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(54) **THERAPEUTIC OR PROPHYLACTIC AGENT, DETECTION METHOD AND DETECTION AGENT FOR METABOLIC SYNDROME, AND METHOD FOR SCREENING OF CANDIDATE COMPOUND FOR THERAPEUTIC AGENT FOR METABOLIC SYNDROME**

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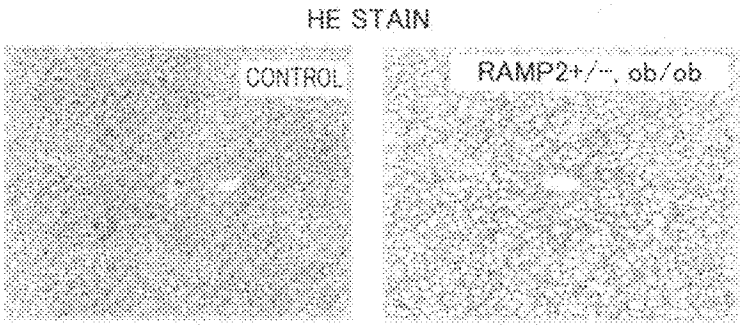
(57) **ABSTRACT**

The object is to provide: a therapeutic or prophylactic agent for metabolic syndrome, which has improved stability in a living body; and others. The therapeutic or prophylactic agent for metabolic syndrome comprises, as an active ingredient, DNA that encodes receptor activity-modifying protein (RAMP) 2 and is selected from the items (a) to (d) below or a polypeptide encoded by the DNA: (a) DNA which has the nucleotide sequence depicted in SEQ ID NO:1; (b) DNA which has a nucleotide sequence capable of hybridizing with the nucleotide sequence depicted in SEQ ID NO:1 under stringent conditions; (c) DNA which has a nucleotide sequence encoding an amino acid sequence having the substitution, deletion and/or addition of one or more amino acid residues in the amino acid sequence depicted in SEQ ID NO:2; and (d) DNA which has a nucleotide sequence having a 90% or more homology with the nucleotide sequence depicted in SEQ ID NO:1.



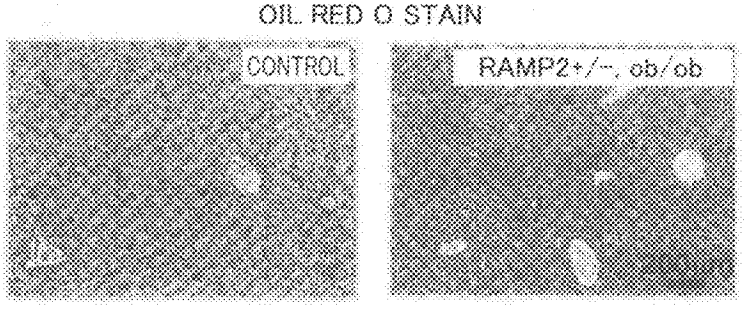


FIG. 1A

HE STAIN

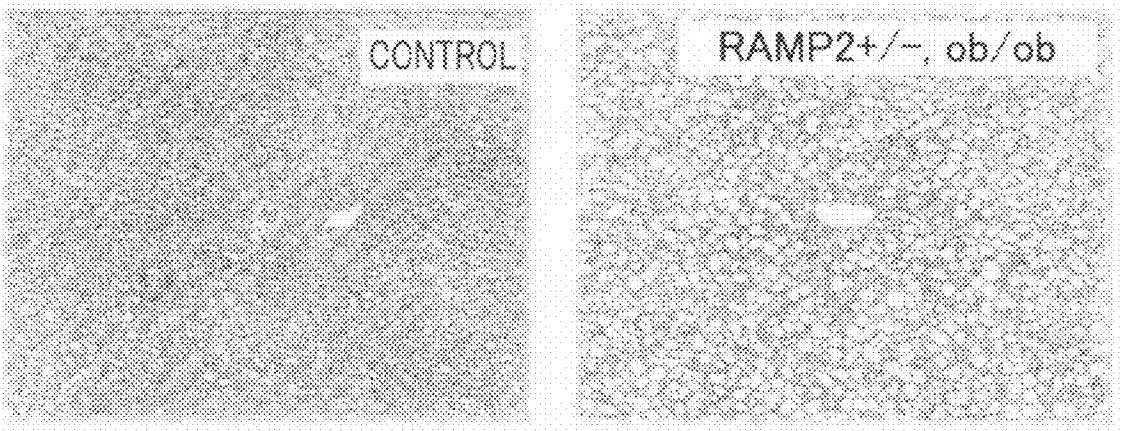


FIG. 1B

OIL RED O STAIN

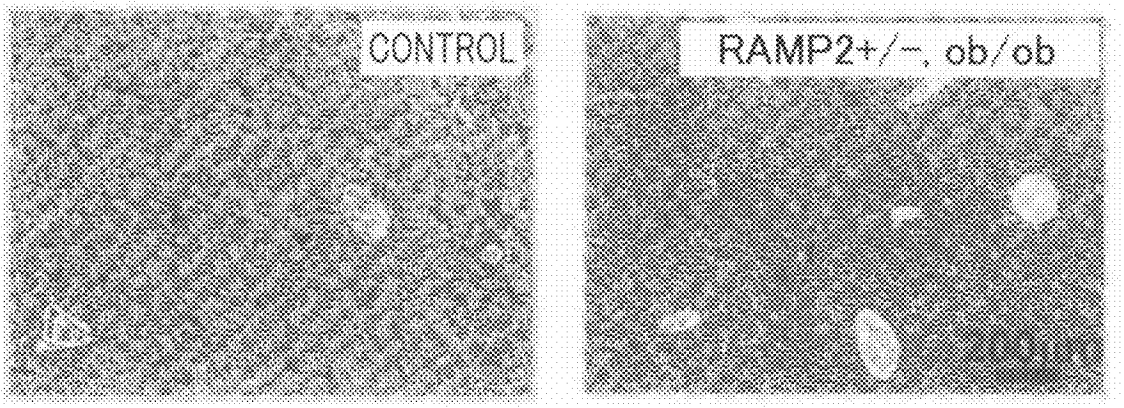


FIG. 2A

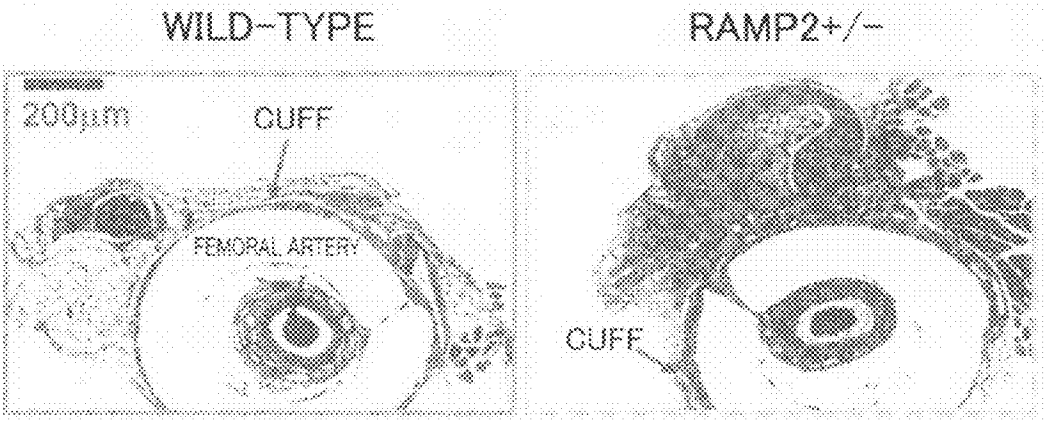

WILD-TYPE
RAMP2+/-
200μm
CUFF
FEMORAL ARTERY
CUFF

FIG. 2B

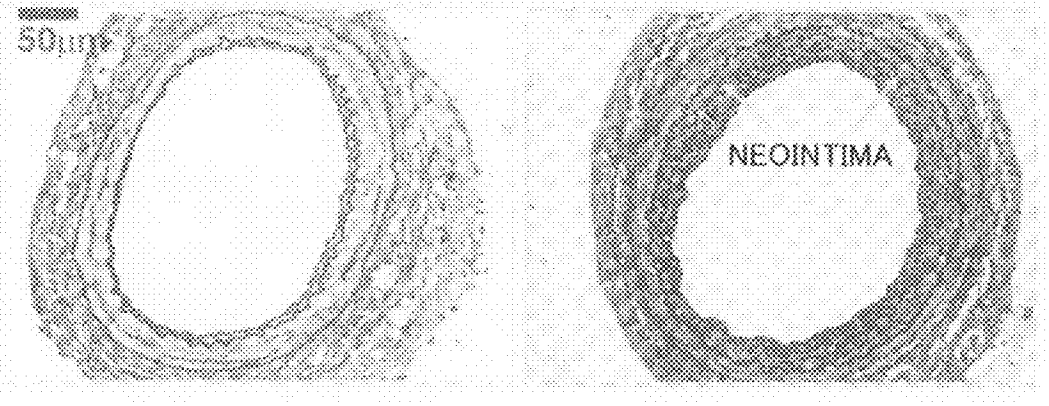

50μm
NEOINTIMA

FIG. 4

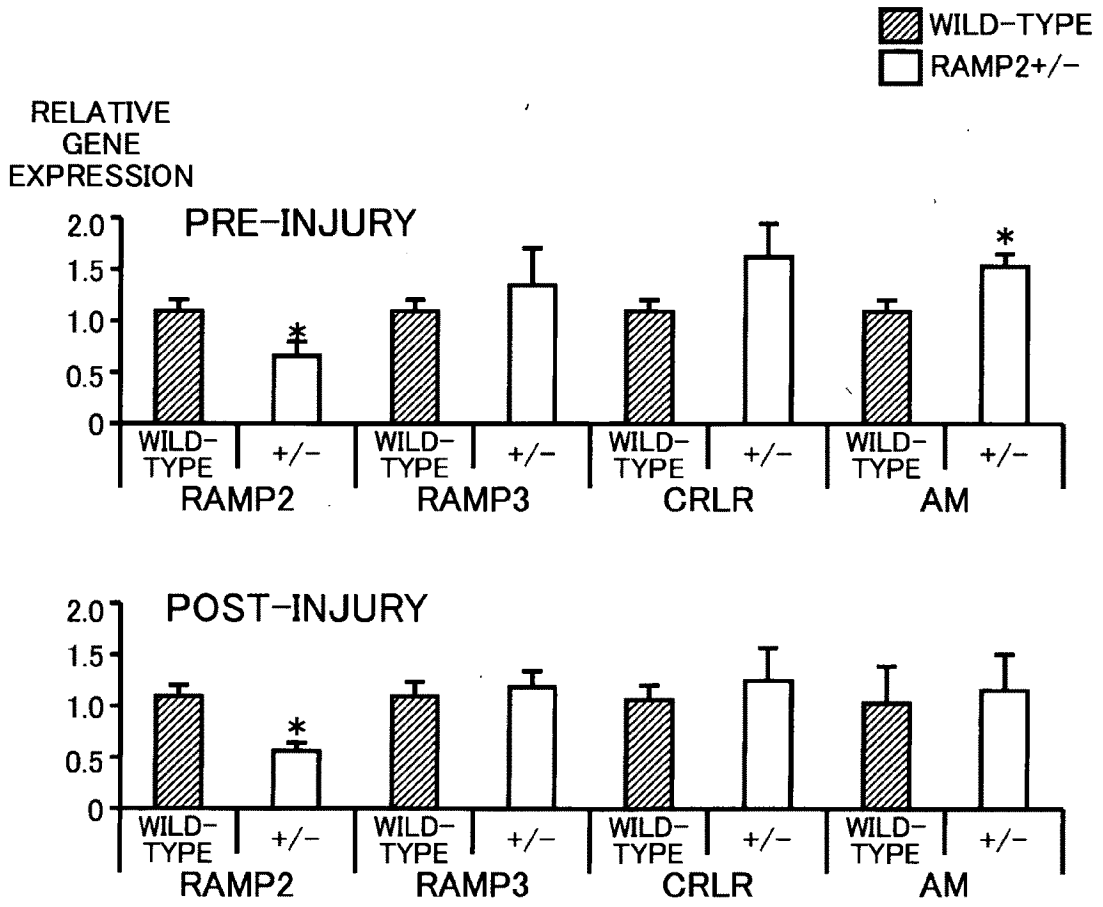

WILD-TYPE
RAMP2+/-
RELATIVE GENE EXPRESSION
PRE-INJURY
2.0
1.5
1.0
0.5
0
*
*
WILD-TYPE
+/-
RAMP2
RAMP3
CRLR
AM
POST-INJURY
*

FIG. 5

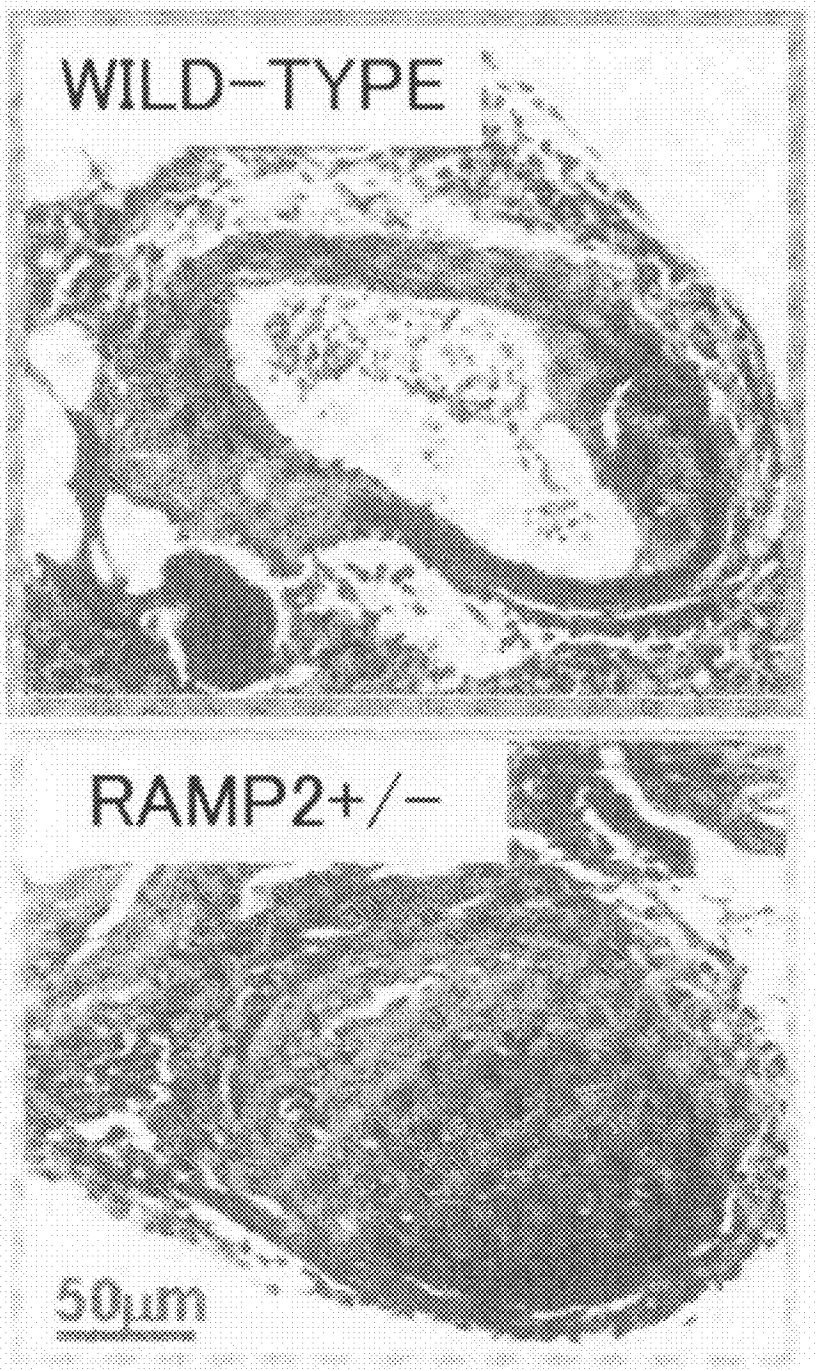


FIG. 6

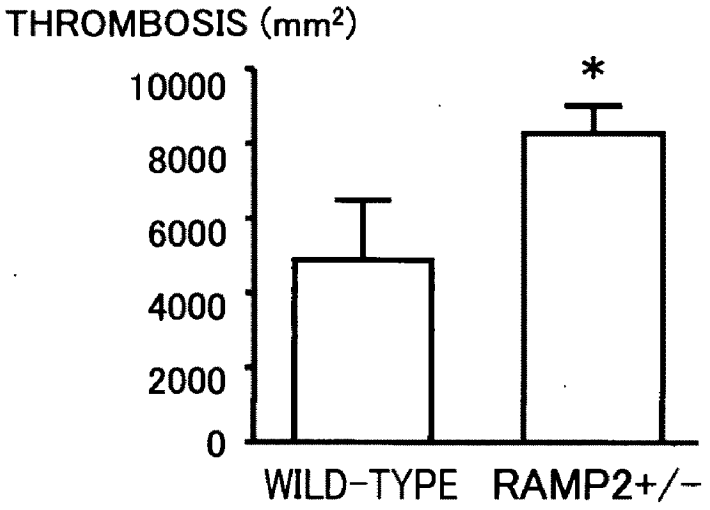

THROMBOSIS (mm2)
10000
8000
6000
4000
2000
0
*
WILD-TYPE
RAMP2+/-

FIG. 7

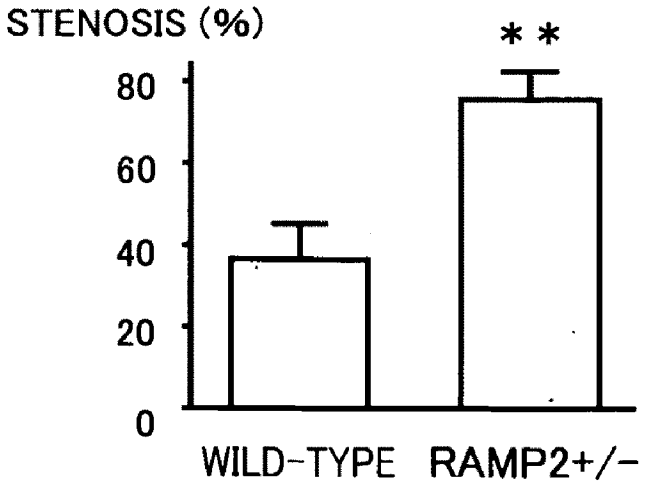

STENOSIS (%)
80
60
40
20
0
* *
WILD-TYPE
RAMP2+/-

FIG. 8

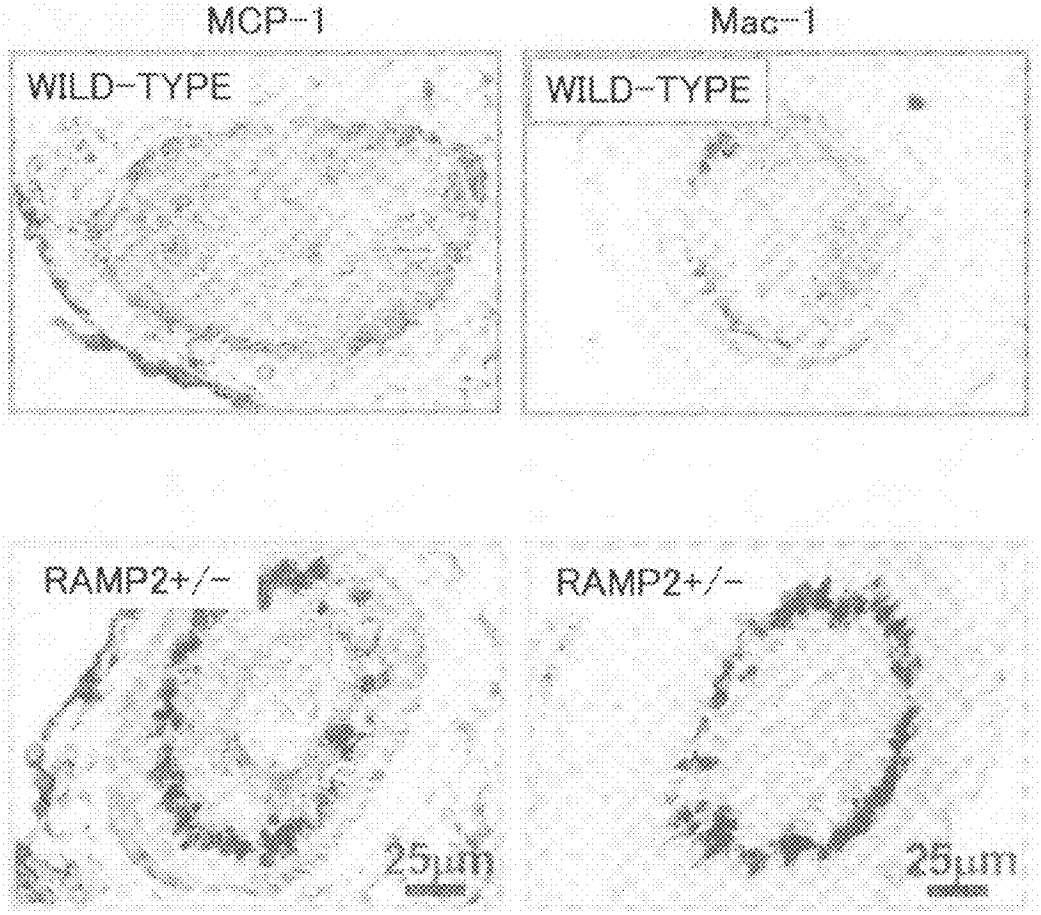


FIG. 9

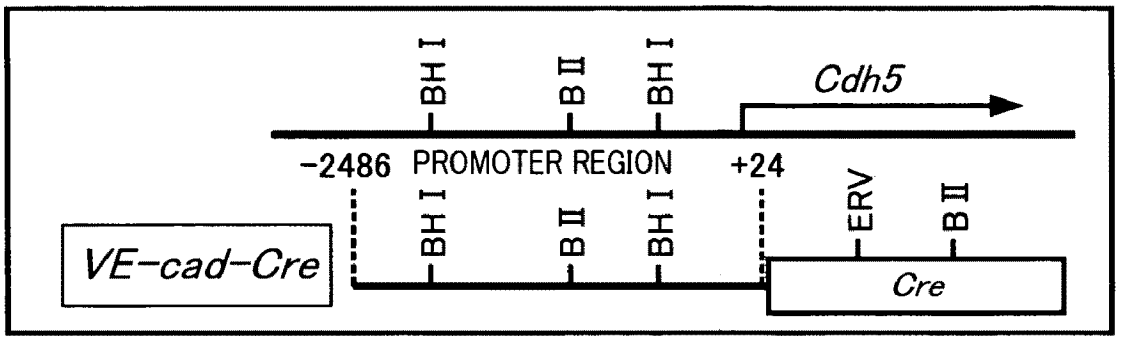

BH I
B II
BH I
Cdh5
-2486
PROMOTER REGION
+24
BH I
B II
BH I
ERV
B II
VE-cad-Cre
Cre

FIG. 10

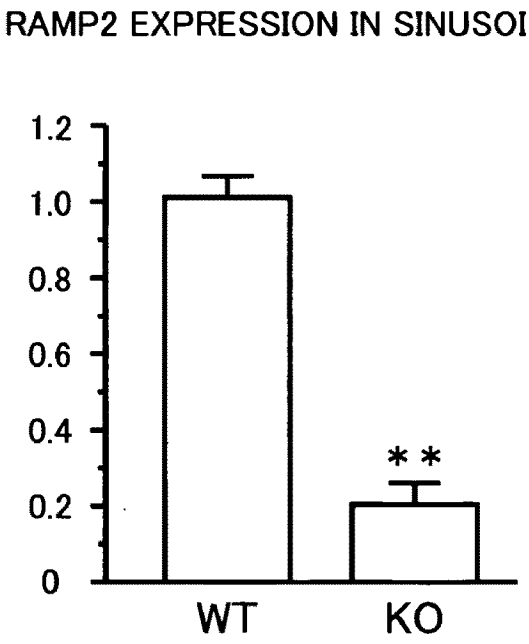

RAMP2 EXPRESSION IN SINUSOIDAL EC
1.2
1.0
0.8
0.6
0.4
0.2
0
**
WT
KO

FIG. 11A

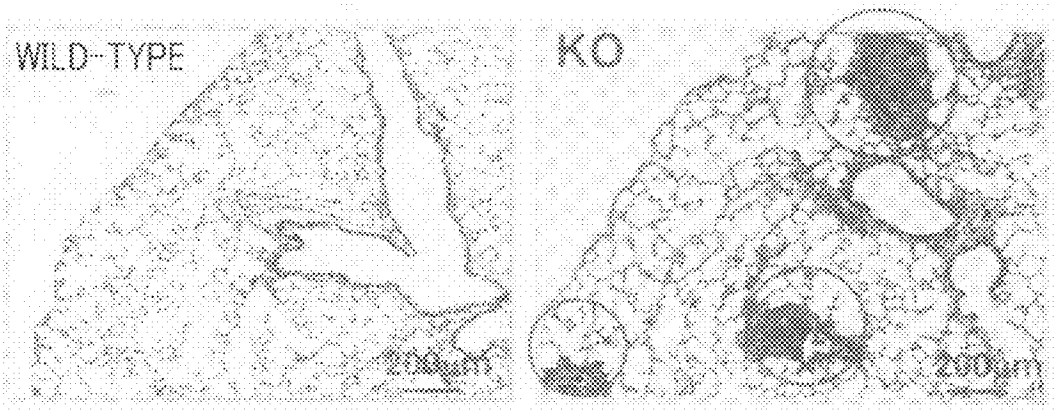


FIG. 11B

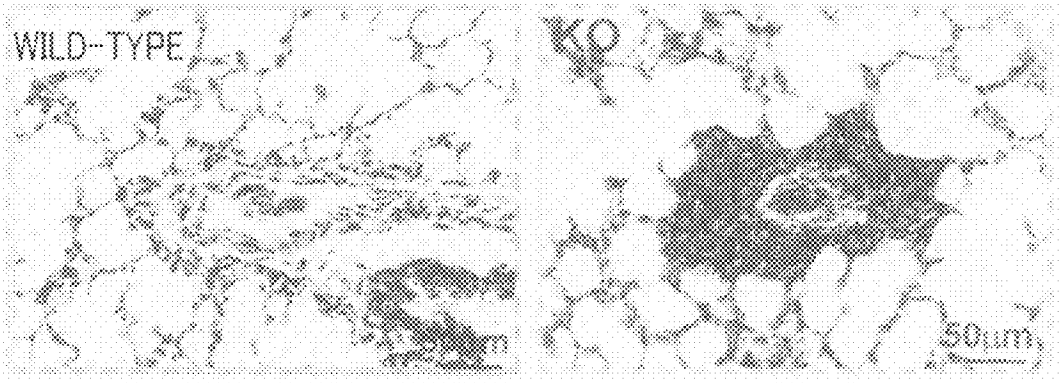


FIG. 12A

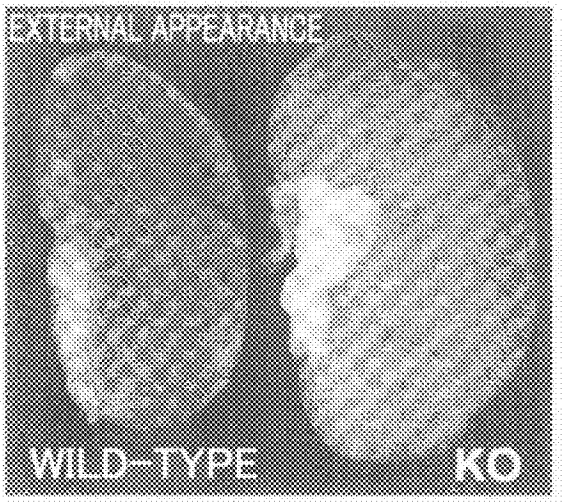


FIG. 12B

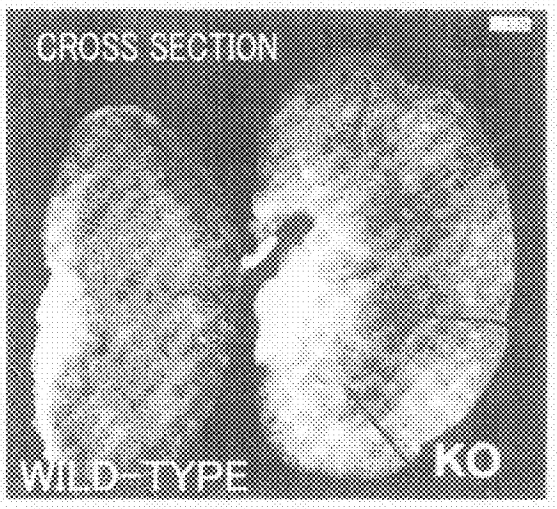


FIG. 12C

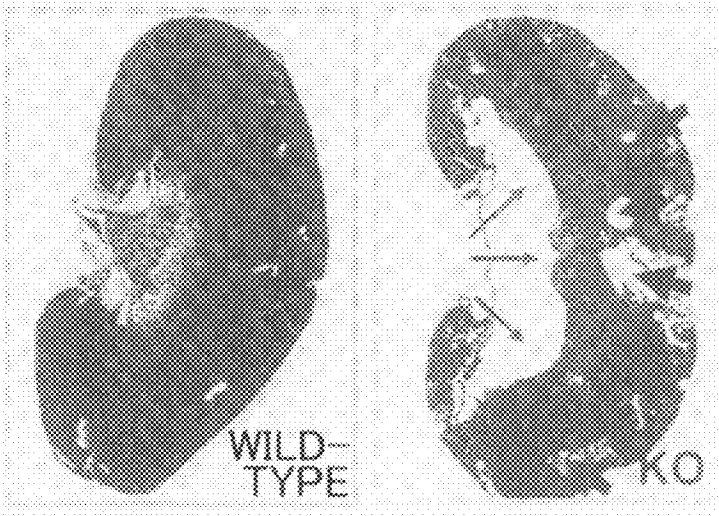


FIG. 13

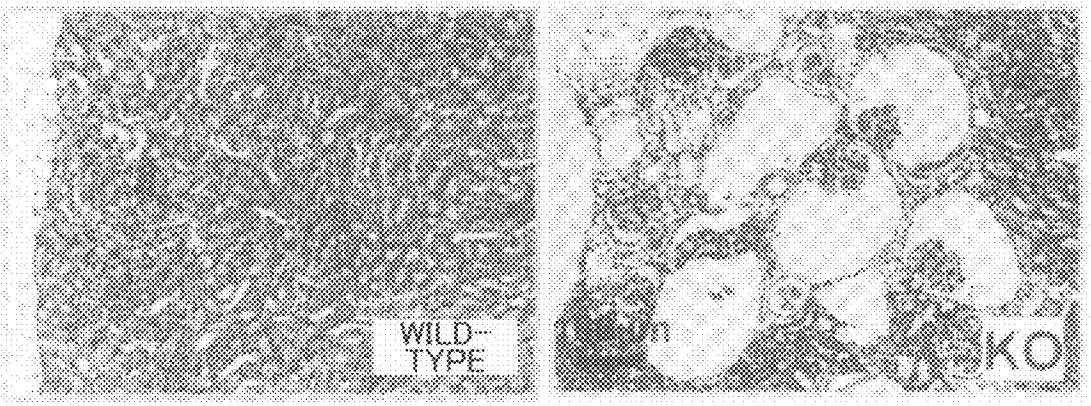


FIG. 14

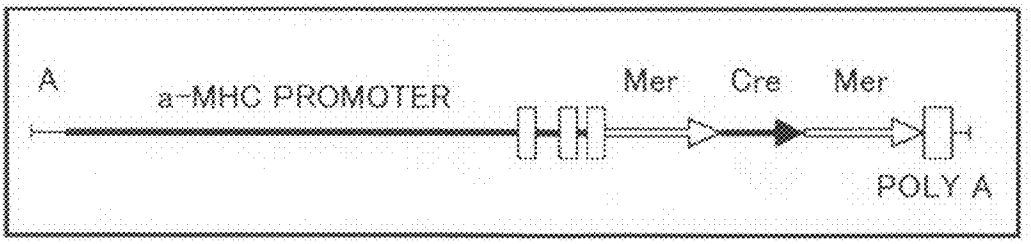


FIG. 15A

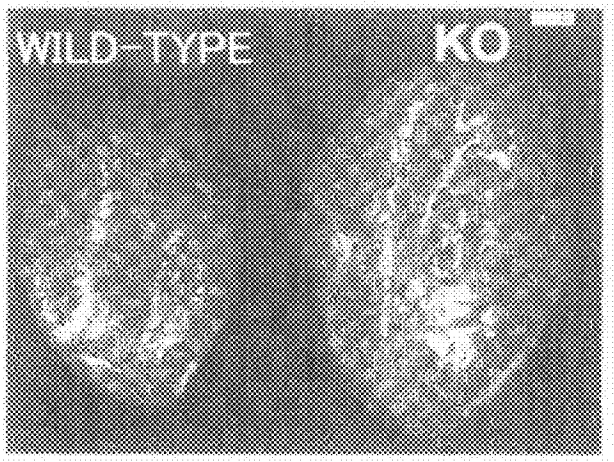


FIG. 15B

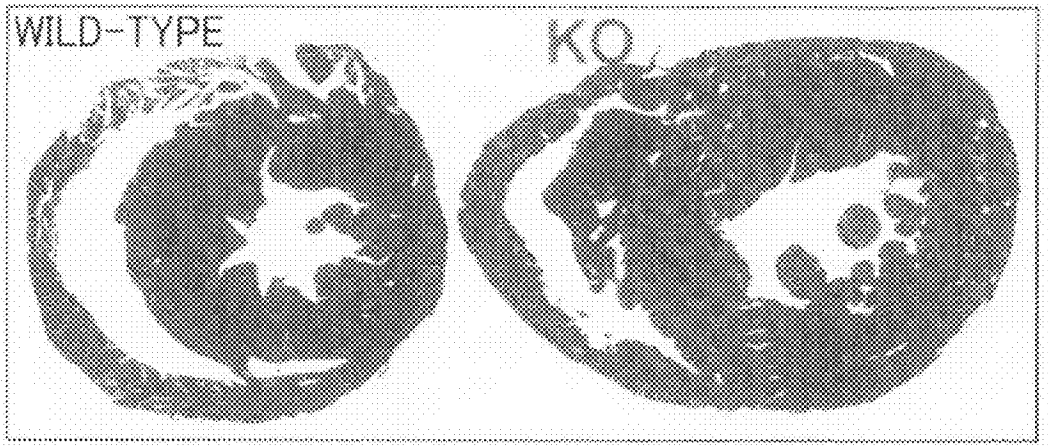
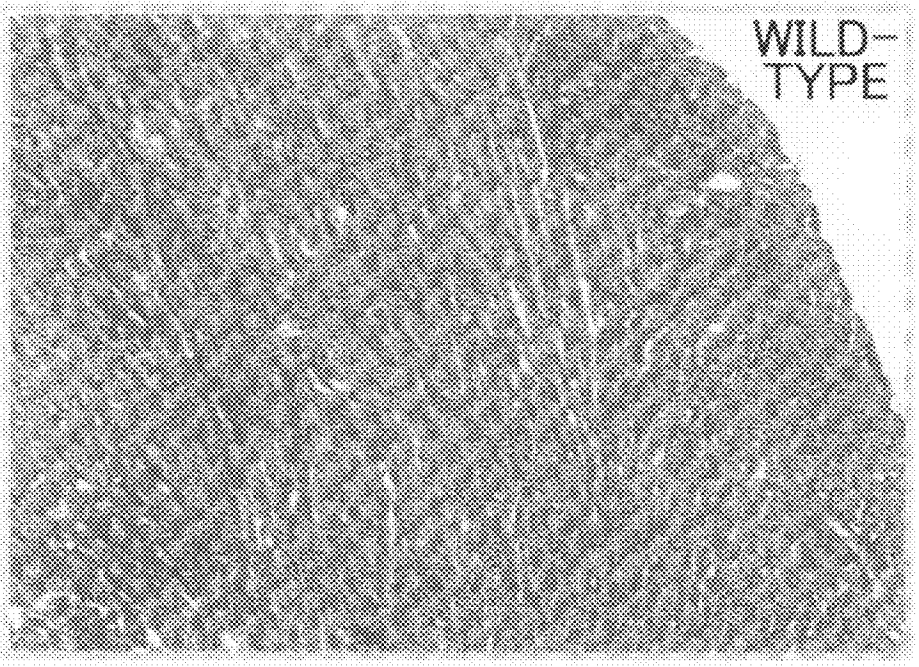


FIG. 16



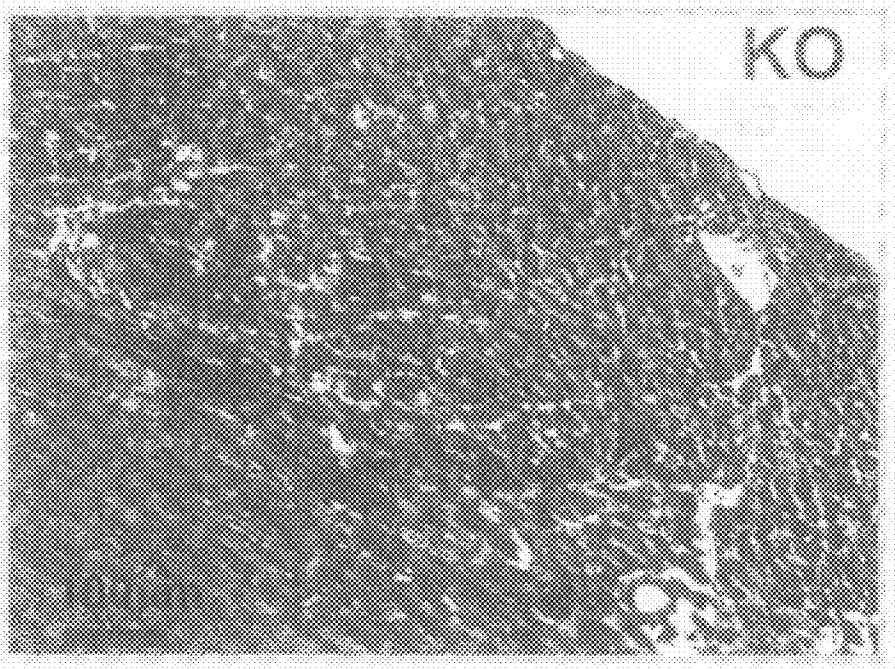
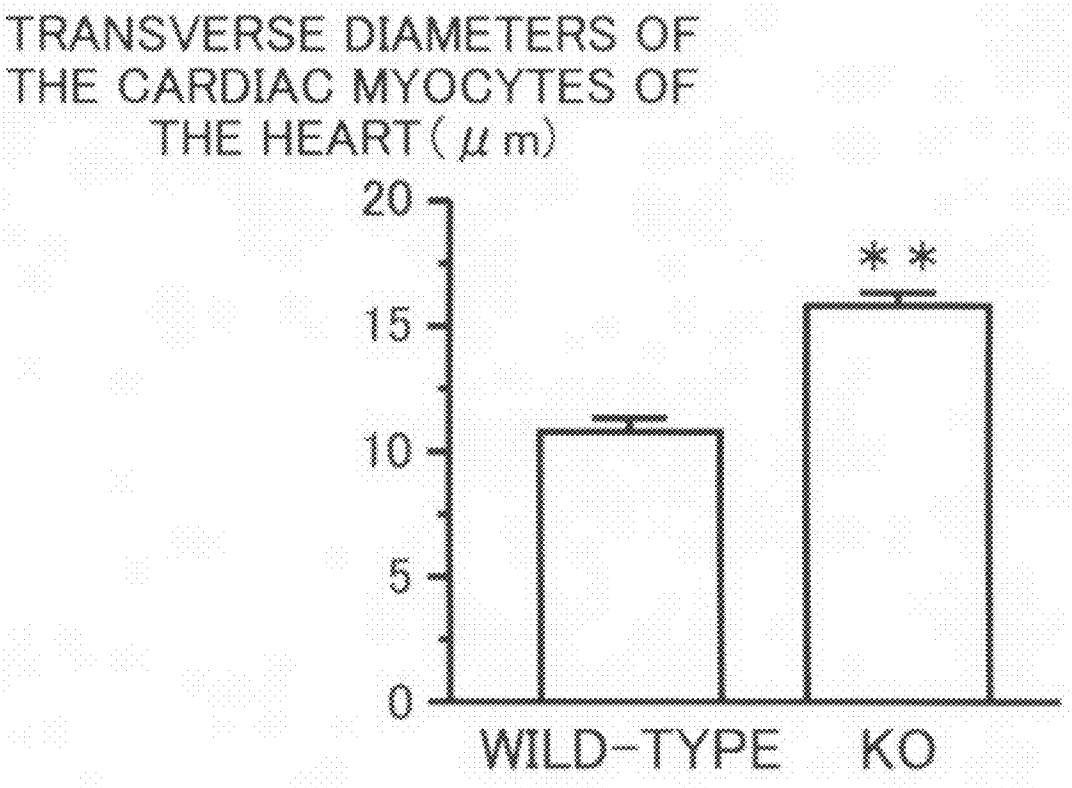


FIG. 17


TRANSVERSE DIAMETERS OF THE CARDIAC MYOCYTES OF THE HEART (μm)
20
15
10
5
0
**
WILD-TYPE
KO

THERAPEUTIC OR PROPHYLACTIC AGENT, DETECTION METHOD AND DETECTION AGENT FOR METABOLIC SYNDROME, AND METHOD FOR SCREENING OF CANDIDATE COMPOUND FOR THERAPEUTIC AGENT FOR METABOLIC SYNDROME

TECHNICAL FIELD

[0001] The present invention relates to a therapeutic or prophylactic agent for metabolic syndrome, a test method, a test drug, and a screening method for candidate compounds for a therapeutic drug for metabolic syndrome.

BACKGROUND ART

[0002] In recent years, patients of metabolic syndrome have increased rapidly especially in advanced countries. Metabolic syndrome is a compound lifestyle disease where visceral fat type obesity (visceral obesity, abdominal obesity), and two or more of hyperglycemia, hypertension, and hyperlipidemia are combined. In accordance with such compound symptoms, a metabolic syndrome can accelerate the progression of heart disease, cerebral stroke or the like, and are the greatest cause of notable deterioration of the healthy lifespan and quality of life of the elderly. Under these circumstances, in order to maintain the vitality of society as a whole and reduce healthcare costs, there is a great social need for overcoming metabolic syndrome.

[0003] As an important molecular basis linking metabolic syndrome with arteriosclerosis, vascular complications, and organ dysfunction, humoral factors having various physiological functions such as adipokines have attracted attention. Humoral factors are produced in various peripheral organs besides the adipose tissue, and these peripheral organs closely cooperate via the humoral factors to maintain homeostasis in vivo, and on the other hand, a failure in the balance of the humoral factors is thought to contribute to arteriosclerosis and organ dysfunction. The present inventors have noted adrenomedullin (AM) and its receptor activity modifying protein (RAMP) system as one of such humoral factors.

[0004] Adrenomedullin (AM) is a peptide produced in the blood vessels and tissues of the whole body. It has become clear that AM has a variety of physiological functions including vasodilation function, fluid volume regulating function, hormone section regulation function, antioxidant function, antiinflammatory function and the like. The present inventors have thus far noted increased blood pressure in heterozygous AM knockout mice, and the aggravation of cardiac hypertrophy, fibrosis, nephropathy and arteriosclerosis, whereas AM-overexpressing mice were, on the contrary, recognized to have lower blood pressure and showed resistance to organ damages and arteriosclerosis; and thus reported that AM has an organ protective function, and antiarteriosclerotic function (refer to Non-Patent Documents 1 to 3).

[0005] Further, the present inventors were the first to clarify that AM is an essential molecule for blood vessel maturation and to stabilize the structure of blood vessels, through the observation that AM knockout mice were lethal at mid-gestation with severe hemorrhage and edema due to insufficient vascular development (refer to Non Patent Document 4). Moreover, it was recently reported that the AM concentration in blood elevates along with obesity and is correlated with the body mass index, and AM is expressed in adipose tissue, and this expression is upregulated with obesity conditions (refer to Non-Patent Documents 5 and 6). On the other hand, in AM knockout mice, with aging, along with obesity, glucose tolerance disorders and the aggravation of in vivo oxidative stress, the development of organ dysfunction was observed, suggesting a relationship between AM and metabolic syndrome.

[0006] [Non-Patent Document 1] T. Shindo et al., Circulation, 2000

[0007] [Non-Patent Document 2] Y. Imai, T. Sindo, et al., ATVB 2002

[0008] [Non-Patent Document 3] P. Niu, T. Shindo, et al., Circulation 2004

[0009] [Non-Patent Document 4] T. Shindo et al., Circulation 2001

[0010] [Non-Patent Document 5] J. Kato et al., Hypertens. Res. 2002

[0011] [Non-Patent Document 6] T. Nambu et al., Regul Pept. 2005

DISCLOSURE OF THE INVENTION

Problems to be Solved by the Invention

[0012] However, because AM has a short half life in blood, it is subject to many constraints for the use as a therapeutic drug for metabolic syndrome which is a chronic malady.

[0013] The present invention was made in consideration of the above circumstances, and has the objective of providing a therapeutic or prophylactic agent for metabolic syndrome for which in vivo stability can be improved. Further, the present invention also has the objective of providing a test method and test drug for metabolic syndrome, and a screening method for candidate compounds for a therapeutic drug for metabolic syndrome.

Means for Solving the Problems

[0014] The present inventors, through a process of carrying out diligent research, attained note of the receptor system of AM. The AM receptor itself is a seven-transmembrane domain receptor called CRLR (calcitonin receptor-like receptor) belonging to the G protein-coupled type receptors, class B. CRLR binds to any of the RAMP (receptor activity modifying protein) 1, 2, and 3 subisoforms which are one-transmembrane domain proteins, and forms a heterodimer.

[0015] The present inventors, by suitably modifying the subisoform of the RAMP binding to the CRLR, discovered that the affinity to AM receptor and ligands can be controlled, thus completing the present invention. Specifically, the present invention provides the following.

[0016] The first aspect of the present invention is a therapeutic or prophylactic agent for metabolic syndrome comprising as an effective component a DNA described in any one of (a) to (d) below, encoding receptor activity modifying 2 (RAMP2) protein, or a polypeptide encoded by the DNA;

[0017] (a) a DNA having a base sequence described in SEQ. ID. No. 1;

[0018] (b) a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1;

[0019] (c) a DNA having a base sequence encoding an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added;

[0020] (d) a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1.

[0021] The second aspect of the present invention is a test method for metabolic syndrome comprising:

[0022] an extraction step of extracting DNA from a cell of a test subject;

[0023] an amplification step of carrying out a polymerase chain reaction using a primer which is capable of selectively amplifying a DNA consisting of the base sequence described in SEQ. ID. No. 3 or part of or all of a DNA of its expression control region, with the extracted DNA as a template;

[0024] a determination step of determinating a base sequence of the amplified DNA; and

[0025] a comparison step of comparing the determined base sequence with the base sequence described in SEQ. ID. No. 3.

[0026] The third aspect of the present invention is a test drug for metabolic syndrome comprising as an effective component a primer which is capable of selectively amplifying a DNA consisting of the base sequence described in SEQ. ID. No. 3 or part of or all of a DNA of its expression control region; or an antibody or antibody fragment which selectively binds to a polypeptide consisting of an amino acid sequence described in SEQ. ID. No. 2.

[0027] The fourth aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

[0028] a step of bringing into contact a test substance and a polypeptide having an amino acid sequence described in SEQ. ID. No. 2, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added; and

[0029] a step of detecting binding between the polypeptide and the test substance.

[0030] The fifth aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome comprising:

[0031] a step of administering a test substance to an animal wherein an endogenous RAMP2 gene has been mutated or knocked out; and

[0032] a step of detecting improvement of symptoms of metabolic syndrome.

[0033] The sixth aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

[0034] a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and

[0035] a step of detecting changes in an expression amount of the DNA.

[0036] The seventh aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

[0037] a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and a step of detecting changes in intracellular localization of a protein synthesized from the DNA.

[0038] The eighth aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

[0039] a step of bringing into coexistence a test substance; a polypeptide having an amino acid sequence described in SEQ. ID. No. 6, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 6, one or a plurality of amino acids are substituted, eliminated, and/or added; and an enzyme which is capable of decomposing this polypeptide;

[0040] a step of measuring a residue of the polypeptide after a predetermined period; and

[0041] a step of comparing the measured value of the residue and a residue measured in an absence of the test substance.

[0042] The ninth aspect of the present invention is a screening method for candidate compounds for a therapeutic drug for metabolic syndrome comprising:

[0043] a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and a DNA having a base sequence described in SEQ. ID. No. 4, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 4, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 4; and

[0044] a step of detecting induction of intracellular signal transmission based on a stimulus of a ligand encoded by a DNA having a base sequence described in SEQ. ID. No. 5, or activation of G protein based on a stimulus of the ligand.

EFFECTS OF THE INVENTION

[0045] According to the present invention, it is possible to effectively treat or prevent metabolic syndrome because RAMP2 or its functional equivalent substance is provided in the body. Moreover, because RAMP2 and its functional equivalent substance are membrane proteins, they have a long half life in the blood, and their stability in the body can be improved.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] FIG. **1**(*a*) is a photograph of HE stain and **1**(*b*) is a photograph of oil red 0 stain of the liver harvested from a RAMP2+/−, ob/ob mouse.

[0047] FIG. **2** (*a*) is a photograph of a slice of the femoral artery and its surroundings, of a RAMP2+/− mouse, and **2**(*b*) an enlargement of a portion of the femoral artery.

[0048] FIG. **3** is a femoral artery sectional photograph of a RAMP2+/− mouse.

[0049] FIG. **4** is a graph showing the expression level of each gene in the femoral artery of a RAMP2+/− mouse.

[0050] FIG. **5** is a different femoral sectional photograph of a RAMP2+/− mouse.

[0051] FIG. **6** is a graph showing the amount of thrombi in the femoral artery of a RAMP2+/− mouse.

[0052] FIG. **7** is a graph showing the amount of vasoconstriction in the femoral artery of a RAMP2+/− mouse.

[0053] FIG. **8** is another femoral sectional photograph of a RAMP2+/− mouse.

[0054] FIG. **9** is an outline diagram of a vector for endothelial cell specific knockout of the RAMP2 gene.

[0055] FIG. **10** is a graph showing the expression level of the RAMP2 gene in cells derived from a vascular endothelial cell-specific RAMP2 knockout mouse.

[0056] FIG. **11**(*a*) is a photograph of a lung section harvested from a vascular endothelial cell-specific RAMP2 knockout mouse, and **11**(*b*) is an enlargement of a portion thereof.

[0057] FIG. **12**(*a*) is an photograph of the external appearance, **12**(*b*) a cross sectional photograph, and **12**(*c*) a section photograph of the kidney harvested from a vascular endothelial cell-specific RAMP2 knockout mouse.

[0058] FIG. **13** is an enlargement of a portion of FIG. **12**(*c*).

[0059] FIG. **14** is a outline diagram of a different vector for cardiac myocyte-specific knockout of the RAMP2 gene.

[0060] FIG. **15**(*a*) is a photograph of the external appearance, and **15**(*b*) a photograph of the section of heart harvested from a cardiac myocyte-specific RAMP2 knockout mouse.

[0061] FIG. **16** is an enlargement of a portion of FIG. **15**(*b*).

[0062] FIG. **17** is a graph showing the transverse diameters of the cardiac myocytes of the heart of FIG. **15**.

PREFERRED MODE FOR CARRYING OUT THE INVENTION

[0063] An embodiment of the present invention is explained below.

Therapeutic Drug•Prophylactic Drug

[0064] The therapeutic or prophylactic drug for metabolic syndrome of the present invention comprises as an effective component a DNA described in any of (a) to (d) below, encoding a receptor activity modifying 2 (RAMP2) protein, or a polypeptide encoded by the DNA.

[0065] (a) a DNA having a base sequence described in SEQ. ID. No. 1;

[0066] (b) a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1;

[0067] (c) a DNA having a base sequence encoding an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added;

[0068] (d) a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1.

[0069] Further, in the present specification, "metabolic syndrome" indicates a condition wherein visceral fat type obesity (visceral obesity, abdominal obesity) and two or more of hyperglycemia, hypertension, hyperlipidemia are combined, and includes complications of arteriosclerosis, ischemic ailments (myocardial infarction, angina pectoris, stroke, arteriosclerosis obliterans), blood vessel failure, organ dysfunction and the like, or is assumed to be one cause of these complications.

[0070] In the present invention "DNA" may be either of the sense strand or the antisense strand (for example, it can be used as a probe), and its form may be either a single strand or a double strand. Further, it may be a genome DNA, it may be a cDNA, or it may be a synthesized DNA.

[0071] The most preferable state of the DNA of the present invention is a DNA having the base sequence described in SEQ. ID. No. 1, but the DNA of the present invention further includes various mutations and homologues having a therapeutic or prophylactic effect for metabolic syndrome. Here, "therapeutic or prophylactic effect for metabolic syndrome" is an effect where at least one type of symptom of visceral fat type obesity (visceral obesity, abdominal obesity), hyperglycemia, hypertension, and hyperlipidemia is improved, or worsening of the symptoms is suppressed, and indicates an effect where the improvement or suppression is statistically significant.

[0072] SEQ. ID. No. 1 is a base sequence encoding the extracellular domain of human RAMP2. Mutants or homologues of the DNA having this base sequence described in SEQ. ID. No. 1, for example, include DNA having a base sequence capable of being hybridized under stringent conditions with the base sequence described in SEQ. ID. No. 1. Herein, as the "stringent conditions", for example, there can be mentioned conditions wherein in a normal hybridization buffer solution, reaction is carried out at 40 to 70° C. (preferably 60 to 65° C.), and washing in a washing solution with a salt concentration of 15 to 300 mM (preferably 15 to 60 mM).

[0073] Further, it is known that SEQ. ID. No. 2 constitutes the extracellular domain of the human RAMP2 protein (Trends in Biochemical Science, Vol. 31, No. 11, pp. 631-638). This also includes DNA having a base sequence encoding an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated and/or added. Herein, "one or a plurality of" is usually 50 amino acids or less, preferably 30 amino acids or less, and more preferably 10 amino acids or less (for example, 5 amino acids or less, 3 amino acids or less, 1 amino acid). In the case of maintaining the ability to activate muscle specific tyrosine kinase, it is desirable that in the mutated amino acid residue, the properties of the amino acid side chain be preserved, and other amino acids be mutated. For example, as the properties of the amino acid side chain, it is possible to mention hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), amino acids having aliphatic side chains (G, A, V, L, I, P), amino acids having hydroxyl group-containing side chains (S, T, Y), amino acids having sulfur atom-containing side chains (C, M), amino acids having carboxylic acid and amide-containing side chains (D, N, E, Q), amino acids having base-containing side chains (R, K, H), and amino acids having aromatic side chains (H, F, Y, W) (the parenthesis all show the one letter designations of amino acids).

[0074] It is known that for a protein having an amino acid sequence modified such that one or a plurality of amino acids are eliminated, added and/or substituted with another amino acid, the biological activity can be maintained (Mark, D. F. et al, Proc. Natl. Acad. Sci. USA (1984) 81, pp. 5662-5666; Zoller, M. J. & Smith, M., Nucleic Acids Research (1982) 10, pp. 6487-6500; Wang A. et al., Science 224 pp. 1431-1433; Dalbadie-McFarland G. et al., Proc. Natl. Acad. Sci. USA (1982) 79, pp. 6409-6413).

[0075] Furthermore, a mutant or homologue of DNA having the base sequence described in SEQ. ID. No. 1 includes

DNA consisting of a base sequence having a high homology with the base sequence described in SEQ. ID. No. 1. Such a DNA has a homology of preferably 90% or more, and more preferably 95% or more (96% or more, 97% or more, 98% or more, 99% or more). The homology of an amino acid sequence or a base sequence can be determined according to the BLAST algorithm by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90: 5873-5877, 1993). Based on this algorithm, the programs called BLASTN and BLASTX have been developed (Altschul et al., J. Mol. Biol. 215: 403-410, 1990). In the case of analysis of a base sequence according to the BLASTN algorithm based on BLAST, the parameters are set to, for example, score=100, wordlength=12. Further, in the case of analysis of an amino acid sequence according to the BLASTX algorithm based on BLAST, the parameters are set to, for example, score=50, wordlength=3. In the case of using the BLAST and Gapped BLAST programs, the default parameters of each program are used. The specific techniques of these analysis methods are publicly known (http://www.ncbi.nlm.nih.gov).

[0076] The method for acquiring the DNA of the present invention is not particularly limited, and a publicly known method method such as a method of obtaining cDNA by reverse transcription from mRNA (for example, the RT-PCR method), method of modification from a genome DNA, method of synthesis by chemical synthesis, and method of isolating from a genome DNA library or cDNA library or the like can be mentioned (for example, refer to Japanese Unexamined Patent Publication No. Hei 11-29599).

Polypeptide

[0077] The polypeptide used as a therapeutic or prophylactic agent in the present invention is encoded by the previously described DNA, and for example, has an amino acid sequence described in SEQ. ID. No. 2, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated and/or added.

[0078] The above described polypeptide, because it does not have all of the transmembrane region or intracellular domain of the human RAMP 2 protein, is expected to be endowed with a high solubility, and can be easily purified by the following method, or the like. Further, the extracellular domain of RAMP2 is understood to be indispensable because it binds to CRLR and forms a complex (data not shown). Because of this, the above described polypeptide forms a complex with CRLR on the cell membrane to which it is administered, and exhibits therapeutic or prophylactic effects for metabolic syndrome.

[0079] The polypeptide encoding the DNA of the present invention can be manufactured using a transformant into which is introduced an expression vector including the above described DNA, for example. Namely, first, this transformant is cultured under appropriate conditions, and a protein (polypeptide) encoding this DNA is synthesized. Then, by retrieving the synthesized protein from the transformant or the culture fluid, the polypeptide of the present invention can be obtained.

[0080] The culture of the transformant, in order to make it possible to obtain the polypeptide in large quantities and easily, is appropriately selected from the publicly known nutrient culture media according to the type and the like of the transformant, and it is possible to carry out appropriate adjustment of the temperature, the pH of the nutrient culture media, culture time and the like (for example, refer to Japanese Unexamined Patent Publication No. Hei 11-29599).

[0081] The isolation method and purification method of the polypeptide are not particularly limited, and a publicly known method such as a method utilizing solubility, a method utilizing differences in molecular weight, a method utilizing electric charge and the like can be mentioned (for example, refer to Japanese Unexamined Patent Publication No. Hei 11-29599). Further, the vector and transformant which can be used in the present invention are explained below.

Vector

[0082] The expression vector can be manufactured by inserting the above described DNA into an appropriate vector. The "appropriate vector" may be a prokaryote or eukaryote capable of reproduction within a host or self-reproduction, and may be appropriately selected in response to the target use. For example, in the case that it is desired to obtain a large amount of DNA, a high copy vector may be selected, and in the case that it is desired to obtain a polypeptide, an expression vector may be selected. Specific examples of these are not particularly limited, and for example, the publicly known vectors described in Japanese Unexamined Patent Publication No. Hei 11-29599 can be mentioned.

Transformant

[0083] The transformant can be manufactured by introducing a vector containing the above described DNA into a host. Such a host may be one which is compatible with the vector of the present invention and is transformed, and specific examples thereof are not particularly limited, but publicly known natural cells such as bacteria, yeast, zooblasts, insect cells and the like, and artificially established cells (refer to Japanese Unexamined Patent Publication No. Hei 11-29599) can be mentioned.

[0084] The insertion method of the vector may be appropriately selected according to the type and the like of the vector and the host. Specific examples thereof are not particularly limited, but publicly known methods such as the protoplast method and competent method (for example, refer to Japanese Unexamined Patent Publication No. Hei 11-29599) can be mentioned.

[0085] The "effective component" in the present specification indicates a component contained in an amount necessary in order to obtain a therapeutic or prophylactic effect for metabolic syndrome, and other components may also be contained so long as the effect is not degraded to below the desired level. Further, the route of administration of the pharmaceutical composition may be either oral or parenteral, and is established as appropriate.

[0086] In the case of oral administration, the pharmaceutical composition may contain additives such as the generally used bonding agents, covering agents, fillers, lubricants, disintegrators, and humectants, and may be formulated in a variety of forms such as pills, granules, subtle granules, powders, capsules and the like. Further, the pharmaceutical composition may also be in a liquid state such as an internal solution, suspension, emulsion syrup and the like, and may be in a dry state which is redissolved upon use.

[0087] In the case of parenteral administration, the pharmaceutical composition may contain additives such as stabilizers, buffers, preservatives, tonicity adjustment agents and the like, and may usually be distributed in a state accommodated

in a unit dose ampule, multiple dose container or a tube. Further, the pharmaceutical composition may be formulated as a powder which is resoluble in a suitable carrier (sterile water or the like) at the time of use.

Test Method, Test Drug

[0088] The above described DNA can be utilized in a test for the presence or absence of an affliction of metabolic syndrome. The test method for metabolic syndrome according to the present invention includes an extraction step, amplification step, determination step and comparison step.

[0089] In the extraction step, DNA is extracted from a cell of the test subject. The extraction technique may follow the usual methods.

[0090] The amplification step carries out a polymerase chain reaction using a primer which is capable of specifically amplifying a DNA consisting of the base sequence described in SEQ. ID. No. 3, or part or all of a DNA of its expression control region, with the extracted DNA as a template. In this way, a DNA having part or all of the SEQ. ID. No. 3 is specifically amplified. Further, SEQ. ID. No. 3 is the full base sequence of the human RAMP2 gene.

[0091] In the determination step, the base sequence of the amplified DNA is determined, and in the comparison step, the determined base sequence is compared with the base sequence of SEQ. ID. No. 3. The determination and comparison of the base sequence may follow the usual methods. As the results of the comparison, it can be judged that if the base sequence of the acquired DNA differ from SEQ. ID. No. 3, the test subject is already afflicted with metabolic syndrome, or could easily become afflicted.

[0092] Further, in the test method of the present invention it is also possible to further apply, for example, a judgment step wherein it is judged whether or not the symptoms relating to metabolic syndrome are improved or their worsening is suppressed, when the DNA amplified in the amplification step is introduced into a cell, tissue, organ or individual where the RAMP2 has been mutated or knocked out. In this way, the test accuracy can be improved because it is possible to exclude cases wherein the differences between the base sequence of the amplified DNA and the base sequence of SEQ. ID. No. 1 are simply polymorphism.

Knockout Body

[0093] By mutating or knocking out the above described DNA, it is possible to manufacture a nonhuman transformed cell, tissue, organ or individual. The nonhuman animal is not particularly limited, but a mouse, rat, guinea pig, hamster, rabbit, goat, pig, dog, cat and the like can be mentioned.

[0094] The manufacturing method of the nonhuman transformed animal is, for example, as follows. First, the DNA, DNA mutation, or DNA homologous recombination of the DNA of the present invention, is introduced into a fertilized egg of a nonhuman mammal. Then, this fertilized egg is transplanted to a female individual's womb and allowed to develop, and in this way the DNA of the present invention can manufacture a transformed nonhuman transformed animal.

[0095] The manufacture of a nonhuman transformed animal can, more specifically, be carried out as follows. First, a female individual made to superovulate by hormone administration is cross bred with a male. Next, a fertilized egg is extracted from the oviduct of the female individual one day after the cross breeding, and a vector including the mutated DNA or DNA which can be homologously recombined with the DNA is introduced by the microinjection method or the like into the fertilized egg. Then, after incubating the fertilized egg after the introduction by an appropriate method, a viable fertilized egg is transplanted to the uterus of a female individual (adoptive parent) made to have a false pregnancy, and a newborn is delivered. It can be confirmed by Southern analysis whether the DNA in this newborn is transformed by extracting DNA from a cell of this newborn.

[0096] In addition, a nonhuman transformed animal may be manufactured by carrying out gene introduction and selection in an embryonic stem cell (ES cell) line, then manufacturing a chimera animal to which the germ cell line is contributed, and cross breeding.

[0097] Another detection method includes a detection step for detecting an expression amount of DNA having the base sequence described in SEQ. ID. No. 1 in a cell derived from the test subject, and a comparison step for comparing the expression amount of the detected DNA and the expression amount of the DNA described in SEQ. ID. No. 1 of a healthy person.

[0098] Herein, the "DNA expression" includes the transcription level (expression of mRNA) and the translation level (expression of protein). Accordingly, the expression amount my be detected by carrying out quantitative RT-PCR using a primer which is capable of specifically amplifying DNA having part of all of the base sequence described in SEQ. ID. No. 1, or detected by carrying out by Western analysis or the like using an antibody or an antibody fragment specifically binding to a polypeptide consisting of the amino acid sequence described in SEQ. ID. No. 2.

[0099] According to these detection methods, in the comparison results, in the case that the expression amount of DNA in the test subject differs significantly from the expression amount of the DNA in a healthy person, it can be judged that the test subject is already afflicted with metabolic syndrome, or could easily become afflicted.

[0100] The test drug for metabolic syndrome according to the present invention contains as an effective component a primer which is capable of specifically amplifying DNA having part or all of the base sequence described in SEQ. ID. No. 1, or the above described antibody or antibody fragment.

Screening Method

[0101] The screening method for candidate compounds for therapeutic drugs for metabolic syndrome according to the present invention includes a step of administering a test substance to an animal where the endogenous RAMP2 gene has been knocked out, and a step for detecting symptom improvement of metabolic syndrome.

[0102] According to this screening method, a test substance for which symptom improvement is detected can complement or substitute the function of RAMP2, and thus it can be specified as a candidate compound for a therapeutic drug for metabolic syndrome.

[0103] Another screening method includes a step of bringing into contact the test substance and a cell expressing a DNA having the base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which can hybridize under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1; and a step of detecting changes in the expression amount of this DNA.

[0104] The test substance for which changes in the expression amount of the DNA described in SEQ. ID. No. 1 are detected is conjectured to be capable of strengthening or decreasing the functioning of RAMP2. Specifically, a substance, which increases the DNA expression level, can strengthen the functioning of RAMP2, and thus is conjectured to be capable of treating metabolic syndrome when administered. On the other hand, a substance which decreases the DNA expression amount is capable of decreasing the functioning of RAMP2, and thus it is conjectured that metabolic syndrome can be treated by administering a substance which inhibits this substance.

[0105] Further, the cell used in this method and in the below described method may be one in which endogenous RAMP2 is expressed, or not expressed. However, a cell which does not express endogenous RAMP2 (for example, COS7) is preferable in the point that it is possible to exclude unclear influence due to the endogenous RAMP2.

[0106] Another screening method includes a step of bringing into contact the test substance and a polypeptide having the amino acid sequence described in SEQ. ID. No. 2, an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added; and a step of detecting binding between the polypeptide and the subject substance.

[0107] According to this screening method, a test substance binding to the polypeptide of SEQ. ID. No. 2 and the like can be obtained. Such a test substance has the possibility of participating in the therapeutic pathway of metabolic syndrome via the polypeptide of SEQ. ID. No. 2 and the like, and is conjectured to be capable of treating metabolic syndrome.

[0108] Another screening method includes a step of bringing into contact the test substance and a cell expressing DNA having the base sequence described in SEQ. ID. No. 1, DNA having a base sequence capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1; and a step of detecting changes in the intracellular localization of a protein synthesized from said DNA.

[0109] According to this screening method, it is possible to obtain a test substance which changes the intracellular localization of a protein synthesized from the SEQ. ID. No. 1 or the like. Here, the RAMP2 is first binded to AM and a receptor, and then is taken into the cell by endocytosis along with the receptor, and if there is no stimulus to the AM from the outside, as long as the cell is alive, it will be stably present on the cell membrane. Further, even if it temporarily taken into the cell temporarily by endocytosis, part of it again returns to the cell membrane and is conjectured to be recycled. Accordingly, the obtained test substance has the possibility of improving the therapeutic effects of metabolic syndrome via RAMP2, and can be anticipated as a therapeutic drug for metabolic syndrome.

[0110] Another screening method includes a step of bringing into coexistence the test substance; a polypeptide having the amino acid sequence described in SEQ. ID. No. 6, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 6, one or a plurality of amino acids are substituted, eliminated, and/or added; and an enzyme capable of decomposing this polypeptide; a step of measuring the residue of the polypeptide after a predetermined period of time; and a step of comparing with a residue measured in the absence of the test substance before the measurement of the residue.

[0111] SEQ. ID. No. 6 is the full amino acid sequence of human adrenomedullin (AM). According to this screening method, it is possible to obtain a substance expected to change the in vivo stability of a polypeptide (AM or the like) having the amino acid sequence described in SEQ. ID. No. 6 or the like. Such a substance has the possibility of improving the therapeutic effects of metabolic syndrome via RAMP2, and may be anticipated as a therapeutic drug of metabolic syndrome. Further, the enzyme used in this method may be specific or non-specific to the above polypeptide, and for example may be endopeptidase.

[0112] Another screening method includes a step of bringing into contact the test substance and a cell expressing the DNA having the base sequence described in SEQ. ID. No. 1, DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and DNA having the base sequence described in SEQ. ID. No. 4, DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 4, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 4; and a step of detecting induction of intracellular signal transmission based on a stimulus of a ligand encoded by the DNA having the base sequence described in SEQ. ID. No. 5, or activation of the G protein based on a stimulus of the ligand.

[0113] SEQ. ID. No. 4 is an amino acid sequence constituting the seven-transmembrane domain receptor called CRLR (calcitonin receptor-like receptor) belonging to the G protein coupled receptors, class B. Further, SEQ. ID. No. 5 is the full base sequence of the human RAMP2 gene.

[0114] According to this screening method, it is possible to obtain a substance which induces intracellular signal transmission based on an AM stimulus, or activates the G protein, in a cell coexpressing RAMP2 and CRLR. Such a substance has the possibility of improving the therapeutic effects of metabolic syndrome by RAMP2, via the induction of intracellular signal transmission, or activation of the G protein, and can be anticipated as a therapeutic drug for metabolic syndrome.

[0115] Further, the induction of intracellular signal transmission can be detected by indicators of changes (especially increases) of the level of intracellular cAMP, Ca, NO, the activity level of PKA, the phosphorylation level of Akt, ERK, P38MAPK, PI3K and the like.

[0116] Another screening method includes the step of specifying the steric constitution of the binding pocket which is formed by a complex of a polypeptide having an amino acid sequence wherein in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated and/or added, and a polypeptide having an amino acid sequence wherein in the amino acid sequence described in SEQ. ID. No. 5, one or a plurality of amino acids are substituted, eliminated and/or added; and a step of classifying on a computer from a previously known structure library a substance predicted to be able to bind via the binding pocket with the complex based on the steric structure.

[0117] According to this screening method, it is possible to greatly narrow down from an enormous number of substances, candidates which are able to bind to a complex of RAMP2 and CRLR. By further limiting the narrowed down candidates using one of the above described screening methods, it is possible to further anticipate as a therapeutic drug for metabolic syndrome one having a high possibility of improving the therapeutic effects of metabolic syndrome by RAMP2. Further, the steps themselves of this in silico screening method may be carried out according to the usual methods.

EXAMPLES

Example 1

Manufacture of a RAMP2 Gene Knockout Mouse

[0118] A targeting vector was manufactured by the following procedure. Namely, from among the genome DNA sequence including RAMP2, as a homologous sequence of the 5' side, a sequence of approximately 3 kb including from the upstream of the exon 1 of RAMP2, to midway of the intron 1 is synthesized by PCR (5' homology arm), and as a homologous sequence of the middle, from midway of the intron 1 to downstream of exon 4 was synthesized by PCR (loxP arm), and as a homologous sequence of the 3' side, a sequence of approximately 3 kb downstream of exon 4 was synthesized by PCR (3' homology arm).

[0119] Next, by subcloning at pBluescript, in order from the 5' side in the direction of the 3' side, the 5' homology arm, loxP, loxP arm, the sequence of the neomycin resistance gene (pGK-neo), loxP, and the 3' homology arm, the targeting vector was manufactured. Namely, exons 2 to 4 of RAMP2 are set to be held between two loxP sites.

[0120] After making the obtained targeting vector linear by restriction enzyme treatment, it was introduced into a mouse ES cell by electroporation. The ES cell clones where recombination occurred were concentrated with neomycin resistance as an indicator, and further, the homolog recombinant ES cells were selected by Southern blotting.

[0121] By microinjecting the selected ES cells into mouse blastocyst, a chimera mouse was manufactured. This chimera mouse was cross bred with a wild type mouse, and a mouse where the loxP site was introduced at the intron 1 and downstream of the exon 4 of the RAMP2 gene in one of the genome sequences (hetero flox mouse) was manufactured.

[0122] By cross breeding the manufactured hetero flox mouse with a mouse which expresses the Cre recombinase gene under a CAG promoter (CAG-Cre mouse), a RAMP2 hetero knockout mouse in which the domain held between the loxP was removed was obtained. By cross breeding these RAMP2 hetero knockout mice among themselves, RAMP2 homo knockout mice were obtained.

Experiment 2: Cross Breeding of Genetically Obese Mice

[0123] By cross breeding the RAMP2 hetero knockout mouse (RAMP2+/−) obtained in Experiment 1 with a leptin deficient (ob/ob) mouse which is a genetically obese mouse, and by back crossing the offspring generation mice (RAMP2+/−, wt/ob) with ob/ob mice, grandchild generation mice (RAMP2+/−, ob/ob) were manufactured.

[0124] The ob/ob mice displayed symptoms of obesity, but the RAMP2+/−, ob/ob mice displayed more severe symptoms of obesity. This result suggests that reduced expression of RAMP2 is a cause of worsening of obesity symptoms.

Experiment 3: Fatty Liver

[0125] Livers were harvested from an ob/ob mouse and RAMP2+/−, ob/ob mouse, and slices thereof were stained. FIG. **1**(*a*) shows a photograph of hematoxylin eosin (HE) staining, and FIG. **1**(*b*) shows a photograph of oil red 0 staining.

[0126] As shown in FIG. **1**(*a*), in a RAMP2+/−, ob/ob mouse, the white flecks of fat drops were observed over a remarkably wider range than in the ob/ob mouse. This result is consistent with the result of FIG. **1**(*b*) where an image of far stronger redness by fat drop staining was obtained in the RAMP2+/−, ob/ob mouse than in the ob/ob mouse. From these results, it is indicated that reduced expression of RAMP2 is a cause of worsened symptoms of fatty liver.

Experiment 3: Arteriosclerosis

Morphology

[0127] Arteriosclerosis was induced by indwelling for four weeks of a cuff of a polyethylene tube surrounding the femoral artery of a RAMP2 hetero knockout mouse (RAMP2+/−) obtained in Experiment 1, and a wild type mouse (RAMP2+/+). FIG. **2**(*a*) shows a photograph at low magnification of a sample slice of the cuff indwelling portion, stained by elastic Van Gieson staining, and FIG. **2**(*b*) shows a magnified photograph of the femoral artery.

[0128] As shown in FIG. **2**(*a*), compared to the wild type mouse, the RAMP2+/− mouse showed enhanced proliferation of smooth muscle cells, infiltration of inflammatory cells, and accumulation of extracellular matrix. Further, as shown in FIG. **2**(*b*), compared to the wild type mouse, the RAMP2+/− mouse was observed to have notable formation of a neointima at the blood vessel lumen. According to the above results, it can be judged that, compared to a wild type mouse, a RAMP2+/− mouse has far worse arteriosclerosis from a morphological viewpoint.

Immunology

[0129] In order to confirm the results of FIG. **2** from the viewpoint of immunology, the expression of the ICAM1, VCAM1, MCP1, and PCNA proteins in the femoral artery of the mouse after the indwelling in Experiment 3 were studied. Specifically, using the antibodies binding to each protein, the femoral artery was immunostained according to the usual methods, and the results are shown in FIGS. **3**(*a*) to (d).

[0130] As shown in FIGS. **3**(*a*) to (*d*), all of ICAM1, VCAM1, MCP1 and PCNA are expressed in notably greater amounts in the RAMP2+/− mouse than in a wild type mouse.

[0131] ICAM1 and VCAM1 are adhesion molecules, which show enhanced expression along with tissue inflammation, and thus suggest that stronger inflammation arises in a RAMP2+/− mouse than in a wild type mouse. This can be supported by the observation that the expression of MCP1, a chemokine, is more enhanced in a RAMP2+/− mouse than in a wild type mouse.

[0132] Further, PCNA is a marker of proliferating cells, thus it was confirmed that the proliferation of smooth muscle cells is more enhanced in the RAMP2+/− mouse than in the wild type mouse.

[0133] FIG. **3**(*f*) is a photograph of a dihydroethidium (DHE)—stained slice of a femoral artery of the mouse after indwelling, and FIG. **3**(*e*) is a photograph of an immunostained slice of the femoral artery by p67phox antibody.

[0134] As shown in FIG. **3**(*f*), the staining of the red color indicates the presence of a superoxide, and in the RAMP2+/− mouse, compared to the wild type mouse, this was observed over a far wider range and more strongly. In this way, it was confirmed that in a RAMP2+/− mouse, the oxidative stress at the pathologically changed portions was more enhanced than in the wild type mouse.

[0135] On the other hand, as shown in FIG. **3**(*e*), p67phox was expressed in a notably greater level in a RAMP2+/− mouse than in a wild type mouse. Based on p67phox being a subunit of NADPH oxidase, which participates in the production of active oxygen, it is suggested that enhanced NADPH oxidase activity is a cause of the enhanced oxidative stress in pathologically changed portions.

[0136] According to the above results, it was judged that in a RAMP2+/− mouse, compared to a wild type mouse, arteriosclerosis is notably worsened also from the viewpoint of immunology.

Molecular Genetics

[0137] In the above described mouse before and after indwelling, the expression of RAMP2, RAMP3, CRLR and AM were studied. First, tissue was harvested from the vicinity of the femoral artery from a mouse before and after indwelling, and with total RNA extracted from this tissue as a template, quantitative real time PCR was carried out using a primer of a sequence specific to each gene. The specific process was according to the usual method. The results are shown in FIG. **4**.

[0138] As shown in FIG. **4**, in a RAMP2+/− mouse, compared to a wild type mouse, the expression of RAMP2 both before and after indwelling is significantly low, while on the other hand the expression of RAMP3 and CRLR were statistically the same. This shows that the result shown by Experiment 3, i.e., the result that arteriosclerosis is further worsened in a RAMP2+/− mouse than in a wild type mouse, is due to the lower expression of RAMP2.

[0139] Further, in the state before the injury, the expression of AM is elevated in a RAMP2+/− mouse. This suggests that, even in an adult, if the expression of RAMP2 is reduced, the expression of AM is increased by positive feedback. In other words, RAMP2 plays a central role in the AM-signaling not only in the blood vessels during embryonic period, but also in the blood vessels of adult.

[0140] It can be considered that the reason why the significant difference between the AM expression in the wild type mouse and the RAMP2+/− mouse vanishes after the injury is because upregulation of AM also occurs in a wild type mouse after the injury. In a RAMP2+/− mouse, regardless of the enhanced expression of AM in the baseline (or, after the injury, regardless of the significant difference of the AM expression vanishes), the severity of arteriosclerosis is enhanced compared with a wild type mouse. It can be considered that the severe arteriosclerosis in a RAMP2+/− mouse is because the expression of RAMP2 is low even if the AM expression is upregulated and antiarteriosclerotic function of AM cannot fully work. This shows that the antiarteriosclerotic function is not sufficiently exerted only by the upregulation of AM, but upregulation of RAMP2 is necessary.

Experiment 4: Blood Vessel Occlusion

Morphology

[0141] The outer circumference of the femoral artery of the RAMP2 hetero knockout mouse (RAMP2+/−) obtained in Experiment 1, and of a wild type mouse (RAMP2+/+) were coated with a 10% iron chloride solution, and a femur portion of each mouse was harvested 24 hours after the coating. Photographs of HE stained, slices thereof are shown in FIG. **5**. Further, a cross section of the thrombus was quantified at transverse slices (n=5) of the harvested femoral arteries. Furthermore, the constriction ratio of the blood vessel was calculated based on the ratio of the cross sectional area of the thrombus and the whole area of the blood vessel lumen. The size of the thrombus and the constriction ratio of the blood vessel are respectively shown in FIG. **6** and FIG. **7**.

[0142] As shown in FIG. **5**, compared to a wild type mouse, in the RAMP2+/− mouse the presence of a large thrombus was observed. This result agrees with the significantly high constriction ratio (FIG. **7**) of the blood vessel and the size of the thrombus (FIG. **6**). From these results, it is suggested that a reduction in the expression of RAMP2 worsens thrombosis, from the morphological viewpoint of accelerating the blood vessel blockage.

Immunology

[0143] The expression of the MCP-1 and Mac-1 proteins in the femoral artery in Experiment 4 was studied. Specifically, the femoral artery was immunostained according to the usual methods, using antibodies binding to each protein, and the results are shown in FIG. **8**.

[0144] As shown in FIG. **8**, in the RAMP2+/− mouse, the MCP-1 and Mac-1 proteins were expressed across a wider range than in the wild type mouse. The MCP-1 and Mac-1 proteins are a chemokine and an adhesion molecule, which show enhanced expression with tissue inflammation, and thus, it is suggested that a stronger inflammation arises at occluded locations in a RAMP2+/− mouse than in a wild type mouse.

Experiment 5: Conditional Gene Targeting

[0145] In order to clarify the pathophysiological significance of the AM-RAMP2 system, a homozygote of a RAMP2 knockout mouse is required, but a homozygote (RAMP2−/−) is embryonic lethal. Thus, conditional gene targeting of the RAMP2 gene was carried out using a Cre-loxP system, and RAMP2 was specifically deleted from each cell type.

Vascular Endothelial Cells

[0146] A flox mouse wherein the RAMP2 gene locus is held between loxP sequences was created according to the usual methods. On the other hand, a Cre mouse was created wherein a promoter sequence of VE-cadherin (Cdh5), which is specifically expressed in vascular endothelial cells, was introduced into a vector (refer to FIG. **9**, and Circulation Research, 2006; 98: 897-904) positioned at the 5' side of the Cre sequence. By cross breeding the flox mouse and the Cre mouse, a mouse where the RAMP2 was knocked out specifically in vascular endothelial cells was obtained.

[0147] Manufactured 8 to 10 week-old adult mice were subjected to abdominal section after anesthesia, and the inside of the liver was perfused with a culture fluid containing collagenase, and isolated sinusoid endothelial cells were cultured. The total RNA extracted from these cells was reverse transcribed to cDNA, and quantitative real time PCR was carried out using a primer having a sequence specific to RAMP2. As a result, as shown in FIG. **10**, it was confirmed that in a blood vessel specific RAMP2 knockout mouse, compared to a wild type mouse, RAMP2 is expressed in far smaller amounts (about 20%).

[0148] Further, the lungs were harvested from a blood vessel specific RAMP2 knockout mouse and a wild type mouse, and photographs of HE stained slices of the lungs are shown in FIG. **11**. As shown in FIG. **11**(*a*) and FIG. **11**(*b*) (an enlargement of a portion of FIG. **11**(*a*)), occurrence of severe inflammation was observed in knockout mouse (the portions surrounded by circles).

[0149] Next, kidneys were harvested from a blood vessel specific RAMP2 knockout mouse and a wild type mouse, and photographs of the external appearance of these kidneys are shown in FIG. **12**(*a*), cross sectional photographs are shown in FIG. **12**(*b*), and photographs of HE stained slices are shown in FIG. **12**(*c*), and enlargements of portions of FIG. **12**(*c*) are respectively shown in FIG. **13**. In the knockout mouse, compared to the wild type mouse, kidney disorders such as overall swelling (refer to FIG. **12**(*a*)), glomerulosclerosis, cyst formation, and hydronephrosis were observed (refer to FIG. **12**(*b*) and (c), and FIG. **13**).

[0150] Based on the results of FIG. **10** to FIG. **13**, it is suggested that a reduction in the expression of RAMP2 can facilitate lesions in various organs.

Cardiac Myocytes

[0151] A Cre mouse was created by introducing a vector wherein a promoter sequence of a-MHC, which is expressed specifically in cardiac myocytes, was positioned at the 5' side of the Cre sequence, and further, a mutant estrogen receptor (Mer) to which tamoxifen binds was positioned at both sides of the Cre (refer to FIG. **14**, Circ. Res. 2001, 89; 20-25). Except for the point of using this Cre mouse, using the same procedure as above, cardiac myocyte-specific RAMP2 knockout mice were obtained.

[0152] The manufactured mice were given an intraabdominal administration of 30 mg/kg/day of tamoxifen, and one week after the start of the administration, the hearts were harvested. FIG. **15**(*a*) shows a photograph of the external appearance of these hearts, and FIG. **15**(*b*) shows a photograph of Masson's trichrome stained slice. Further, FIG. **17** shows the results of measuring the transverse diameter of the cardiac myocytes in a cross sectional tissue slice of the hearts. As shown by FIGS. **15**(*a*) and **15**(*b*), in the cardiac myocyte-specific RAMP2 knockout mice, compared to the wild type mice, it was confirmed that the heart is enlarged, and this heart enlargement accompanies the enlargement of the cardiac myocytes (FIG. **17**). These symptoms are conjectured to be caused by a reduction in the RAMP2 expression.

[0153] An enlargement of a portion of FIG. **15**(*b*) is shown in FIG. **16**. As shown in FIG. **16**, in the cardiac myocyte-specific RAMP2 knockout mice, many fibrotic lesions were observed, on the other hand, such lesions were not observed in the wild type mice. This fibrosis of the heart can be conjectured to be caused by a reduction in the expression of RAMP2.

INDUSTRIAL APPLICABILITY

[0154] From the above results, it is indicated that various physiological actions of AM are regulated by RAMP2, in other words, by targeting RAMP2, it is possible to artificially manipulate the physiological functions of AM, and it is possible to comprehensively treat or prevent metabolic syndrome. Because RAMP2 is a low molecular, one-transmembrane protein, it not only has a long half life in blood and excellent in vivo stability, but also its structural analysis and the search for low molecular compound which can artificially control this system, and the like, are easy. Because of this, RAMP2 is very promising as a therapeutic target molecule.

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Ala Val Leu Asn Pro His Glu Ala Leu Ala Gln Pro Leu Pro Thr Thr
        35                  40                  45

Gly Thr Pro Gly Ser Glu Gly Gly Thr Val Lys Asn Tyr Glu Thr Ala
    50                  55                  60

Val Gln Phe Cys Trp Asn His Tyr Lys Asp Gln Met Asp Pro Ile Glu
65                  70                  75                  80

Lys Asp Trp Cys Asp Trp Ala Met Ile Ser Arg Pro Tyr Ser Thr Leu
                85                  90                  95

Arg Asp Cys Leu Glu His Phe Ala Glu Leu Phe Asp Leu Gly Phe Pro
            100                 105                 110

Asn Pro Leu Ala Glu Arg Ile Ile Phe Glu Thr His Gln Ile His Phe
        115                 120                 125

Ala Asn Cys Ser Leu Val Gln Pro Thr Phe Ser Asp Pro Pro Glu Asp
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Pro Arg Thr Arg Val Gly Arg Pro Ala Ala Val Arg Leu Leu Leu Leu
15                  20                  25                  30

ctg ggc gct gtc ctg aat ccc cac gag gcc ctg gct cag cct ctt ccc      206
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acc aca ggc aca cca ggg tca gaa ggg ggg acg gtg aag aac tat gag      254
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atc gaa aag gat tgg tgc gac tgg gcc atg att agc agg cct tat agc      350
Ile Glu Lys Asp Trp Cys Asp Trp Ala Met Ile Ser Arg Pro Tyr Ser
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Phe Pro Asn Pro Leu Ala Glu Arg Ile Ile Phe Glu Thr His Gln Ile
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cac ttt gcc aac tgc tcc ctg gtg cag ccc acc ttc tct gac ccc cca      494
His Phe Ala Asn Cys Ser Leu Val Gln Pro Thr Phe Ser Asp Pro Pro
            130                 135                 140

gag gat gta ctc ctg gcc atg atc ata gcc ccc atc tgc ctc atc ccc      542
Glu Asp Val Leu Leu Ala Met Ile Ile Ala Pro Ile Cys Leu Ile Pro
        145                 150                 155

ttc ctc atc act ctt gta gta tgg agg agt aaa gac agt gag gcc cag      590
Phe Leu Ile Thr Leu Val Val Trp Arg Ser Lys Asp Ser Glu Ala Gln
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gcc tag ggggcacgag cttctcaaca accatgttac tccacttccc caccccacc       646
Ala
175

aggcctccct cctcccctcc tactcccttt tctcactctc atccccacca cagatccctg    706

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Gln Gln Ala Glu Gly Val Tyr Cys Asn Arg Thr Trp Asp Gly Trp Leu
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Cys Trp Asn Asp Val Ala Ala Gly Thr Glu Ser Met Gln Leu Cys Pro
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gat tac ttt cag gac ttt gat cca tca gaa aaa gtt aca aag atc tgt      820
Asp Tyr Phe Gln Asp Phe Asp Pro Ser Glu Lys Val Thr Lys Ile Cys
90                  95                  100                 105

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Asp Gln Asp Gly Asn Trp Phe Arg His Pro Ala Ser Asn Arg Thr Trp
                110                 115                 120

aca aat tat acc cag tgt aat gtt aac acc cac gag aaa gtg aag act      916
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Phe Ser Asn Ser Glu Ala Leu Arg Ser Ala Ser Tyr Thr Val Ser Thr
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Arg Ser Lys Ile Ser Pro Gln Gly Tyr
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Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
        35                  40                  45

Pro Gln Gly Tyr
    50
```

1. A therapeutic or prophylactic agent for metabolic syndrome comprising as an effective component a DNA described in any one of (a) to (d) below, encoding receptor activity modifying 2 (RAMP2) protein, or a polypeptide encoded by the DNA;

(a) a DNA having a base sequence described in SEQ. ID. No. 1;

(b) a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1;

(c) a DNA having a base sequence encoding an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added;

(d) a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1.

2. A test method for metabolic syndrome comprising:

an extraction step of extracting DNA from a cell of a test subject;

an amplification step of carrying out a polymerase chain reaction using a primer which is capable of selectively amplifying a DNA consisting of the base sequence described in SEQ. ID. No. 3 or part of or all of a DNA of its expression control region, with the extracted DNA as a template;

a determination step of determinating a base sequence of the amplified DNA; and

a comparison step of comparing the determined base sequence with the base sequence described in SEQ. ID. No. 3.

3. A test drug for metabolic syndrome comprising as an effective component a primer which is capable of selectively amplifying a DNA consisting of the base sequence described in SEQ. ID. No. 3 or part of or all of a DNA of its expression control region; or an antibody or antibody fragment which selectively binds to a polypeptide consisting of an amino acid sequence described in SEQ. ID. No. 2.

4. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

a step of bringing into contact a test substance and a polypeptide having an amino acid sequence described in SEQ. ID. No. 2, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 2, one or a plurality of amino acids are substituted, eliminated, and/or added; and

a step of detecting binding between the polypeptide and the test substance.

5. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome comprising:

a step of administering a test substance to an animal wherein an endogenous RAMP2 gene has been mutated or knocked out; and

a step of detecting improvement of symptoms of metabolic syndrome.

6. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and

a step of detecting changes in an expression amount of the DNA.

7. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and

a step of detecting changes in intracellular localization of a protein synthesized from the DNA.

8. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome, comprising:

a step of bringing into coexistence a test substance; a polypeptide having an amino acid sequence described in SEQ. ID. No. 6, or an amino acid sequence wherein, in the amino acid sequence described in SEQ. ID. No. 6, one or a plurality of amino acids are substituted, eliminated, and/or added; and

an enzyme which is capable of decomposing this polypeptide;

a step of measuring a residue of the polypeptide after a predetermined period; and

a step of comparing the measured value of the residue and a residue measured in an absence of the test substance.

9. A screening method for candidate compounds for a therapeutic drug for metabolic syndrome comprising:

a step of bringing into contact a test substance and a cell expressing a DNA having a base sequence described in

SEQ. ID. No. 1, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 1, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 1, and a DNA having a base sequence described in SEQ. ID. No. 4, a DNA having a base sequence which is capable of hybridizing under stringent conditions with the base sequence described in SEQ. ID. No. 4, or a DNA consisting of a base sequence having a homology of 90% or more with the base sequence described in SEQ. ID. No. 4; and

a step of detecting induction of intracellular signal transmission based on a stimulus of a ligand encoded by a DNA having a base sequence described in SEQ. ID. No. 5, or activation of G protein based on a stimulus of the ligand.

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