEQ ID NO: 12)

[0112] These polypeptide sequences were obtained from each of the three corresponding cDNA sequences, in rats, mice and humans, which will be described in detail later. These polypeptide sequences were obtained from each of the three corresponding cDNA sequences, in rats, mice and humans, which will be described in detail later. The nomenclature used to designate these cDNA sequences, which reflects their length in terms of nucleotides, as well as the sequence identifier numbers of their respective sequences in the listing included, are presented in Table 2b below.

TABLE 2b

cDNAc	Rat	Mouse	Human
α subunit	LSR-Rn-2097 (SEQ ID NO: 1)	LSR-Mm-1886 (SEQ ID NO: 13)	LSR-Hs-2062 (SEQ ID NO: 7)
α' subunit	LSR-Rn-2040 (SEQ ID NO: 3)	LSR-Mm-1829 (SEQ ID NO: 14)	LSR-Hs-2005 (SEQ ID NO: 9)
β subunit	LSR-Rn-1893 (SEQ ID NO: 5)	LSR-Mm-1682 (SEQ ID NO: 15)	LSR-Hs-1858 (SEQ ID NO: 11)

[0113] The protein sequence, corresponding to the α subunit of the LSR receptor, deduced from the LSR-Hs-2062 sequence has a length of 649 amino acids. It is aligned with the protein sequences deduced from LSR-Mm-1886, 594 amino acids long, and from LSR-Rn-2097, 593 amino acids long (FIG. **2**). The conservation of the protein sequences is very high (respectively 80.2% and 82.2% identity for 591 and 590 overlapping amino acids). The functional domains identified in the protein sequence of the rat LSR α are found in the human LSR α sequence as well as in that of the murine LSR α (FIG. **2**).

[0114] The human proteins corresponding to the LSR-Hs-2005 (α') and LSR-Hs-1858 (β) forms have a predicted size of 630 and 581 amino acids respectively. The rat proteins corresponding to the LSR-Rn-2040 (α') and LSR-Rn-1893 (β) forms have a predicted size of 574 and 525 amino acids respectively. The mouse proteins corresponding to the LSR-Mm-1829 (α') and LSR-Mm-1682 (β) forms have a predicted size of 575 and 526 amino acids respectively. The alignment of the three human forms (FIG. **3**), of the three forms described in rats (FIG. **4**) and of the three forms described in mice (FIG. **5**) shows that in the three species, all the protein forms conserve the NPGY signal for binding to clathrin and the RSRS motif. The human (product of LSR-Hs-2062), rat (product of LSR-Rn-2097) and mouse (product of LSR-Mm-1886) long forms (α) exhibit all the functional characteristics of LSR. The three short forms (β) (respective products of LSR-Hs-1817, LSR-Rn-1893 and LSR-Mm-1682) lose the di-leucine domain for lysosomal addressing, the transmembrane domain and the cytokine receptor signature. It is also possible to observe that the three intermediate forms (α') (product of LSR-Hs-2005, of LSR-Rn-2040 and LSR-Mn-1829) lose the di-leucin domain, the transmembrane domain and the domain corresponding to the cytokine receptor signature being conserved (FIGS. **3**, **4** and **5**). FIG. **6** finally represents the proteins derived from the three cDNA forms identified in humans, and the motifs carried by each of them as a result of the splicing from which each is derived.

[0115] Table 3 below lists the different domains or motifs described above, as well as the positions of the start and end of the said domains or motifs, or of regions carrying the said domains or motifs, as indicated in the mouse SEQ ID NO:16 sequence.

TABLE 3

Domain or motif	Position on SEQ ID NO: 16 Start	End	Presence on: α	α'	β
Potential fatty acid binding site	23	41	X	X	X
Potential clathrin binding site	104	107	X	X	X
Signal for transport:					
LI	183	184	X	X	X
LL	195	196	X		
Transmembrane domain	204	213	X	X	
Potential cytokine receptor site	214	249	X	X	
RSRS motif	470	473	X	X	X
Potential lipoprotein ligand binding site	544	558	X	X	X

[0116] Table 4 below lists the different domains or motifs described above, as well as the positions of the start and end of the said domains or motifs, or of regions carrying the said domains or motifs, as indicated in the human SEQ ID NO:8 sequence.

TABLE 4

Domain or motif	Position on SEQ ID NO: 8 Start	End	Presence on: α	α'	β
Potential fatty acid binding site	76	94	X	X	X
Potential clathrin binding site	157	160	X	X	X
Signal for transport:					
LI	236	237	X	X	X
LL	248	249	X		
Transmembrane domain	257	266	X	X	
Potential cytokine receptor site	267	302	X	X	
RSRS motif	527	530	X	X	X
Potential lipoprotein ligand binding site	601	613	X	X	X

[0117] In conclusion, the similarity in the sequence and structure of LSR which is described above makes it possible to extrapolate to humans the observations made in rats and/or mice.

[0118] Homologous polypeptide will be understood to mean the polypeptides exhibiting, compared with the natural polypeptide, certain modifications such as in particular a deletion, truncation, extension, chimeric fusion and/or mutation, in particular a point mutation. Among the homologous polypeptides, those in which the amino acid sequence exhibits at least 80%, preferably 90%, homology with the amino acid sequences of the polypeptides according to the invention are preferred.

[0119] Equivalent polypeptide will be understood to mean a polypeptide having at least one of the activities of the LSR receptor, in particular the activity of the receptor for lipoproteins or chylomicrons, the activity of the receptor for cytokine, in particular leptin, or the activity of the receptor for the gC1q-R protein or one of its analogous proteins. Equivalent polypeptide will also be understood to mean any polypeptide resulting from the alternative splicing of the genomic nucleic sequence encoding the polypeptides according to the invention.

[0120] Variant polypeptide (or protein variant) will be understood to mean all the mutated polypeptides which may exist, in particular in human beings, and which correspond in particular to truncations, deletions and/or additions of amino acid residues, substitutions or mutations, in particular point mutations, as well as the artificial variant polypeptides which will nevertheless be called variant polypeptides. In the present case, the variant polypeptides will be in particular partly associated with the onset and with the development of obesity or anorexia. They may also be associated with the onset and/or development of the risks or complications associated with obesity, in particular at the cardiovascular level, and/or of pathologies associated with abnormalities in the metabolism of cytokines.

[0121] Polypeptide fragment is understood to mean a polypeptide or a peptide encoded by a nucleic sequence comprising a minimum of 15 nucleotides or bases, preferably 20 bases or 30 bases. These fragments may comprise in particular a point mutation, compared with the normal polypeptide sequence, or may correspond to specific amino acid sequences of variant polypeptides, artificial or existing in humans, such as those linked to a polymorphism linked in particular to obesity or to the abovementioned pathologies.

[0122] Biologically active fragment will be understood to mean in particular a fragment of an amino acid sequence of a polypeptide:

[0123] exhibiting at least one of the LSR receptor activities, in particular the lipoprotein receptor activity, or the cytokine, particularly leptin, receptor activity and/or cell signalling activity, and/or

[0124] capable of being recognized by an antibody specific for the receptor according to the invention, and/or

[0125] capable of being recognized by a compound capable, for example by neutralizing the binding of a ligand specific for the said receptor, of modulating the activity of the LSR receptor, and/or

[0126] capable of modulating the addressing and/or cellular location of the LSR receptor, and/or

[0127] more generally constituting a biologically active domain or motif of the LSR receptor.

[0128] Among the preferred biologically active fragments according to the invention, there are in particular:

[0129] the fragments comprising a clathrin binding site,

[0130] the fragments comprising a fatty acid binding site, in particular a fatty acid binding site comprising a hydrophobic amino acid sequence separated into two parts by two contiguous prolines, which induce a hairpin structure whose arms consist of hydrophobic amino acids,

[0131] the fragments comprising a hydrophobic region constituting a transmembrane domain,

[0132] the fragments comprising a region capable of controlling endocytosis and intracellular addressing of the proteins in the peripheral membrane system, in particular a fragment comprising a site containing the LI and LL motifs,

[0133] the fragments comprising a cytokine binding site, in particular a site including a cysteine-rich region,

[0134] the fragments comprising a region defining a potential binding site for lipoprotein ligands such as ApoB and ApoE, in particular a region comprising a sequence of amino acids alternately charged + and −, and

[0135] the fragments comprising an RSRS motif.

[0136] There are in particular among these fragments polypeptides as defined in Tables 1, 2 and 4, or any fragments of the nucleotides of SEQ ID NO:2, 8 or 16, comprising the said polypeptides, and any equivalent, homologous or variant fragments.

[0137] Other preferred fragments include antigenic peptides such as those having the sequences SEQ ID NOs:29 and 30.

Nucleotide Sequences of the LSR Receptor

[0138] The subject of the present invention is isolated nucleic acid sequences, characterized in that they encode an LSR receptor or a polypeptide according to the invention.

[0139] More particularly, the invention relates to a purified nucleic acid, characterized in that it comprises at least 8, preferably at least 10 and more particularly at least 15 consecutive nucleotides of the polynucleotide of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0140] The invention also relates to a purified nucleic acid, characterized in that it comprises at least 8, preferably at least 10 and more particularly at least 15 consecutive nucleotides

of the polynucleotide of SEQ ID NO:41, as well as the nucleic acid sequences complementary to this nucleic acid.

[0141] The invention also relates to a purified nucleic acid encoding the human LSR receptor, characterized in that it comprises a nucleotide sequence corresponding to nucleotides 1898 to 21094, particularly to nucleotides 2001 to 20979, more particularly to nucleotides 2145 to 20979 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0142] The invention also relates to the nucleic acid sequences contained in the gene encoding the human LSR receptor, in particular each of the exons of the said gene or a combination of exons of the said gene, or alternatively a polynucleotide extending over a portion of one or more exons. Preferably, these nucleic acids encode one or more biologically active fragments of the human LSR receptor.

[0143] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence corresponding to nucleotides 1 to 1897 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0144] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence corresponding to nucleotides 21095 to 22976 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0145] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence chosen from the group comprising the sequences of SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11, as well as the nucleic acid sequences complementary to this nucleic acid.

[0146] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence chosen from the group comprising the sequences of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, as well as the nucleic acid sequences complementary to this nucleic acid.

[0147] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence chosen from the group comprising the sequences of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15, as well as the nucleic acid sequences complementary to this nucleic acid.

[0148] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence corresponding to nucleotides 1898 to 2001 of SEQ ID NO:19 or preferably to nucleotides 1898 to 2144 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0149] The invention also relates to a purified nucleic acid, characterized in that it comprises a nucleotide sequence corresponding to nucleotides 20980 to 21094 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid.

[0150] Among the nucleic acids according to the invention, the nucleic acids having the nucleotide sequence chosen from the group comprising the sequences of SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11, the sequences of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, as well as the sequences of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15, as well as their complementary sequences, are preferred.

[0151] Also forming part of the invention are the variant, mutated, equivalent or homologous sequences of the sequences according to the invention, as well as their fragments and the nucleic sequences capable of hybridizing specifically with the sequences according to the invention.

Human Genomic Sequence

[0152] The invention therefore relates to the genomic sequence of the human LSR receptor, preferably the sequence of SEQ ID NO:19, as well as their complementary sequences or one of their allelic variants, the mutated, equivalent or homologous sequences, or one of their fragments.

[0153] The gene for human LSR (SEQ ID NO:19) comprises 10 exons distributed over 21 094 bp. The size of the exons is respectively: 356, 345, 120, 57, 147, 174, 60, 132, 626 and 141 bp (Table 5).

TABLE 5

EXON	START	END	5' SPLIC.	BL 5'	3' SPLIC.	BL 3'
Ex1	1898	2253	—	—	GTACGG	+2
Ex2	3437	3781	CAG	+1	GTATGT	+1
Ex3	12067	12186	CAG	+2	GTGAGT	+1
Ex4	15047	15103	CAG	+2	GTACGG	+1
Ex5	15668	15814	CAG	+2	GTAAGT	+1
Ex6	19481	19654	CAG	+2	GTGAGG	+1
Ex7	19801	19860	CAG	+2	GTGAGA	+1
Ex8	19958	20089	TAG	+2	GTAAGC	+1
Ex9	20231	20856	CAG	+2	GTGAGG	0
Ex10	20946	21094	CAG	0	—	

[0154] The EXON column indicates the exons numbered from 1 to 10 in the 5'-3' order of their position on the genomic sequence. The START and END columns indicate respectively the position of the first and of the last nucleotide of the exon considered. The sequences of the splicing site bordering the exon in 5' and 3 are indicated in the 5'SPLIC. and 3'SPLIC columns. The BL 5' and BL 3' columns indicate the number of bases in 5' and in 3', respectively, of an exon which will be used in the reading frame of the messenger only after splicing. For example: as exon 7 has a free base in 3', this exon can be joined to the 5' end of exon 8 which has 2 free bases in 5'. The combination 1 base+2 bases constitutes the codon which was destroyed by the intron in the genomic sequence. Exon 7 may be joined by its 3' end to any exon having two free bases in 5'; if the new codon created does not correspond to a stop codon, the open reading frame will be conserved.

[0155] Exons 1 and 2 as well as 9 and 10 are necessarily co-spliced, thus forming a 5' block corresponding to exons 1 and 2 and a 3' block corresponding to exons 9 and 10. The functional minimal messenger, corresponding to the product of these four exons, could therefore have a size of about 1 331 bp. For the other exons, all the possible combinations make it possible to conserve the open reading frame.

[0156] The size of the noncoding exons in 5' could not be determined with precision. Indeed, the rat 5' UTR sequences are too divergent from those of humans to finalize the analysis of these sequences and to identify the real 5' end of the human LSR cDNA. This can be carried out by isolating the 5' end of the human LSR messengers by the 5' end capture methods developed by the inventors (WO 96/34981). The polyadeny-

lation site described below is the only one which is present before the USF2 gene, situated in 3' of the human LSR gene. It is therefore very likely that the untranslated 3' region of this gene is very short (of an estimated size of about 100 bp). All the sizes given in relation to the human LSR cDNA molecules will therefore have to be adjusted according to the size of the untranslated 5' end. The human cDNA sequence obtained taking into account all the exons deduced from the analysis of the genomic sequence have a size of 2 158 bp. This form could correspond to the LSR-Rn-2097 form.

[0157] The location of some of the signals for expression of the nucleotide sequence of SEQ ID NO:19 is presented in Table 6 which follows.

TABLE 6

Signal	Start	End
preferred ATG	2145	2147
other possible ATG	2001	2003
STOP	20977	20979
POLY Ad	21065	21070

[0158] The characteristic elements of the messenger RNA molecule are described in the Signal column: Initiation of translation (ATG), termination of translation (STOP) and polyadenylation signal (POLY Ad). The Start and End columns indicate the position as nucleotide for the start and end of these signals on the genomic sequence SEQ ID NO:19. An ATG signal for initiation of translation is preferred to another because it provides an environment which is more suitable for initiation.

[0159] The present invention also relates to the purified nucleic acid sequences encoding one or more elements for regulating the expression of the human LSR gene. Also included in the invention are the nucleic acid sequences of the promoter and/or regulator of the gene encoding the receptor according to the invention, or one of their allelic variants, the mutated, equivalent or homologous sequences, or one of their fragments.

[0160] The invention relates more particularly to a purified nucleic acid situated in 5' of the coding sequence of the LSR gene. This nucleic acid is characterized in that it comprises a nucleotide sequence corresponding to nucleotides 1 to 1897 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid. Shorter fragments of this nucleic acid may also be used as promoters for expression of the LSR gene or of any other sequence encoding a heterologous polypeptide.

[0161] The invention also relates to a purified nucleic acid situated in 3' of the transcribed sequence of the LSR gene. This nucleic acid is characterized in that it comprises a nucleotide sequence corresponding to nucleotides 21095 to 22976 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid. Shorter fragments of this nucleic acid can also be used as elements regulating the expression of genes.

[0162] Finally, the invention also relates to the genomic sequence of the human LSR receptor, preferably the sequence of SEQ ID NO:41, as well as their complementary sequences, or one or their allelic variants, the mutated, equivalent or homologous sequences, or one of their fragments.

Comparison of the Genomic Organizations in Humans, Rats and Mice

[0163] It is advantageous to note that a syntheny (conservation of the organization of certain chromosomal regions between species) between the mouse chromosome 7 region where the Lisch7 gene is located, in the immediate vicinity of USF2, and the human chromosome 19 region 19q13, carrying LSR, is well described. The organization of the two Lisch7/LSR and USF2 genes is conserved between species. Likewise, Apo E, which is of a more centromeric location relative to these genes, exists both in mice and in humans. It is remarkable that the LSR lipoprotein receptor and one of their ligands ApoE are located in the same chromosomal region. Indeed, the receptor and the ligand are frequently co-regulated. Such a situation would make it possible to envisage that the phenomena observed in mice are applicable to humans.

Human, Rat and Mouse cDNA Sequences

[0164] The invention relates, in addition, to 3 different cDNAs derived from the LSR receptor gene by alternative splicing. These 3 cDNAs have been identified in humans, rats and mice (Table 2b). They encode the three types of LSR receptor subunits, α (long), α' (intermediate) and β (short). The longest cDNA contains the totality of the 10 exons of the gene. The intermediate cDNA does not comprise exon 4. Finally, the shortest cDNA does not contain exons 4 and 5.

[0165] The human LSR-Hs-2062 cDNA nucleotide sequence, encoding the α subunit of the LSR receptor, and the rat LSR-Rn-2097 cDNA nucleotide sequence are 78.6% identical over 1 955 bp which overlap. These figures are respectively 78.8% and 1 851 bp when the murine LSR-Mm-1886 sequence (long form) is aligned with the human sequence. This reflects a very high conservation of the nucleic sequences between species. The highest divergence levels are observed in the untranslated 5' end (when the sequence is available), in the first coding exon and in the untranslated 3' end (FIGS. **7**A, **7**B, **7**C, **7**D and **7**E).

[0166] The invention therefore also relates to a purified nucleic acid, characterized in that it is chosen from the group comprising the sequences of SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11, the sequences SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, and the sequences SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15, as well as the nucleic acid sequences complementary to this nucleic acid, or one of their allelic variants, the mutated, equivalent or homologous sequences, or one of their fragments.

[0167] The nucleic acids constituting the coding frames of the abovementioned nucleic acids, between the codons for initiation and for termination of translation, also form part of the invention.

[0168] The nucleic acids encoding the polypeptide fragments according to the invention are also part of the invention. It will be particularly noted that the nucleic acids encode the fragments described in Tables 1, 3 and 4.

[0169] Thus, Table 7 describes the position of such nucleic acid fragments on the human sequence of SEQ ID NO:7.

TABLE 7

	Position on the cDNA of SEQ NO: 7	
Domain or motif	Start	End
Potential fatty acid binding site	329	385
Potential clathrin binding site	572	583
Signal for transport:		
LI	809	814
LL	845	850
Transmembrane domain	872	901

TABLE 7-continued

Domain or motif	Position on the cDNA of SEQ NO: 7 Start	End
Potential cytokine receptor site	902	1009
RSRS motif	1682	1693
Potential lipoprotein ligand binding site	1904	1942

[0170] The invention also relates to a purified nucleic acid corresponding to the sequence of the 5'UTR of the cDNAs encoding the human LSR receptor. This nucleic acid is characterized in that it comprises a nucleotide sequence corresponding to nucleotides 1898 to 2001 of SEQ ID NO:19 or preferably to nucleotides 1898 to 2144 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid. Shorter fragments of this nucleic acid can also be used.

[0171] The invention also relates to a purified nucleic acid corresponding to the sequence of the 3'UTR of the cDNAs encoding the LSR receptor. This nucleic acid is characterized in that it comprises a nucleotide sequence corresponding to nucleotides 20980 to 21094 of SEQ ID NO:19, as well as the nucleic acid sequences complementary to this nucleic acid. Shorter fragments of this nucleic acid can also be used.

[0172] The invention also relates to the purified nucleic acids corresponding respectively to the sequences of the 5'UTR or of the 3'UTR of the cDNAs encoding the rat or mouse LSR receptor. Shorter fragments of this nucleic acid can also be used.

[0173] The 5'UTR and 3'UTR may contain elements ("responsive elements" and "enhancers") which are involved in the regulation of transcription and of translation. These regions have in particular a role in the stability of the mRNAs. Furthermore, the 5'UTR comprises the Shine-Delgarno motif which is essential for the translation of the mRNA.

[0174] Nucleic acid, nucleic sequence or nucleic acid sequence are understood to mean an isolated natural, or a synthetic, DNA and/or RNA fragment comprising, or otherwise, non-natural nucleotides, designating a precise succession of nucleotides, modified or otherwise, allowing a fragment, a segment or a region of a nucleic acid to be defined.

[0175] Equivalent nucleic sequences are understood to mean nucleic sequences encoding the polypeptides according to the invention taking into account the degeneracy of the genetic code, the complementary DNA sequences and the corresponding RNA sequences, as well as the nucleic sequences encoding the equivalent polypeptides.

[0176] Homologous nucleic sequences are understood to mean the nucleic sequences encoding the homologous polypeptides and/or the nucleic sequences exhibiting a level of homology of at least 80%, preferably 90%. According to the invention, the homology is only of the statistical type, which means that the sequences have a minimum of 80%, preferably 90%, of nucleotides in common. They are preferably sequences capable of hybridizing specifically with a sequence of the invention. Preferably, the specific hybridization conditions will be like those found in the examples, or such that they ensure at least 95% homology.

[0177] The length of these nucleic sequences for hybridization can vary from 8, 10, 15, 20 or 30 to 200 nucleotides, particularly from 20 to 50 nucleotides, more particularly from 20 to 30 nucleotides.

[0178] Allele or allelic variant will be understood to mean the natural mutated sequences corresponding to polymorphisms present in human beings and, in particular, to polymorphisms which can lead to the onset and/or to the development of obesity or of anorexia. These polymorphisms can also lead to the onset and/or to the development of risks or complications associated with obesity, in particular at the cardiovascular level, and/or of pathologies associated with abnormalities in the metabolism of cytokines.

[0179] Mutated nucleic sequences are understood to mean the nucleic sequences comprising at least one point mutation compared with the normal sequence.

[0180] While the sequences according to the invention are in general normal sequences, they are also mutated sequences since they comprise at least one point mutation and preferably at most 10% of mutations compared with the normal sequence.

[0181] Preferably, the present invention relates to mutated nucleic sequences in which the point mutations are not silent, that is to say that they lead to a modification of the amino acid encoded in relation to the normal sequence. Still more preferably, these mutations affect amino acids which structure the LSR complex and/or receptor or the corresponding domains and fragments thereof. These mutations may also affect amino acids carried by the regions corresponding to the receptor sites, for lipoproteins or cytokines, in particular leptin, or to sites for binding of cofactors, in particular or free fatty acids, or alternatively to phosphorylation sites. These mutations may also affect the sequences involved in the transport, addressing and membrane anchorage of LSR.

[0182] In general, the present invention relates to the normal LSR polypeptides, the mutated LSR polypeptides as well as fragments thereof and to the corresponding DNA and RNA sequences, the LSR polypeptides designating polypeptides of the receptor according to the invention.

[0183] According to the invention, the fragments of nucleic sequences may in particular encode domains of receptors and polypeptides possessing a function or a biological activity as defined above, contain domains or regions situated upstream or downstream of the coding sequence and containing elements for regulating the expression of the LSR gene or alternatively possessing a sequence allowing their use as a probe or as a primer in methods of detection, identification or amplification of nucleic sequences. These fragments preferably have a minimum size of 8, of 10 bases, and fragments of 20 bases, and preferably of 30 bases, will be preferred.

[0184] Among the nucleic fragments which may be of interest, in particular for diagnosis, there should be mentioned, for example, the genomic intron sequences of the gene for the LSR complex, such as in particular the joining sequences between the introns and the exons, normal or mutated.

[0185] The nucleic acid sequences which can be used as sense or antisense oligonucleotides, characterized in that their sequences are chosen from the sequences according to the invention, also form part of the invention.

[0186] Among the nucleic acid fragments of interest, there should thus be mentioned, in particular the antisense oligonucleotides, that is to say whose structure ensures, by hybridization with the target sequence, inhibition of the expression of the corresponding product. There should also be mentioned the sense oligonucleotides which, by interaction with

the proteins involved in the regulation of the expression of the corresponding product, will induce either inhibition, or activation of this expression.

[0187] The sequences carrying mutations which may be involved in the promoter and/or regulatory sequences of the genes for the LSR complex, which may have effects on the expression of the corresponding proteins, in particular on their level of expression, also form part of the preceding sequences according to the invention.

[0188] The nucleic sequences which can be used as primer or probe, characterized in that their nucleic sequence is a sequence of the invention, also form part of the invention.

[0189] The present invention relates to all the primers which may be deduced from the nucleotide sequences of the invention and which may make it possible to detect the said nucleotide sequences of the invention, in particular the mutated sequences, using in particular a method of amplification such as the PCR method, or a related method.

[0190] The present invention relates to all the probes which may be deduced from the nucleotide sequences of the invention, in particular sequences capable of hybridizing with them, and which may make it possible to detect the said nucleotide sequences of the invention, in particular to discriminate between the normal sequences and the mutated sequences.

[0191] The invention also relates to the use of a nucleic acid sequence according to the invention as a probe or a primer for the detection and/or the amplification of a nucleic acid sequence according to the invention.

[0192] All the probes and primers according to the invention may be labelled by methods well known to persons skilled in the art, in order to obtain a detectable and/or quantifiable signal.

[0193] The present invention also relates to the nucleotide sequences which may comprise non-natural nucleotides, in particular sulphur-containing nucleotides, for example, or nucleotides of α or β structure.

[0194] The present invention relates, of course, to both the DNA and RNA sequences, as well as the sequences which hybridize with them, as well as the corresponding double-stranded DNAs.

[0195] In the text which follows, the preceding DNA sequences will be called genes for the LSR complex, whether they are normal or pathologic sequences.

[0196] It should be understood that the present invention does not relate to the genomic nucleotide sequences in their natural chromosomal environment, that is to say in the natural state. They are sequences which have been isolated, that is to say that they have been collected directly or indirectly, for example by copying (cDNA), their environment having been at least partially modified.

[0197] Thus, this may also be both cDNA and genomic DNA, partially modified or carried by sequences which are at least partially different from the sequences carrying them naturally.

[0198] These sequences may also be termed non-natural.

[0199] The invention also comprises methods for screening cDNA and genomic DNA libraries, for the cloning of the isolated cDNAs, and/or the genes coding for the receptor according to the invention, and for their promoters and/or regulators, characterized in that they use a nucleic sequence according to the invention. Among these methods, there may be mentioned in particular:

[0200] the screening of cDNA libraries and the cloning of the isolated cDNAs (Sambrook et al., 1989; Suggs et al., 1981; Woo et al., 1979), with the aid of the nucleic sequences according to the invention,

[0201] the screening of 5' end tag libraries (WO 96/34981) for nucleic sequences according to the invention, and thus the isolation of tags allowing the cloning of complete cDNAs and the corresponding promoters from genomic DNA libraries,

[0202] the screening of genomic libraries, for example of BACs, (Chumakov et al., 1992; Chumakov et al., 1995) and, optionally, a genetic analysis by FISH (Cherif et al., 1990) with the aid of sequences according to the invention, allowing isolation and chromosomal location, and then the complete sequencing of the genes encoding the LSR receptor.

[0203] Also included in the invention is a sequence, in particular a genomic sequence encoding a receptor or a polypeptide according to the invention, or a nucleic acid sequence of a promoter and/or regulator of a gene encoding a receptor or a polypeptide according to the invention, or one of their allelic variants, a mutated, equivalent or homologous sequence, or one of their fragments, characterized in that it is capable of being obtained by one of the preceding methods according to the invention, or a sequence capable of hybridizing with the said sequences.

Vectors, Host Cells and Transgenic Animals

[0204] The invention also comprises the cloning and/or expression vectors containing a nucleic acid sequence according to the invention.

[0205] The vectors according to the invention, characterized in that they comprise the elements allowing the expression and/or the secretion of the said sequences in a host cell, also form part of the invention.

[0206] The vectors characterized in that they comprise a promoter and/or regulator sequence according to the invention, or a sequence for cellular addressing according to the invention, or one of their fragments, also form part of the invention.

[0207] The said vectors will preferably comprise a promoter, signals for initiation and termination of translation, as well as appropriate regions for regulation of transcription. They must be able to be stably maintained in the cell and may optionally possess particular signals specifying the secretion of the translated protein.

[0208] These different control signals are chosen according to the cellular host used. To this end, the nucleic acid sequences according to the invention may be inserted into autonomously replicating vectors inside the chosen host, or integrative vectors of the chosen host.

[0209] Among the autonomously replicating systems, there will be preferably used according to the host cell, systems of the plasmid or viral type, it being possible for the viral vectors to be in particular adenoviruses (Perricaudet et al., 1992), retroviruses, poxviruses or herpesviruses (Epstein et al., 1992). Persons skilled in the art know the technologies which can be used for each of these systems.

[0210] When the integration of the sequence into the chromosomes of the host cell is desired, it will be possible to use, for example, systems of the plasmid or viral type; such viruses will be, for example, retroviruses (Temin, 1986), or AAVs (Carter, 1993).

[0211] Such vectors will be prepared according to the methods commonly used by persons skilled in the art, and the clones resulting therefrom may be introduced into an appropriate host by standard methods such as, for example, lipofection, electroporation or heat shock.

[0212] The invention comprises, in addition, the host cells, in particular eukaryotic and prokaryotic cells, transformed by the vectors according to the invention, as well as transgenic animals, except humans, comprising one of the said transformed cells according to the invention.

[0213] Among the cells which can be used for these purposes, there may of course be mentioned bacterial cells (Olins and Lee, 1993), but also yeast cells (Buckholz, 1993), as well as animal cells, in particular mammalian cell cultures (Edwards and Aruffo, 1993), and in particular Chinese hamster ovary cells (CHO), but also insect cells in which it is possible to use methods using baculoviruses, for example (Luckow, 1993). A preferred cellular host for the expression of the proteins of the invention consists of the CHO cells.

[0214] Among the mammals according to the invention, there will be preferred animals such as mice, rats or rabbits, expressing a polypeptide according to the invention, the phenotype corresponding to the normal or variant LSR receptor, in particular mutated of human origin.

[0215] Among the animal models more particularly of interest here, there are in particular:

[0216] transgenic animals exhibiting a deficiency in one of the components of LSR. They are obtained by homologous recombination on embryonic stem cells, transfer of these stem cells to embryos, selection of the chimeras affected at the level of the reproductive lines, and growth of the said chimeras;

[0217] transgenic mice overexpressing one or more of the genes for the LSR complex of murine and/or human origin. The mice are obtained by transfection of multiple copies of the genes for the LSR complex under the control of a strong promoter of an ubiquitous nature, or selective for a type of tissue, preferably the liver;

[0218] transgenic animals (preferably mice) made deficient in one or more of the genes for the LSR complex, by inactivation with the aid of the LOXP/CRE recombinase system (Rohlmann et al., 1996) or any other system for inactivating the expression of a gene at a precise age of the animal;

[0219] animals (preferably rats, rabbits, mice) overexpressing one or more of the genes for the LSR complex, after viral transcription or gene therapy;

[0220] crossings of animals deficient in LSR (in particular mice) with animals deficient in, or overexpressing:

[0221] the LDL receptor (Herz et al., 1995; Ishibashi et al., 1993)

[0222] hepatic lipase (Homanics et al., 1995; Kobayashi et al., 1996)

[0223] apoprotein B (Purcellhuynh et al., 1995; Fan et al., 1995)

[0224] apoprotein E (Plump et al., 1992; Zhang et al., 1992; Huang et al., 1996)

[0225] apoCIII (Aalto-Setälä et al., 1992; Ito et al., 1990; Maeda et al., 1994).

[0226] The production of transgenic animals, and the viral or nonviral transfections will be preferably carried out on the following rat and mouse lines:

[0227] Zucker rat (fa/fa) (Iida et al., 1996)

[0228] AKR/J mouse (West et al., 1992)

[0229] ob/ob mouse (Zhang et al., 1994)

[0230] ob^2j/ob^2j mouse (ibid)

[0231] tubby mouse (Kleyn et al., 1996; Nobben-Trauth et al., 1996)

[0232] fat/fat (Heldin et al., 1995)

[0233] agouti mouse (Lu et al., 1994; Manne et al., 1995)

[0234] db/db mouse (Chen et al., 1996).

[0235] The cells and mammals according to the invention can be used in a method for the production of a polypeptide according to the invention, as described below, and can also serve as a model for analysis and screening.

[0236] The transformed cells or mammals as described above can also be used as models so as to study the interactions between the polypeptides of the LSR complex, between these and their partners, chemical or protein compounds, which are involved directly or indirectly in the activities of the receptor for lipoproteins or the receptor for cytokines, and in particular for leptin, and in order to study the different mechanisms and interactions called into play according to the type of activity, or according to whether a normal complex is involved, or a complex in which at least one of the domains is a variant.

[0237] In particular, they may be used for the selection of products which interact with the LSR complex, or one of its normal or variant domains, as cofactor or as inhibitor, in particular a competitive inhibitor, or alternatively having an agonist or antagonist activity on the conformational changes in the LSR complex. Preferably, the said transformed cells will be used as a model allowing, in particular, the selection of products which make it possible to combat obesity or the pathologies mentioned above. The said cells may also serve for the detection of the potential risks posed by certain compounds.

Production of Polypeptides Derived from the LSR Receptor

[0238] The invention also relates to the synthesis of synthetic or recombinant polypeptides of the invention, in particular by chemical synthesis or by the use of a nucleic acid sequence according to the invention.

[0239] The polypeptides according to the present invention can be obtained by chemical synthesis using any of the numerous known peptide syntheses, for example the techniques using solid phases or techniques using partial solid phases, by condensation of fragments or by a conventional synthesis in solution.

[0240] When the compounds according to the present invention are synthesized by the solid phase method, the C-terminal amino acid is bound to an inert solid support and comprises groups protecting its amino group at the alpha position (and if necessary, protection on its functional side groups).

[0241] At the end of this step, the group protecting the amino-terminal group is removed and the second amino acid, it too comprising the necessary protection, is bound.

[0242] The N-terminal protecting groups are removed after each amino acid has been bound; on the other hand, the protection is of course maintained on the side chains. When the polypeptide chain is complete, the peptide is cleaved from its support and the side protecting groups are removed.

[0243] The solid phase synthesis technique is well known to a person skilled in the art. See in particular Stewart et al. (1984) and Bodansky (1984).

[0244] The polypeptides obtained by chemical synthesis and which may comprise corresponding non-natural amino acids are also included in the invention.

[0245] The method for the production of a polypeptide of the invention in recombinant form is itself included in the present invention, and is characterized in that the transformed cells, in particular the cells or mammals of the present invention, are cultured under conditions allowing the expression of a recombinant polypeptide encoded by a nucleic acid sequence according to the invention, and in that the said recombinant polypeptide is recovered.

[0246] Also forming part of the invention is a method for the production of a heterologous polypeptide, characterized in that it uses a vector or a host cell containing at least one of the promoter and/or regulatory sequences according to the invention, or at least one of the sequences for cellular addressing according to the invention, or one of their fragments.

[0247] The recombinant polypeptides, characterized in that they are capable of being obtained by the said method of production, also form part of the invention.

[0248] The recombinant polypeptides obtained as indicated above may be both in glycosylated and nonglycosylated form and may or may not have the natural tertiary structure.

[0249] These polypeptides may be produced from the nucleic acid sequences defined above, according to techniques for the production of recombinant polypeptides known to persons skilled in the art. In this case, the nucleic acid sequence used is placed under the control of signals allowing its expression in a cellular host.

[0250] An effective system of production of a recombinant polypeptide requires having a vector and a host cell according to the invention.

[0251] These cells may be obtained by introducing into the host cells a nucleotide sequence inserted into a vector as defined above, and then culturing the said cells under conditions allowing the replication and/or expression of the transfected nucleotide sequence.

[0252] The methods for the purification of a recombinant polypeptide which are used are known to persons skilled in the art. The recombinant polypeptide may be purified from cell lysates and extracts, from the culture medium supernatant, by methods used individually or in combination, such as fractionation, chromatographic methods, immunoaffinity techniques with the aid of specific mono- or polyclonal antibodies, and the like.

[0253] A preferred variant consists in producing a recombinant polypeptide fused with a "carrier" protein (chimeric protein). The advantage of this system is that it allows a stabilization and a reduction in proteolysis of the recombinant product, an increase in solubility during in vitro renaturation and/or simplification of the purification when the fusion partner has affinity for a specific ligand.

Antibodies

[0254] The mono- or polyclonal antibodies or fragments thereof, chimeric or immunoconjugated antibodies, characterized in that they are capable of specifically recognizing a polypeptide or receptor according to the invention, also form part of the invention.

[0255] Specific polyclonal antibodies may be obtained from a serum of an animal immunized against, for example:

[0256] the LSR receptor purified from membranes of cells carrying the said LSR receptor, by methods well known to persons skilled in the art such as affinity chromatography using, for example, recombinant leptin as specific ligand, or

[0257] a polypeptide according to the invention, in particular produced by genetic recombination or by peptide synthesis, according to the customary procedures, from a nucleic acid sequence according to the invention.

[0258] There may be noted in particular the advantage of antibodies specifically recognizing certain polypeptides, variants or fragments, which are in particular biologically active, according to the invention.

[0259] The specific monoclonal antibodies may be obtained according to the conventional hybridoma culture method described by Kohler and Milstein, 1975.

[0260] The antibodies according to the invention are, for example, chimeric antibodies, humanized antibodies, Fab or F(ab')2 fragments. They may also be in the form of immunoconjugates or of labelled antibodies so as to obtain a detectable and/or quantifiable signal.

[0261] The invention also relates to methods for the detection and/or purification of a polypeptide according to the invention, characterized in that they use an antibody according to the invention.

[0262] The invention comprises, in addition, purified polypeptides, characterized in that they are obtained by a method according to the invention.

[0263] Moreover, in addition to their use for the purification of polypeptides, the antibodies of the invention, in particular the monoclonal antibodies, may also be used for the detection of these polypeptides in a biological sample.

[0264] They thus constitute a means for the immunocytochemical or immunohistochemical analysis of the expression of the polypeptide of the LSR receptor on specific tissue sections, for example by immunofluorescence, gold labelling, enzymatic immunoconjugates.

[0265] They make it possible in particular to detect abnormal expression of these polypeptides in the biological tissues or samples, which makes them useful for the detection of abnormal expression of the LSR receptor or for monitoring the progress of the method of prevention or treatment.

[0266] More generally, the antibodies of the invention may be advantageously used in any situation where the expression of a polypeptide of the LSR receptor, normal or mutated, needs to be observed.

Detection of Allelic Variability and Diagnosis

[0267] Also forming part of the invention are the methods for the determination of an allelic variability, a mutation, a deletion, a loss of heterozygosity or a genetic abnormality, characterized in that they use a nucleic acid sequence or an antibody according to the invention.

[0268] These methods relate to, for example, the methods for the diagnosis of predisposition to obesity, to the associated risks, or to pathologies associated with abnormalities in the metabolism of cytokines, by determining, in a biological sample from the patient, the presence of mutations in at least one of the sequences described above. The nucleic acid sequences analysed may be either the genomic DNA, the cDNA or the mRNA.

[0269] It will also be possible to use nucleic acids or antibodies based on the present invention in order to allow a positive and differential diagnosis in a patient taken in isolation. The nucleic sequences will be preferably used for a pre-symptomatic diagnosis in an at risk subject, in particular with a familial history. It is also possible to envisage an ante-natal diagnosis.

[0270] In addition, the detection of a specific mutation may allow an evolutive diagnosis, in particular as regards the intensity of the pathology or the probable period of its appearance.

[0271] The methods allowing the detection of a mutation in a gene compared with the natural gene are, of course, highly numerous. They can essentially be divided into two large categories. The first type of method is that in which the presence of a mutation is detected by comparing the mutated sequence with the corresponding nonmutated natural sequence, and the second type is that in which the presence of the mutation is detected indirectly, for example by evidence of the mismatches due to the presence of the mutation.

[0272] These methods can use the probes and primers of the present invention which are described. They are generally purified nucleic sequences for hybridization comprising at least 8 nucleotides, characterized in that they can hybridize specifically with a nucleic sequence chosen from the group comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14 SEQ ID NO:15, SEQ ID NO:19 and SEQ ID NO:41. Preferably, the specific hybridization conditions are like those defined in the examples, or such that they ensure at least 95% homology. The length of these nucleic sequences for hybridization can vary from 8, 10, 15, 20 or 30 to 200 nucleotides, particularly from 20 to 50 nucleotides, more particularly from 20 to 30 nucleotides.

[0273] Among the methods for the determination of an allelic variability, a mutation, a deletion, a loss of heterozygocity or a genetic abnormality, the methods comprising at least one stage for the so-called PCR (polymerase chain reaction) or PCR-like amplification of the target sequence according to the invention likely to exhibit an abnormality with the aid of a pair of primers of nucleotide sequences according to the invention are preferred. The amplified products may be treated with the aid of an appropriate restriction enzyme before carrying out the detection or assay of the targeted product.

[0274] PCR-like will be understood to mean all methods using direct or indirect reproductions of nucleic acid sequences, or alternatively in which the labelling systems have been amplified, these techniques are of course known, in general they involve the amplification of DNA by a polymerase; when the original sample is an RNA, it is advisable to carry out a reverse transcription beforehand. There are currently a great number of methods allowing this amplification, for example the so-called NASBA "Nucleic Acid Sequence Based Amplification" (Compton 1991), TAS "Transcription based Amplification System" (Guatelli et al., 1990), LCR "Ligase Chain Reaction" (Landegren et al., 1988), "Endo Run Amplification" (ERA), "Cycling Probe Reaction" (CPR), and SDA "Strand Displacement Amplification" (Walker et al., 1992), methods well known to persons skilled in the art.

[0275] The invention comprises, in addition, methods for the diagnosis of pathologies and/or pathogeneses correlated with abnormal expression of a polypeptide and/or a receptor according to the invention, characterized in that an antibody according to the invention is brought into contact with the biological material to be tested, under conditions allowing the possible formation of specific immunological complexes between the said polypeptide and the said antibody, and in that the immunological complexes possibly formed are detected.

[0276] Mutations in one or more genes of the LSR complex may be responsible for various modifications of their product (s), which modifications can be used for a diagnostic approach. Indeed, modifications of antigenicity can allow the development of specific antibodies. The discrimination between the various conformations of LSR can be achieved by these methods. All these modifications may be used in a diagnostic approach by virtue of several well-known methods based on the use of mono- or polyclonal antibodies recognizing the normal polypeptide or mutated variants, such as for example using RIA or ELISA.

[0277] These diagnostic methods also relate to the methods of diagnosis by imaging in vivo or ex vivo using the monoclonal or polyclonal antibodies according to the invention, particularly those labelled and corresponding to all or part of the mutated polypeptides (imaging with the aid of antibodies coupled to a molecule which is detectable in PET-scan type imaging, for example).

Screening of Compounds of Interest

[0278] Also included in the invention are the methods for selecting the chemical or biochemical compound capable of interacting, directly or indirectly, with the receptor according to the invention, and/or allowing the expression or the activity of the said receptor to be modulated, characterized in that they use a receptor, a nucleic acid, a polypeptide, a vector, a cell or a mammal according to the invention.

Screening of Compounds Modifying the Activity of the LSR Receptor

[0279] The invention relates to a method for screening compounds modifying the activity of the LSR receptor, consisting in measuring the effect of candidate compounds on various parameters reflecting, directly or indirectly, taken independently or in combination, an LSR receptor activity.

[0280] For the screening of compounds capable of modulating the LSR activity for lipoprotein clearance, the preferred principal effect is the effect of the compound on the activity of binding, internalization and degradation of the lipoproteins by the LSR receptor.

[0281] This effect can be analysed in the absence or in the presence of free fatty acids, or of any other agent known to induce or to inhibit the activity of LSR on the clearance of lipoproteins, or in the absence or the presence of leptin, or of any other agent capable of inducing or of inhibiting the LSR function of cytokine clearance. It can, in addition, be measured in the absence or in the presence of agents capable of promoting or reducing the lipase activities, either intracellular or extracellular, as well as in the presence or in the absence of alternative known routes of degradation of lipoproteins.

[0282] Various indirect parameters can also be measured, including the following:

[0283] the change in weight induced by the administration of the compound

[0284] the food intake induced by the administration of the compound

[0285] the postprandial lipemic response induced by the administration of the compound, before, during or after ingestion of a meal, for example high in fat.

[0286] The selection of compounds capable of influencing the plasma triglyceride concentrations, and/or the binding, internalization and hepatic degradation of lipoproteins or particles high in triglycerides, will be preferred.

[0287] For the screening of compounds capable of modulating the LSR activity of clearance of cytokines, in particular of leptin, the preferred principal effect is the effect of the compound on the activity of binding, internalization and hepatic degradation of cytokines by the LSR receptor, in the absence or in the presence of free fatty acids.

[0288] The measurement of the binding, internalization and/or degradation of lipids or of cytokines can be carried out, for example, on hepatocytes or fibroblasts in culture, or on any other cell expressing the LSR receptor at its surface. The cells will be preferably cells expressing a recombinant LSR receptor, more particularly cells expressing a recombinant LSR receptor and whose endogenous LSR receptor would be inactivated or absent. These cells may or may not express the LDL receptor.

[0289] The screening of compounds modulating the LSR activity preferably uses cells or model animals according to the invention, in particular mice, rats or humans, more particularly those described above and in the examples which follow.

Screening of Compounds Modifying the Expression of the LSR Receptor

[0290] Screening may be used to test compounds capable of modifying the level and/or the specificity of expression of the LSR receptor either by binding competitively to the sites for binding of transcription factors situated in the LSR promoter or by binding directly to the transcription factors.

[0291] The level of expression of the LSR receptor and its location can be analysed by hybridization in solution with large probes as indicated in Patent PCT WO 97/05277, the teaching of this document being incorporated by reference. Briefly, a cDNA or the genomic DNA for the LSR receptor or alternatively a fragment thereof is inserted at a cloning site situated directly downstream of a bacteriophage (T3, T7 or SP6) RNA polymerase promoter in order to produce an antisense RNA. Preferably, the insert comprises at least 100 consecutive nucleotides of the genomic sequence of the LSR receptor or of one of the cDNAs of the present invention, more particularly one or more of the cDNAs of SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13. The plasmid is linearized and transcribed in the presence of ribonucleotides comprising modified ribonucleotides such as Biotin-UTP and Digoxigenin-UTP. An excess of this labelled RNA is hybridized in solution with the mRNAs isolated from cells or from tissues of interest. The hybridizations are carried out under stringent conditions (40-50° C. for 16 h in a solution containing 80% formamide and 0.4 M NaCl, pH 7-8). The non-hybridized probe is eliminated by digestion with ribonucleases specific for single-stranded RNAs (CL3, T1, PhyM, U2 or A RNases). The presence of modified nucleotides biotin-UTP allows the capture of the hybrids on microtitre plates carrying streptavidine. The presence of the DIG modification allows the detection and quantification of the hybrids by ELISA using anti-DIG antibodies coupled to alkaline phosphatase.

[0292] A quantitative analysis of the expression of the gene for the LSR receptor can also be carried out using DNA templates, the term DNA templates designating a one-dimensional, two-dimensional or multi-dimensional arrangement of a plurality of nucleic acids having a sufficient length to allow a specific detection of the expression of mRNAs capable of hybridizing thereto. For example, the DNA templates may contain a plurality of nucleic acids derived from genes for which it is desired to estimate the level of expression. The DNA templates may include the genomic sequences of LSR, that of a cDNA of the present invention, more particularly one or more of the cDNAs of SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13, any sequences complementary thereto or any fragments thereof. Preferably, the fragments comprise at least 15, at least 25, at least 50, at least 100 or at least 500 consecutive nucleotides of the nucleic sequences from which they are derived.

[0293] For example, a quantitative analysis of the expression of the LSR receptor can be carried out with a DNA template having the cDNA for the LSR receptor as described in Schena et al. (1995 and 1996). cDNAs for the LSR receptor or fragments thereof are amplified by PCR and bound in the form of a template from a 96-well microplate onto a sylated microscope slide using a very fast automated machine. The DNA template thus produced is incubated in a humid chamber in order to allow its rehydratation. It is then rinsed once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in a sodium borohydride solution. The template is then submerged in water for 2 min at 95° C., transferred into 0.2% SDS for 1 min, rinsed twice with water, dried and stored in the dark at 25° C.

[0294] The mRNAs of cells and of tissues are isolated or obtained from a commercial source, for example the company Clontech. The probes are prepared by a reverse transcription cycle. The probes are then hybridized with the DNA template of 1 cm^2 under a glass coverslip of 14×14 mm for 6-12 hours at 60° C. The template is washed for 5 min at 25° C. in a washing buffer at low stringency (1×SSC/0.2% SDS) and then for 10 min at room temperature in a highly stringent buffer (0.1×SSC/0.2% SDS). The template is analysed in 0.1×SSC using a laser fluorescence microscope with a set of appropriate filters. Measurements of precise differential expression are obtained by taking the mean of the ratios of two independent hybridizations.

[0295] A quantitative analysis of the expression of the LSR receptor can also be carried out with cDNAs for the LSR receptor or fragments thereof on DNA templates according to the description by Pietu et al. (1996). The cDNAs for the LSR receptor or fragments thereof are amplified by PCR and bound to membranes. The mRNAs obtained from different tissues or cells are labelled with radioactive nucleotides. After hybridization and washing under controlled conditions, the hybridized mRNAs are detected with a Phosphor Imager or by autoradiography. The experiments are carried out in duplicate and a quantitative analysis of the differentially expressed mRNAs can be carried out.

[0296] Alternatively, the analysis of the expression of the LSR receptor can be made with DNA templates at high density as described by Lockhart et al. (1996) and Sosnowski et al. (1997). Oligonucleotides of 15 to 50 nucleotides, preferably about 20 nucleotides, extracted from genomic DNA or cDNA sequences for the LSR receptor or of their complementary sequences are synthesized directly on a chip or synthesized and then addressed onto the chip.

[0297] LSR cDNA probes labelled with an appropriate compound such as biotin, digoxigenin or a fluorescent molecule are synthesized from a population of mRNA and are fragmented into oligonucleotides of 50 to 100 nucleotides on average. The probes thus obtained are then hybridized to a chip. After washing as described in Lockhart et al (1996) and an application of various electric fields (Sosnowski et al. 1997), the labelled compounds are detected and quantitied. The hybridizations are duplicated. A comparative analysis of

the intensity of the signals generated by the probes on the same target oligonucleotide in various cDNA samples indicates a differential expression of the mRNAs for the LSR receptor.

[0298] The techniques mentioned above allow the analysis of the levels of expression of the LSR receptor, in the same cell or the same tissue depending on various conditions, for example of induction or of noninduction, but also the analysis of the tissue specificity of this expression, under conditions which can also vary. It will be possible, by virtue of these techniques, to analyse the expression of either of the subunits of the LSR receptor, and more generally of different forms derived from alternative splicing, by adequately defining the probes.

[0299] The effect of compounds which are candidates for modulating the level or the specificity of expression, or of splicing of the different forms of the LSR receptor can thus be analysed on a large scale by exposing the cells which are the source of messenger RNA, in particular the model cells according to the invention, whether they express LSR naturally or whether they are recombinant cells, to the said candidate compounds.

Screening of Compounds Interacting with the LSR Receptor

[0300] Another aspect of the present invention consists in methods of identifying molecules capable of binding to the LSR receptor. Such molecules can be used to modulate the activity of the LSR receptor. For example, such molecules can be used to stimulate or reduce the degradation of lipoproteins, preferably of lipoproteins high in triglycerides, or of cytokines, preferably of leptin. Such molecules can also be used to inhibit the activation by leptin or the activation by free fatty acids of the LSR activity.

[0301] Numerous methods exist for identifying ligands for the LSR receptor. One of these methods is described in U.S. Pat. No. 5,270,170, whose teaching is incorporated by reference. Briefly, a library is constructed which consists of random peptides, comprising a plurality of vectors each encoding a fusion between a peptide which is a candidate for binding to the LSR receptor and a protein binding to DNA such as the Lac repressor encoded by the lad gene. The vectors for the library of random peptides also contain binding sites for the proteins binding to DNA such as the LacO site when the protein is the Lac repressor. The library of random peptides is introduced into a host cell in which the fusion protein is expressed. The host cell is then lysed under conditions allowing the binding of the fusion protein to the sites of the vector.

[0302] The vectors which have bound the fusion protein are brought into contact with the immobilized LSR receptor, a subunit of the immobilized LSR receptor or a fragment of the immobilized LSR receptor under conditions allowing the peptides to bind specifically. For example, the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids can be immobilized by binding to a surface such as a plate or a plastic particle.

[0303] The vectors which encode the peptides capable of binding to the LSR receptor are specifically retained at the surface by interactions between the peptide and the LSR receptor, a subunit of the receptor or a fragment thereof.

[0304] Alternatively, molecules capable of interacting with the LSR receptor can be identified using a double hybrid system such as the Matchmaker Two Hybrid System 2. According to the instructions of the manual accompanying the Matchmaker Two Hybrid System 2 (Catalogue No. K1604-1, Clontech), whose teaching is incorporated by reference, the nucleic acids encoding the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids are inserted into an expression vector so that they are in phase with the DNA encoding the DNA binding domain of the transcription activator of yeast GAL4. The nucleic acids of a library encoding proteins or peptides capable of interacting with the LSR receptor are inserted into a second expression vector so that they are in phase with the DNA encoding the activation domain of the GAL4 activator. The yeasts are transformed with the two expression plasmids and they are placed in a medium which makes it possible to select the cells expressing markers contained in each of the vectors as well as those expressing the HIS3 gene whose expression is dependent on GAL4. The transformed cells capable of growing on a histidine-free medium are analysed for expression of LacZ under the dependence of GAL4. The cells which grow in the absence of histidine and express LacZ contain a plasmid which encodes proteins or peptides which interact with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids thereof.

[0305] To study the interaction of the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids with small molecules such as those generated by combinatory chemistry, it is possible to use an HPLC-coupled microdialysis as described in Wang et al. (1997), or an affinity capillary electrophoresis as described in Busch et al. (1997), the teaching of these documents being incorporated by reference.

[0306] In other methods, the peptides or small molecules capable of interacting with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids may be linked to detectable markers such as radioactive, fluorescent or enzymatic markers. These labelled molecules are brought into contact with the immobilized LSR receptor, an immobilized subunit thereof or an immobilized fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids under conditions allowing a specific interaction. After elimination of the molecules which are not specifically bound, the bound molecules are detected by appropriate means.

[0307] These methods may allow in particular the identification of fatty acids or analogues capable of binding to the fatty acid binding site on the LSR, of lipoproteins or analogues, capable of binding to the lipoprotein binding site on the LSR receptor, of leptin derivatives or analogues capable of binding to the leptin binding site on the LSR, and of derivatives of the gC1qR receptor or analogues capable of binding to the gC1qR binding site on the LSR.

[0308] In addition, the peptides or small molecules which bind to LSR, preferably to the binding sites on the LSR receptor for fatty acids, lipoproteins, cytokines, in particular leptin, or gC1qR or one of its analogous proteins, can be identified by competition experiments. In such experiments, the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids is immobilized on a surface such as a plastic support. Increasing quantities of peptides or of small molecules are brought into contact with the immobilized LSR receptor, an immobilized subunit thereof or an immobilized

fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids in the presence of a labelled ligand for the receptor, it being possible for this ligand to be, for example, leptin, oleate, the LDLs or gC1qR. The ligand for the LSR receptor may be labelled with a radioactive, fluorescent or enzymatic marker. The capacity of the molecule tested to interact with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids is determined by measuring the quantity of labelled ligand bound in the presence of the molecule tested. A decrease in the quantity of bound ligand when the molecule tested is present indicates that the latter is capable of interacting with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids.

[0309] These methods can in particular allow the identification of fatty acids or analogues capable of binding to the fatty acid binding site on the LSR, of lipoproteins or analogues, capable of binding to the lipoprotein binding site on the LSR receptor, of leptin derivatives or analogues capable of binding to the leptin binding site on the LSR, and of derivatives of the gC1qR receptor or analogues capable of binding to the gC1qR binding site on the LSR. The capacity of such compounds, or of any other candidate compound, to compete with the binding of oleates, lipoproteins, leptin or gC1qR to LSR can be measured in particular.

[0310] The BIACORE technology can also be used to carry out the screening of compounds capable of interacting with the LSR receptor. This technology is described in Szabo et al. (1995) and in Edwards and Leartherbarrow (1997), of which the teaching is incorporated by reference, and makes it possible to detect interactions between molecules in real time without the use of labelling. It is based on the phenomenon of SPR (surface plasmon resonance). Briefly, the molecule to be analysed is bound to a surface (typically using a carboxymethyl dextran matrix). A light ray is directed onto the face of the surface which does not contain the sample and is reflected by the said surface. The SPR phenomenon causes a reduction in the intensity of the reflected light with a specific combination of angle and of wavelength. The molecule binding events cause a change in the refractive index at the surface which is detected as a modification of the SPR signal. To carry out a screening of compounds capable of interacting with the LSR receptor, the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids, is immobilized on a surface. This surface constitutes one face of a cell through which passes the molecule to be tested. The binding of the molecule to the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids is detected by a change in the SPR signal. The molecules tested may be proteins, peptides, carbohydrates, lipids or small molecules generated, for example, by combinatory chemistry. The candidate proteins can be extracted from any tissue, obtained from any species. The BIACORE technology can also be used by immobilizing eukaryotic or prokaryotic cells or lipid vesicles having an endogenous or recombinant LSR receptor at their surface.

[0311] One of the main advantages of this method is that it allows the determination of the association constants between the LSR receptor and the interacting molecules. Thus, it is possible to specifically select the molecules interacting with high or low association constants.

[0312] The proteins or other molecules interacting with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids can be identified using affinity columns which contain the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids. The LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids may be attached to the column using conventional techniques including chemical coupling to an appropriate column matrix such as agarose, Affi Gel, or other matrices known to a person skilled in the art. In another aspect of the invention, the affinity column may contain chimeric proteins in which the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids would be fused, for example, with glutathione S-transferase. The molecules to be tested which are described above are then deposited on the column. The molecules interacting with the LSR receptor, a subunit thereof or a fragment thereof comprising at least 10, at least 20, at least 30, or more than 30 consecutive amino acids are retained by the column and can be isolated by elution. In the case where the molecules tested are proteins, they can then be analysed on a 2-D electrophoresis gel as described in Ramunsen et al, (1997), of which the teaching is incorporated by reference. Alternatively, the proteins or the other molecules retained by the affinity column can be purified by electrophoresis and sequenced. A similar method can be used to isolate antibodies, to screen "phage display" products or "phage display" derived human antibodies.

Screening of Compounds Interacting with the Promoter and/or Regulatory Sequences of the LSR Receptor

[0313] The invention also relates to a method of screening compounds interacting with the promoter and/or regulatory sequences of the LSR receptor.

[0314] The nucleic acids encoding proteins interacting with the promoter and/or regulatory sequences of the LSR receptor gene, more particularly a nucleotide sequence corresponding to nucleotides 1 to 1897 of SEQ ID NO:19 or a fragment thereof, can be identified using a single hybrid system such as that described in the manual accompanying the Matchmaker One-Hybrid System from Clontech (Catalogue No. K1603-1), of which the teaching is incorporated by reference. Briefly, the target nucleotide sequence is cloned upstream of a selectable marker gene and integrated into a yeast genome. The yeasts containing the integrated marker gene are transformed by a library containing fusions between cDNAs encoding candidate proteins for binding to the promoter and/or regulatory regions of the gene for the LSR receptor and the yeast transcription factor activating domain such as GAL4. The yeasts are placed in a medium which makes it possible to select the cells expressing the marker gene. The yeasts selected contain a fusion protein capable of binding to the promoter and/or regulatory target region. The cDNAs of the genes encoding the fusion proteins are then sequenced. The corresponding inserts can then be cloned into expression or transcription vectors in vitro. The binding of the polypeptides thus encoded to the promoter target sequences can be confirmed by techniques familiar to persons skilled in the art, including gel retardation or protection to DNAse experiments.

[0315] The screening of compounds capable of modifying the expression of the LSR receptor by binding to its regula-

tory and/or promoter sequences can also be carried out with the aid of "reporter" genes. For example, a genomic region situated in 5' of the coding sequence of the LSR receptor, more particularly a nucleotide sequence corresponding to nucleotides 1 to 1897 of SEQ ID NO:19 or a fragment thereof, can be cloned into a vector such as pSEAP-Basic, pSEAP-Enhancer, pβgal-Basic, pβgal-Enhancer, or pEGFP-1 available from Clontech. Briefly, each of these vectors contains multiple cloning sites situated upstream of a marker gene encoding an easily detectable protein such as alkaline phosphatase, β-galactosidase or GFP (green fluorescent protein). After insertion of the genomic region situated in 5' of the coding sequence of the LSR receptor, more particularly a nucleotide sequence corresponding to nucleotides 1 to 1897 of SEQ ID NO:19 or a fragment thereof, the level of expression of the marker proteins is measured and compared with a vector containing no insert. The effect of candidate compounds on the expression resulting from the regulatory and/or promoter sequences of LSR can thus be evaluated.

[0316] The screening of the compounds capable of binding to the regulatory and/or promoter regions of the gene for the LSR receptor can also be carried out by gel retardation experiments well known to persons skilled in the art and described in Fried and Crothers (1981), Garner and Revzin (1981) and Dent and Latchman (1993), of which the teaching is incorporated by reference. These experiments are based on the principle that a DNA fragment bound to a protein migrates more slowly than the same fragment without protein. Briefly, the target nucleotide sequence is labelled. It is then brought into contact either with a nuclear or total cell extract prepared so as to contain the transcription factors, or with various compounds to be tested. The interaction between the regulatory and/or promoter region of the gene for the LSR receptor and the transcription factor or compound is detected after electrophoresis by retardation of migration.

Compounds

[0317] The chemical or biochemical compounds, characterized in that they make it possible to modulate the expression or the activity of the receptor according to the invention, also form part of the invention.

[0318] The chemical or biochemical compounds, characterized in that they are capable of interacting, directly or indirectly, with the receptor according to the invention, also form part of the invention.

The chemical or biochemical compounds, characterized in that they are selected by the said methods defined above, also form part of the invention.

[0319] In particular, among these compounds according to the invention, a leptin or one of its derived compounds, preferably one of its protein variants, or leptins which are chemically modified or which are obtained by genetic recombination, or one of their fragments, are preferred.

[0320] Compounds which make it possible to modulate the expression or the activity of the receptor are understood to mean the compounds which make it possible in particular to reduce, stabilize or increase the number, the recycling rate and/or the change in the conformation of the receptor according to the invention, or to promote or inhibit the overall activity or the activity of one of the domains of the said receptor or alternatively to reestablish normal expression of the said receptor in the case, for example, where a genetic abnormality is observed. These compounds may, for example, interact as ligands specific for the said receptor or for one of its domains as cofactor, or as inhibitor, in particular a competitive inhibitor, or alternatively having an agonist or antagonist activity on the conformational changes in the complex. These compounds may also interact by neutralizing the natural ligands specific for the said receptor and by thereby inhibiting the receptor activity induced by these ligands.

[0321] Among these compounds, the compounds which make it possible to modulate the number of polypeptides of the said receptor, its recycling rate and/or the selectivity of their activity, are preferred.

[0322] Also preferred are the compounds according to the invention, characterized in that they allow an increase in the total activity or in the expression of the receptor according to the invention, and/or a specific increase in the clearance activity for cytokines, in particular leptin, of the said receptor, and/or a specific increase in the clearance activity for lipoproteins, of the said receptor.

[0323] Also preferred are the compounds characterized in that they allow a decrease in the total activity or in the expression of the receptor according to the invention, and/or a specific decrease in the clearance activity for cytokines, in particular leptin, of the said receptor, and/or a specific decrease in the clearance activity for lipoproteins, of the said receptor.

[0324] Also preferred are the compounds characterized in that they allow modulation of the elimination of the cytokines, in particular leptin, and/or modulation of the elimination of the lipoproteins, chylomicron residues, and/or triglycerides.

[0325] The invention also comprises the compounds according to the invention, characterized in that they allow modulation of the level of cytokines, in particular leptinemia, and/or modulation of the level of lipoproteins, chylomicron residues, and/or triglycerides.

[0326] The compounds according to the invention, characterized in that they allow control of the level of cytokines, in particular leptinemia, are more particularly preferred.

[0327] Still preferably, the invention comprises the compounds according to the invention, characterized in that they allow control, preferably a decrease, of the level of lipoproteins, a decrease in the plasma concentration of chylomicron residues, and/or a decrease in triglyceridemia.

[0328] Among the compounds which are most preferred, there are preferred those characterized in that they are chosen from:

[0329] a. an antibody according to the invention;

[0330] b. a polypeptide according to the invention;

[0331] c. a polypeptide according to the invention, characterized in that it corresponds to a soluble form of the receptor according to the invention;

[0332] d. a vector according to the invention;

[0333] e. a vector according to the invention, characterized in that it has on its outer surface a site for specific recognition of hepatic cells;

[0334] f. a vector according to the invention, characterized in that the product of expression of the nucleic acid inserted by the vector into the target cell is either anchored in or excreted by the said transformed target cell;

[0335] g. a sense or antisense oligonucleotide according to the invention;

[0336] h. a leptin, or one of its protein variants, or a leptin which is chemically modified or which is modified by genetic recombination, or one of their fragments.

[0337] The invention finally relates to the compounds according to the invention as a medicament.

[0338] The compounds according to the invention as a medicament for the prevention and/or treatment of pathologies and/or of pathogeneses linked to disorders in dietary habit are preferred in particular.

[0339] The compounds according to the invention as a medicament for the prevention and/or treatment of pathologies and/or of pathogeneses linked to disorders in the metabolism of cytokines are also preferred.

[0340] Preferably, the invention also relates to the compounds according to the invention as medicament for the prevention or treatment of obesity or anorexia.

[0341] The compounds according to the invention as a medicament for the prevention and/or treatment of pathologies and/or of pathogeneses associated with, or induced by obesity, are the preferred compounds.

[0342] In particular, there are preferred the compounds according to the invention, as a medicament for the prevention and/or treatment of cardiac insufficiency, of coronary insufficiency, of cerebrovascular accidents, of atheromatous disease, of atherosclerosis, of high blood pressure, of non-insulin-dependent diabetes, of hyperlipidemia and/or of hyperuricemia.

[0343] The most preferred are the compounds according to the invention, as a medicament for the prevention and/or treatment of atheromatous disease and/or of atherosclerosis.

[0344] Finally, the invention comprises compounds according to the invention for the prevention and/or treatment by gene therapy, of pathologies and/or of pathogeneses linked to disorders in dietary habit, of obesity and/or of pathologies and/or of pathogeneses associated with, or induced by, obesity.

[0345] The compounds of the invention as active ingredients of a medicament will be preferably in soluble form, combined with a pharmaceutically acceptable vehicle.

[0346] Such compounds which can be used as a medicament offer a new approach for preventing and/or treating pathologies and/or pathogeneses linked to disorders in dietary habit such as obesity or anorexia, and the related risks and/or complications.

[0347] Preferably, these compounds will be administered by the systemic route, in particular by the intravenous route, by the intramuscular or intradermal route or by the oral route.

[0348] Their modes of administration, optimum dosages and galenic forms can be determined according to the criteria generally taken into account in establishing a treatment suited to a patient, such as for example the age or body weight of the patient, the seriousness of his general condition, the tolerance to treatment and the side effects observed, and the like.

[0349] As mentioned above, depending on the cases, it may be advisable to amplify the activity of LSR, by promoting, for example, the expression of its genes or by increasing the activity of their expression products, in pathological cases resulting from the fact that at least one of these genes is not expressed, is insufficiently expressed or is expressed in an abnormal form which does not allow the expression product to carry out its functions, or on the contrary to repress an overexpression or an abnormal expression of these genes. It is therefore advisable in general to compensate for the deficiency or the overexpression of expression products of this gene by a so-called "replacement" therapy allowing the amplification or the reduction in the activities of the LSR complex.

[0350] The replacement therapy may be carried out by gene therapy, that is to say by introducing the nucleic acid sequences according to the invention and/or the corresponding genes with the elements which allow their expression in vivo, in the case where one of the genes is insufficiently expressed for example, or alternatively when it is expressed in an abnormal form.

[0351] The principles of gene therapy are known. It is possible to use viral vectors according to the invention; it is also possible to envisage nonviral, that is to say synthetic, vectors which mimic viral sequences or alternatively which consist of naked RNA or DNA according to the technique developed in particular by the company VICAL.

[0352] In most cases, it is necessary to envisage targeting elements ensuring expression specific for the liver so as to be able to limit the zones of expression of the proteins which remain involved in the clearance of leptin and that of lipoproteins. It is even advantageous, in some cases, to have vectors for transient expression or at least for controlled expression which it will be possible to block when necessary.

[0353] Other characteristics and advantages of the invention appear in the remainder of the description with the examples and figures whose legends are represented below.

LEGEND TO THE FIGURES

[0354] FIG. **1**: Schematic representation of the three forms of the rat LSR protein: LSR 66 (α subunit), LSR 64 (α' subunit), and LSR 58 (β subunit).

[0355] FIG. **2**: Alignment of the protein sequences of the long forms (α subunits) of the human LSR (LSR1.Hs; SEQ ID NO: 8), rat LSR (LSR1.Rn; SEQ ID NO: 2) and mouse LSR (LSR1.Mm; SEQ ID NO: 16). The (*) symbols placed under the alignments indicate the conserved amino acids, the (.) symbols indicate the conservative substitutions of amino acids. Boxed, from the NH_2-terminal end to the COOH-terminal end, the potential fatty acid (FFA) binding site boxed, the clathrin binding site [NPGY], the lyosomal addressing consensus: di-leucine LI-X10-LL, the transmembrane TM domain overlined, the motif [RSRS], the potential lipoprotein binding site (+−+−) boxed. Overlined, the signature of the TNF receptor with (arrow); indicated, the amino acids conserved in the signature. The transmembrane domain is situated between the last di-leucine and the TNF signature.

[0356] A: Alignment shown from amino acid positions 1 to 539 of SEQ ID NO:8.

[0357] B: Alignment shown from amino acid positions 540 to 649 of SEQ ID NO: 8.

[0358] FIG. **3**: Alignment of the protein sequences of the three types of subunits of the human LSR (α: LSR1.Hs, SEQ ID NO:8; α': LSR2.Hs, SEQ ID NO:10; β: LSR3.Hs, SEQ ID NO:12). The meaning of the symbols, of the boxes and of the overlines is the same as that in FIGS. **2**A and **2**B.

[0359] A: Alignment shown from amino acid positions 1 to 540 of SEQ ID NO:8.

[0360] B: Alignment shown from amino acid positions 541 to 649 of SEQ ID NO:8.

[0361] FIG. **4**: Alignment of the protein sequences of the three types of subunits of rat LSR.

(α: LSR1.Rn, SEQ ID NO:2; α': LSR2.Rn, SEQ ID NO:4; β: LSR3.Rn, SEQ ID NO:6).

[0362] The meaning of the symbols, of the boxes and of the overlines is the same as that in FIGS. **2**A and **2**B.

[0363] FIG. **5**: Alignment of the protein sequences of the three types of subunits of mouse LSR (α: LSR1.Mm, SEQ ID

NO:16; α': LSR2.Mm, SEQ ID NO:17; β: LSR3.Mm, SEQ ID NO:18). The meaning of the symbols, of the boxes and of the overlines is the same as that in FIGS. **2**A and **2**B.

[0364] A: Alignment shown from amino acid positions 1 to 540 of SEQ ID NO:16.

[0365] B: Alignment shown from amino acid positions 541 to 594 SEQ ID NO: 16.

[0366] FIG. **6**: Schematic representation of the three LSR forms identified in humans, indicating the motifs conserved on each of them.

[0367] A: Schematic representation of the genomic organization of the human LSR starting from the first coding exon. The exons are indicated by boxes, the introns by interrupted bars. The size, in nucleotides, of the exons and introns is indicated above them. The elements characterizing the messenger and the encoded protein are presented in this figure. The box on the right gives the meaning of the symbols used.

[0368] B: Structure of the LSR-Hs-2062 form of human LSR. This form encodes a protein of 649 amino acids.

[0369] C: Structure of the LSR-Hs-2005 form of human LSR. This form encodes a protein of 630 amino acids.

[0370] D: Structure of the LSR-Hs-1858 form of human LSR. This form encodes a protein of 581 amino acids.

[0371] FIG. **7**: Alignment of the nucleotide sequences of the long forms of cDNA (encoding the α subunit) or portions thereof for human LSR (Isr1.Hs; nucleotides 1 to 2062 of SEQ ID NO:7), rat LSR (Isr1.rRn; SEQ ID NO:1) and mouse LSR (Isr1.Mm; SEQ ID NO:13). The nucleotides conserved in the three sequences are identified by an * sign placed under the sequences. Dashes are added inside the sequences when the optimum alignment of the sequences cannot be achieved without creating microdeletions.

[0372] A: Alignment shown from amino acid position 1 to 486 of SEQ ID NO:1.

[0373] B: Alignment shown from amino acid position 487 to 1026 of SEQ ID NO:1.

[0374] C: Alignment shown from amino acid position 1027 to 1551 of SEQ ID NO:1.

[0375] D: Alignment shown from amino acid position 1552 to 2080 of SEQ ID NO:1.

[0376] E: Alignment shown from amino acid position 2081 to 2097 of SEQ ID NO:1.

[0377] FIG. **8**: Identification of the LSR receptor by ligand and Western blotting on solubilized proteins of rat liver membranes (lanes 1, 2 and 4), or on the partially purified protein of 240 kD (lane 3).

[0378] Lanes 1, 2 and 3: Ligand blotting. Lane 1: in the absence of oleate and of ^{125}I-LDL; lane 2: in the presence of oleate and of ^{125}I-LDL; lane 3: in the presence of oleate and of ^{125}I-LDL.

[0379] Lane 4: Western blotting with anti-LSR antibodies.

[0380] FIG. **9**: Effect of anti-LSR antibodies on the LSR activity.

[0381] A. Binding of ^{125}I-LDL onto the plasma membranes of rat hepatocytes in the presence of oleate and of increasing concentrations of anti-LSR antibody (v) or of control antibody (o), expressed as % of the total quantity of ^{125}I-LDL bound in the absence of antibodies.

[0382] B. Binding, incorporation and degradation of ^{125}I-LDL in rat hepatocytes in primary culture in the presence of oleate and of anti-LSR antibody (v) or of control antibody (o), expressed respectively as % of the binding, incorporation and total degradation of ^{125}I-LDL in the presence of non-specific antibodies.

[0383] FIG. **10**: Identification of the LSR receptor by immunoprecipitation of ^{35}S-methionine- and ^{35}S-cysteine-labelled hepatocyte lysates, in the presence of control antibodies (lane 1), or of anti-LSR antibodies (lanes 2 to 4), after separation by electrophoresis under nonreducing (lanes 2 and 3) or reducing (lanes 1 and 3) conditions.

[0384] FIG. **11**: Cloning of the cDNA encoding α and β-LSR.

[0385] A. Northern-blot analysis showing several sizes of LSR messenger RNA.

[0386] B. Multi-tissue Northern-blot analysis of LSR mRNA with a probe specific for LSR and a control probe specific for β-actin.

[0387] C. RT-PCR analysis of LSR mRNA using 5 pairs of primers covering the entire sequence and identification of three forms derived from alternative splicing in the amplification fragment obtained by means of the bc' primers. The diagram represents the results of sequence analysis of the three corresponding forms of LSR cDNA: the squared region is absent from the two short forms, the hatched region is absent only from the shortest form.

[0388] FIG. **12**: Translation in vitro of the two complete cDNAs encoding the longest (66 kDa, lane 2) and the shortest (58 kDa, lane 3) forms of rat LSR, and of a control cDNA, an antisense of the cDNA encoding the longest form of LSR (lane 1).

[0389] The products of translation in vitro, labelled with ^{35}S-methionine, are analysed after electrophoresis under nonreducing conditions.

[0390] FIG. **13**: Identification of the α- and β-LSR subunits as being responsible for the LSR activity.

[0391] A. Diagram showing the location and the sequence of LSR N-terminal peptide used to generate anti-LSR peptide antibodies.

[0392] B. Effect of antibodies directed against a synthetic LSR peptide on the LSR activity of rat liver plasma membranes. The LSR activity is measured in the presence of a control antibody (o) or of the anti-LSR peptide antibody (v).

[0393] C. Western and Ligand blotting of the α and β subunits of LSR. The Western blotting is carried out using the anti-LSR (lane 1) or anti-LSR peptide (lane 2) antibody. The ligand blotting is carried out in the presence of ^{125}I-LDL, with (lane 4) or without (lane 3) oleate.

[0394] FIG. **14**: Identification of the subunits of the LSR receptor and inhibitory effect of antibodies directed against a C-terminal synthetic peptide derived from LSR.

[0395] A—Diagram showing the location and the sequence of the synthetic peptide 170,

[0396] B—Western blotting of rat hepatocyte lysates using antibodies directed against the synthetic peptide 170 (lane 2), or a control antibody (lane 1); lane 3: molecular weight markers.

[0397] C—Binding of ^{125}I-LDL by the LSR receptor in the presence of oleate and of control antibodies or antibodies directed against the LSR 170 peptide.

[0398] FIG. **15**: Effect of a transient transfection of CHO-K1 cells with the plasmids expressing the α and β subunits of the LSR receptor on the binding of LDLs in the presence or in the absence of oleate. Increasing concentration of α plasmid alone (○□); fixed concentration of a plasmid and increasing concentration of β plasmid (●■).

[0399] FIG. **16**: Effect of a transient transfection of CHO-K1 cells with plasmids expressing the α and β subunits of the LSR receptor on the internalization and degradation of LDLs.

Increasing concentration of a plasmid alone (■); fixed concentration of α plasmid and increasing concentration of β plasmid (●). The results are expressed as the difference between the measurements in the presence and in the absence of oleate.

[0400] FIG. **17**: Characterization of the LSR activity obtained in CHO-K1 cells transiently transfected with the nucleic sequences encoding the α and β subunits of the LSR receptor, compared with the LSR activity obtained in the same cells not transfected (control).

[0401] A—Binding of ^{125}I-LDL in the presence of a control antibody or of an anti-LSR antibody.

[0402] B—Binding of ^{125}I-LDL in the presence of increasing concentrations of unlabelled lipoproteins; rat chylomicrons (υ), human VLDL (■), LDL (□), HDL (σ), LDLs treated with pronase (○), or LDLs modified with cyclohexanedione (LDL-chd, ●).

[0403] FIG. **18**: Effect of oleate, of RAP-39, of anti-LSR antibodies and of chloroquine on the specific degradation of leptin in primary cultures of rat hepatocytes.

[0404] FIG. **19**: Western blot analysis with anti-LSR antibodies, of the fraction of rat liver plasma membrane proteins retained on an affinity chromatography column containing leptin.

[0405] FIG. **20**: Clearance of ^{125}I-leptin on control (o), ob/ob (v) and db/db (⩓) mice in the liver and the kidney. The results are expressed as the difference between the quantities of ^{125}I-leptin and ^{125}I-β2-microglobulin found in the liver and in the kidney.

[0406] FIG. **21**: Apparent number of LSR receptors expressed in the liver of control, ob/ob and db/db mice.

[0407] FIG. **22**: Effect of anti-LSR antibodies on the proportion between the quantities of ^{125}I-leptin distributed in the liver and in the kidney.

[0408] FIG. **23**: Effect of increasing leptin concentrations on the LSR activity of rat hepatocytes in primary cultures. The results represent the differences in activity which are obtained between the cells incubated with and without oleate in the presence either of ^{125}I-LDL, or of ^{125}I -VLDL.

[0409] FIG. **24**: Capacity for inducing, by leptin, the LSR activity of rat hepatocytes in primary culture.

[0410] A. Apparent number of receptors expressed at the surface of the hepatocytes in the presence or in the absence of leptin, estimated by the measurement of the quantity of ^{125}I-LDL bound in the presence of oleate.

[0411] B. Effect of cycloheximide, of colchicine and of cytochalasin B on the induction, by leptin, of the LSR activity.

[0412] FIG. **25**: Effect of leptin on the postprandial lipemic response in control (o), ob/ob (v) and db/db (o) mice, reflected by the variation in the plasma concentration of triglycerides (TG) after ingestion of a high-fat meal, with (B) and without (A) injection of murine recombinant leptin.

[0413] FIG. **26**: Effect of leptin, in the presence and in the absence of lactoferrin, on the postprandial lipemic response of ob/ob mice, expressed by the measurement of the plasma concentration of triglycerides (TG) after ingestion of a high-fat meal.

[0414] FIG. **27**: Effect of leptin injection on the apparent number of LSR receptors expressed in the liver of ob/ob and db/db mice.

[0415] FIG. **28**: Postprandial lipemic response and LSR activity in control (C57BL6), ob/ob and db/db mice.

[0416] A—Weight of control, ob/ob and db/db male mice.

[0417] B—Postprandial lipemic response in control, ob/ob and db/db mice.

[0418] C— Apparent number of LSR receptors estimated by measurement of the binding of LDL and expressed in arbitrary unit by comparison with the 5'-nucleotidase activity in each plasma membrane preparation.

D—Northern blot on an extract of liver total RNA. GAPDH is used as control.

[0419] FIG. **29**: Effect of a long-term treatment by leptin on ob/ob mice.

[0420] A—Weight change over 30 days

[0421] B—Postprandial lipemic response on the 29th day of treatment

[0422] C—Apparent number of LSR receptors on day 30, estimated by the measurement of the binding of LDL and expressed in arbitrary unit by comparison with the 5'-nucleotidase activity in each plasma membrane preparation

[0423] D—Nothern blot analysis of the expression of LSR established on a total extract of liver RNA. GAPDH and actin are used as controls.

[0424] FIG. **30**: Effect of the oleates on the binding and internalization of the ^{125}I-LDL in normal human fibroblasts, under normal conditions.

[0425] FIG. **31**: Effect of increasing concentrations of leptin on the LSR activity of human fibroblasts HF (familial hypercholesterolemia).

[0426] FIG. **32**: Inhibitory effect of antibodies directed against an NH_2-terminal (v) or COOH-terminal (O) peptide of gC1qR, or of control antibodies (o) on the LSR activity of plasma membranes of rat hepatocytes, expressed as a percentage of the quantity of ^{125}I-LDL bound in the absence of antibodies.

[0427] FIG. **33**: Effect of increasing concentrations of C1q on the binding, internalization and degradation of ^{125}I-LDL on rat hepatocytes in primary culture, in the presence (v) or in the absence (o) of oleate.

[0428] FIG. **34**: Effect of 25 ng/ml of recombinant AdipoQ on the LSR activity in a primary culture of rat hepatocytes.

[0429] FIG. **35**: Effect of two successive injections of 1 mg of AdipoQ on the postprandial lipemic response in rats after ingestion of a high-fat meal.

[0430] FIG. **36**: Effect of an intraperitoneal administration of AdipoQ for 3 days on the weight and the concentrations of plasma triglycerides in rats on a normal diet or on a fatty diet.

[0431] FIG. **37**: Effect of a daily injection of 100 μg of AdipoQ over 5 days, on food intake in ob/ob and db/db obese mice.

EXAMPLES

Experimental Procedures

Materials

[0432] Na^{125}I is provided by Amersham (Les Ulis, France). Oleic acid, bovine serum albumin (A 2153) (BSA) and Triton X100 are obtained from Sigma (St Quentin Fallavier, France). Human lactoferrin (Serva) and sodium heparin are provided by Biowhittaker (Fontenay sous Bois, France) and Choay laboratories (Gentilly, France) respectively. The enzymatic kits for the determination of triglycerides (TG) are obtained from Boehringer Mannheim (Meylan, France). Suramin sodium is obtained from CBC Chemicals (Wood-

burg, Conn.). Dulbecco's modified Eagle medium (DMEM), trypsin and foetal calf serum are provided by Life Technologies, Inc. (Eragny, France).

Animals

[0433] The mice C57BL/6J of the wild type, C57BL/6J ob/ob, C57BL/Ks of the wild type and C57BL/Ks db/db are obtained from R. Janvier Breeding Center (Le Genest St Isle, France).

Cells

[0434] Normal fibroblasts (GM08333) and HF (GM00486A, GM007001B, GM00488C) are provided by the NIGMS human genetic mutant cell repository (Camden, N.J.). The cells were plated on Petri dishes of 36 mm as described above (300,000 normal fibroblasts per well, 150, 000 HF fibroblasts per well), and are cultured in a humidified CO_2 incubator, in DMEM medium containing 10% (normal fibroblasts) or 20% (HF fibroblasts) foetal calf serum, 2 mM glutamine, 100 U/ml of penicillin and 100 U/ml of streptomycin.

[0435] The hepatocytes in primary culture are obtained according to the procedure described above (Mann et al., 1995). The cells are then plated at 900,000 cells per well or 22×10^6 cells per flask of 165 cm^2. The cells are used for the studies after 48 hours in culture.

Preparation and Radiolabelling of the Lipoproteins

[0436] The VLDLs ($d<1.006$ g/ml) and LDLs ($1.025<d<1.055$ g/ml) are isolated by sequential ultracentrifugation of fresh plasma from volunteers (Bihain and Yen, 1992; Goldstein et al., 1983) and used before 2 weeks. The lipoproteins are radioiodinated (Bilheimer et al., 1972) and used less than one week after the labelling. ^{125}I-LDL and ^{125}I-VLDL are filtered (0.22 μm membrane, Gelman) immediately before use.

Preparation and Radiolabelling of Mouse Recombinant Leptin

[0437] The leptin cDNA is obtained from the mRNA of adipose tissue of the mouse C57BL/6J by PCR. The 5' PCR primer introduces an initiation codon after the signal sequence which is deleted and a sequence encoding a hexahistidine end. The modified sequence encoding murine leptin is cloned into an expression vector pSE280 (Invitrogen, France) and expressed in *E. coli*. The sequencing of the plasmid DNA confirms the coding sequence. The bacteria are cultured at 37° C. and the synthesis of the protein is induced by 1 mM isopropyl β-D-thiogalactopyranoside. The bacteria, recovered after gentle centrifugation, are lysed by freeze-thaw and the DNA is digested with a deoxyribonuclease I. The cellular membranes are extracted with the aid of a detergent and the inclusion bodies are separated after centrifugation. After 3 washes in 1% sodium deoxycholate in PBS, the inclusion bodies are solubilized in a 6 M guanidine HCl solution. The renaturation of the recombinant protein is achieved by diluting 1/100 in PBS. The renatured protein is then purified and concentrated on a nickel-based chelate metal affinity chromatography column (Probond, Invitrogen). The elution is carried out with imidazole. The purity of the recombinant leptin is controlled by SDS-PAGE electrophoresis and its activity by the evaluation of satiety in mice C57BL/6J ob/ob after intraperitoneal injection of 25 μg of leptin. The recombinant leptin is then radiolabelled using Iodobeads (Pierce) and according to the method recommended by the manufacturer.

Cloning of the AdipoQ mRNA. Production and Purification of Recombinant AdipoQ Proteins

Cloning of the cDNA into an Expression Vector

[0438] Mouse adipose tissue is obtained from C57BI/6J mice and the mRNA is extracted with the aid of polydTs bound to magnetic beads (Dynabeads, Dynal, France). A cDNA library is constructed from mouse adipose tissue by reverse transcription at 40° C. using a commercial kit (Superscript Life Technologies) using the supplier's instructions. The cDNA specific for AdipoQ is amplified using the following two primers:

(SEQ ID NO: 37)
5' CTACATGGATCCAGTCATGCCGAAGAT 3'

(SEQ ID NO: 38)
5' CGACAACTCGAGTCAGTTGGTATCATGG 3'.

[0439] The amplification product is then digested with the restriction enzymes BamHI and XhoI and inserted into an expression vector pTRC HisB (Invitrogen, France) at the corresponding sites. The B version of pTRC allows the expression of heterogeneous sequences downstream of a hexahistidine peptide which carries a recognition site for an enterokinase and an epitope for the anti-Xpress antibody.

Bacterial Transfection and Checking of the Construct

[0440] The plasmid thus obtained is transfected into *E. coli* DII5 α. Furthermore, the DNA of the plasmid is extracted and the heterologous insert is sequenced.

Cell Culture, Extraction and Purification of the Recombinant Protein

[0441] The recombinant bacterial cells are cultured at 37° C. in an LB medium containing antibiotics until the OD at 600 nm reaches 0.2. The production of recombinant protein is then induced by adding 1 mM isopropyl-β-D-thiogalactopyranoside to the culture medium. The bacterial culture is continued for 16 h at 37° C. The cells are recovered by centrifugation. The cells are lysed using lysozyme in a Tris buffer pH 7.4 in the presence of NaCl, PMSF and sodium deoxycholate. The DNA is degraded by sonication. After centrifugation, the recombinant protein is separated from the supernatant using a Probond column (Invitrogen, France). This column contains charged nickel which has affinity for the hexahistidine peptides. The elution is carried out in the presence of imidazole. The protein concentration is estimated by the Lowry method after having dialysed the product of the elution. The purity of the protein obtained is tested by SDS-PAGE electrophoresis, which shows a single band.

Example 1

Identification of the Protein Complex Responsible for the LSR Activity: Partial Purification and Characterization by Means of Polyclonal Antibodies

[0442] The technique of ligand blotting was used to identify the protein complex responsible for the LSR activity. This technique, described in detail by Mann et al., 1995, is detailed below.

Ligand Blotting

[0443] The technique consists in isolating, by differential centrifugation (Belcher et al., 1987) rat liver membranes, and

in solubilizing the membrane proteins in a solution containing 125 mM octylglucoside, 20 mM Tris and 2 mM EDTA, pH 8. The proteins thus solubilized are separated under non-denaturing conditions on a preparative SDS gel (thickness 5 mm) consisting of a gradient from 4 to 12% polyacrylamide (35-50 mg of protein per gel). For part of the gel, the proteins are then electrotransferred (semi-dry transfer, 21 V, 45 min, Biorad) onto a nitrocellulose membrane. After blocking the free sites of this membrane in a PBS solution containing 3% albumin, the membrane is incubated with 40 μg/ml of ^{125}I-LDL in the presence (FIG. **8**, lane 2) or in the absence (FIG. **8**, lane 1) of 0.8 mM oleate. The membrane is then washed five times for 10 minutes in PBS containing 0.5% (v/v) Triton X100, and exposed on a Phosphor Imager screen.

[0444] Analysis of the image obtained in the presence (FIG. **8**, lane 2) or in the absence (FIG. **8**, lane 1) oleate shows the presence of 3 main bands which have bound the LDLs. The apparent MW of the first band is about 240 kDa, that of the second is 115 kDa and that of the third is 90 kDa. On the basis of these observations, two hypotheses are formulated: on the one hand, the LSR activity is linked to the presence of several distinct proteins; on the other hand, the same type of image can be explained by a multimeric organization of a protein complex.

[0445] In order to check this hypothesis, the inventors undertook the purification of the band having the highest apparent molecular weight (240 kDa). The partial purification of this protein, designated "band A", is carried out by preparative electrophoresis as follows.

Partial Purification of LSR

[0446] The technique consists in isolating, by differential centrifugation (Belcher et al., 1987) rat liver membranes, and in solubilizing the membrane proteins in a solution containing 125 mM octylglucoside, 20 mM Tris and 2 mM EDTA, pH 8. The proteins thus solubilized are separated under non-denaturing conditions on a preparative SDS gel (thickness 5 mm) consisting of a gradient from 4 to 12% polyacrylamide (35-50 mg per gel). For part of the gel, the proteins are then electrotransferred (semi-dry transfer, 21 V, 45 min, Biorad) onto a nitrocellulose membrane. After blocking the free sites of this membrane in a PBS solution containing 3% albumin, the membrane is incubated with 40 μg/ml of ^{125}I-LDL in the presence (FIG. **8**, lane 2) or in the absence (FIG. **8**, lane 1) of 0.8 mM oleate. The membrane is then washed five times for 10 minutes in PBS containing 0.5% (v/v) Triton X100, and exposed on a Phosphor Imager screen. The proteins of interest are electroeluted (Eletroeluter, Biorad).

[0447] The rat liver plasma membrane proteins were prepared and separated on a polyacrylamide gel as above. The precise location of band A was established by ligand blotting carried out after electrotransfer of preparative gel sample removed at various levels.

[0448] The gel fragments containing band A are then collected, electroeluted and concentrated (speedvac), and then tested for their capacity to bind the LDLs in the presence of oleate after electrophoresis and transfer onto nitrocellulose membranes (FIG. **8**, lane 3; 80 μg of protein/lane).

[0449] The proteins thus obtained were also used to produce polyclonal antibodies whose specificity was tested by Western blotting (FIG. **8**, lane 4).

Preparation of Polyclonal Antibodies

[0450] The LSR proteins used as antigens for the production of anti-LSR antibodies were prepared as indicated above.

[0451] The antigen preparation is injected subcutaneously into a rabbit in the presence of complete Freund's adjuvant, followed by a conventional immunization procedure. The titer of the antibody directed against the rat proteins is determined regularly (dot-blot technique). When the latter is judged to be sufficient, the specificity of the antibodies obtained is tested by Western blotting on a preparation of solubilized proteins of rat liver membranes as described above, with anti-rabbit IgG goat antibodies labelled with iodine I^{125} as second antibodies.

[0452] The Western blot results after electrophoresis under nonreducing conditions indicate that the antibodies produced from the proteins of band A bind to 3 main protein bands (240 kDa, 115 kDa and 90 kDa) which bind the ^{125}I-LDL in the presence of oleate (FIG. **8**, lane 4). To verify the link between these protein complexes and the LSR activity, the effect of these polyclonal antibodies on the LSR activity was tested.

[0453] The methods used are described in detail below (Mann et al., 1995; Troussard et al., 1995). The LSR activity is estimated by measuring the binding of lipoproteins to plasma membranes and by measuring the binding, internalization and degradation of the lipoproteins on primary cultures of rat hepatocytes.

Measurement of the Binding of Lipoproteins on Plasma Membranes

[0454] The LSR activity is measured on a preparation of rat liver plasma membranes (Bartles and Hubbard, 1990). These membranes exhibit 10 to 15-fold enrichment with 5-nucleotidase (marker specific for plasma membranes). 100 μg aliquots of proteins are incubated for 30 minutes at 37° C. in the presence or in the absence of 0.8 mM oleate in a final volume of 250 μl supplemented with 100 mM PBS, 2 mM EDTA, 350 mM NaCl, pH 8 (buffer A). The oleate is added in a volume of 5 to 10 μl of isopropanol. The excess and unbound oleate is then removed by 6 washes. The pellets are resuspended in 250 μl of incubation buffer, sonicated for 5 seconds, power 1.90% in the active cycle, and then centrifuged for 15 min at 18,000 rpm. The activated membranes are incubated for 1 hour at 4° C. with various concentrations of antibody and then with 5 μg/ml of ^{125}I-LDL (1 hour at 4° C.). 25 μl of 2% BSA are added to the incubation mixture. The quantity of ^{125}I-LDL bound to the membranes is measured by sedimenting the membranes by centrifugation after having deposited 200 μl of the incubation mixture on a layer of 5% (W/V) of BSA in buffer A. The supernatants are removed by aspiration, the tube bottoms are cut off and their radioactivity is counted in a γ counter.

[0455] The inhibitory effect of anti-LSR antibodies on the LSR activity, compared to that of any preparation of rabbit immunoglobulins is shown in FIG. **9** A. The inhibition of the LSR activity by the anti-LSR antibodies confirms that the multimeric complex described above is responsible for the activity of the receptor and validates the ligand blotting technique used to identify it. The effect of the anti-band A antibodies was, in addition, tested on the other steps of the activity of the receptor: the internalization and the degradation of lipoproteins by the LSR expressed at the surface of hepatocytes in primary cultures.

Measurement of the Binding, Internalization and Degradation of Lipoproteins by Hepatocytes

[0456] The LSR activity in the primary cultures of rat hepatocytes is measured by the binding, internalization and deg-

radation of ^{125}I-LDL and ^{125}I-VLDL (LDL: low-density lipoprotein; VLDL: very low-density lipoprotein), as described in Bihain and Yen, 1992 and Mann et al., 1995.

[0457] To measure the effect of the anti-LSR antibodies on the binding, internalization and degradation of LDLs by LSR, primary cultures of rat hepatocytes (48 h after plating) are incubated in the presence of 20 ng of leptin/well for 30 min at 37° C., followed by the addition of anti-LSR antibodies in the presence or in the absence of oleate. After incubating at room temperature for 30 min, ^{125}I-LDL (20 μg/ml) is added and then the cells are incubated for 4 h at 37° C. The binding, incorporation and degradation of ^{125}I-LDL are measured as described in Bihain and Yen, 1992 and Mann, et al., 1995.

[0458] The data in FIG. **9** B show that the anti-band A antibodies inhibit most of the activity of binding of the LDLs to the LSRs present at the level of the hepatocytes. This inhibition induces a decrease in the same proportions in the internalization and proteolytic degradation of the lipoproteins.

[0459] The anti-band A antibodies are thus characterized as anti-LSR. Their relative specificity was defined by a selective immunoprecipitation method. Extracts of hepatocytes in primary culture are immunoprecipitated by means of the anti-LSR antibodies described above, according to the protocol described below.

Immunoprecipitation of Extracts of Hepatocytes in the Presence of Specific Antibodies

[0460] Primary cultures of rat hepatocyte (Oukka et al., 1997) are incubated for 60 minutes to 2 hours in the presence of a mixture of ^{35}S-methionine and ^{35}S-cysteine (Promix, Amersham). This medium is then removed and the cells are washed and then incubated in PBS containing 1% of Triton X100. This cellular lysate is then incubated in the presence of non-specific antibodies and then of protein A. The equivalent of 40 μg of specific anti-LSR antibodies is then added and the LSR-antibody complexes are precipitated with the aid of a second preparation of protein A. After washing, the complexes are dissociated in the presence of 1% SDS supplemented or otherwise with 5% β-mercaptoethanol, incubated at 100° C. for 5-10 minutes, and separated on a 10% acrylamide gel. The gels are dried and exposed on a Phosphor Imager screen. Each of the lanes contains the equivalent of a 165 cm^2 flask, that is to say 22×10^6 cells.

[0461] Analysis of the immunoprecipitation results indicates that under nonreducing conditions (FIG. **10**, lanes 2—without incubation at 100° C.—and 3—with incubation at 100° C.—), the antibodies reveal 3 principal protein bands: 2 of apparent molecular weight 240 kDa and 180 kDa, 1 of apparent molecular weight 68 kDa. The presence of 2 bands of weaker intensity, corresponding to a molecular weight of 115 kDa and 90 kDa, can also be noted. This experimental approach therefore essentially identifies the same protein elements as those identified by the ligand blotting method. It can be observed, moreover, that under reducing conditions (FIG. **10**, lanes 1 and 4), the elements of high molecular weight dissociate into 3 elements of apparent molecular weight 68 kDa, 56 kDa and 35 kDa, respectively.

[0462] The relative intensity of the 68 kDa and 56 kDa bands is similar whereas that of the 35 kDa band is about ¼ of that of the other two.

Example 2

Cloning of the c-DNA Encoding the α- and β-LSR

[0463] The screening of an expression library by means of the anti-LSR antibodies described above was carried out as indicated below.

Screening of an Expression Library

[0464] After infection of bacteria with lambda GT11 bacteriophages containing rat liver cDNA (commercially obtained from Clontech Laboratories Inc.) (5' Strech Plus c-DNA Library), the cells are plated on LB $MgSO_4$ medium. After 4 hours of culture at 42° C., a nitrocellulose membrane, previously incubated in a 10 mM IPTG solution, is deposited in the Petri dishes. Four hours later, the first membrane is removed and a second is applied to the Petri dish.

[0465] Each membrane is immersed in a Petri dish containing blocking buffer kept stirring for one hour. Next, the antibody is added to a final concentration of 10 μg/ml of blocking buffer (Huynh et al., 1984; Young and Davis, 1983a and 1983b). The membranes are then washed three times for 10 minutes with TNT (10 mM Tris, 150 mM NaCl, 0.05% Tween 20).

[0466] The membranes are incubated in the presence of secondary antibodies (alkaline phosphatase-conjugated affinipure F(ab') 2 fragment goat anti-rabbit IgG; Immunotech) at a final concentration of 0.08 μg/ml of blocking buffer (TNT+5% powdered skimmed milk, Pâturage trademark).

[0467] After washing the membranes in TNT, they are incubated in the presence of BCIP (5-bromo-4-chloro-3-indolyl phosphate) and of NBT (nitro blue tetrazolium) until a colour is obtained.

[0468] The positive clones are then recovered on the dishes, titrated and subjected to the same immunoscreening procedure so as to confirm that they are true positives (secondary screening). Optionally, a tertiary screening may be carried out. The phage DNA of the selected clones, isolated from a bacterial lysate (Clontech protocol), and digested with the restriction enzyme EcoR1 is inserted at the EcoR1 site of the plasmid pBluescript SK+.

[0469] Two clones containing an insert of 1.8 kb were thus obtained, and proved to be of identical sequences. The hybridization of rat liver mRNA (2 μg of polyA+ mRNA) with a probe corresponding to the BgIII-XbaI fragment of this insert revealed two bands of sizes 1.9 kb and 2.1 kb (FIG. **11**A) respectively. Northern blot analysis, with a probe corresponding to the XbaI-XbaI fragment of this insert, of the tissue distribution of the corresponding messengers showed that they are preferably expressed in the liver (FIG. **11** B). The Northern blotting was carried out according to the following protocol.

Northern Blotting

[0470] The membranes containing the mRNAs of different rat tissues (Clontech) were hybridized with fragments of the cDNA for the LSR gene and of the cDNA for human β-actin (Clontech), labelled with [^{33}P]dCTP, in 5×SSPE, 10×Denhardt buffer containing 0.5% SDS, 100 μg/ml of salmon sperm DNA, 50% deionized formamide, at 42° C. for 16 hours. The membranes were then washed in 2×SSC, 0.5% SDS at room temperature and in 1×SSC, 0.1% SDS at 65° C., and then exposed on the Phospor Imager (Molecular Dynamics).

[0471] A cDNA corresponding to the 1.9 kb band was reconstructed by 5'RACE PCR from the 1.8 kb fragment and sequenced.

[0472] In order to elucidate the presence of multiple bands in Northern blotting, several pairs of primers defining fragments of a rat cDNA sequence were synthesized and used as primers for a PCR amplification (FIG. **11** C). The sequences of the oligonucleotides used are listed below:

(SEQ ID NO: 20)
a: 5'-GTTACAGAATTCGCCGCGATGGCGCCGGCG-3'

(SEQ ID NO: 21)
b: 5'-GCCAGGACAGTGTACGCACT-3'

(SEQ ID NO: 22)
c: 5'-ACCTCAGGTGTCCCGAGCAT-3'

(SEQ ID NO: 23)
d: 5'-GAAGATGACTGGCGATCGAG-3'

(SEQ ID NO: 24)
e: 5'-ACCTCTATGACCCGGACGAT-3'

(SEQ ID NO: 25)
b': 5'-CACCACCCTGACAGTGCGTA-3'

(SEQ ID NO: 26)
c': 5'-CTGGGGGCATAGATGCTCGG-3'

(SEQ ID NO: 27)
d': 5'-GCCCTGGAAGGCCTCGATCG-3'

(SEQ ID NO: 28)
e': 5'-CAAGTCCCTAGGATCGTCCG-3'

[0473] Whereas each pair of primers shows a single fragment, the bc' pair makes it possible to amplify three fragments of different sizes. Analysis of the sequences of these fragments makes it possible to reconstitute the sequence of three complete cDNAs for rat LSR, having sizes of 2097 bp (SEQ ID NO:1), 2040 bp (SEQ ID NO:3) and 1893 bp (SEQ ID NO:5) respectively, and all three corresponding to the same precursor messenger by alternative splicing.

[0474] These three cDNAs contain an open reading frame starting with an AUG codon at position 219 surrounded by a Kozak consensus sequence (Kozak, 1987 and 1990). The predicted molecular weights of the proteins encoded by these three cDNAs are 66 kDa, 64 kDa and 58 kDa, respectively.

[0475] The two cDNAs encoding respectively the longest and the shortest forms of rat LSR were then translated in vitro as indicated below.

Translation In Vitro

[0476] The cDNAs are subcloned into the plasmid pcDNA3; transcription and translation in vitro are carried out using the Promega TNT kit. The products of translation, labelled with ^{35}S-methionine and ^{35}S-cysteine, are visualized after electrophoresis on a polyacrylamide gradient gel (10%) and exposure on Phospor Imager.

[0477] The molecular weights of the products obtained, that is to say 68 kDa and 56 kDa (FIG. **12**), correspond closely to those of the α and β subunits of LSR.

[0478] To define if the products of these mRNAs are responsible for the receptor activity, three different experimental approaches were used.

[0479] Firstly, two peptides corresponding to residues 169-186 (SAQDLDGNNEAYAELIVLGR: SEQ ID NO:29) of the LSR produced from the mRNA of size 2097 bp and to residues 556-570 (EEGQYPPAPPPYSET: SEQ ID NO:30) were synthesized. The sequence of these peptides is common to the three proteins identified above. Antibodies directed against these synthetic peptides were obtained according to the protocols indicated above. FIGS. **13** C and **14** C show that these anti-LSR peptide antibodies have an inhibitory effect on the binding of the LDLs to the LSRs present on rat plasma membranes, measured according to the protocol described in Example 1.

[0480] Secondly, a partial purification of the α and β subunits was obtained by selective solublization with the aid of sarkosyl; a study using Western and ligand blotting showed that the α and β components bind the anti-LSR polyclonal antibodies (FIG. **13** B, lane 1), the anti-LSR peptide antibodies (FIG. **13** B, lane 2 and FIG. **14** B, lane 2), and the LDLs after incubation with oleates (FIG. **13** B, lane 4). Ligand blotting was carried out according to the protocol described in Example 1; Western blotting was carried out as indicated below.

Western Blotting

[0481] Primary cultures of rat hepatocytes are prepared as indicated in "Experimental procedures". The cells harvested after 48 hours of culture are washed and lysed in PBS containing 1% Triton X100. The lysates are deposited on a 10% SDS-PAGE gel under reducing conditions (2% SDS, 5% β-mercaptoethanol and 20 mM DTT, at 56° C. for 1 h). After transferring onto a nitrocellulose membrane, the Western blotting is carried out with IgG antibodies directed against the LSR receptor.

[0482] Thirdly, the labelled proteins LSR 66 and 58 obtained by in vitro translation from the cDNAs LSR-Rn-2097 and LSR-Rn-1893 are used to estimate the effect of oleate on the binding of the LDLs according to the protocol detailed below.

Binding of the LDLs onto the LSR Proteins Expressed In Vitro ("Flotation")

[0483] The ^{35}S-cysteine or ^{35}S-methionine labelled products of translation in vitro (17 μl) are incubated for 1 hour at 37° C. in the presence of 100 μg/ml of LDL, 1 mM oleate in buffer A, in a final volume of 400 μl. An equal volume of 8% (w/v) BSA is added. The density is adjusted to 1.21 g/ml (assuming an initial density of 1.025 g/ml), with sodium bromide. The samples are then deposited on a sodium bromide solution at 1.063 g/ml, and then centrifuged for 20 hours at 4° C. (Beckman SW41 rotor). A volume of 1 ml is collected at the surface, dialysed against electrophoresis elution buffer, and the radioactivity is counted (Beckman p counter).

[0484] Oleate increases the binding of LDL to LSR 56 (respectively LSR 68) by a factor of 2 (5 respectively). It can thus be shown that the α and β subunits of rat LSR, encoded respectively by the cDNAs LSR-Rn-2097 and LSR-Rn-1893 (LSR 56 and LSR 68), preferably bind the LDLs after incubation with oleate.

[0485] All these results indicate that the cDNAs LSR-Rn-2097 and LSR-Rn-2040 encode two proteins which are indistinguishable by electrophoresis and whose apparent molecular weight is 68 kDa; these proteins correspond to the band comprising the α and α' subunits of LSR, which is identified after immunoprecipitation under reducing conditions. The β subunit of LSR is presumably the product of translation of the cDNA LSR-Rn-1893. The analyses of stoichiometry after immunoprecipitation indicate that the multimeric complex of

apparent molecular weight 240 kDa is the result of an assembly of an α subunit with three β subunits. Analysis of the various domains of the proteins corresponding to the α- and β-LCRs is compatible with a lipoprotein receptor function.

Example 3

Analysis of the Activity of a Recombinant LSR Receptor, and its Subunits, in Transfected Cells

[0486] The inventors also expressed a recombinant LSR receptor in CHO cells according the following protocol.

Transfection with cDNA Sequences Encoding the LSR Receptor

[0487] In order to study the activity of each of the recombinant subunits of LSR, as well as the activity of a reconstituted receptor, the inventors used the expression plasmid pcDNA3 (No et al., 1996) to study the expression, in animal cells, of either cDNA encoding the α subunit (α plasmid), or of a cDNA encoding the β subunit (β plasmid), of rat LSR. The LSR cDNAs were subcloned into the plasmid pcDNA3 (Invitrogen) using the EcoRI and/or NotI restriction sites. Once obtained, these constructs are used to transfect CHO (Chinese hamster ovary) animal cells.

[0488] After 48 hours of culture, CHO (Chinese hamster ovary) cells (CHO-K1, CCL-61, ATCC, Rockville, Md.) were distributed into 6-well plates (Falcon) at $2.5–2.75\times10^5$ cells/well. After 24 h of culture in a Ham F-12 medium containing 10% (v/v) FBS, 2 mM glutamine and 100 units/ml of penicillin and streptomycin, a maximum of 2 μg of plasmid/well were transfected using Superfect (Qiagen) according to the supplier's instructions (10 μl Superfect/well, 2 h at 37° C. in a Ham F-12 medium free of serum). The plates were then washed in PBS in order to remove the transfection reagents and the cells were then cultured in a Ham F-12 medium containing serum. The LSR activity was measured 48 h after transfection according to the protocols detailed in Example 1.

[0489] The inventors tested the effect of a co-transfection with the α and β plasmids compared with that of a transfection with the α plasmid alone, or with the β plasmid alone, on the three stages of the activity of the LSR receptor according to the protocols detailed below. FIGS. **15** and **16** show the comparisons between the LSR activities obtained on the recombinant cells expressing the β subunit alone, or the two α and β subunits; similar results are obtained for the β versus α+β comparison, which is compatible with the comparative analysis of the primary sequences of each of the subunits (each of them also carrying the potential binding sites for lipoprotein ligands and fatty acids, such as oleate).

Effect of a Transfection with the LSR (α) Plasmid Alone, or of a Co-Transfection with the LSR (α) and LSR (β) Plasmid, on the Binding, Internalization and Degradation of the LDLs

[0490] The CHO-K1 cells were transiently transfected with increasing concentrations of α plasmid and co-transfected with 0.4 μg of α plasmid and increasing concentrations of β plasmid. After 48 h of culture, the cells were washed once with PBS and incubated for 3 h at 37° C. with 20 μg/ml ^{125}I-LDL in the presence or in the absence of 1 mM oleate in DMEM containing 0.2% BSA, 5 mM Hepes, and 2 mM $CaCl_2$, pH 7.5, Next, the cells were washed as described above and incubated at 4° C. for 1 h with 10 mM suramin in PBS.

[0491] To measure the binding of the LDLs (FIG. **15**), the medium was recovered and passed through a γ counter in order to evaluate the quantity of bound ^{125}I-LDL. The results are the mean values of two measurements. For the measurement of the internalization and the degradation of LDLs (FIG. **16**), the quantity of ^{125}I-LDL internalized and degraded was measured according to the protocols detailed in Example 1.

[0492] The co-transfection with α and β plasmids makes it possible to establish three stages of LSR activity (FIGS. **15** and **16**).

[0493] The inventors also observed that the co-transfection with the α and β plasmids increases the LSR activity compared with a transfection with only an α plasmid. The results suggesting a more efficient activity of the LSR when the ([β]/[α]) ratio between the concentrations of β and α subunits expressed increases, is compatible with the observation that the LSR receptor might consist of the assembly of an α (or α') subunit, and of several, probably three, β subunits.

[0494] The results show that only the co-transfection of the β and α subunits allows the overexpression of a completely functional LSR receptor in the sense that it allows the complete proteolytic degradation of the protein.

[0495] In order to characterize the lipoprotein degradation activity obtained above in cells transfected with the LSR cDNAs, the inventors finally tested the capacity of anti-LSR antibodies to inhibit the binding of LDLs as measured above, as well as the substrate-specificity thereof.

Characterization of the Lipoprotein Degradation Activity Obtained in Transfected Cells Expressing a Recombinant LSR Receptor

[0496] The CHO cells were transfected with the α end β plasmids in a concentration ratio of 1 to 3.

[0497] FIG. **17**A shows that the LDL binding activity obtained in the transfected cells (expressed relative to the same activity observed in nontransfected control cells) is specifically inhibited by the anti-LSR antibodies.

[0498] FIG. **17**B shows the LDL binding activity obtained in the cells transfected in the presence of various nonlabelled lipoproteins acting as competitive ligands. The results show a ligand specificity similar to that observed for the endogenous LSR activity in rats (Mann et al., 1995): the rat chylomicrons are the preferred substrates for the rat recombinant LSR; then come in particular, in decreasing order of specificity, the VLDLs and then the LDLs.

Example 4

Involvement of LSR in the Clearance of Cytokines

[0499] The analysis of the sequence of the α subunit of LSR reveals a cysteine-rich region which corresponds to a Tumor Necrosis Factor type cytokine receptor signature. LSR is, however, distinguishable from the cytokine receptors by the presence of signals allowing rapid endocytosis of the receptor/ligand complex (clathrin motif).

[0500] The inventors formulated the hypothesis that this receptor could serve for the removal of cytokines, and in particular of leptin; in order to verify this hypothesis they analysed the degradation of recombinant leptin by hepatocytes in primary culture according to the protocol below.

Degradation of Leptin by Hepatocytes in Primary Culture

[0501] Primary cells of rat hepatocytes are incubated for 4 hours at 37° C. with 20 ng/ml of ^{125}I-leptin in the absence or in the presence of 0.5 mM oleate, 75 μg/ml of RAP, 200 μg/ml

of non-specific antibodies or anti-LSR specific antibodies, or 50 μM chloroquinine. The medium is then recovered and the quantity of ^{125}I-leptin degraded is measured.

[0502] As indicated in FIG. **18**, the degradation of leptin by hepatocytes in primary culture is inhibited by:

a) polyclonal antibodies directed against LSR. These antibodies also inhibit, in the same proportions, the LSR activity,

b) the 39 kD Receptor Associated Protein (RAP); this protein blocks the LSR activity in vitro and retards the clearance of chylomicrons in vivo (Troussard et al., 1995; Willow et al., 1994)

c) chloroquine; this cellular poison prevents the acidification of the endocytosis vesicles and inhibits the activity of the lysosomal proteases,

d) oleate; this free fatty acid induces a change in the conformation of LSR which unmasks the lipoprotein binding site.

[0503] This indicates that the FAF (Fatty Acid Free) conformation of LSR is probably the only one which is compatible with the role of binding followed by degradation of leptin. The non-specific immunoglobulins are without effect on the degradation of leptin (FIG. **18**).

[0504] In order to verify the binding of leptin to LSR, the rat liver plasma membrane proteins were deposited on an affinity chromatography column containing recombinant leptin, according to the protocol detailed below.

Leptin Affinity Chromatography

[0505] A Hi-trap column (Pharmacia) is used: 5 mg of leptin are bound onto 1 ml of column according to the methods recommended by the manufacturer. The plasma membrane proteins are solubilized from rat livers as indicated above (Mann et al., 1995), and then dialysed overnight against PBS pH 7.4, 0.1% Tween 20. The column is washed in the same buffer and the protein extract is deposited at a rate of 0.2 ml/minute. The column is washed with 6 ml of the same buffer. It is then eluted with the same buffer supplemented with 100 mM glycine pH 3; 20 fractions of 500 μl are then neutralized with 5 μl of PBS, 0.1% Tween 20, pH 8. 50 μl of each fraction are deposited on a nitrocellulose membrane for dot-blot analysis by means of anti-LSR antibodies. The positive fractions (1, 3, 4, 7 and 8) are dialysed against 24 mM ammonium bicarbonate, 0.01% Tween 20, pooled and concentrated in a Speedvac in a final volume of 300 μl. 40 μl of the final product are analysed by Western blotting by means of anti-LSR antibodies.

[0506] FIG. **19** shows that the anti-LSR antibodies specifically recognize the α subunit which, after binding to leptin, was released by the glycine buffer.

[0507] Experiments of stable transfection of the α subunit will make it possible to measure the affinity of leptin for this new receptor.

[0508] All these results suggest that LSR represents one of the pathways for the degradation and elimination of leptin. The in vivo injection of radiolabelled recombinant leptin showed, both in the obese mice and in the control mice, a rapid speed of clearance and a preferential capture of leptin by the liver and the kidney: 50% of the injected dose is found after 10 minutes in these two organs. In order to analyse the mechanisms for the selective capture of leptin, the inventors compared the quantities of leptin and of β2-microglobulin (soluble protein having a molecular weight close to that of leptin, chosen as control) present in the kidney and liver of normal mice and of two obese mouse lines 5 minutes after injection of the same tracer dose of these two radiolabelled proteins.

Measurement of the Clearance of Leptin in Mice

[0509] The female control, ob/ob, or db/db mice (6-8 weeks), on an empty stomach, are anaesthetized and receive via the saphenous vein an injection of 80 ng of murine recombinant ^{125}I-leptin or of ^{125}I-β_2-microglobulin (Sigma, labelled by the Iodobeads method, like leptin). Five minutes later, the animals are infused with a physiological saline solution (15 ml, at 4° C.). The tissues are collected and counted for their radioactivity (Gamma counter). In some cases, an anti-LSR antibody or a control protein are injected 30 minutes after injection of ^{125}I-leptin. It is important to note that the labelling of leptin with ^{125}I has no effect on its biological activity.

[0510] The results presented in FIG. **20** show that the quantity of leptin selectively captured by the liver is reduced in the obese mice, compared with the control mice; moreover, no difference is observed between the various lines as regards the renal capture of leptin.

[0511] The inventors then measured the number of LSR receptors in control, ob/ob and db/db mice according to the following protocol.

Measurement of the Apparent Number of LSR Receptors on Plasma Membranes

[0512] The apparent number of LSR receptors on plasma membranes is measured as previously described (Mann et al., 1995) by estimating the quantity of LDL bound to a plasma membrane preparation. The plasma membranes (100 μg) are incubated with 1 mM oleate; they are then washed three times as indicated above, and then incubated for 1 hour at 37° C. with 40 μg/ml of ^{125}I-LDL. The quantity of ^{125}I-LDL bound to the plasma membranes is then determined by counting. The mean is established on 3 measurements per animal for 3 different animals in each of the groups.

[0513] FIG. **21** shows that the number of LSR receptors in obese animals exhibiting either a deficiency in leptin (ob/ob), or a deficiency in the ob receptor (db/db), is significantly reduced. The reduction in the selective hepatic capture of leptin in obese mice coincides with the reduction, in these animals, of the apparent number of LSR receptors.

[0514] The inventors finally tested, according to the protocol presented below, the effect of anti-LSR antibodies on the distribution of leptin between the liver and the kidney, 5 minutes after injection of a tracer dose.

Measurement of the Distribution of Leptin Between the Liver and the Kidney in the Presence of Anti-LSR Antibodies

[0515] Control mice are anaesthetized and then they are injected intravenously with 1 mg of non-specific IgG antibody or of anti-LSR IgG antibody. After 30 minutes, 80 ng of ^{125}I-leptin are injected and, after 5 minutes, an infusion of physiological saline solution at 4° C. The tissues are removed immediately and the radioactivity is measured. The results represent the mean and the standard deviation obtained for 3 animals for each of the groups.

[0516] As shown in FIG. **22**, the hepatic capture of leptin is reduced and the renal capture is increased by the anti-LSR antibodies, compared with the control immunoglobulins.

[0517] These results therefore indicate that LSR is responsible for the selective hepatic capture of leptin and that a reduction in the number of receptors is observed in the obese animals. Such a reduction may explain the leptin-resistance syndrome and the increase in the plasma concentration of leptin which is observed in most obese human subjects.

[0518] It is also possible that the LSR receptor serves as degradation pathway for other cytokines, in particular those produced by the adipose tissue. The importance of Tumor Necrosis Factor α and Nerve Growth Factor will be noted in particular. These two cytokines exert a significant slimming effect when they are injected into human subjects (Cytokines and their receptors, 1996).

Example 5

Control of the LSR Activity by Cytokines

[0519] The α subunit of the LSR receptor binds leptin and possesses potential phosphorylation sites. This makes it a receptor which not only mediates endocytosis, but could also serve in cell signalling.

[0520] The inventors therefore tested the hypothesis according to which leptin modulates the activity of LSR, as described below.

Measurement of the LSR Activity of Binding, Internalization and Degradation of Lipoproteins in the Presence of Leptin

[0521] Rat hepatocytes in primary culture are incubated at 37° C. for 30 min with an increasing concentration of leptin, and then incubated at 37° C. for 4 hours with either 50 μg/ml of ^{125}I-LDL (specific activity: 209 cpm/ng) or 50 μg/ml of ^{125}I-VLDL (specific activity: 157 cpm/ng) in the absence or in the presence of 500 μM oleate. The cells are then washed and the quantities of ^{125}I-lipoproteins bound, incorporated and degraded are measured as described above in Example 1 (Bihain and Yen, 1992). The results shown in FIG. **23** represent the differences obtained between the cells incubated with or without oleate. Each point represents the mean of 3 measurements. The standard deviation for each point is included in the symbol.

[0522] The addition of increasing concentrations of leptin to hepatocytes in culture increases the binding, internalization and degradation of VLDLs and LDLs (FIG. **23**).

Analysis of the Capacity for Inducing the LSR Activity by Leptin Measurement, in the Presence of Leptin, of the Apparent Number of LSR Receptors Expressed at the Surface of Rat Hepatocytes in Primary Culture

[0523] Primary cultures of rat hepatocytes are incubated for 30 min at 37° C. in the presence or in the absence of 20 ng/ml of leptin, for 10 min at 37° C. in the presence of 0.8 mM oleate. The cells are washed with PBS buffer precooled to 4° C., and then incubated for 2 hours at 4° C. in the presence of increasing concentrations of ^{125}I-LDL. The cells are then washed, lysed and the quantity of bound ^{125}I-LDL is measured.

Comparative Effects of Leptin in the Presence of Cycloheximide, Colchicine and Cytochalasin B

[0524] The initial conditions are identical to those described above; after incubation with leptin, the cells are incubated for 30 min at 37° C. with 5 μM cycloheximide, 5 μM colchicine or 2.5 μM cytochalasin B. The cells are then incubated for 10 min at 37° C. in the presence of 0.8 mM oleate. The cells are then washed with PBS buffer precooled to 4° C., and then incubated for 2 hours at 4° C. in the presence of 50 μg/ml of ^{125}I-LDL. 2 measurements are carried out, and the mean results are presented.

[0525] It is thus shown that the increase in the LSR activity by leptin is obtained through an increase in the apparent number of receptors expressed at the surface of the hepatocytes (FIG. **24** A). This increase results, on the one hand, from an increase in protein synthesis (it is partially inhibited by cycloheximide, an inhibitor of protein synthesis). It involves, on the other hand, the mobilization of the endocytosis vesicles by the microtubule system (it is indeed inhibited by cytochalasin B which blocks microtubular transport) (FIG. **24** B).

[0526] In order to check the in vivo effect of leptin on the LSR activity, the inventors characterized the postprandial triglyceridemic response of control, ob/ob and db/db mice after a force-fed test meal according to the following protocols.

Measurement of the Postprandial Lipemic Response in Mice

[0527] Control, ob/ob and db/db mice, starved since the day before, are force-fed with a meal which is very high in fat [60% fat (37% saturated, 27% monounsaturated and 36% polyunsaturated fatty acids), 20% protein and 20% carbohydrate] providing 56 kcal of energy/kg of the weight of the animal. Immediately after the meal (time=0 hour), the mice are injected intravenously with 200 μl of physiological saline solution. At various times, 20 μl of blood are collected via the caudal vein in tubes containing 90 μg of disodium EDTA, and after separating the plasma by centrifugation, the plasma concentration of triglyceridemia is determined with the aid of an enzymatic assay kit. Each point on the curves presented corresponds to the mean with standard deviation obtained for 3 measurements per animal and for 3 different animals.

Measurement of the Effect of Leptin on the Postprandial Lipemic Response in Mice

[0528] The procedure is the same as above, except that immediately after the meal (time=0 hour), the mice are injected intravenously with either 200 μl of physiological saline solution, or 200 μl of the same solution containing 50 μg of murine recombinant leptin.

Measurement of the Postprandial Lipemic Response in Mice in the Presence of Lactoferrin and/or Leptin

[0529] ob/ob mice, starved since the day before, are force-fed with a meal identical to that described above. Immediately after the meal (time=0 hour), the mice are injected intravenously with 200 μl of saline solution containing either no supplement, or 0.5 μg of leptin, or 2.5 mg of lactoferrin or alternatively a mixture of 0.5 μg of leptin and 2.5 mg of lactoferrin. Blood is collected between 2 and 3 hours after the meal and the plasma concentration of triglycerides (TG) is measured. The values obtained represent the mean with standard deviation obtained for 4 measurements per animal and for 2 different animals [$p<0.02$ (ob/ob compared with ob/ob+leptin), $p<0.01$ (ob/ob compared with ob/ob+lactoferrin), NS (ob/ob+lactoferrin compared with ob/ob+leptin+lactoferrin)].

[0530] In agreement with the reduction in the number of LSR receptors observed in the obese mice, an amplification of the postprandial lipemic response also exists in the untreated

obese mice. The administration of leptin by the intravenous route, at the same time as the test meal, makes it possible to reduce the postprandial lipemic response in the two obese mouse lines and in the control mice (FIG. **25**).

[0531] This reduction in the lipemic response induced by leptin is suppressed by the administration of lactoferrin (FIG. **26**), which blocks the activity of LSR (Yen et al., 1994; Mann et al., 1995). This strongly suggests that the reduction in the lipemic response is explained by an increase in the LSR activity.

[0532] Finally, also in vivo, the administration of leptin induces an increase in the apparent number of LSR receptors expressed at the level of the surface of the hepatocytes. This increase is significant both in the ob/ob mice and in the db/db mice (FIG. **27**).

[0533] Leptin and probably other cytokines are therefore regulators of the activity of LSR. A syndrome of resistance to leptin or to other cytokines can lead to hypertriglyceridemia, which is either permanent or limited to the postprandial phase.

Example 6

Effect of Leptin on the Expression of LSR; Therapeutic Effects

[0534] To reinforce correlation between the administration of leptin, the reduction in the postprandial lipemic response, and an enhanced expression or activity of the LSR receptor, and to better evaluate the possible therapeutic implications of the induction of the activity of hepatic clearance of lipoproteins by leptin, the inventors supplemented the preceding analysis with monitoring of the weight variation, of the LSR activity and of the expression of LSR mRNA, in control or obese animals treated with leptin or otherwise.

Postprandial Lipemic Response and LSR Activity in Control and Obese Mice

[0535] Control male mice (C57BL6) (n=8) and obese male mice (ob/ob, n=8—animals deficient in the leptin gene—and db/db, n=8—animals deficient in the gene for the leptin receptor—) (aged 17 weeks old) were weighed in order to quantitatively establish the differences in weight between lines (FIG. **28**A). The postprandial lipemic responses of the animals of each line were measured in the absence of treatment with leptin as described above. The apparent number of LSR receptors expressed at the surface of the hepatic cells was measured on 4 animals of each line, as described above, and expressed in comparison with the 5'-nucleotidase activity (enzyme selectively measured at the level of the plasma membranes; Sigma kit). Finally, Northern blotting made it possible to estimate the level of expression of the LSR receptor in three animals of each line, according to the protocol described above.

[0536] The higher postprandial lipemic response in the obese animals (FIG. **28**B) is in agreement with the smaller apparent number of hepatic LSR receptors in these same animals (FIG. **28**C). Furthermore, the Northern blotting results (FIG. **28**D) indicate that this reduction in the apparent number of LSR receptors in the obese animals is accompanied by a reduction in the level of expression of the said receptor in the same animals. The inventors have shown that indeed, a reduction in the number of mRNA encoding the LSR receptor is observed in the obese mice ob/ob and db/db.

[0537] The inventors also studied the effect of a long-term treatment of a treatment with leptin on ob/ob mice (FIG. **29**).

Effect of a Long-Term Treatment with Leptin on ob/ob Mice

[0538] The ob/ob obese mice received a daily injection of either leptin, or of an equivalent volume of sterile PBS, for 30 days. The injected doses are 50 μg/animal from day 0 to day 4, 100 μg/animal from day 5 to day 17, and 150 μg/animal from day 18 to day 30. Several parameters indicated below are measured:

[0539] the weight (FIG. **29** A): the change in weight is measured for 6 animals, over the duration of the treatment;

[0540] the postprandial lipemic response (FIG. **29** B): it is measured according to the protocol detailed in Example 5 on three animals in each group, on day 29;

[0541] the apparent number of LSR receptors (FIG. **29** C): it is measured according to the protocol detailed in Example 4 on three animals in each group, on day 30;

[0542] the quantity of LSR mRNA (FIG. **23** D): it is estimated by Northern blotting as indicated in the protocol of Example 2.

[0543] The inventors thus observed a very significant loss of weight in the ob/ob obese mice treated over 30 days with leptin. Furthermore, the treatment with leptin causes a clear reduction in the postprandial lipemic response. This reduction in the postprandial lipemic response is correlated with an increase in the apparent number of LSR receptors at the surface of the cells and with an increase in the quantity of mRNA encoding the subunits of the LSR receptor.

[0544] These results establish in vivo that LSR represents the limiting step in the elimination of dietary lipids. Furthermore, the treatment of this obesity inducing a weight loss causes an increase in the activity of hepatic degradation of dietary lipids, and a reduction in the postprandial lipemic response.

Example 7

Characterization of the Human LSR Receptor

Northern-Blot Analysis

[0545] Nucleic probes for rat LSR were used to carry out Northern-blot analyses with a membrane (Human Multiple Tissue Northern Blot, Clontech #7760-1) comprising human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas poly A RNAs. A band of about 2 kbp is detected in the liver and in the kidney. Approximate quantification of the hybridization results indicate that LSR is expressed in the liver at least 5 times more than in the kidney.

Cloning of the cDNA; Study of the Splicing Zone

[0546] Reverse transcription-PCR experiments on the mRNA made it possible to determine with greater precision the size of exon 1 on the 5' side and splicing sites between exons 1 and 2. However, it is not certain that this end constitutes the start of this exon. In addition, a second initiation site exists in exon 1 which is more downstream from the first and which exhibits a greater probability than the latter. The splicing between exons 1 and 2 was different between the human RNA and the rat RNA.

[0547] The amplification was carried out with several pairs of primers:

(SEQ ID NO: 31)
a: 5'-ATGCAACAGGACGGACTTGGA-3'
exon 1

(SEQ ID NO: 32)
b: 5'-TCAGACGACTAAACTTTCCCGACTCAGG-3'
exon 10

(SEQ ID NO: 33)
c: 5'-CTACAACCCCTACGTTGAGT-3'
exon 2

(SEQ ID NO: 34)
d: 5'-TCGTGACCTGACCTTTGACCAGAC-3'
exon 3

(SEQ ID NO: 35)
e: 5'-CCTGAGCTACTCCTGTCAACGTCT-3'
exon 6

(SEQ ID NO: 36)
f: 5-AGGCCGAGATCGCCAGTCGT-3'
exon 9

[0548] The amplification carried out with the ab pair of primers led to two products 1.8 kb and 2 kb in size after separation on an electrophoresis gel. Given that the sizes of these two products can be explained by an alternative splicing similar to that described in rats, the other amplification primers were drawn. These primers made it possible to identify the three forms of cDNA resulting from the alternative splicing of the RNA.

[0549] The first cDNA which contains the totality of the ten exons is called LSR-Hs-2062 and corresponds to SEQ ID NO:7. It corresponds to the rat cDNA LSR-Rn-2097. The second cDNA contains exons 1, 2, 3, 5, 6, 7, 8, 9 and 10, and is called LSR-Hs-2005. It corresponds to SEQ ID NO:9. This cDNA corresponds to the rat cDNA LSR-Rn-2040. Finally, the cDNA containing exons 1, 2, 3, 6, 7, 8, 9 and 10 is called LSR-Hs-1858 and its sequence is listed in SEQ ID NO:11. It corresponds to the rat cDNA LSR-Rn-1893.

[0550] It should be noted that it was possible to demonstrate a slippage of the splicing site at the boundary of exon 8. This slippage, of the triplet TAG at position 19953-19955 of SEQ ID NO:19 to the contiguous triplet AAG at position 19956-19958 of SEQ ID NO:19, results in the loss of the Glu residue at position 386 of the cDNA of SEQ ID NO:8.

[0551] The sequences of the proteins encoded by the cDNA LSR-Hs-2062, LSR-Hs-2005 and LSR-Hs-1858 correspond respectively to SEQ ID NOs:8, 10 and 12. The biological protein sequences can start at the first ATG codon observed in the reading frame (position 35 of the protein sequence). However, the preferred codon for initiation of translation is more downstream at position 83 of the protein sequence. Furthermore, it is quite possible that this initiation codon is more upstream in the 5' region of exon 1 not yet determined or in a possible exon preceding the latter.

[0552] Finally, FIGS. **3**A and **3**B represents a schematic representation of the various protein forms identified in humans, indicating the conserved motifs.

[0553] This analysis makes it possible to conclude that three α, α' and β subunits of LSR, which are equivalent to the LSR 66, LSR 64 and LSR 58 forms in rats, exists in humans.

Identification and Isolation of the Genomic Sequence for Human LSR

[0554] Screening of public data banks of nucleic sequences (Genbank, version: 101) both with the sequence of mouse lisch7 (Accession No.: U49507) and with that of rat LSR 2097 isolated by the inventors made it possible to isolate two human genomic DNA sequences. They are cosmids whose accession numbers are AC002128 and AD000684, of respective sizes 45,328 bp and 41,936 bp. These two cosmids partially overlap. The 3' end of the cosmid AC002128 overlaps, over 12838 bp, the 5' end of the cosmid AD000684. On the common portion of 12,838 bp, the sequences are 100% identical, apart from two deletions at positions 822 and 3170 of the cosmid AD000684. The human LSR gene is distributed over the two cosmids. To facilitate the study of this region, a complete genomic sequence was reconstituted: the 45,328 bp of the cosmid AC002128 were added to the sequence of the cosmid AD000684 between the 12,839 base and the 41,936 base. The combination constitutes a sequence of 74,426 bp. A genomic sequence covering the LSR gene, was extracted (SEQ ID NO:19).

[0555] The putative exons of the LSR gene were determined after alignment of the sequence described above with the sequences of the RNAs for mouse Lisch7 and rat LSR. The validity of the splicing sites on either side of the putative exons was verified.

[0556] Moreover, a human genomic library consisting of BACs was screened by the methods described in Chumakov et al., 1995; the clones thus isolated were contiged, subcloned and then sequenced in order to obtain the human genomic sequence encoding LSR (SEQ ID NO:41).

[0557] The two sequences thus obtained (SEQ ID NOs:19 and 41) carry minor differences which are mentioned in the accompanying listings.

Example 8

LSR Activity in Humans

[0558] Primary cultures of human fibroblasts, isolated from subjects having a deletion affecting the promoter and the first exon of the LDL receptor gene, were obtained.

[0559] The incubation of these cells in the presence and in the absence of oleate shows that the latter induces LDL binding, internalization and degradation activity which follows a saturation kinetics (Bihain and Yen, 1992). The affinity of this receptor, induced by oleate, is maximum for the particles high in triglycerides (VLDL and chylomicrons) as well as for triolein and phosphatidylcholine supplemented with recombinant apoprotein E. The affinity of the LDLs for the receptor is lower than that of the VLDLs and the chylomicrons but, however, higher than those of triolein and phosphatidylcholine particles not containing ApoE, or than those of VLDLs isolated from a subject with type III hyperlipidemia and the ApoE $E_{2/2}$ phenotype (Yen et al., 1994).

[0560] It was also possible to measure the LSR activity in fibroblasts of normal human subjects (FIG. **30**), according to the protocol below.

Measurement of the Binding, Internalization and Degradation of LDLs by Fibroblasts.

[0561] The fibroblasts are cultured beforehand for one week as described above, except that the medium contains 20% foetal bovine serum (Goldstein et al., 1983). Next, they are incubated with increasing concentrations of ^{125}I-LDL in

the absence or in the presence of 1 mM oleate. The cells are then washed, lysed and counted for their radioactivity.

Example 9

Effect of Leptin on the LSR Activity in Humans

[0562] The LSR activity of human fibroblasts HF (familial hypercholesterolemia) is also increased after incubation with leptin (FIG. **31**), suggesting that as in rats, LSR participates, in humans, in the clearance of cytokines, and its activity is modulated by the latter. The corresponding measurements were carried out as indicated below.

Effect of Leptin on the LSR Activity on Human Fibroblasts

[0563] The fibroblasts HF are incubated for 30 minutes at 37° C. with increasing concentrations of leptin, and then for 2 hours at 37° C. with 50 μg/ml of ^{125}I-LDL, in the presence of 500 μM oleate. The binding, internalization and degradation of the LDLs are measured as indicated in Example 1.

Example 10

Cloning of the cDNA for Mouse LSR; Analysis of the Products of Alternative Splicing

[0564] The cloning of the cDNA for mouse LSR was carried out using a mouse liver mRNA library. The cloning method used is the same as that for the cDNA for human LSR. The mRNAs were purified and a reverse transcription PCR amplification was carried out with the specific DNA primers. The amplification fragment was cloned to a TA cloning vector (Introgene).

[0565] A study of the products of alternative splicing with primers situated in exon 2 and in exon 9 was also carried out in a manner similar to that carried out for the human LSR.

[0566] Three products of alternative splicing were observed: LSR-Mm-1886, LSR-Mm-1829 and LSR-Mm-1682. LSR-Mm-1886 contains all the exons from 1 to 10. LSR-Mm-1829 and LSR-Mm-1682 lack exon 4 and exons 4 and 5, respectively. These three biological forms of cDNA indeed correspond to what was observed in humans and rats. The nucleotide sequences of the cDNAs LSR-Mm-1886, LSR-Mm-1829 and LSR-Mm-1682 are illustrated in SEQ ID NOs:13, 14 and 15, respectively. The protein sequences encoded by the cDNAs LSR-Mm-1886, LSR-Mm-1829 and LSR-Mm-1682 are illustrated in SEQ ID NOs:16, 17 and 18.

Example 11

Identification of the γ Subunit of LSR

[0567] The α and β subunits of LSR were identified as indicated above. Analysis of the products of translation of the RNAs encoding these two subunits does not allow the presence of a third subunit of molecular weight ≈35 kDa to be explained. This subunit is detected only after reduction of the LSR complex (FIG. **10**, lane 4).

[0568] We purified and obtained the NH_2-terminal sequence of this γ subunit.

[0569] The purification was carried out by immunoaffinity chromatography according to the following procedure.

Purification of the γ Subunit of LSR

[0570] Anti-LSR antibodies (band A) are coupled to a resin [2.5 mg of IgG per 3.5 ml of affi-gel Hz immunoaffinity kit resin (Biorad 153-6060)] which is then incubated with proteins solubilized from total membranes of rat liver (20 mM Tris buffer, 2 mM EDTA, 0.125 M octyl glucoside (5×CMC), 1% inhibitor cocktail, pH=7.4: 160 mg of membrane proteins give 41.3 mg of solubilized proteins (SP) in a volume of 17 ml.

[0571] The incubation is carried out for 12 hours: 17 ml filled to 50 ml with 20 mM Tris buffer, 2 mM EDTA, pH 7.4 and the 3.5 ml of resin, with rotary shaking, at room temperature. The resin is washed with 40 ml of 20 mM Tris buffer, 2 mM EDTA, pH 7.4 and then eluted with 20 mM Tris buffer, 2 mM EDTA, 200 mM glycine, pH 2.5 in 30 fractions of 500 μl. The pH of each fraction is neutralized with 100 μl per tube of 1 M Tris buffer, 2 mM EDTA, pH 9. 50 μl of each fraction are deposited on a nitrocellulose membrane for dot-blot analysis: incubation with anti-LSR antibody, and then with a second antibody coupled to alkaline phosphatase.

[0572] The positive fractions from 7 to 28 are pooled in pairs and concentrated 2.5-fold in a Speedvac. Western blotting is carried out on the pooled, concentrated and separated fractions on a 10% PAGE-SDS gel. Bands are observed in fractions 7 to 14 (the fractions are pooled).

[0573] The two pools are dialysed against 24 mM ammonium bicarbonate and then freeze-dried in a Speedvac. The powder is taken up in 80 μl of 20 mM Tris buffer, 2 mM EDTA, 2% SDS, 3% urea, pH 7.4 and reduced in the presence of 5% β-mercaptoethanol for 30 minutes at 100° C.

[0574] After migration and wet transfer in 50 mM Tris, 50 mM borate on a sequencing membrane (PVDF) at 30 mA, the membrane is stained with amido black.

[0575] A band with an apparent MW of about 35 kDa was thus identified and sent for sequencing according to the Edman method.

[0576] The sequence obtained is LHTGDKAFVEFLTDEIKEE. This sequence corresponds identically to that of a protein of molecular weight 33 kDa identified above as a protein of the cellular surface which binds the globular heads of C1q (gC1q-R) (Ghebrehiwet et al., 1994). A more recent observation indicates that this potential receptor for C1q is also located in the vesicles situated under the cellular surface (van den Berg et al., 1997). This protein also corresponds to a protein previously identified as p34, and which combines with a lamin receptor. This receptor possesses a long NH_2-terminal segment oriented inwards in the cell nucleus as well as 8 transmembrane domains. This receptor binds to lamin in a manner which depends on the degree of phosphorylation. Finally, gC1q-R combines with "splicing factor 2" (Honoré et al., 1993). The lamin receptor and "splicing factor 2" have in common the characteristic of containing a repeated sequence of serine and arginine (RSRS) situated at the level of the NH_2-terminal segment in the case of the lamin receptor and at the level of the carboxy-terminal segment in the case of SF2.

[0577] It is remarkable to observe that both α-LSR and β-LSR exhibit repeated segments high in serine and arginine (FIG. **1**). Our hypothesis is that the γ-LSR protein represents a molecular chaperone which combines with the α and β subunits of LSR via their RSRS domain.

[0578] In order to verify this hypothesis, we obtained polyclonal antibodies directed against two synthetic peptides whose sequence was situated at the carboxy- or NH_2-terminal end of the gC1q-R protein:

NH_2-terminal peptide of gC1q-R:
(SEQ ID NO: 39)
LRCVPRVLGSSVAGY*
(amino acids 5 to 19 of gC1q-R)

COOH-terminal peptide of gC1q-R:
(SEQ ID NO: 40)
C*YITFLEDLKSFVKSQ.
(amino acids 268 to 282 of gC1q-R)

*amino acids differing from the protein sequence, so as to optimize the antigenicity of the peptides.

[0579] FIG. **32** shows these antibodies specifically inhibit the activity of LSR. The antibody directed against the COOH-terminal end appears to be the most effective.
These results indicate that gC1q-R, or one of its structurally similar homologues, represents a molecular chaperone non-covalently combined with the LSR multimeric complex.

Example 12

Regulation of the LSR Activity by C1q and its Homologues

[0580] It has been shown that gC1q-R could bind the globular head of complement factor 1. We sought to use this property of C1q to displace gC1q-R combined with LSR, and we measured the effect of increasing doses of C1q on the binding, internalization and degradation of the LDLs by hepatocytes in primary culture. FIG. **33** shows an increase in the capture and degradation of LDLs induced by human C1q, even in the absence of oleate.

[0581] A less substantial, but nevertheless significant, increase is also observed in the presence of oleate. However, under these conditions, the maximum effect is obtained for lower concentrations of C1q.

[0582] It therefore appears that gC1q-R exerts on LSR an inhibitory effect which is comparable to that induced by the 39 kD RAP for LRP, the LDL receptor and LSR (Troussard et al., 1995). The displacement of the chaperone gC1q-R using its capacity to bind to complement C1q makes it possible to lift the inhibitory effect. Analysis of the gC1q-R sequence shows that it may be a typical membrane receptor. Indeed, the protein possesses no hydrophobic sequence capable of crossing the phospholipid bilayer.

[0583] The effect of complement C1q on the activity of LSR opens major perspectives in the context of the genetics of obesity. It is possible, indeed, that mutations affecting either the gene for C1q, that for gC1q-R, or alternatively that for their analogues such as for example AdipoQ, cerebellin, collagen alpha 1-10, SPA and SPD (pulmonary surfactant proteins), mannan-binding protein, and the scavenger receptor or its homologue LRP (Hu et al., 1996; Drickamer et al., 1986; Krieger and Herz, 1994; Elomaa et al., 1995) modulate the activity of LSR, both as regards clearance of lipoproteins and as regards that of leptin.

[0584] Several proteins can interact with gC1q-R because they exhibit homologies with complement C1q. In particular, two proteins isolated in mice, AdipoQ (Hu et al., 1996) and acrp30 (Scherer et al., 1995), and a human protein APM1 (Maeda et al., 1996) exhibit marked homologies. These three proteins, like the components of complement C1q (C1q A, B, C), are secreted proteins; they have an NH_2-terminal end which resembles collagen (repetition of Gly-X-Y motifs) and a COOH-terminal end corresponding to the globular domain of complement C1q. These three proteins are preferably expressed in the adipose tissue. There are only 3 amino acids differing between AdipoQ and acrp30. APM1, a protein whose messenger has been characterized as being highly expressed in adipocytes, exhibits 79.7% nucleic acid identity and 80.6% amino acid identity with AdipoQ. APM1 is therefore certainly the human homologue of AdipoQ.

Example 13

Screening of Compounds Modifying the Activity of the LSR Receptor

[0585] As described above, the inventors formulated the hypothesis that the LSR "γ band", a protein which is highly homologous to gC1qR, might interact with the LSR receptor like a molecular chaperone and might thus form an "LSR complex", comprising the α or α' and β subunits of the LSR receptor and a gC1qR type molecule. gC1qR has been previously identified as a cell surface protein which binds the globular heads of the complement factor C1q. In addition to C1q, several proteins exhibiting homologies with the C1q proteins, in particular AdipoQ and acrp30 in mice and APM1 in humans, are capable of interacting with the protein homologous to gC1qR in the LSR complex and of modifying the LSR activity.

Screening Parameters

[0586] The screening of a compound such as C1q or AdipoQ was carried out through the measurement of various parameters of which the most important is the measurement of the effect of the compound on the activity of the LSR receptor. The various parameters are the following:

[0587] change in weight

[0588] food intake

[0589] postprandial lipemic response

[0590] binding, internalization and/or degradation of lipoproteins such as the LDLs.

Change in Weight

[0591] Osmotic pumps were surgically inserted into the abdominal cavities of 12 Sprague-Dawley male rats of 400-450 g. The osmotic pumps contained either 2 ml of PBS (phosphate buffered saline), pH 7.4 (control 6 rats), or 2 ml of recombinant AdipoQ protein (5 mg/ml PBS, 6 rats). These pumps were designed to deliver 10 μl/h (50 μg AdipoQ/h). The animals are weighed and individually housed in metabolic cages. 3 animals in each group are subjected ad libitum either to a normal diet or to a fatty diet (day 0). The fatty diet consists of a normal diet supplemented with 2% (w/w) cholesterol, 10% (w/w) saturated fatty acid in the form of vegetaline, [lacuna] % (w/w) sunflower oil and 15% (w/w) sucrose. On day 3, the animals are weighed and blood samples are obtained from the caudal vein. The quantity of plasma triglycerides was measured using an enzymatic kit.

Food Intake

[0592] Recombinant AdipoQ protein (100 μg) or PBS alone were injected daily for 5 days through the caudal vein of ob/ob or db/db mice kept in a metabolic cage. The mice are weighed each day and the quantity of food consumed was also

measured. The results correspond to a mean food intake and a standard deviation for 4 mice in each group.

Postprandial Lipemic Response

[0593] Male Sprague-Dawley rats (400-450 g), starved since the day before, were force-fed with a meal which was very high in fat (t=0) (60% fatty acid of which 37% saturated, 27% monounsaturated and 36% polyunsaturated, 20% protein and 20% carbohydrate, the total providing 56 kcal/kg of body weight) and received immediately afterwards an intravenous injection (femoral vein) of either 300 μl of PBS alone or of the same volume containing 1 mg of mouse recombinant AdipoQ protein. Blood samples were collected at various times (0, 2, 4 and 6 h). The quantity of plasma triglycerides was measured using an enzymatic kit. The results are presented as mean values and standard deviations on 3 animals.

LSR Activity or Binding, Internalization and Degradation of Lipoproteins

[0594] Primary cultures of rat hepatocytes were prepared and distributed into 6-well plates (9000,000 cells/well). After 48 h, the cells were washed once with PBS (2 ml/well) and incubated for 30 min at 37° C. with 20 ng/ml of recombinant murine leptin. The cells were then incubated for 4 h at 37° C. with increasing concentrations of recombinant murine AdipoQ proteins and 20 μg/ml ^{125}I-LDL in the presence or in the absence of 0.5 mM oleate. The binding, internalization and degradation of lipoproteins were measured as indicated in Example 1.

C1q

[0595] The compound C1q was tested for its capacity to modulate the activity of the LSR receptor (binding, internalization and degradation of lipoproteins). FIG. **33** shows that the compound C1q exhibits the property of increasing the activity in the presence and in the absence of oleate. Thus, it was possible for this compound C1q to be selected as modulator of the LSR activity through the test of activity described above.

AdipoQ

[0596] The compound AdipoQ was tested according to the four parameters presented above.

[0597] FIG. **34** shows that the compound AdipoQ modulates the LSR activity in the presence of oleate. Indeed, at the concentration of 25 ng/ml, it increases the LSR activity.

[0598] FIG. **35** shows that the administration of AdipoQ makes it possible to massively reduce the postprandial lipemic response.

[0599] FIG. **36** shows that a 3-day ip infusion treatment with AdipoQ causes a loss in weight which is much greater when the rat is subjected to a fatty diet. Furthermore, the inventors observed that the level of plasma triglycerides is reduced in the animals treated with AdipoQ.

[0600] FIG. **37** shows that an injection of AdipoQ reduces the food intake in obese animals.

[0601] The increase in the LSR activity induced by 25 ng/ml of AdipoQ can explain the reduction in the postprandial lipemic response and the weight loss.

[0602] Thus, the AdipoQ protein is a very valuable compound which could be used in particular in the treatment of obesity. The selection of this protein as a candidate molecule in the treatment of obesity validates the parameters for screening a compound of interest modulating the LSR activity, the most important parameter consisting in measuring the LSR activity.

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SEQUENCE LISTING

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ggggacgcgc gggcaccgtc gctgctagac ggccgcg atg gcg ccg gcg gcc ggc     235
                                        Met Ala Pro Ala Ala Gly
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gcg tgt gct ggg gcg cct gac tcc cac cca gct acc gtg gtc ttc gtg      283
Ala Cys Ala Gly Ala Pro Asp Ser His Pro Ala Thr Val Val Phe Val
            10                  15                  20

tgt ctc ttt ctc atc att ttc tgc cca gac cct gcc agt gcc atc cag      331
Cys Leu Phe Leu Ile Ile Phe Cys Pro Asp Pro Ala Ser Ala Ile Gln
        25                  30                  35

gtg act gtg tct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg      379
Val Thr Val Ser Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val
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acc ctg ccc tgc acc tat cag atg agc aac act ctc aca gtc ccc atc      427
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55                  60                  65                  70

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Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala
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Phe Ser Pro Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala
            90                  95                  100

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Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg
        105                 110                 115

act gtc agg gtg gtg gcc acc aaa cag ggc aat gcg gtg acc ctg gga      619
Thr Val Arg Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly
    120                 125                 130

gac tac tac caa ggc agg agg atc acc ata aca gga aat gct gac ctg      667
Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu
135                 140                 145                 150

acc ttc gag cag aca gcc tgg gga gac agt gga gtg tat tac tgc tct      715
Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser
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Phe Arg Ala Gly Pro Leu Glu Asp Trp Leu Phe Val Val Val Val Cys
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ctg gcg agc ctc ctc ctc ttc ctc ctc ctg ggc atc tgc tgg tgc cag      907
Leu Ala Ser Leu Leu Leu Phe Leu Leu Leu Gly Ile Cys Trp Cys Gln
215                 220                 225                 230

tgc tgt cct cac acc tgc tgc tgc tat gtc cga tgt ccc tgc tgc cca      955
Cys Cys Pro His Thr Cys Cys Cys Tyr Val Arg Cys Pro Cys Cys Pro
                235                 240                 245

gac aag tgc tgt tgc cct gag gct ctt tat gct gct ggc aaa gca gcc     1003
Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr Ala Ala Gly Lys Ala Ala
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acc tca ggt gtc ccg agc atc tat gcc ccc agc atc tat acc cac ctc     1051
Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr His Leu
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tca cct gcc aag acc cca cca cct ccg cct gcc atg att ccc atg ggc     1099
Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro Ala Met Ile Pro Met Gly
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cct ccc tat ggg tac cct gga gac ttt gac aga cat agc tca gtt ggt     1147
Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp Arg His Ser Ser Val Gly
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Gly His Ser Ser Gln Val Pro Leu Leu Arg Asp Val Asp Gly Ser Val
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tct tca gaa gta cga agt ggc tac agg atc cag gct aac cag caa gat     1243
Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn Gln Gln Asp
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gac tcc atg agg gtc cta tac tat atg gag aaa gag cta gcc aac ttt     1291
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gac cct tcc cga cct ggc cct ccc aat ggc aga gtg gaa cgg gcc atg     1339
Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly Arg Val Glu Arg Ala Met
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agt gaa gta acc tcc ctc cat gaa gat gac tgg cga tcg agg cct tcc     1387
Ser Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser
375                 380                 385                 390

agg gct cct gcc ctc acc ccc atc agg gat gag gag tgg aat cgc cac     1435
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tcc cca cag agt ccc aga aca tgg gag cag gaa ccc ctt caa gaa caa     1483
Ser Pro Gln Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln Glu Gln
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Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly Arg Ser
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455                 460                 465                 470

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Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg Ser Arg Asp Pro Arg Asp
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Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu Leu Glu
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Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys
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Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Ser Asn
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Thr Leu Thr Val Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
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Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
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Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
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Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
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Thr Gly Asn Ala Asp Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

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Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr
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Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro
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Ser Ile Tyr Thr His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro
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Ala Met Ile Pro Met Gly Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp
    290                 295                 300

Arg His Ser Ser Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg
305                 310                 315                 320

Asp Val Asp Gly Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile
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Gln Ala Asn Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu
            340                 345                 350

Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly
        355                 360                 365

Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp
    370                 375                 380

Trp Arg Ser Arg Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp
385                 390                 395                 400

Glu Glu Trp Asn Arg His Ser Pro Gln Ser Pro Arg Thr Trp Glu Gln
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Glu Pro Leu Gln Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro
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Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser
        435                 440                 445

Thr Glu Ser Gly Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg
    450                 455                 460

Ala Tyr Ala Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro
465                 470                 475                 480

Asp Asp Pro Arg Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp
                485                 490                 495

Asp Ile Arg Ser Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg
            500                 505                 510

Ser Arg Asp Pro Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr
        515                 520                 525

Asp Gly Arg Leu Leu Glu Glu Ala Leu Lys Lys Lys Gly Ser Gly Glu
    530                 535                 540

Arg Arg Arg Val Tyr Arg Glu Glu Glu Glu Glu Glu Glu Gly Gln Tyr
545                 550                 555                 560

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                                          Met Ala Pro Ala Ala Gly
                                          1               5

gcg tgt gct ggg gcg cct gac tcc cac cca gct acc gtg gtc ttc gtg      283
Ala Cys Ala Gly Ala Pro Asp Ser His Pro Ala Thr Val Val Phe Val
            10                  15                  20

tgt ctc ttt ctc atc att ttc tgc cca gac cct gcc agt gcc atc cag      331
Cys Leu Phe Leu Ile Ile Phe Cys Pro Asp Pro Ala Ser Ala Ile Gln
        25                  30                  35

gtg act gtg tct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg      379
Val Thr Val Ser Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val
    40                  45                  50

acc ctg ccc tgc acc tat cag atg agc aac act ctc aca gtc ccc atc      427
Thr Leu Pro Cys Thr Tyr Gln Met Ser Asn Thr Leu Thr Val Pro Ile
55                  60                  65                  70

gtg atc tgg aag tac aag tca ttc tgc cgg gac cgt att gcc gat gcc      475
Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala
                75                  80                  85

ttc tct cct gcc agt gtg gac aac cag cta aat gcc cag ttg gca gct      523
Phe Ser Pro Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala
            90                  95                  100

ggc aac ccc ggc tac aac ccc tat gtg gag tgc cag gac agt gta cgc      571
Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg
        105                 110                 115

act gtc agg gtg gtg gcc acc aaa cag ggc aat gcg gtg acc ctg gga      619
Thr Val Arg Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly
    120                 125                 130

gac tac tac caa ggc agg agg atc acc ata aca gga aat gct gac ctg      667
Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu
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acc ttc gag cag aca gcc tgg gga gac agt gga gtg tat tac tgc tct      715
Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser
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gtg gtc tcg gcc caa gat ctg gat gga aac aac gag gcg tac gca gag      763
Val Val Ser Ala Gln Asp Leu Asp Gly Asn Asn Glu Ala Tyr Ala Glu
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ctc atc gtc ctt gat tgg ctc ttt gtg gtc gtg gtc tgc ctg gcg agc      811
Leu Ile Val Leu Asp Trp Leu Phe Val Val Val Val Cys Leu Ala Ser
        185                 190                 195

ctc ctc ctc ttc ctc ctc ctg ggc atc tgc tgg tgc cag tgc tgt cct      859
Leu Leu Leu Phe Leu Leu Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro
    200                 205                 210

cac acc tgc tgc tgc tat gtc cga tgt ccc tgc tgc cca gac aag tgc      907
His Thr Cys Cys Cys Tyr Val Arg Cys Pro Cys Cys Pro Asp Lys Cys
215                 220                 225                 230
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gtc ccg agc atc tat gcc ccc agc atc tat acc cac ctc tca cct gcc     1003
Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr His Leu Ser Pro Ala
            250                 255                 260

aag acc cca cca cct ccg cct gcc atg att ccc atg ggc cct ccc tat     1051
Lys Thr Pro Pro Pro Pro Pro Ala Met Ile Pro Met Gly Pro Pro Tyr
        265                 270                 275

ggg tac cct gga gac ttt gac aga cat agc tca gtt ggt ggc cac agc     1099
Gly Tyr Pro Gly Asp Phe Asp Arg His Ser Ser Val Gly Gly His Ser
    280                 285                 290

tcc caa gta ccc ctg ctg cgt gac gtg gat ggc agt gta tct tca gaa     1147
Ser Gln Val Pro Leu Leu Arg Asp Val Asp Gly Ser Val Ser Ser Glu
295                 300                 305                 310

gta cga agt ggc tac agg atc cag gct aac cag caa gat gac tcc atg     1195
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            330                 335                 340

cga cct ggc cct ccc aat ggc aga gtg gaa cgg gcc atg agt gaa gta     1291
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        345                 350                 355

acc tcc ctc cat gaa gat gac tgg cga tcg agg cct tcc agg gct cct     1339
Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Ala Pro
    360                 365                 370

gcc ctc acc ccc atc agg gat gag gag tgg aat cgc cac tcc cca cag     1387
Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp Asn Arg His Ser Pro Gln
375                 380                 385                 390

agt ccc aga aca tgg gag cag gaa ccc ctt caa gaa caa cca agg ggt     1435
Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln Glu Gln Pro Arg Gly
                395                 400                 405

ggt tgg ggg tct gga cgc cct cgg gcc cgc tct gtg gat gct cta gat     1483
Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp
            410                 415                 420

gat atc aac cgg cct ggc tcc act gaa tca gga cgg tct tct ccc cca     1531
Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly Arg Ser Ser Pro Pro
        425                 430                 435

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Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala Pro Pro Arg Ser Arg Ser
    440                 445                 450

cgg gat gac ctc tat gac ccg gac gat cct agg gac ttg cca cat tcc     1627
Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro Arg Asp Leu Pro His Ser
455                 460                 465                 470

cga gat ccc cac tat tat gac gac atc agg tct aga gat cca cgt gct     1675
Arg Asp Pro His Tyr Tyr Asp Asp Ile Arg Ser Arg Asp Pro Arg Ala
                475                 480                 485

gac ccc aga tcc cgt cag cga tcc cga gat cct cgg gat gct ggc ttc     1723
Asp Pro Arg Ser Arg Gln Arg Ser Arg Asp Pro Arg Asp Ala Gly Phe
            490                 495                 500

agg tca agg gac cct cag tat gat ggg cga cta tta gaa gag gct tta     1771
Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu Leu Glu Glu Ala Leu
        505                 510                 515

aag aaa aag ggg tcg ggc gag aga agg agg gtt tac agg gag gaa gaa     1819
Lys Lys Lys Gly Ser Gly Glu Arg Arg Arg Val Tyr Arg Glu Glu Glu
    520                 525                 530
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535                 540                 545                 550

act gac tcg cag gcc tca cgg gag agg agg ctg aaa aag aat ttg gcc     1915
Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala
                555                 560                 565

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Leu Ser Arg Glu Ser Leu Val Val  *
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<400> SEQUENCE: 4

Met Ala Pro Ala Ala Gly Ala Cys Ala Gly Ala Pro Asp Ser His Pro
1               5                   10                  15

Ala Thr Val Val Phe Val Cys Leu Phe Leu Ile Ile Phe Cys Pro Asp
            20                  25                  30

Pro Ala Ser Ala Ile Gln Val Thr Val Ser Asp Pro Tyr His Val Val
        35                  40                  45

Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Ser Asn
    50                  55                  60

Thr Leu Thr Val Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
                85                  90                  95

Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
            100                 105                 110

Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
        115                 120                 125

Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
    130                 135                 140

Thr Gly Asn Ala Asp Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Asp Gly Asn
                165                 170                 175

Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Asp Trp Leu Phe Val Val
            180                 185                 190

Val Val Cys Leu Ala Ser Leu Leu Leu Phe Leu Leu Leu Gly Ile Cys
        195                 200                 205

Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys Tyr Val Arg Cys Pro
    210                 215                 220

Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr Ala Ala Gly
225                 230                 235                 240

Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr
                245                 250                 255

Thr His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro Ala Met Ile
            260                 265                 270

Pro Met Gly Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp Arg His Ser
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        275                 280                 285

Ser Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg Asp Val Asp
    290                 295                 300

Gly Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn
305                 310                 315                 320

Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu
                325                 330                 335

Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly Arg Val Glu
            340                 345                 350

Arg Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser
        355                 360                 365

Arg Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp
    370                 375                 380

Asn Arg His Ser Pro Gln Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu
385                 390                 395                 400

Gln Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg
                405                 410                 415

Ser Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser
            420                 425                 430

Gly Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala
        435                 440                 445

Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro
    450                 455                 460

Arg Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp Asp Ile Arg
465                 470                 475                 480

Ser Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg Ser Arg Asp
                485                 490                 495

Pro Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg
            500                 505                 510

Leu Leu Glu Glu Ala Leu Lys Lys Lys Gly Ser Gly Glu Arg Arg Arg
        515                 520                 525

Val Tyr Arg Glu Glu Glu Glu Glu Glu Glu Gly Gln Tyr Pro Pro Ala
    530                 535                 540

Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg
545                 550                 555                 560

Leu Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu Val Val
                565                 570


<210> SEQ ID NO 5
<211> LENGTH: 1893
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 5

accgctcacc aggtcagttg tccccggaaa gccgaaggca tgagcttcgc ccaagttctt     60

tttatgggtt agaactcctc cagagcgggg gaaaaaggac ttggaatagg ggcgggacgg    120

agcacgcacc cttctccgcc ttggttctcg ccgcgccccc tactctcggg atacttggga    180

ggggacgcgc gggcaccgtc gctgctagac ggccgcg atg gcg ccg gcg gcc ggc     235
                                          Met Ala Pro Ala Ala Gly
                                          1               5

gcg tgt gct ggg gcg cct gac tcc cac cca gct acc gtg gtc ttc gtg      283
Ala Cys Ala Gly Ala Pro Asp Ser His Pro Ala Thr Val Val Phe Val
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           10                  15                  20

tgt ctc ttt ctc atc att ttc tgc cca gac cct gcc agt gcc atc cag      331
Cys Leu Phe Leu Ile Ile Phe Cys Pro Asp Pro Ala Ser Ala Ile Gln
        25                  30                  35

gtg act gtg tct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg      379
Val Thr Val Ser Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val
    40                  45                  50

acc ctg ccc tgc acc tat cag atg agc aac act ctc aca gtc ccc atc      427
Thr Leu Pro Cys Thr Tyr Gln Met Ser Asn Thr Leu Thr Val Pro Ile
55                  60                  65                  70

gtg atc tgg aag tac aag tca ttc tgc cgg gac cgt att gcc gat gcc      475
Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala
                75                  80                  85

ttc tct cct gcc agt gtg gac aac cag cta aat gcc cag ttg gca gct      523
Phe Ser Pro Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala
            90                  95                  100

ggc aac ccc ggc tac aac ccc tat gtg gag tgc cag gac agt gta cgc      571
Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg
        105                 110                 115

act gtc agg gtg gtg gcc acc aaa cag ggc aat gcg gtg acc ctg gga      619
Thr Val Arg Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly
    120                 125                 130

gac tac tac caa ggc agg agg atc acc ata aca gga aat gct gac ctg      667
Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu
135                 140                 145                 150

acc ttc gag cag aca gcc tgg gga gac agt gga gtg tat tac tgc tct      715
Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser
                155                 160                 165

gtg gtc tcg gcc caa gat ctg gat gga aac aac gag gcg tac gca gag      763
Val Val Ser Ala Gln Asp Leu Asp Gly Asn Asn Glu Ala Tyr Ala Glu
            170                 175                 180

ctc atc gtc ctt gtt tat gct gct ggc aaa gca gcc acc tca ggt gtc      811
Leu Ile Val Leu Val Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val
        185                 190                 195

ccg agc atc tat gcc ccc agc atc tat acc cac ctc tca cct gcc aag      859
Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr His Leu Ser Pro Ala Lys
    200                 205                 210

acc cca cca cct ccg cct gcc atg att ccc atg ggc cct ccc tat ggg      907
Thr Pro Pro Pro Pro Pro Ala Met Ile Pro Met Gly Pro Pro Tyr Gly
215                 220                 225                 230

tac cct gga gac ttt gac aga cat agc tca gtt ggt ggc cac agc tcc      955
Tyr Pro Gly Asp Phe Asp Arg His Ser Ser Val Gly Gly His Ser Ser
                235                 240                 245

caa gta ccc ctg ctg cgt gac gtg gat ggc agt gta tct tca gaa gta     1003
Gln Val Pro Leu Leu Arg Asp Val Asp Gly Ser Val Ser Ser Glu Val
            250                 255                 260

cga agt ggc tac agg atc cag gct aac cag caa gat gac tcc atg agg     1051
Arg Ser Gly Tyr Arg Ile Gln Ala Asn Gln Gln Asp Asp Ser Met Arg
        265                 270                 275

gtc cta tac tat atg gag aaa gag cta gcc aac ttt gac cct tcc cga     1099
Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg
    280                 285                 290

cct ggc cct ccc aat ggc aga gtg gaa cgg gcc atg agt gaa gta acc     1147
Pro Gly Pro Pro Asn Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr
295                 300                 305                 310

tcc ctc cat gaa gat gac tgg cga tcg agg cct tcc agg gct cct gcc     1195
Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Ala Pro Ala
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               315                 320                 325

ctc acc ccc atc agg gat gag gag tgg aat cgc cac tcc cca cag agt     1243
Leu Thr Pro Ile Arg Asp Glu Glu Trp Asn Arg His Ser Pro Gln Ser
            330                 335                 340

ccc aga aca tgg gag cag gaa ccc ctt caa gaa caa cca agg ggt ggt     1291
Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln Glu Gln Pro Arg Gly Gly
        345                 350                 355

tgg ggg tct gga cgc cct cgg gcc cgc tct gtg gat gct cta gat gat     1339
Trp Gly Ser Gly Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp
    360                 365                 370

atc aac cgg cct ggc tcc act gaa tca gga cgg tct tct ccc cca agt     1387
Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly Arg Ser Ser Pro Pro Ser
375                 380                 385                 390

agt gga cgg aga gga cgg gcc tat gca cct cca aga agt cgc agc cgg     1435
Ser Gly Arg Arg Gly Arg Ala Tyr Ala Pro Pro Arg Ser Arg Ser Arg
                395                 400                 405

gat gac ctc tat gac ccg gac gat cct agg gac ttg cca cat tcc cga     1483
Asp Asp Leu Tyr Asp Pro Asp Asp Pro Arg Asp Leu Pro His Ser Arg
            410                 415                 420

gat ccc cac tat tat gac gac atc agg tct aga gat cca cgt gct gac     1531
Asp Pro His Tyr Tyr Asp Asp Ile Arg Ser Arg Asp Pro Arg Ala Asp
        425                 430                 435

ccc aga tcc cgt cag cga tcc cga gat cct cgg gat gct ggc ttc agg     1579
Pro Arg Ser Arg Gln Arg Ser Arg Asp Pro Arg Asp Ala Gly Phe Arg
    440                 445                 450

tca agg gac cct cag tat gat ggg cga cta tta gaa gag gct tta aag     1627
Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu Leu Glu Glu Ala Leu Lys
455                 460                 465                 470

aaa aag ggg tcg ggc gag aga agg agg gtt tac agg gag gaa gaa gag     1675
Lys Lys Gly Ser Gly Glu Arg Arg Arg Val Tyr Arg Glu Glu Glu Glu
                475                 480                 485

gaa gag gag ggc caa tac ccc cca gca cct cca cct tac tca gag act     1723
Glu Glu Glu Gly Gln Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr
            490                 495                 500

gac tcg cag gcc tca cgg gag agg agg ctg aaa aag aat ttg gcc ctg     1771
Asp Ser Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu
        505                 510                 515

agt cgg gaa agt tta gtc gtc tga tccacgtttt gtatgtagct tttgtacttt    1825
Ser Arg Glu Ser Leu Val Val  *
    520                 525

ttttttaatt ggaatcaata ttgatgaaac ttcaagccta ataaaatgtc taatcacaaa   1885

aaaaaaaa                                                            1893


<210> SEQ ID NO 6
<211> LENGTH: 525
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 6

Met Ala Pro Ala Ala Gly Ala Cys Ala Gly Ala Pro Asp Ser His Pro
1               5                   10                  15

Ala Thr Val Val Phe Val Cys Leu Phe Leu Ile Ile Phe Cys Pro Asp
            20                  25                  30

Pro Ala Ser Ala Ile Gln Val Thr Val Ser Asp Pro Tyr His Val Val
        35                  40                  45

Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Ser Asn
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    50                  55                  60

Thr Leu Thr Val Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
                85                  90                  95

Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
            100                 105                 110

Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
        115                 120                 125

Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
    130                 135                 140

Thr Gly Asn Ala Asp Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Asp Gly Asn
                165                 170                 175

Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Val Tyr Ala Ala Gly Lys
            180                 185                 190

Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr
        195                 200                 205

His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro Ala Met Ile Pro
    210                 215                 220

Met Gly Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp Arg His Ser Ser
225                 230                 235                 240

Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg Asp Val Asp Gly
                245                 250                 255

Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn Gln
            260                 265                 270

Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala
        275                 280                 285

Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly Arg Val Glu Arg
    290                 295                 300

Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg
305                 310                 315                 320

Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp Asn
                325                 330                 335

Arg His Ser Pro Gln Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln
            340                 345                 350

Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg Ser
        355                 360                 365

Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly
    370                 375                 380

Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala Pro
385                 390                 395                 400

Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro Arg
                405                 410                 415

Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp Asp Ile Arg Ser
            420                 425                 430

Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg Ser Arg Asp Pro
        435                 440                 445

Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu
    450                 455                 460
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Leu Glu Glu Ala Leu Lys Lys Lys Gly Ser Gly Glu Arg Arg Arg Val
465                 470                 475                 480

Tyr Arg Glu Glu Glu Glu Glu Glu Glu Gly Gln Tyr Pro Pro Ala Pro
                485                 490                 495

Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg Leu
            500                 505                 510

Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu Val Val
        515                 520                 525


<210> SEQ ID NO 7
<211> LENGTH: 2158
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 1259..1261
<223> OTHER INFORMATION: Potential splicing site AAG
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 1657
<223> OTHER INFORMATION: Potential insertion of a AGG

<400> SEQUENCE: 7

tggagtgtgg ctcggaggac cgcggcgggt caagcacctt tctcccccat atctgaaagc     60

atgccctttg tccacgtcgt ttacgctcat taaaacttcc aga atg caa cag gac      115
                                              Met Gln Gln Asp
                                              1

gga ctt gga gta ggg aca agg aac gga agt ggg aag ggg agg agc gtg      163
Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys Gly Arg Ser Val
5                   10                  15                  20

cac ccc tcc tgg cct tgg tgc gcg ccg cgc ccc cta agg tac ttt gga      211
His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu Arg Tyr Phe Gly
                25                  30                  35

agg gac gcg cgg gcc aga cgc gcc cag acg gcc gcg atg gcg ctg ttg      259
Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala Met Ala Leu Leu
            40                  45                  50

gcc ggc ggg ctc tcc aga ggg ctg ggc tcc cac ccg gcc gcc gca ggc      307
Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro Ala Ala Ala Gly
        55                  60                  65

cgg gac gcg gtc gtc ttc gtg tgg ctt ctg ctt agc acc tgg tgc aca      355
Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser Thr Trp Cys Thr
    70                  75                  80

gct cct gcc agg gcc atc cag gtg acc gtg tcc aac ccc tac cac gtg      403
Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn Pro Tyr His Val
85                  90                  95                  100

gtg atc ctc ttc cag cct gtg acc ctg ccc tgt acc tac cag atg acc      451
Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Thr
                105                 110                 115

tcg acc ccc acg caa ccc atc gtc atc tgg aag tac aag tct ttc tgc      499
Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys
            120                 125                 130

cgg gac cgc atc gcc gat gcc ttc tcc ccg gcc agc gtc gac aac cag      547
Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln
        135                 140                 145

ctc aat gcc cag ctg gca gcc ggg aac cca ggc tac aac ccc tac gtt      595
Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val
    150                 155                 160

gag tgc cag gac agc gtg cgc acc gtc agg gtc gtg gcc acc aag cag      643
Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln
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165                 170                 175                 180

ggc aac gct gtg acc ctg gga gat tac tac cag ggc cgg agg att acc      691
Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr
                185                 190                 195

atc acc gga aat gct gac ctg acc ttt gac cag acg gcg tgg ggg gac      739
Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr Ala Trp Gly Asp
            200                 205                 210

agt ggt gtg tat tac tgc tcc gtg gtc tca gcc cag gac ctc cag ggg      787
Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Gln Gly
        215                 220                 225

aac aat gag gcc tac gca gag ctc atc gtc ctt ggg agg acc tca ggg      835
Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Gly Arg Thr Ser Gly
    230                 235                 240

gtg gct gag ctc tta cct ggt ttt cag gcg ggg ccc ata gaa gac tgg      883
Val Ala Glu Leu Leu Pro Gly Phe Gln Ala Gly Pro Ile Glu Asp Trp
245                 250                 255                 260

ctc ttc gtg gtt gtg gta tgc ctg gct gcc ttc ctc atc ttc ctc ctc      931
Leu Phe Val Val Val Val Cys Leu Ala Ala Phe Leu Ile Phe Leu Leu
                265                 270                 275

ctg ggc atc tgc tgg tgc cag tgc tgc ccg cac act tgc tgc tgc tac      979
Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys Tyr
            280                 285                 290

gtc agg tgc ccc tgc tgc cca gac aag tgc tgc tgc ccc gag gcc ctg     1027
Val Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu
        295                 300                 305

tat gcc gcc ggc aaa gca gcc acc tca ggt gtt ccc agc att tat gcc     1075
Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala
    310                 315                 320

ccc agc acc tat gcc cac ctg tct ccc gcc aag acc cca ccc cca cca     1123
Pro Ser Thr Tyr Ala His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro
325                 330                 335                 340

gct atg att ccc atg ggc cct gcc tac aac ggg tac cct gga gga tac     1171
Ala Met Ile Pro Met Gly Pro Ala Tyr Asn Gly Tyr Pro Gly Gly Tyr
                345                 350                 355

cct gga gac gtt gac agg agt agc tca gct ggt ggc caa ggc tcc tat     1219
Pro Gly Asp Val Asp Arg Ser Ser Ser Ala Gly Gly Gln Gly Ser Tyr
            360                 365                 370

gta ccc ctg ctt cgg gac acg gac agc agt gtg gcc tct gaa gtc cgc     1267
Val Pro Leu Leu Arg Asp Thr Asp Ser Ser Val Ala Ser Glu Val Arg
        375                 380                 385

agt ggc tac agg att cag gcc agc cag cag gac gac tcc atg cgg gtc     1315
Ser Gly Tyr Arg Ile Gln Ala Ser Gln Gln Asp Asp Ser Met Arg Val
    390                 395                 400

ctg tac tac atg gag aag gag ctg gcc aac ttc gac cct tct cga cct     1363
Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro
405                 410                 415                 420

ggc ccc ccc agt ggc cgt gtg gag cgg gcc atg agt gaa gtc acc tcc     1411
Gly Pro Pro Ser Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser
                425                 430                 435

ctc cac gag gac gac tgg cga tct cgg cct tcc cgg ggc cct gcc ctc     1459
Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Gly Pro Ala Leu
            440                 445                 450

acc ccg atc cgg gat gag gag tgg ggt ggc cac tcc ccc cgg agt ccc     1507
Thr Pro Ile Arg Asp Glu Glu Trp Gly Gly His Ser Pro Arg Ser Pro
        455                 460                 465

agg gga tgg gac cag gag ccc gcc agg gag cag gca ggc ggg ggc tgg     1555
Arg Gly Trp Asp Gln Glu Pro Ala Arg Glu Gln Ala Gly Gly Gly Trp
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    470                 475                 480

cgg gcc agg cgg ccc cgg gcc cgc tcc gtg gac gcc ctg gac gac ctc     1603
Arg Ala Arg Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Leu
485                 490                 495                 500

acc ccg ccg agc acc gcc gag tca ggg agc agg tct ccc acg agt aat     1651
Thr Pro Pro Ser Thr Ala Glu Ser Gly Ser Arg Ser Pro Thr Ser Asn
                505                 510                 515

ggt ggg aga agc cgg gcc tac atg ccc ccg cgg agc cgc agc cgg gac     1699
Gly Gly Arg Ser Arg Ala Tyr Met Pro Pro Arg Ser Arg Ser Arg Asp
            520                 525                 530

gac ctc tat gac caa gac gac tcg agg gac ttc cca cgc tcc cgg gac     1747
Asp Leu Tyr Asp Gln Asp Asp Ser Arg Asp Phe Pro Arg Ser Arg Asp
        535                 540                 545

ccc cac tac gac gac ttc agg tct cgg gag cgc cct cct gcc gac ccc     1795
Pro His Tyr Asp Asp Phe Arg Ser Arg Glu Arg Pro Pro Ala Asp Pro
    550                 555                 560

agg tcc cac cac cac cgt acc cgg gac cct cgg gac aac ggc tcc agg     1843
Arg Ser His His His Arg Thr Arg Asp Pro Arg Asp Asn Gly Ser Arg
565                 570                 575                 580

tcc ggg gac ctc ccc tat gat ggg cgg cta ctg gag gag gct gtg agg     1891
Ser Gly Asp Leu Pro Tyr Asp Gly Arg Leu Leu Glu Glu Ala Val Arg
                585                 590                 595

aag aag ggg tcg gag gag agg agg aga ccc cac aag gag gag gag gaa     1939
Lys Lys Gly Ser Glu Glu Arg Arg Arg Pro His Lys Glu Glu Glu Glu
            600                 605                 610

gag gcc tac tac ccg ccc gcg ccg ccc ccg tac tcg gag acc gac tcg     1987
Glu Ala Tyr Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser
        615                 620                 625

cag gcg tcc cga gag cgc agg ctc aag aag aac ttg gcc ctg agt cgg     2035
Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu Ser Arg
    630                 635                 640

gaa agt tta gtc gtc tga tctgacgttt tctacgtagc ttttgtattt            2083
Glu Ser Leu Val Val  *
645

ttttttttaa tttgaaggaa cactgatgaa gccctgccat acccctcccg agtctaataa   2143

aacgtataat cacaa                                                    2158


<210> SEQ ID NO 8
<211> LENGTH: 649
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 386
<223> OTHER INFORMATION: Potential deletion of a Glu
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 518
<223> OTHER INFORMATION: Potential insertion of a Arg

<400> SEQUENCE: 8

Met Gln Gln Asp Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys
1               5                   10                  15

Gly Arg Ser Val His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu
            20                  25                  30

Arg Tyr Phe Gly Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala
        35                  40                  45

Met Ala Leu Leu Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro
    50                  55                  60
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Ala Ala Ala Gly Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser
65                  70                  75                  80

Thr Trp Cys Thr Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn
                85                  90                  95

Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr
            100                 105                 110

Tyr Gln Met Thr Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr
        115                 120                 125

Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser
    130                 135                 140

Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr
145                 150                 155                 160

Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val
                165                 170                 175

Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly
            180                 185                 190

Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr
        195                 200                 205

Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln
    210                 215                 220

Asp Leu Gln Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Gly
225                 230                 235                 240

Arg Thr Ser Gly Val Ala Glu Leu Leu Pro Gly Phe Gln Ala Gly Pro
                245                 250                 255

Ile Glu Asp Trp Leu Phe Val Val Val Val Cys Leu Ala Ala Phe Leu
            260                 265                 270

Ile Phe Leu Leu Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro His Thr
        275                 280                 285

Cys Cys Cys Tyr Val Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys
    290                 295                 300

Pro Glu Ala Leu Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro
305                 310                 315                 320

Ser Ile Tyr Ala Pro Ser Thr Tyr Ala His Leu Ser Pro Ala Lys Thr
                325                 330                 335

Pro Pro Pro Pro Ala Met Ile Pro Met Gly Pro Ala Tyr Asn Gly Tyr
            340                 345                 350

Pro Gly Gly Tyr Pro Gly Asp Val Asp Arg Ser Ser Ser Ala Gly Gly
        355                 360                 365

Gln Gly Ser Tyr Val Pro Leu Leu Arg Asp Thr Asp Ser Ser Val Ala
    370                 375                 380

Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Ser Gln Gln Asp Asp
385                 390                 395                 400

Ser Met Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp
                405                 410                 415

Pro Ser Arg Pro Gly Pro Pro Ser Gly Arg Val Glu Arg Ala Met Ser
            420                 425                 430

Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg
        435                 440                 445

Gly Pro Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp Gly Gly His Ser
    450                 455                 460
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Pro Arg Ser Pro Arg Gly Trp Asp Gln Glu Pro Ala Arg Glu Gln Ala
465                 470                 475                 480

Gly Gly Gly Trp Arg Ala Arg Arg Pro Arg Ala Arg Ser Val Asp Ala
                485                 490                 495

Leu Asp Asp Leu Thr Pro Pro Ser Thr Ala Glu Ser Gly Ser Arg Ser
            500                 505                 510

Pro Thr Ser Asn Gly Gly Arg Ser Arg Ala Tyr Met Pro Pro Arg Ser
        515                 520                 525

Arg Ser Arg Asp Asp Leu Tyr Asp Gln Asp Asp Ser Arg Asp Phe Pro
    530                 535                 540

Arg Ser Arg Asp Pro His Tyr Asp Asp Phe Arg Ser Arg Glu Arg Pro
545                 550                 555                 560

Pro Ala Asp Pro Arg Ser His His His Arg Thr Arg Asp Pro Arg Asp
                565                 570                 575

Asn Gly Ser Arg Ser Gly Asp Leu Pro Tyr Asp Gly Arg Leu Leu Glu
            580                 585                 590

Glu Ala Val Arg Lys Lys Gly Ser Glu Glu Arg Arg Arg Pro His Lys
        595                 600                 605

Glu Glu Glu Glu Glu Ala Tyr Tyr Pro Pro Ala Pro Pro Pro Tyr Ser
    610                 615                 620

Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu
625                 630                 635                 640

Ala Leu Ser Arg Glu Ser Leu Val Val
                645


<210> SEQ ID NO 9
<211> LENGTH: 2101
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

tggagtgtgg ctcggaggac cgcggcgggt caagcacctt tctcccccat atctgaaagc     60

atgccctttg tccacgtcgt ttacgctcat taaaacttcc aga atg caa cag gac      115
                                               Met Gln Gln Asp
                                               1

gga ctt gga gta ggg aca agg aac gga agt ggg aag ggg agg agc gtg      163
Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys Gly Arg Ser Val
5                   10                  15                  20

cac ccc tcc tgg cct tgg tgc gcg ccg cgc ccc cta agg tac ttt gga      211
His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu Arg Tyr Phe Gly
                25                  30                  35

agg gac gcg cgg gcc aga cgc gcc cag acg gcc gcg atg gcg ctg ttg      259
Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala Met Ala Leu Leu
            40                  45                  50

gcc ggc ggg ctc tcc aga ggg ctg ggc tcc cac ccg gcc gcc gca ggc      307
Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro Ala Ala Ala Gly
        55                  60                  65

cgg gac gcg gtc gtc ttc gtg tgg ctt ctg ctt agc acc tgg tgc aca      355
Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser Thr Trp Cys Thr
    70                  75                  80

gct cct gcc agg gcc atc cag gtg acc gtg tcc aac ccc tac cac gtg      403
Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn Pro Tyr His Val
85                  90                  95                  100

gtg atc ctc ttc cag cct gtg acc ctg ccc tgt acc tac cag atg acc      451
Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Thr
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                105                 110                 115

tcg acc ccc acg caa ccc atc gtc atc tgg aag tac aag tct ttc tgc      499
Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys
            120                 125                 130

cgg gac cgc atc gcc gat gcc ttc tcc ccg gcc agc gtc gac aac cag      547
Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln
        135                 140                 145

ctc aat gcc cag ctg gca gcc ggg aac cca ggc tac aac ccc tac gtt      595
Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val
    150                 155                 160

gag tgc cag gac agc gtg cgc acc gtc agg gtc gtg gcc acc aag cag      643
Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln
165                 170                 175                 180

ggc aac gct gtg acc ctg gga gat tac tac cag ggc cgg agg att acc      691
Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr
                185                 190                 195

atc acc gga aat gct gac ctg acc ttt gac cag acg gcg tgg ggg gac      739
Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr Ala Trp Gly Asp
            200                 205                 210

agt ggt gtg tat tac tgc tcc gtg gtc tca gcc cag gac ctc cag ggg      787
Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Gln Gly
        215                 220                 225

aac aat gag gcc tac gca gag ctc atc gtc ctt gac tgg ctc ttc gtg      835
Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Asp Trp Leu Phe Val
    230                 235                 240

gtt gtg gta tgc ctg gct gcc ttc ctc atc ttc ctc ctc ctg ggc atc      883
Val Val Val Cys Leu Ala Ala Phe Leu Ile Phe Leu Leu Leu Gly Ile
245                 250                 255                 260

tgc tgg tgc cag tgc tgc ccg cac act tgc tgc tgc tac gtc agg tgc      931
Cys Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys Tyr Val Arg Cys
                265                 270                 275

ccc tgc tgc cca gac aag tgc tgc tgc ccc gag gcc ctg tat gcc gcc      979
Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr Ala Ala
            280                 285                 290

ggc aaa gca gcc acc tca ggt gtt ccc agc att tat gcc ccc agc acc     1027
Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Thr
        295                 300                 305

tat gcc cac ctg tct ccc gcc aag acc cca ccc cca cca gct atg att     1075
Tyr Ala His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Ala Met Ile
    310                 315                 320

ccc atg ggc cct gcc tac aac ggg tac cct gga gga tac cct gga gac     1123
Pro Met Gly Pro Ala Tyr Asn Gly Tyr Pro Gly Gly Tyr Pro Gly Asp
325                 330                 335                 340

gtt gac agg agt agc tca gct ggt ggc caa ggc tcc tat gta ccc ctg     1171
Val Asp Arg Ser Ser Ser Ala Gly Gly Gln Gly Ser Tyr Val Pro Leu
                345                 350                 355

ctt cgg gac acg gac agc agt gtg gcc tct gaa gtc cgc agt ggc tac     1219
Leu Arg Asp Thr Asp Ser Ser Val Ala Ser Glu Val Arg Ser Gly Tyr
            360                 365                 370

agg att cag gcc agc cag cag gac gac tcc atg cgg gtc ctg tac tac     1267
Arg Ile Gln Ala Ser Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr
        375                 380                 385

atg gag aag gag ctg gcc aac ttc gac cct tct cga cct ggc ccc ccc     1315
Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro
    390                 395                 400

agt ggc cgt gtg gag cgg gcc atg agt gaa gtc acc tcc ctc cac gag     1363
Ser Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu His Glu
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405                 410                 415                 420

gac gac tgg cga tct cgg cct tcc cgg ggc cct gcc ctc acc ccg atc     1411
Asp Asp Trp Arg Ser Arg Pro Ser Arg Gly Pro Ala Leu Thr Pro Ile
                425                 430                 435

cgg gat gag gag tgg ggt ggc cac tcc ccc cgg agt ccc agg gga tgg     1459
Arg Asp Glu Glu Trp Gly Gly His Ser Pro Arg Ser Pro Arg Gly Trp
            440                 445                 450

gac cag gag ccc gcc agg gag cag gca ggc ggg ggc tgg cgg gcc agg     1507
Asp Gln Glu Pro Ala Arg Glu Gln Ala Gly Gly Gly Trp Arg Ala Arg
        455                 460                 465

cgg ccc cgg gcc cgc tcc gtg gac gcc ctg gac gac ctc acc ccg ccg     1555
Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Leu Thr Pro Pro
    470                 475                 480

agc acc gcc gag tca ggg agc agg tct ccc acg agt aat ggt ggg aga     1603
Ser Thr Ala Glu Ser Gly Ser Arg Ser Pro Thr Ser Asn Gly Gly Arg
485                 490                 495                 500

agc cgg gcc tac atg ccc ccg cgg agc cgc agc cgg gac gac ctc tat     1651
Ser Arg Ala Tyr Met Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr
                505                 510                 515

gac caa gac gac tcg agg gac ttc cca cgc tcc cgg gac ccc cac tac     1699
Asp Gln Asp Asp Ser Arg Asp Phe Pro Arg Ser Arg Asp Pro His Tyr
            520                 525                 530

gac gac ttc agg tct cgg gag cgc cct cct gcc gac ccc agg tcc cac     1747
Asp Asp Phe Arg Ser Arg Glu Arg Pro Pro Ala Asp Pro Arg Ser His
        535                 540                 545

cac cac cgt acc cgg gac cct cgg gac aac ggc tcc agg tcc ggg gac     1795
His His Arg Thr Arg Asp Pro Arg Asp Asn Gly Ser Arg Ser Gly Asp
    550                 555                 560

ctc ccc tat gat ggg cgg cta ctg gag gag gct gtg agg aag aag ggg     1843
Leu Pro Tyr Asp Gly Arg Leu Leu Glu Glu Ala Val Arg Lys Lys Gly
565                 570                 575                 580

tcg gag gag agg agg aga ccc cac aag gag gag gag gaa gag gcc tac     1891
Ser Glu Glu Arg Arg Arg Pro His Lys Glu Glu Glu Glu Glu Ala Tyr
                585                 590                 595

tac ccg ccc gcg ccg ccc ccg tac tcg gag acc gac tcg cag gcg tcc     1939
Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser
            600                 605                 610

cga gag cgc agg ctc aag aag aac ttg gcc ctg agt cgg gaa agt tta     1987
Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu
        615                 620                 625

gtc gtc tga tctgacgttt tctacgtagc ttttgtattt ttttttttaa             2036
Val Val  *
    630

tttgaaggaa cactgatgaa gccctgccat acccctcccg agtctaataa aacgtataat   2096

cacaa                                                               2101


<210> SEQ ID NO 10
<211> LENGTH: 630
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Gln Gln Asp Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys
1               5                   10                  15

Gly Arg Ser Val His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu
            20                  25                  30
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Arg Tyr Phe Gly Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala
        35                  40                  45

Met Ala Leu Leu Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro
    50                  55                  60

Ala Ala Ala Gly Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser
65                  70                  75                  80

Thr Trp Cys Thr Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn
                85                  90                  95

Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr
            100                 105                 110

Tyr Gln Met Thr Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr
        115                 120                 125

Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser
    130                 135                 140

Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr
145                 150                 155                 160

Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val
                165                 170                 175

Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly
            180                 185                 190

Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr
        195                 200                 205

Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln
    210                 215                 220

Asp Leu Gln Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Asp
225                 230                 235                 240

Trp Leu Phe Val Val Val Val Cys Leu Ala Ala Phe Leu Ile Phe Leu
                245                 250                 255

Leu Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys
            260                 265                 270

Tyr Val Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala
        275                 280                 285

Leu Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr
    290                 295                 300

Ala Pro Ser Thr Tyr Ala His Leu Ser Pro Ala Lys Thr Pro Pro Pro
305                 310                 315                 320

Pro Ala Met Ile Pro Met Gly Pro Ala Tyr Asn Gly Tyr Pro Gly Gly
                325                 330                 335

Tyr Pro Gly Asp Val Asp Arg Ser Ser Ser Ala Gly Gly Gln Gly Ser
            340                 345                 350

Tyr Val Pro Leu Leu Arg Asp Thr Asp Ser Ser Val Ala Ser Glu Val
        355                 360                 365

Arg Ser Gly Tyr Arg Ile Gln Ala Ser Gln Gln Asp Asp Ser Met Arg
    370                 375                 380

Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg
385                 390                 395                 400

Pro Gly Pro Pro Ser Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr
                405                 410                 415

Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Gly Pro Ala
            420                 425                 430

Leu Thr Pro Ile Arg Asp Glu Glu Trp Gly Gly His Ser Pro Arg Ser
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        435                 440                 445

Pro Arg Gly Trp Asp Gln Glu Pro Ala Arg Glu Gln Ala Gly Gly Gly
    450                 455                 460

Trp Arg Ala Arg Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp
465                 470                 475                 480

Leu Thr Pro Pro Ser Thr Ala Glu Ser Gly Ser Arg Ser Pro Thr Ser
                485                 490                 495

Asn Gly Gly Arg Ser Arg Ala Tyr Met Pro Pro Arg Ser Arg Ser Arg
            500                 505                 510

Asp Asp Leu Tyr Asp Gln Asp Asp Ser Arg Asp Phe Pro Arg Ser Arg
        515                 520                 525

Asp Pro His Tyr Asp Asp Phe Arg Ser Arg Glu Arg Pro Pro Ala Asp
    530                 535                 540

Pro Arg Ser His His His Arg Thr Arg Asp Pro Arg Asp Asn Gly Ser
545                 550                 555                 560

Arg Ser Gly Asp Leu Pro Tyr Asp Gly Arg Leu Leu Glu Glu Ala Val
                565                 570                 575

Arg Lys Lys Gly Ser Glu Glu Arg Arg Arg Pro His Lys Glu Glu Glu
            580                 585                 590

Glu Glu Ala Tyr Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp
        595                 600                 605

Ser Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu Ser
    610                 615                 620

Arg Glu Ser Leu Val Val
625                 630


<210> SEQ ID NO 11
<211> LENGTH: 1954
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

tggagtgtgg ctcggaggac cgcggcgggt caagcacctt tctcccccat atctgaaagc     60

atgccctttg tccacgtcgt ttacgctcat taaaacttcc aga atg caa cag gac      115
                                                Met Gln Gln Asp
                                                1

gga ctt gga gta ggg aca agg aac gga agt ggg aag ggg agg agc gtg      163
Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys Gly Arg Ser Val
5                   10                  15                  20

cac ccc tcc tgg cct tgg tgc gcg ccg cgc ccc cta agg tac ttt gga      211
His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu Arg Tyr Phe Gly
                25                  30                  35

agg gac gcg cgg gcc aga cgc gcc cag acg gcc gcg atg gcg ctg ttg      259
Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala Met Ala Leu Leu
            40                  45                  50

gcc ggc ggg ctc tcc aga ggg ctg ggc tcc cac ccg gcc gcc gca ggc      307
Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro Ala Ala Ala Gly
        55                  60                  65

cgg gac gcg gtc gtc ttc gtg tgg ctt ctg ctt agc acc tgg tgc aca      355
Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser Thr Trp Cys Thr
    70                  75                  80

gct cct gcc agg gcc atc cag gtg acc gtg tcc aac ccc tac cac gtg      403
Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn Pro Tyr His Val
85                  90                  95                  100
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gtg atc ctc ttc cag cct gtg acc ctg ccc tgt acc tac cag atg acc      451
Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr Tyr Gln Met Thr
                105                 110                 115

tcg acc ccc acg caa ccc atc gtc atc tgg aag tac aag tct ttc tgc      499
Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys
            120                 125                 130

cgg gac cgc atc gcc gat gcc ttc tcc ccg gcc agc gtc gac aac cag      547
Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln
        135                 140                 145

ctc aat gcc cag ctg gca gcc ggg aac cca ggc tac aac ccc tac gtt      595
Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val
    150                 155                 160

gag tgc cag gac agc gtg cgc acc gtc agg gtc gtg gcc acc aag cag      643
Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln
165                 170                 175                 180

ggc aac gct gtg acc ctg gga gat tac tac cag ggc cgg agg att acc      691
Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr
                185                 190                 195

atc acc gga aat gct gac ctg acc ttt gac cag acg gcg tgg ggg gac      739
Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr Ala Trp Gly Asp
            200                 205                 210

agt ggt gtg tat tac tgc tcc gtg gtc tca gcc cag gac ctc cag ggg      787
Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Gln Gly
        215                 220                 225

aac aat gag gcc tac gca gag ctc atc gtc ctt gtg tat gcc gcc ggc      835
Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Val Tyr Ala Ala Gly
    230                 235                 240

aaa gca gcc acc tca ggt gtt ccc agc att tat gcc ccc agc acc tat      883
Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Thr Tyr
245                 250                 255                 260

gcc cac ctg tct ccc gcc aag acc cca ccc cca cca gct atg att ccc      931
Ala His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Ala Met Ile Pro
                265                 270                 275

atg ggc cct gcc tac aac ggg tac cct gga gga tac cct gga gac gtt      979
Met Gly Pro Ala Tyr Asn Gly Tyr Pro Gly Gly Tyr Pro Gly Asp Val
            280                 285                 290

gac agg agt agc tca gct ggt ggc caa ggc tcc tat gta ccc ctg ctt     1027
Asp Arg Ser Ser Ser Ala Gly Gly Gln Gly Ser Tyr Val Pro Leu Leu
        295                 300                 305

cgg gac acg gac agc agt gtg gcc tct gaa gtc cgc agt ggc tac agg     1075
Arg Asp Thr Asp Ser Ser Val Ala Ser Glu Val Arg Ser Gly Tyr Arg
    310                 315                 320

att cag gcc agc cag cag gac gac tcc atg cgg gtc ctg tac tac atg     1123
Ile Gln Ala Ser Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met
325                 330                 335                 340

gag aag gag ctg gcc aac ttc gac cct tct cga cct ggc ccc ccc agt     1171
Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Ser
                345                 350                 355

ggc cgt gtg gag cgg gcc atg agt gaa gtc acc tcc ctc cac gag gac     1219
Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu His Glu Asp
            360                 365                 370

gac tgg cga tct cgg cct tcc cgg ggc cct gcc ctc acc ccg atc cgg     1267
Asp Trp Arg Ser Arg Pro Ser Arg Gly Pro Ala Leu Thr Pro Ile Arg
        375                 380                 385

gat gag gag tgg ggt ggc cac tcc ccc cgg agt ccc agg gga tgg gac     1315
Asp Glu Glu Trp Gly Gly His Ser Pro Arg Ser Pro Arg Gly Trp Asp
    390                 395                 400
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cag gag ccc gcc agg gag cag gca ggc ggg ggc tgg cgg gcc agg cgg     1363
Gln Glu Pro Ala Arg Glu Gln Ala Gly Gly Gly Trp Arg Ala Arg Arg
405                 410                 415                 420

ccc cgg gcc cgc tcc gtg gac gcc ctg gac gac ctc acc ccg ccg agc     1411
Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Leu Thr Pro Pro Ser
                425                 430                 435

acc gcc gag tca ggg agc agg tct ccc acg agt aat ggt ggg aga agc     1459
Thr Ala Glu Ser Gly Ser Arg Ser Pro Thr Ser Asn Gly Gly Arg Ser
            440                 445                 450

cgg gcc tac atg ccc ccg cgg agc cgc agc cgg gac gac ctc tat gac     1507
Arg Ala Tyr Met Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp
        455                 460                 465

caa gac gac tcg agg gac ttc cca cgc tcc cgg gac ccc cac tac gac     1555
Gln Asp Asp Ser Arg Asp Phe Pro Arg Ser Arg Asp Pro His Tyr Asp
    470                 475                 480

gac ttc agg tct cgg gag cgc cct cct gcc gac ccc agg tcc cac cac     1603
Asp Phe Arg Ser Arg Glu Arg Pro Pro Ala Asp Pro Arg Ser His His
485                 490                 495                 500

cac cgt acc cgg gac cct cgg gac aac ggc tcc agg tcc ggg gac ctc     1651
His Arg Thr Arg Asp Pro Arg Asp Asn Gly Ser Arg Ser Gly Asp Leu
                505                 510                 515

ccc tat gat ggg cgg cta ctg gag gag gct gtg agg aag aag ggg tcg     1699
Pro Tyr Asp Gly Arg Leu Leu Glu Glu Ala Val Arg Lys Lys Gly Ser
            520                 525                 530

gag gag agg agg aga ccc cac aag gag gag gag gaa gag gcc tac tac     1747
Glu Glu Arg Arg Arg Pro His Lys Glu Glu Glu Glu Glu Ala Tyr Tyr
        535                 540                 545

ccg ccc gcg ccg ccc ccg tac tcg gag acc gac tcg cag gcg tcc cga     1795
Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg
    550                 555                 560

gag cgc agg ctc aag aag aac ttg gcc ctg agt cgg gaa agt tta gtc     1843
Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu Val
565                 570                 575                 580

gtc tga tctgacgttt tctacgtagc ttttgtattt ttttttttaa tttgaaggaa      1899
Val  *

cactgatgaa gccctgccat acccctcccg agtctaataa aacgtataat cacaa       1954


<210> SEQ ID NO 12
<211> LENGTH: 581
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Gln Gln Asp Gly Leu Gly Val Gly Thr Arg Asn Gly Ser Gly Lys
1               5                   10                  15

Gly Arg Ser Val His Pro Ser Trp Pro Trp Cys Ala Pro Arg Pro Leu
            20                  25                  30

Arg Tyr Phe Gly Arg Asp Ala Arg Ala Arg Arg Ala Gln Thr Ala Ala
        35                  40                  45

Met Ala Leu Leu Ala Gly Gly Leu Ser Arg Gly Leu Gly Ser His Pro
    50                  55                  60

Ala Ala Ala Gly Arg Asp Ala Val Val Phe Val Trp Leu Leu Leu Ser
65                  70                  75                  80

Thr Trp Cys Thr Ala Pro Ala Arg Ala Ile Gln Val Thr Val Ser Asn
                85                  90                  95

Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu Pro Cys Thr
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           100                 105                 110

Tyr Gln Met Thr Ser Thr Pro Thr Gln Pro Ile Val Ile Trp Lys Tyr
        115                 120                 125

Lys Ser Phe Cys Arg Asp Arg Ile Ala Asp Ala Phe Ser Pro Ala Ser
    130                 135                 140

Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr
145                 150                 155                 160

Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg Val Val
                165                 170                 175

Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly
            180                 185                 190

Arg Arg Ile Thr Ile Thr Gly Asn Ala Asp Leu Thr Phe Asp Gln Thr
        195                 200                 205

Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln
    210                 215                 220

Asp Leu Gln Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Val
225                 230                 235                 240

Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala
                245                 250                 255

Pro Ser Thr Tyr Ala His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro
            260                 265                 270

Ala Met Ile Pro Met Gly Pro Ala Tyr Asn Gly Tyr Pro Gly Gly Tyr
        275                 280                 285

Pro Gly Asp Val Asp Arg Ser Ser Ser Ala Gly Gly Gln Gly Ser Tyr
    290                 295                 300

Val Pro Leu Leu Arg Asp Thr Asp Ser Ser Val Ala Ser Glu Val Arg
305                 310                 315                 320

Ser Gly Tyr Arg Ile Gln Ala Ser Gln Gln Asp Asp Ser Met Arg Val
                325                 330                 335

Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro
            340                 345                 350

Gly Pro Pro Ser Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser
        355                 360                 365

Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Gly Pro Ala Leu
    370                 375                 380

Thr Pro Ile Arg Asp Glu Glu Trp Gly Gly His Ser Pro Arg Ser Pro
385                 390                 395                 400

Arg Gly Trp Asp Gln Glu Pro Ala Arg Glu Gln Ala Gly Gly Gly Trp
                405                 410                 415

Arg Ala Arg Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Leu
            420                 425                 430

Thr Pro Pro Ser Thr Ala Glu Ser Gly Ser Arg Ser Pro Thr Ser Asn
        435                 440                 445

Gly Gly Arg Ser Arg Ala Tyr Met Pro Pro Arg Ser Arg Ser Arg Asp
    450                 455                 460

Asp Leu Tyr Asp Gln Asp Asp Ser Arg Asp Phe Pro Arg Ser Arg Asp
465                 470                 475                 480

Pro His Tyr Asp Asp Phe Arg Ser Arg Glu Arg Pro Pro Ala Asp Pro
                485                 490                 495

Arg Ser His His His Arg Thr Arg Asp Pro Arg Asp Asn Gly Ser Arg
            500                 505                 510
```

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```
Ser Gly Asp Leu Pro Tyr Asp Gly Arg Leu Leu Glu Glu Ala Val Arg
        515                 520                 525

Lys Lys Gly Ser Glu Glu Arg Arg Arg Pro His Lys Glu Glu Glu Glu
    530                 535                 540

Glu Ala Tyr Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser
545                 550                 555                 560

Gln Ala Ser Arg Glu Arg Arg Leu Lys Lys Asn Leu Ala Leu Ser Arg
                565                 570                 575

Glu Ser Leu Val Val
            580


<210> SEQ ID NO 13
<211> LENGTH: 1886
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

gcaccgtcgc tgctagacgg ccgcg atg gcg ccg gcg gcc agc gcg tgt gct       52
                           Met Ala Pro Ala Ala Ser Ala Cys Ala
                           1               5

ggg gcg cct ggc tcc cac ccg gcc acc acg atc ttc gtg tgt ctt ttt      100
Gly Ala Pro Gly Ser His Pro Ala Thr Thr Ile Phe Val Cys Leu Phe
10                  15                  20                  25

ctc atc att tac tgc cca gac cgt gcc agt gcc atc cag gtg acc gtg      148
Leu Ile Ile Tyr Cys Pro Asp Arg Ala Ser Ala Ile Gln Val Thr Val
                30                  35                  40

cct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg aca cta cac      196
Pro Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu His
            45                  50                  55

tgc acc tac cag atg agc aat acc ctc aca gcc cct atc gtg atc tgg      244
Cys Thr Tyr Gln Met Ser Asn Thr Leu Thr Ala Pro Ile Val Ile Trp
        60                  65                  70

aag tat aag tcg ttc tgt cgg gac cgt gtt gcc gac gcc ttc tcc cct      292
Lys Tyr Lys Ser Phe Cys Arg Asp Arg Val Ala Asp Ala Phe Ser Pro
    75                  80                  85

gcc agc gtg gac aac cag ctc aac gcc cag ctg gcg gct ggc aac ccc      340
Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro
90                  95                  100                 105

ggc tac aac ccc tat gtg gag tgc cag gac agc gta cgc act gtc agg      388
Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg
                110                 115                 120

gtg gtg gcc acc aaa cag ggc aat gct gtg acc ctg gga gac tac tac      436
Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr
            125                 130                 135

cag ggc agg aga atc acc atc aca gga aat gct ggc ctg acc ttc gag      484
Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Gly Leu Thr Phe Glu
        140                 145                 150

cag acg gcc tgg gga gac agt gga gtg tat tac tgc tcc gtg gtc tca      532
Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser
    155                 160                 165

gcc caa gat ctg gat ggg aac aac gag gcg tac gca gag ctc att gtc      580
Ala Gln Asp Leu Asp Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val
170                 175                 180                 185

ctt ggc agg acc tca gaa gcc cct gag ctc cta cct ggt ttt cgg gcg      628
Leu Gly Arg Thr Ser Glu Ala Pro Glu Leu Leu Pro Gly Phe Arg Ala
                190                 195                 200
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ggg ccc ttg gaa gat tgg ctc ttt gtg gtc gtg gtc tgc ctg gca agc      676
Gly Pro Leu Glu Asp Trp Leu Phe Val Val Val Val Cys Leu Ala Ser
            205                 210                 215

ctc ctc ttc ttc ctc ctc ctg ggc atc tgc tgg tgc cag tgc tgt ccc      724
Leu Leu Phe Phe Leu Leu Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro
        220                 225                 230

cac acc tgc tgc tgc tat gtc aga tgt ccc tgc tgc cca gac aag tgc      772
His Thr Cys Cys Cys Tyr Val Arg Cys Pro Cys Cys Pro Asp Lys Cys
    235                 240                 245

tgt tgc cct gag gcc ctt tat gct gct ggc aaa gca gcc acc tca ggt      820
Cys Cys Pro Glu Ala Leu Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly
250                 255                 260                 265

gtg cca agc atc tat gcc ccc agc atc tat acc cac ctc tct cct gcc      868
Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr His Leu Ser Pro Ala
                270                 275                 280

aag act ccg cca cct ccg cct gcc atg att ccc atg cgt cct ccc tat      916
Lys Thr Pro Pro Pro Pro Ala Met Ile Pro Met Arg Pro Pro Tyr
            285                 290                 295

ggg tac cct gga gac ttt gac agg acc agc tca gtt ggt ggc cac agc      964
Gly Tyr Pro Gly Asp Phe Asp Arg Thr Ser Ser Val Gly Gly His Ser
        300                 305                 310

tcc cag gtg ccc ctg ctg cgt gaa gtg gat ggg agc gta tct tca gaa     1012
Ser Gln Val Pro Leu Leu Arg Glu Val Asp Gly Ser Val Ser Ser Glu
    315                 320                 325

gta cga agt ggc tac agg atc cag gct aac cag caa gat gac tcc atg     1060
Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn Gln Gln Asp Asp Ser Met
330                 335                 340                 345

agg gtc cta tac tat atg gag aag gag cta gcc aac ttc gat cct tcc     1108
Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser
                350                 355                 360

cgg cct ggc cct ccc aat ggc cga gtg gaa cgg gcc atg agt gaa gta     1156
Arg Pro Gly Pro Pro Asn Gly Arg Val Glu Arg Ala Met Ser Glu Val
            365                 370                 375

acc tcc ctc cat gaa gat gac tgg cga tct cgg cct tcc agg gct cct     1204
Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Ala Pro
        380                 385                 390

gcc ctc aca ccc atc agg gat gag gag tgg aat cgc cac tcc cct cgg     1252
Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp Asn Arg His Ser Pro Arg
    395                 400                 405

agt ccc aga aca tgg gag cag gaa ccc ctt caa gaa cag cca agg ggt     1300
Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln Glu Gln Pro Arg Gly
410                 415                 420                 425

ggt tgg ggg tct ggg cgg cct cgg gcc cgc tct gtg gat gct cta gat     1348
Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp
                430                 435                 440

gac atc aac cgg cct ggc tcc act gaa tca gga agg tct tct ccc cca     1396
Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly Arg Ser Ser Pro Pro
            445                 450                 455

agt agt gga cgg aga ggg cgg gcc tat gca cct ccg aga agt cgc agc     1444
Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala Pro Pro Arg Ser Arg Ser
        460                 465                 470

cgg gat gac ctc tat gac ccc gac gat cct aga gac ttg cca cat tcc     1492
Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro Arg Asp Leu Pro His Ser
    475                 480                 485

cga gat ccc cac tat tat gat gat ttg agg tct agg gat cca cgt gct     1540
Arg Asp Pro His Tyr Tyr Asp Asp Leu Arg Ser Arg Asp Pro Arg Ala
490                 495                 500                 505
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gac ccc aga tcc cgt cag cga tcc cac gat cct cgg gat gct ggc ttc     1588
Asp Pro Arg Ser Arg Gln Arg Ser His Asp Pro Arg Asp Ala Gly Phe
                510                 515                 520

agg tca cgg gac cct cag tat gat ggg cga ctc tta gaa gag gct tta     1636
Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu Leu Glu Glu Ala Leu
            525                 530                 535

aag aaa aaa ggg gct ggg gag aga aga cgc gtt tac agg gag gaa gaa     1684
Lys Lys Lys Gly Ala Gly Glu Arg Arg Arg Val Tyr Arg Glu Glu Glu
        540                 545                 550

gaa gaa gaa gag gag ggc cac tat ccc cca gca cct ccg cct tac tct     1732
Glu Glu Glu Glu Glu Gly His Tyr Pro Pro Ala Pro Pro Pro Tyr Ser
    555                 560                 565

gag act gac tcg cag gcc tcg agg gag cgg agg atg aaa aag aat ttg     1780
Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg Met Lys Lys Asn Leu
570                 575                 580                 585

gcc ctg agt cgg gaa agt tta gtc gtc tga tcccacgttt tgttatgtag       1830
Ala Leu Ser Arg Glu Ser Leu Val Val  *
                590

cttttatact tttttaattg gaatattgat gaaactcttc accaagccta ataaaa       1886


<210> SEQ ID NO 14
<211> LENGTH: 1829
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

gcaccgtcgc tgctagacgg ccgcg atg gcg ccg gcg gcc agc gcg tgt gct       52
                            Met Ala Pro Ala Ala Ser Ala Cys Ala
                            1               5

ggg gcg cct ggc tcc cac ccg gcc acc acg atc ttc gtg tgt ctt ttt      100
Gly Ala Pro Gly Ser His Pro Ala Thr Thr Ile Phe Val Cys Leu Phe
10                  15                  20                  25

ctc atc att tac tgc cca gac cgt gcc agt gcc atc cag gtg acc gtg      148
Leu Ile Ile Tyr Cys Pro Asp Arg Ala Ser Ala Ile Gln Val Thr Val
                30                  35                  40

cct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg aca cta cac      196
Pro Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu His
            45                  50                  55

tgc acc tac cag atg agc aat acc ctc aca gcc cct atc gtg atc tgg      244
Cys Thr Tyr Gln Met Ser Asn Thr Leu Thr Ala Pro Ile Val Ile Trp
        60                  65                  70

aag tat aag tcg ttc tgt cgg gac cgt gtt gcc gac gcc ttc tcc cct      292
Lys Tyr Lys Ser Phe Cys Arg Asp Arg Val Ala Asp Ala Phe Ser Pro
    75                  80                  85

gcc agc gtg gac aac cag ctc aac gcc cag ctg gcg gct ggc aac ccc      340
Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro
90                  95                  100                 105

ggc tac aac ccc tat gtg gag tgc cag gac agc gta cgc act gtc agg      388
Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg
                110                 115                 120

gtg gtg gcc acc aaa cag ggc aat gct gtg acc ctg gga gac tac tac      436
Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr
            125                 130                 135

cag ggc agg aga atc acc atc aca gga aat gct ggc ctg acc ttc gag      484
Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Gly Leu Thr Phe Glu
        140                 145                 150

cag acg gcc tgg gga gac agt gga gtg tat tac tgc tcc gtg gtc tca      532
Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser
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    155                 160                 165

gcc caa gat ctg gat ggg aac aac gag gcg tac gca gag ctc att gtc      580
Ala Gln Asp Leu Asp Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val
170                 175                 180                 185

ctt gat tgg ctc ttt gtg gtc gtg gtc tgc ctg gca agc ctc ctc ttc      628
Leu Asp Trp Leu Phe Val Val Val Val Cys Leu Ala Ser Leu Leu Phe
                190                 195                 200

ttc ctc ctc ctg ggc atc tgc tgg tgc cag tgc tgt ccc cac acc tgc      676
Phe Leu Leu Leu Gly Ile Cys Trp Cys Gln Cys Cys Pro His Thr Cys
            205                 210                 215

tgc tgc tat gtc aga tgt ccc tgc tgc cca gac aag tgc tgt tgc cct      724
Cys Cys Tyr Val Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro
        220                 225                 230

gag gcc ctt tat gct gct ggc aaa gca gcc acc tca ggt gtg cca agc      772
Glu Ala Leu Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser
    235                 240                 245

atc tat gcc ccc agc atc tat acc cac ctc tct cct gcc aag act ccg      820
Ile Tyr Ala Pro Ser Ile Tyr Thr His Leu Ser Pro Ala Lys Thr Pro
250                 255                 260                 265

cca cct ccg cct gcc atg att ccc atg cgt cct ccc tat ggg tac cct      868
Pro Pro Pro Pro Ala Met Ile Pro Met Arg Pro Pro Tyr Gly Tyr Pro
                270                 275                 280

gga gac ttt gac agg acc agc tca gtt ggt ggc cac agc tcc cag gtg      916
Gly Asp Phe Asp Arg Thr Ser Ser Val Gly Gly His Ser Ser Gln Val
            285                 290                 295

ccc ctg ctg cgt gaa gtg gat ggg agc gta tct tca gaa gta cga agt      964
Pro Leu Leu Arg Glu Val Asp Gly Ser Val Ser Ser Glu Val Arg Ser
        300                 305                 310

ggc tac agg atc cag gct aac cag caa gat gac tcc atg agg gtc cta     1012
Gly Tyr Arg Ile Gln Ala Asn Gln Gln Asp Asp Ser Met Arg Val Leu
    315                 320                 325

tac tat atg gag aag gag cta gcc aac ttc gat cct tcc cgg cct ggc     1060
Tyr Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly
330                 335                 340                 345

cct ccc aat ggc cga gtg gaa cgg gcc atg agt gaa gta acc tcc ctc     1108
Pro Pro Asn Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu
                350                 355                 360

cat gaa gat gac tgg cga tct cgg cct tcc agg gct cct gcc ctc aca     1156
His Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Ala Pro Ala Leu Thr
            365                 370                 375

ccc atc agg gat gag gag tgg aat cgc cac tcc cct cgg agt ccc aga     1204
Pro Ile Arg Asp Glu Glu Trp Asn Arg His Ser Pro Arg Ser Pro Arg
        380                 385                 390

aca tgg gag cag gaa ccc ctt caa gaa cag cca agg ggt ggt tgg ggg     1252
Thr Trp Glu Gln Glu Pro Leu Gln Glu Gln Pro Arg Gly Gly Trp Gly
    395                 400                 405

tct ggg cgg cct cgg gcc cgc tct gtg gat gct cta gat gac atc aac     1300
Ser Gly Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Ile Asn
410                 415                 420                 425

cgg cct ggc tcc act gaa tca gga agg tct tct ccc cca agt agt gga     1348
Arg Pro Gly Ser Thr Glu Ser Gly Arg Ser Ser Pro Pro Ser Ser Gly
                430                 435                 440

cgg aga ggg cgg gcc tat gca cct ccg aga agt cgc agc cgg gat gac     1396
Arg Arg Gly Arg Ala Tyr Ala Pro Pro Arg Ser Arg Ser Arg Asp Asp
            445                 450                 455

ctc tat gac ccc gac gat cct aga gac ttg cca cat tcc cga gat ccc     1444
Leu Tyr Asp Pro Asp Asp Pro Arg Asp Leu Pro His Ser Arg Asp Pro
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        460                 465                 470

cac tat tat gat gat ttg agg tct agg gat cca cgt gct gac ccc aga     1492
His Tyr Tyr Asp Asp Leu Arg Ser Arg Asp Pro Arg Ala Asp Pro Arg
    475                 480                 485

tcc cgt cag cga tcc cac gat cct cgg gat gct ggc ttc agg tca cgg     1540
Ser Arg Gln Arg Ser His Asp Pro Arg Asp Ala Gly Phe Arg Ser Arg
490                 495                 500                 505

gac cct cag tat gat ggg cga ctc tta gaa gag gct tta aag aaa aaa     1588
Asp Pro Gln Tyr Asp Gly Arg Leu Leu Glu Glu Ala Leu Lys Lys Lys
                510                 515                 520

ggg gct ggg gag aga aga cgc gtt tac agg gag gaa gaa gaa gaa gaa     1636
Gly Ala Gly Glu Arg Arg Arg Val Tyr Arg Glu Glu Glu Glu Glu Glu
            525                 530                 535

gag gag ggc cac tat ccc cca gca cct ccg cct tac tct gag act gac     1684
Glu Glu Gly His Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp
        540                 545                 550

tcg cag gcc tcg agg gag cgg agg atg aaa aag aat ttg gcc ctg agt     1732
Ser Gln Ala Ser Arg Glu Arg Arg Met Lys Lys Asn Leu Ala Leu Ser
    555                 560                 565

cgg gaa agt tta gtc gtc tga tcccacgttt tgttatgtag cttttatact        1783
Arg Glu Ser Leu Val Val  *
570                 575

tttttaattg gaatattgat gaaactcttc accaagccta ataaaa                  1829


<210> SEQ ID NO 15
<211> LENGTH: 1682
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

gcaccgtcgc tgctagacgg ccgcg atg gcg ccg gcg gcc agc gcg tgt gct       52
                            Met Ala Pro Ala Ala Ser Ala Cys Ala
                            1               5

ggg gcg cct ggc tcc cac ccg gcc acc acg atc ttc gtg tgt ctt ttt      100
Gly Ala Pro Gly Ser His Pro Ala Thr Thr Ile Phe Val Cys Leu Phe
10                  15                  20                  25

ctc atc att tac tgc cca gac cgt gcc agt gcc atc cag gtg acc gtg      148
Leu Ile Ile Tyr Cys Pro Asp Arg Ala Ser Ala Ile Gln Val Thr Val
                30                  35                  40

cct gac ccc tac cac gta gtg atc ctg ttc cag cca gtg aca cta cac      196
Pro Asp Pro Tyr His Val Val Ile Leu Phe Gln Pro Val Thr Leu His
            45                  50                  55

tgc acc tac cag atg agc aat acc ctc aca gcc cct atc gtg atc tgg      244
Cys Thr Tyr Gln Met Ser Asn Thr Leu Thr Ala Pro Ile Val Ile Trp
        60                  65                  70

aag tat aag tcg ttc tgt cgg gac cgt gtt gcc gac gcc ttc tcc cct      292
Lys Tyr Lys Ser Phe Cys Arg Asp Arg Val Ala Asp Ala Phe Ser Pro
    75                  80                  85

gcc agc gtg gac aac cag ctc aac gcc cag ctg gcg gct ggc aac ccc      340
Ala Ser Val Asp Asn Gln Leu Asn Ala Gln Leu Ala Ala Gly Asn Pro
90                  95                  100                 105

ggc tac aac ccc tat gtg gag tgc cag gac agc gta cgc act gtc agg      388
Gly Tyr Asn Pro Tyr Val Glu Cys Gln Asp Ser Val Arg Thr Val Arg
                110                 115                 120

gtg gtg gcc acc aaa cag ggc aat gct gtg acc ctg gga gac tac tac      436
Val Val Ala Thr Lys Gln Gly Asn Ala Val Thr Leu Gly Asp Tyr Tyr
            125                 130                 135
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cag ggc agg aga atc acc atc aca gga aat gct ggc ctg acc ttc gag      484
Gln Gly Arg Arg Ile Thr Ile Thr Gly Asn Ala Gly Leu Thr Phe Glu
        140                 145                 150

cag acg gcc tgg gga gac agt gga gtg tat tac tgc tcc gtg gtc tca      532
Gln Thr Ala Trp Gly Asp Ser Gly Val Tyr Tyr Cys Ser Val Val Ser
    155                 160                 165

gcc caa gat ctg gat ggg aac aac gag gcg tac gca gag ctc att gtc      580
Ala Gln Asp Leu Asp Gly Asn Asn Glu Ala Tyr Ala Glu Leu Ile Val
170                 175                 180                 185

ctt gtt tat gct gct ggc aaa gca gcc acc tca ggt gtg cca agc atc      628
Leu Val Tyr Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile
                190                 195                 200

tat gcc ccc agc atc tat acc cac ctc tct cct gcc aag act ccg cca      676
Tyr Ala Pro Ser Ile Tyr Thr His Leu Ser Pro Ala Lys Thr Pro Pro
            205                 210                 215

cct ccg cct gcc atg att ccc atg cgt cct ccc tat ggg tac cct gga      724
Pro Pro Pro Ala Met Ile Pro Met Arg Pro Pro Tyr Gly Tyr Pro Gly
        220                 225                 230

gac ttt gac agg acc agc tca gtt ggt ggc cac agc tcc cag gtg ccc      772
Asp Phe Asp Arg Thr Ser Ser Val Gly Gly His Ser Ser Gln Val Pro
    235                 240                 245

ctg ctg cgt gaa gtg gat ggg agc gta tct tca gaa gta cga agt ggc      820
Leu Leu Arg Glu Val Asp Gly Ser Val Ser Ser Glu Val Arg Ser Gly
250                 255                 260                 265

tac agg atc cag gct aac cag caa gat gac tcc atg agg gtc cta tac      868
Tyr Arg Ile Gln Ala Asn Gln Gln Asp Asp Ser Met Arg Val Leu Tyr
                270                 275                 280

tat atg gag aag gag cta gcc aac ttc gat cct tcc cgg cct ggc cct      916
Tyr Met Glu Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro
            285                 290                 295

ccc aat ggc cga gtg gaa cgg gcc atg agt gaa gta acc tcc ctc cat      964
Pro Asn Gly Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu His
        300                 305                 310

gaa gat gac tgg cga tct cgg cct tcc agg gct cct gcc ctc aca ccc     1012
Glu Asp Asp Trp Arg Ser Arg Pro Ser Arg Ala Pro Ala Leu Thr Pro
    315                 320                 325

atc agg gat gag gag tgg aat cgc cac tcc cct cgg agt ccc aga aca     1060
Ile Arg Asp Glu Glu Trp Asn Arg His Ser Pro Arg Ser Pro Arg Thr
330                 335                 340                 345

tgg gag cag gaa ccc ctt caa gaa cag cca agg ggt ggt tgg ggg tct     1108
Trp Glu Gln Glu Pro Leu Gln Glu Gln Pro Arg Gly Gly Trp Gly Ser
                350                 355                 360

ggg cgg cct cgg gcc cgc tct gtg gat gct cta gat gac atc aac cgg     1156
Gly Arg Pro Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Ile Asn Arg
            365                 370                 375

cct ggc tcc act gaa tca gga agg tct tct ccc cca agt agt gga cgg     1204
Pro Gly Ser Thr Glu Ser Gly Arg Ser Ser Pro Pro Ser Ser Gly Arg
        380                 385                 390

aga ggg cgg gcc tat gca cct ccg aga agt cgc agc cgg gat gac ctc     1252
Arg Gly Arg Ala Tyr Ala Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu
    395                 400                 405

tat gac ccc gac gat cct aga gac ttg cca cat tcc cga gat ccc cac     1300
Tyr Asp Pro Asp Asp Pro Arg Asp Leu Pro His Ser Arg Asp Pro His
410                 415                 420                 425

tat tat gat gat ttg agg tct agg gat cca cgt gct gac ccc aga tcc     1348
Tyr Tyr Asp Asp Leu Arg Ser Arg Asp Pro Arg Ala Asp Pro Arg Ser
                430                 435                 440
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cgt cag cga tcc cac gat cct cgg gat gct ggc ttc agg tca cgg gac     1396
Arg Gln Arg Ser His Asp Pro Arg Asp Ala Gly Phe Arg Ser Arg Asp
            445                 450                 455

cct cag tat gat ggg cga ctc tta gaa gag gct tta aag aaa aaa ggg     1444
Pro Gln Tyr Asp Gly Arg Leu Leu Glu Glu Ala Leu Lys Lys Lys Gly
        460                 465                 470

gct ggg gag aga aga cgc gtt tac agg gag gaa gaa gaa gaa gaa gag     1492
Ala Gly Glu Arg Arg Arg Val Tyr Arg Glu Glu Glu Glu Glu Glu Glu
    475                 480                 485

gag ggc cac tat ccc cca gca cct ccg cct tac tct gag act gac tcg     1540
Glu Gly His Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser
490                 495                 500                 505

cag gcc tcg agg gag cgg agg atg aaa aag aat ttg gcc ctg agt cgg     1588
Gln Ala Ser Arg Glu Arg Arg Met Lys Lys Asn Leu Ala Leu Ser Arg
                510                 515                 520

gaa agt tta gtc gtc tga tcccacgttt tgttatgtag cttttatact            1636
Glu Ser Leu Val Val  *
            525

tttttaattg gaatattgat gaaactcttc accaagccta ataaaa                  1682


<210> SEQ ID NO 16
<211> LENGTH: 594
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

Met Ala Pro Ala Ala Ser Ala Cys Ala Gly Ala Pro Gly Ser His Pro
1               5                   10                  15

Ala Thr Thr Ile Phe Val Cys Leu Phe Leu Ile Ile Tyr Cys Pro Asp
            20                  25                  30

Arg Ala Ser Ala Ile Gln Val Thr Val Pro Asp Pro Tyr His Val Val
        35                  40                  45

Ile Leu Phe Gln Pro Val Thr Leu His Cys Thr Tyr Gln Met Ser Asn
    50                  55                  60

Thr Leu Thr Ala Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Val Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
                85                  90                  95

Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
            100                 105                 110

Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
        115                 120                 125

Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
    130                 135                 140

Thr Gly Asn Ala Gly Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Asp Gly Asn
                165                 170                 175

Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Gly Arg Thr Ser Glu Ala
            180                 185                 190

Pro Glu Leu Leu Pro Gly Phe Arg Ala Gly Pro Leu Glu Asp Trp Leu
        195                 200                 205

Phe Val Val Val Val Cys Leu Ala Ser Leu Leu Phe Phe Leu Leu Leu
    210                 215                 220
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Gly Ile Cys Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys Tyr Val
225                 230                 235                 240

Arg Cys Pro Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr
                245                 250                 255

Ala Ala Gly Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro
            260                 265                 270

Ser Ile Tyr Thr His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro
        275                 280                 285

Ala Met Ile Pro Met Arg Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp
    290                 295                 300

Arg Thr Ser Ser Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg
305                 310                 315                 320

Glu Val Asp Gly Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile
                325                 330                 335

Gln Ala Asn Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu
            340                 345                 350

Lys Glu Leu Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly
        355                 360                 365

Arg Val Glu Arg Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp
    370                 375                 380

Trp Arg Ser Arg Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp
385                 390                 395                 400

Glu Glu Trp Asn Arg His Ser Pro Arg Ser Pro Arg Thr Trp Glu Gln
                405                 410                 415

Glu Pro Leu Gln Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro
            420                 425                 430

Arg Ala Arg Ser Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser
        435                 440                 445

Thr Glu Ser Gly Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg
    450                 455                 460

Ala Tyr Ala Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro
465                 470                 475                 480

Asp Asp Pro Arg Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp
                485                 490                 495

Asp Leu Arg Ser Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg
            500                 505                 510

Ser His Asp Pro Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr
        515                 520                 525

Asp Gly Arg Leu Leu Glu Glu Ala Leu Lys Lys Lys Gly Ala Gly Glu
    530                 535                 540

Arg Arg Arg Val Tyr Arg Glu Glu Glu Glu Glu Glu Glu Glu Gly His
545                 550                 555                 560

Tyr Pro Pro Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser
                565                 570                 575

Arg Glu Arg Arg Met Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu
            580                 585                 590

Val Val
```

<210> SEQ ID NO 17
<211> LENGTH: 575
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 17

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Ala Thr Thr Ile Phe Val Cys Leu Phe Leu Ile Ile Tyr Cys Pro Asp
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Arg Ala Ser Ala Ile Gln Val Thr Val Pro Asp Pro Tyr His Val Val
        35                  40                  45

Ile Leu Phe Gln Pro Val Thr Leu His Cys Thr Tyr Gln Met Ser Asn
    50                  55                  60

Thr Leu Thr Ala Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Val Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
                85                  90                  95

Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
            100                 105                 110

Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
        115                 120                 125

Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
    130                 135                 140

Thr Gly Asn Ala Gly Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Asp Gly Asn
                165                 170                 175

Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Asp Trp Leu Phe Val Val
            180                 185                 190

Val Val Cys Leu Ala Ser Leu Leu Phe Phe Leu Leu Leu Gly Ile Cys
        195                 200                 205

Trp Cys Gln Cys Cys Pro His Thr Cys Cys Cys Tyr Val Arg Cys Pro
    210                 215                 220

Cys Cys Pro Asp Lys Cys Cys Cys Pro Glu Ala Leu Tyr Ala Ala Gly
225                 230                 235                 240

Lys Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr
                245                 250                 255

Thr His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro Ala Met Ile
            260                 265                 270

Pro Met Arg Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp Arg Thr Ser
        275                 280                 285

Ser Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg Glu Val Asp
    290                 295                 300

Gly Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn
305                 310                 315                 320

Gln Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu
                325                 330                 335

Ala Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly Arg Val Glu
            340                 345                 350

Arg Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser
        355                 360                 365

Arg Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp
    370                 375                 380

Asn Arg His Ser Pro Arg Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu
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Gln Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg
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Ser Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser
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Gly Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala
        435                 440                 445

Pro Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro
    450                 455                 460

Arg Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp Asp Leu Arg
465                 470                 475                 480

Ser Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg Ser His Asp
                485                 490                 495

Pro Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg
            500                 505                 510

Leu Leu Glu Glu Ala Leu Lys Lys Lys Gly Ala Gly Glu Arg Arg Arg
        515                 520                 525

Val Tyr Arg Glu Glu Glu Glu Glu Glu Glu Glu Gly His Tyr Pro Pro
    530                 535                 540

Ala Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg
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Arg Met Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu Val Val
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<210> SEQ ID NO 18
<211> LENGTH: 526
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

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Arg Ala Ser Ala Ile Gln Val Thr Val Pro Asp Pro Tyr His Val Val
        35                  40                  45

Ile Leu Phe Gln Pro Val Thr Leu His Cys Thr Tyr Gln Met Ser Asn
    50                  55                  60

Thr Leu Thr Ala Pro Ile Val Ile Trp Lys Tyr Lys Ser Phe Cys Arg
65                  70                  75                  80

Asp Arg Val Ala Asp Ala Phe Ser Pro Ala Ser Val Asp Asn Gln Leu
                85                  90                  95

Asn Ala Gln Leu Ala Ala Gly Asn Pro Gly Tyr Asn Pro Tyr Val Glu
            100                 105                 110

Cys Gln Asp Ser Val Arg Thr Val Arg Val Val Ala Thr Lys Gln Gly
        115                 120                 125

Asn Ala Val Thr Leu Gly Asp Tyr Tyr Gln Gly Arg Arg Ile Thr Ile
    130                 135                 140

Thr Gly Asn Ala Gly Leu Thr Phe Glu Gln Thr Ala Trp Gly Asp Ser
145                 150                 155                 160

Gly Val Tyr Tyr Cys Ser Val Val Ser Ala Gln Asp Leu Asp Gly Asn
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Asn Glu Ala Tyr Ala Glu Leu Ile Val Leu Val Tyr Ala Ala Gly Lys
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Ala Ala Thr Ser Gly Val Pro Ser Ile Tyr Ala Pro Ser Ile Tyr Thr
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His Leu Ser Pro Ala Lys Thr Pro Pro Pro Pro Pro Ala Met Ile Pro
    210                 215                 220

Met Arg Pro Pro Tyr Gly Tyr Pro Gly Asp Phe Asp Arg Thr Ser Ser
225                 230                 235                 240

Val Gly Gly His Ser Ser Gln Val Pro Leu Leu Arg Glu Val Asp Gly
                245                 250                 255

Ser Val Ser Ser Glu Val Arg Ser Gly Tyr Arg Ile Gln Ala Asn Gln
            260                 265                 270

Gln Asp Asp Ser Met Arg Val Leu Tyr Tyr Met Glu Lys Glu Leu Ala
        275                 280                 285

Asn Phe Asp Pro Ser Arg Pro Gly Pro Pro Asn Gly Arg Val Glu Arg
    290                 295                 300

Ala Met Ser Glu Val Thr Ser Leu His Glu Asp Asp Trp Arg Ser Arg
305                 310                 315                 320

Pro Ser Arg Ala Pro Ala Leu Thr Pro Ile Arg Asp Glu Glu Trp Asn
                325                 330                 335

Arg His Ser Pro Arg Ser Pro Arg Thr Trp Glu Gln Glu Pro Leu Gln
            340                 345                 350

Glu Gln Pro Arg Gly Gly Trp Gly Ser Gly Arg Pro Arg Ala Arg Ser
        355                 360                 365

Val Asp Ala Leu Asp Asp Ile Asn Arg Pro Gly Ser Thr Glu Ser Gly
    370                 375                 380

Arg Ser Ser Pro Pro Ser Ser Gly Arg Arg Gly Arg Ala Tyr Ala Pro
385                 390                 395                 400

Pro Arg Ser Arg Ser Arg Asp Asp Leu Tyr Asp Pro Asp Asp Pro Arg
                405                 410                 415

Asp Leu Pro His Ser Arg Asp Pro His Tyr Tyr Asp Asp Leu Arg Ser
            420                 425                 430

Arg Asp Pro Arg Ala Asp Pro Arg Ser Arg Gln Arg Ser His Asp Pro
        435                 440                 445

Arg Asp Ala Gly Phe Arg Ser Arg Asp Pro Gln Tyr Asp Gly Arg Leu
    450                 455                 460

Leu Glu Glu Ala Leu Lys Lys Lys Gly Ala Gly Glu Arg Arg Arg Val
465                 470                 475                 480

Tyr Arg Glu Glu Glu Glu Glu Glu Glu Glu Gly His Tyr Pro Pro Ala
                485                 490                 495

Pro Pro Pro Tyr Ser Glu Thr Asp Ser Gln Ala Ser Arg Glu Arg Arg
            500                 505                 510

Met Lys Lys Asn Leu Ala Leu Ser Arg Glu Ser Leu Val Val
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<210> SEQ ID NO 19
<211> LENGTH: 22976
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
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<223> OTHER INFORMATION: Potential variant splicing site AAG

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<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 20

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<210> SEQ ID NO 21
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<400> SEQUENCE: 23

gaagatgact ggcgatcgag 20

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acctctatga cccggacgat 20

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<210> SEQ ID NO 26
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<210> SEQ ID NO 27
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<400> SEQUENCE: 27

gccctggaag gcctcgatcg 20

<210> SEQ ID NO 28
<211> LENGTH: 20
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<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 28

caagtcccta ggatcgtccg 20

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus
<220> FEATURE:
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 169..188
<223> OTHER INFORMATION: Position in SEQID2

<400> SEQUENCE: 29

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1 5 10 15

Val Leu Gly Arg
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<210> SEQ ID NO 30
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<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus
<220> FEATURE:
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 556..570
<223> OTHER INFORMATION: Position in SEQID2

<400> SEQUENCE: 30

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1 5 10 15

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<210> SEQ ID NO 31
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

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<210> SEQ ID NO 32
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

tcagacgact aaactttccc gactcagg 28

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

ctacaacccc tacgttgagt 20

<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

tcgtgacctg acctttgacc agac 24

<210> SEQ ID NO 35
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<400> SEQUENCE: 35

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<400> SEQUENCE: 36

aggccgagat cgccagtcgt 20

<210> SEQ ID NO 37
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<400> SEQUENCE: 37

ctacatggat ccagtcatgc cgaagat 27

<210> SEQ ID NO 38
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<400> SEQUENCE: 38

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cgacaactcg agtcagttgg tatcatgg 28

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Leu Arg Cys Val Pro Arg Val Leu Gly Ser Ser Val Ala Gly Tyr
1 5 10 15

<210> SEQ ID NO 40
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<212> TYPE: PRT
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<220> FEATURE:
<221> NAME/KEY: Misc_Feature
<222> LOCATION: 2..15
<223> OTHER INFORMATION: Identical to 268 .. 282 in ref swissprot
:Q07021

<400> SEQUENCE: 40

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1 5 10 15

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<221> NAME/KEY: exon
<222> LOCATION: 12064..12183
<221> NAME/KEY: exon
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genbank:AD000684
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<223> OTHER INFORMATION: diverging insertion, T in ref genbank:AD000684
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<223> OTHER INFORMATION: diverging insertion, C in ref genbank:AD000684
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<223> OTHER INFORMATION: diverging insertion, G in ref genbank:AD000684
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<221> NAME/KEY: Misc_Feature
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tgcactattt acaatagtca aaaggtagaa acaacctagg tccatccaca aatgaatgga 180

taaataaaac gtagcatata catacaatgg tacactagtc cgctgtaaaa agaaattttg 240

atcttactgc atgctacatg gcttcgacat actacaacat ggatggacct tgaaaacatt 300

attctttgtg aaataaacta gacacaggac aaatgttaga cgattccact tatatgaggc 360

acctagaatg ggcaatttgg taagcaaagt agaatagaaa ttactagggg cacaggtagc 420

agggaatggg gagttactgt ttaatggtca cagagtttat gttggggatg atgaaacagt 480

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atatttttt aaacgatgaa gggacgtgaa cgggttgaaa ttttataaaa agtggccagg 660

gaaggtgtca ctgcaatggt gtcctacagg aggaggaaga tcatgtggac atctccggga 720

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gccggccgga gaacgtgccc cgcgcagcca ccgcccgcct gcgtgcgcgc cccggccccg  21300

cccaggcgtg cgcatgcgcc ccggccctcc gccttcgcgc accgcaggct ggccgccggg  21360

agcgcgcgcg cgctcctctc cccttccagc ccatcccccc cagcccccca ccgacctact  21420

ttactgtctc caaactcggg cagcccacct ggcccccgac gaccccagcc cctgctccgg  21480

gtaccccgac gttccatcca gacccgcgtt tcaccagggc ggcgcgcggc gacctcgcgc  21540

cccgcggagc cccgggctcg cgcgcgcccg cccgcccccg gagacagaca gcgcgcgcgc  21600

tcccgggccg cctcccccca gcgcgcgtcc gccccgggct cgcgccgccg ccgccgccgc  21660

cgccgcgcgc gcgcagctca agtaaaggag gaaaaaaaaa aggggaaaa atagaaagcg  21720

g                                                                  21721
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We claim:

1. A composition of matter comprising:

a) a purified polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18 or comprising a polypeptide that is at least 90% identical to SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18;

b) a purified nucleic acid sequence comprising: SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14 or 15; or encoding: a polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18; or comprising a polypeptide that is at least 90% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18;

c) a vector comprising a nucleic acid comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14 or 15 or encoding: a polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18; or comprising a polypeptide that is at least 90% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18;

d) a recombinant cell comprising:

i) SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14 or 15;

ii) a vector comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14 or 15; or

iii) a nucleic sequence encoding: a polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18; or comprising a polypeptide that is at least 90% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18; or

e) an isolated antibody that specifically binds to a polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 16, 17 or 18.

2. The composition of matter according to claim **1**, wherein said polypeptide is a purified or recombinant Lipolysis Stimulated Receptor and said receptor comprises at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18.

3. The composition of matter according to claim **1**, wherein said polypeptide comprises at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18.

4. The composition of matter according to claim **3**, wherein said polypeptide comprises SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18.

5. The composition of matter according to claim **1**, wherein said polypeptide is at least 90% identical to SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18.

6. The composition of matter according to claim **5**, wherein said polypeptide comprises SEQ ID NO: 2, 4, 6, 10, 16, 17 or 18.

7. The composition of matter according to claim **1**, wherein said nucleic acid encodes polypeptide comprising at least 10 to 15 consecutive amino acids of SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18.

8. The composition of matter according to claim **1**, wherein said nucleic acid encodes a polypeptide that is at least 90% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18.

9. The composition of matter according to claim **1**, wherein said nucleic acid encodes SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 17 or 18.

10. The composition of matter according to claim **1**, wherein said nucleic acid is SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14 or 15.

11. A method for selecting a compound useful for enhancing lipoprotein uptake in cells comprising the steps:

a) contacting the recombinant cell of claim **1** with a candidate compound in the presence of a lipoprotein; and

b) determining whether the amount of internalized lipoprotein is greater in the presence of said compound than in the absence of said compound, wherein a determination that said amount of internalized lipoprotein is greater in the presence of said compound indicates that said compound is useful for enhancing lipoprotein uptake in cells.

12. The method according to claim **11**, wherein said candidate compound is a small molecule.

13. A method for selecting a compound useful for enhancing lipoprotein uptake in cells comprising the steps:

a) contacting the recombinant cell of claim **1** with a candidate compound in the presence of a lipoprotein; and

b) determining whether the amount of internalized lipoprotein is greater in the presence of said compound than in the absence of said compound, wherein a determination that said amount of internalized lipoprotein is greater in the presence of said compound indicates that said compound is useful for enhancing lipoprotein uptake in cells.

14. The method according to claim **13**, wherein said candidate compound is a small molecule.

15. A composition comprising the composition of matter according to claim **1** and a physiologically acceptable carrier.

16. A method of making a polypeptide comprising:

a) obtaining a recombinant cell according to claim **1** that expresses said polypeptide;

b) growing said recombinant cells under conditions suitable to produce said polypeptide; and

c) isolating said polypeptide produced by said recombinant cell.

17. The method according to claim **16**, wherein said cell is prokaryotic.

18. The method according to claim **16**, wherein said cell is eukaryotic.

19. The method according to claim **16**, further comprising purifying said polypeptide produced by said cell.

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