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(54) **CONSTRUCT COMPRISING RECOGNITION DOMAIN OF ANTIBODY AGAINST VON WILLEBRAND FACTOR-SPECIFIC CLEAVING ENZYME**

(57) The present invention provides an epitope recognized by an antibody (hereinafter, also referred to as an anti-ADAMTS-13 antibody) against a cleaving protease (hereinafter, also referred to as ADAMTS-13) specific to von Willebrand factor (hereinafter, also referred to as vWF), and a polypeptide comprising the epitope region. The present invention also provides a polypeptide located in a region from position 449 to position 687 in an amino acid sequence composing the ADAMTS-13, which is recognized by the anti-ADAMTS-13 antibody, or a peptide fragment derived from the polypeptide.

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Description

Technical Field

[0001] The present invention relates to the field of ethical drugs. Specifically, the present invention relates to an epitope recognized by an antibody (hereinafter, also referred to as an anti-ADAMTS-13 antibody) against cleaving protease (hereinafter, also referred to as ADAMTS-13) specific to von Willebrand factor (hereinafter, also referred to as vWF) involved in blood coagulation, and to a polypeptide comprising the epitope region. The present invention is also relates to an antibody that recognizes the polypeptide.

[0002] The polypeptide or a peptide fragment thereof provided by the present invention, which comprises an epitope region recognized by an antibody against ADAMTS-13 opens up the possibility of diagnosis given on the presence or absence of an autoantibody against ADAMTS-13 or an absorbing agent for the autoantibody or ADAMTS-13 replacement therapy for a patient with diseases associated with positivity for the autoantibody.

Background Art

[0003] vWF is a blood coagulation factor that is produced in a vascular endothelial cell and a megakaryocyte and is present as a multimer structure (with a molecular weight of 500 to 20,000 kDa) where single subunits each composed of 2050 amino acid residues (approximately 250-kDa monomers) are bound through a S-S bond. The concentration of vWF in blood is approximately 10 μg/ml, and in general, vWF with a higher molecular weight has higher specific activity.

[0004] The vWF has two major functions as a blood coagulation factor, one of which is a function as a carrier protein that binds to and thereby stabilizes blood coagulation factor VIII and another of which is a function of helping platelets adhere and aggregate to the tissue beneath vascular endothelial cells of injured vascular walls to form platelet thrombi.

[0005] Thrombotic thrombocytopenic purpura (hereinafter, also referred to as TTP) is a disease that causes platelet thrombi in body tissue arterioles and capillary vessels in the whole body. In spite of the current progression of medical technology, the mortality associated with the disease has increased approximately threefold from 1971 to 1991. Pathologically, the TTP is considered to be caused by vascular endothelia cell injury and platelet aggregation in blood vessels. Immunohistologically, the presence of vWF in large amounts is observed in generated platelet thrombi, and the vWF is considered to play a crucial role in the pathogenesis of the disease. TTP is broadly divided into familial (congenital) TTP likely to have an inheritance factor and acquired (idiopathic) TTP developed especially in adults. Normal or high-molecular-weight vWF multimer structures are dominant in TTP patients. Especially, unusually large vWF multimer (ULvWFM) and large vWF multimer (LvWFM) are presumed to play a crucial role in the promotion of platelet aggregation and microthrombus formation under high shearing stress. On the other hand, vWF has been known to undergo digestion at the location between 842Tyr and 843Met by the action of vWF-cleaving protease under high shearing stress in circulating blood of healthy individuals. Thus, a probable scenario of how TTP is caused is as follows: the activity of the protease in plasma is reduced for some reason and ULvWFM or LvWFM is increased to accelerate platelet aggregation, followed by platelet thrombus formation in the blood vessel.

[0006] In 2001, a gene encoding vWF-cleaving protease also known as ADAMTS-13 that is an active body having the activity of the protease described above was cloned by the present inventors (JP Patent Publication (Kokai) No. 2003-284570). The findings about the molecular structure of ADAMTS-13 are summarized below. The location of a residue number that is numbered from methionine encoded by an initiation codon (ATG) is shown as a rough guide within parentheses (see SEQ ID No. 1).

[0007] The domain structure of ADAMTS-13 has a signal peptide preceding a propeptide that ends in a RQRR sequence as a furin cleavage motif, followed by a metalloprotease domain containing a reprolysin-type zinc-chelating region consisting of HEXXHXXGXXHD as a consensus sequence (to amino acid residue No. 284 (p285X)); via a disintegrin-like domain as found in snake venom metalloprotease (to amino acid residue No. 386 (W387X)), there exists a first Tspl motif (Tspl-1) consisting of approximately 50 to 60 residues generally considered to be important for molecular recognition (to amino acid residue No. 448 (Q449X)), which continues to a Cys-rich region containing a RGDS sequence as one of cell adhesion motifs (to amino acid residue No. 580 (T581X)); and, through a spacer domain consisting of approximately 130 amino acid residues with no cysteine residue (to amino acid residue No. 687 (W688X)), additional Tspl motif repeats (Tspl-2 to Tspl-8) follow, which is followed by a CUB domain-1, and -2 that are said to be first found in a complement component Clr or CLs.

[0008] By the way, no finding about a major neutralizing epitope region in ADAMTS-13 has been obtained so far. In addition, a convenient diagnostic method for a patient positive for an autoantibody against the protease has not been established.

[0009] In light of such circumstances, a first object of the present invention is directed to an invention relating to the identification of a neutralizing epitope present on ADAMTS-13 and a neutralizing/absorbing agent for an antibody

thereby proposed, which is mainly intended for an autoantibody.

[0010] A second object of the present invention is to provide a method of producing the neutralizing/absorbing agent.

[0011] A third object of the present invention is to provide an application of the neutralizing/absorbing agent.

[0012] A fourth object of the present invention is to provide a method of producing a full-length or partially modified molecule of vWF-specific cleaving protease, which is obtained by modifying the epitope.

[0013] A fifth object of the present invention is to provide an application of the full-length or partially modified molecule of vWF-specific cleaving protease, which is obtained by modifying the epitope.

[0014] Up to now, plasmapheresis has been provided as treatment to a patient congenitally deficient in vWF-specific cleaving protease and a patient with acquired production of an antibody against the protease. Therefore, there is a demand for the establishment of replacement therapy with pure vWF-specific cleaving protease such as the protease purified or genetically altered. It has been reported that a familial TTP patient is congenitally deficient in vWF-specific cleaving protease and non-familial TTP is caused by the acquired production of an autoantibody against the protease. Thus, replacement therapy with the protease is preferred for a familial TTP patient (in reality, plasma administration is given to the patient), while a non-familial TTP patient requires the removal of an autoantibody by plasma exchange as well as the supplementation of the protease.

[0015] However, in the administration of ADAMTS-13 for supplementation to a patient positive for an autoantibody, an antibody against the protease, that is, an autoantibody, present in blood of the patient neutralizes the administered protease. As a result, the protease loses enzyme activity and has substantial reduction in concentration. However, the use of a neutralizing region identified by a method of determining an epitope for an antibody against ADAMTS-13 disclosed in a previous application (JP Patent Application No. 2002-279924) or identified by the present invention as well as the preparation of a partially modified molecule of a neutralizing epitope region that can be newly identified by Western blotting of competitive inhibition assay used in the present invention allows the administration of the protease to a patient positive for an antibody against the protease; or alternatively, allows the absorption of the antibody by a polypeptide or the like containing the neutralizing region provided by the present invention.

Disclosure of the Invention

[0016] As a result of conducting diligent studies for attaining the isolation and identification of vWF-cleaving protease under the above-described circumstances, the present inventors successfully purified and isolated heretofore unreported vWF-cleaving protease of interest and identified an amino acid sequence of a mature protein thereof and a gene encoding the amino acid sequence in the previous application (JP Patent Publication (Kokai) No. 2003-284570).

[0017] Based on the findings obtained using genetic recombination techniques described in the previous application (JP Patent Publication (Kokai) No. 2003-284570), the present inventors identified a region likely to be essential for activity expression (JP Patent Application No. 2002-279924). From the results of using a mutant molecule prepared on the basis of these findings to analyze a major neutralizing region recognized by an autoantibody against anti-ADAMTS-13 in an acquired TTP patient in the present invention, it has been revealed that the region recognized by the autoantibody is in agreement with the above-described region likely to be essential for activity expression and is located in a region from a Cys-rich region (at approximately position 499) to a spacer region (at approximately position 687). Accordingly, a principal requirement of a major neutralizing epitope region provided by the present invention for an anti-ADAMTS-13 antibody is the region from the Cys-rich region (at approximately position 499) to the spacer region (at approximately position 687) in a polypeptide composing ADAMTS-13 or a peptide fragment having an equivalent amino acid sequence. That is, the present invention relates to a polypeptide comprising a neutralizing epitope region in von Willebrand factor-specific cleaving protease (hereinafter, also referred to as vWFCP or ADAMTS-13), which is recognized by an antibody against the protease, or a peptide fragment derived from the polypeptide. The neutralizing epitope region in the polypeptide or the peptide fragment derived from the polypeptide that is claimed in claim 1 is located in a region from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1. The present invention is further intended to a polypeptide comprising an amino acid sequence from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1, or a peptide fragment derived from the polypeptide. The present invention is also intended to a polypeptide comprising an amino acid sequence from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1 where one or several amino acids are deleted, substituted, or added, the polypeptide being recognized by an antibody against von Willebrand factor-specific cleaving protease, or a peptide fragment derived from the polypeptide. One or several amino acids used herein refers to one to five amino acids, preferably one to three amino acids, more preferably one or two amino acids.

[0018] Using, as an antigen, a polypeptide or the like of the neutralizing epitope region, which is prepared on the basis of the amino acid sequence of ADAMTS-13 obtained by this finding, for example, monoclonal and polyclonal antibodies can be created by a typical immunization method (Current Protocols in Molecular Biology, edited by F.M. Ausbel et al., (1987); Antibody Engineering: A PRACTICAL APPROACH, edited by J. McCAFFERTY et al., (1996); Antibodies: A Laboratory Manual, edited by Harlow David Lane (1988); or ANTIBODY ENGINEERING second edition,

edited by Carl A.K. BORREBAECK (1995)). Alternatively, an antibody binding to the protein (ADAMTS-13) can be created by a technique of creating an antibody by use of a phage display technique (Phage Display of Peptides and Proteins: A Laboratory Manual, edited by Brian K. Kay et al., (1996); Antibody Engineering: A PRACTICAL APPROACH, edited by J. McCAFFERTY et al., (1996); or ANTIBODY ENGINEERING second edition, edited by Carl A.K. BORREBAECK (1995)). Based on these techniques, a neutralizing antibody for the activity of the protease of the present invention or an antibody simply binding to the protease can also be isolated from a sample from a TTP patient positive for an autoantibody against the protease of the present invention. In addition, the use of these antibodies allows the application of the present invention to diagnosis and treatment for a disease associated with variations in the amount of the protease of the present invention, for example, TTP. The present invention also encompasses these antibodies.

[0019] In one embodiment, the present invention relates to a method of diagnosing a patient with a TTP-like disease or a patient at risk of developing vWF-dependent thrombosis, and the method comprises steps below.

[0020] Diagnostic assay for a disease associated with variations in the amount of the protease of the present invention is performed using a biological sample from the patient. Such a sample can be used directly in the assay or, in some cases, can need to be subjected prior to the assay to treatment such as the removal of possible interfering substances in the sample. Examples of suitable biological samples include blood, urine, sweat, tissue, or serum. The method involves detecting an autoantibody against vWF-cleaving protease in the biological sample. The steps of the method are as follows:

(a) bringing a biological sample obtained from the patient into contact with a solid support in which ADAMTS-13 or a partial peptide fragment thereof is immobilized;
(b) bringing the solid support into contact with an anti-human immunoglobulin antibody labeled with a developer; and
(c) detecting the label of the specifically bound developer in the step (b) in order to obtain a value corresponding to the concentration of an anti-ADAMTS-13 antibody in the sample.

[0021] The above-described diagnosis can be conducted by immunoassay known in the art. Solid supports that can be used include beads and plates made of a resin such as polystyrene. Developers that can be used include radioisotopes, enzymes such as peroxidase and alkaline phosphatase, and fluorescent substances.

[0022] In an alternative embodiment of the present invention, the polypeptide of the present invention is also useful as a neutralizing agent for an autoantibody by administering the polypeptide to a patient positive for an anti-ADAMTS-13 antibody or as a removing agent for an autoantibody. In this case, the neutralization of the autoantibody refers to binding to the autoantibody, thereby inhibiting the binding of the autoantibody to vWF-cleaving protease. In this method, the polypeptide is optionally immobilized in a suitable support or the like using a method known in the art. Subsequently, a sample containing an anti-ADAMTS-13 antibody to be removed, for example, blood from the patient is brought into contact with the immobilized polypeptide to thereby remove the autoantibody from the sample from the patient. On this occasion, a carrier bound with a ligand specific to the anti-ADAMTS-13 antibody is brought into contact with blood or plasma from the patient and the anti-ADAMTS-13 antibody in the blood or the plasma is bound to the ligand to thereby remove the antibody from the blood or the plasma. Subsequently, the blood or the plasma from which the antibody have been removed may be reinjected to the patient. The above-described polypeptide or peptide fragment derived from the polypeptide can be used as the ligand specific to the anti-ADAMTS-13 antibody. The contact may be performed, for example, by allowing the blood or plasma from the patient to pass through the carrier bound with the ligand. The present invention further encompasses a method of producing blood or plasma free from an anti-ADAMTS 13 antibody by bringing a carrier bound with the above-described polypeptide or peptide fragment derived from the polypeptide into contact with blood or plasma from a patient positive for an anti-ADAMTS-13 antibody and binding an anti-ADAMTS-13 antibody in the blood or the plasma to the ligand to remove the antibody from the blood or the plasma.

[0023] A neutralizing agent for an autoantibody administered to a patient positive for an anti-ADAMTS-13 antibody is a pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody comprising, as an active ingredient, the above-described polypeptide or peptide fragment derived from the polypeptide. The pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody also includes a pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody comprising, as an active ingredient, a polypeptide or a peptide fragment derived from the polypeptide composed of the above-described polypeptide or peptide fragment derived from the polypeptide, which lacks reactivity with an anti-ADAMTS-13 antibody by modification such as molecular substitution, deletion, or insertion. The modification such as molecular substitution, deletion, or insertion used herein refers to the deletion, substitution, or addition of one or several amino acids, for example, in an amino acid sequence of the above-described polypeptide or peptide fragment derived from the polypeptide. Such modification allows, for example, alteration in the structure of the polypeptide or the peptide fragment and therefore allows the loss of the epitope. Accordingly, the polypeptide or the peptide fragment loses reactivity with the anti-ADAMTS-13 antibody. When the polypeptide of the present invention recognized by an ADAMTS-13 antibody is used, for example, as a

neutralizing agent for an autoantibody by administering the polypeptide to a patient positive for an anti-ADAMTS-13 antibody, the polypeptide can be diluted with a saline, a buffer solution, or the like, and made into a preparation to obtain a pharmaceutical composition. The pH of the preparation is preferably in the pH range from weakly-acidic pH to neutral pH, which is close to body fluid pH; a lower limit thereof is preferably from pH 5.0 to 6.4 and an upper limit thereof is preferably from pH 6.4 to 7.4. The preparation can also be provided in a form storable for a long period such as a freeze-dried form. In this case, when used, the preparation can be dissolved in water, a saline, a buffer solution, or the like, and then used at a desired concentration. The preparation of the present invention may contain pharmacologically acceptable additives (e.g., carriers, excipients, and diluents) and stabilizers that are usually used in drugs or other ingredients that are pharmaceutically required. The stabilizers are exemplified by monosaccharides such as glucose, disaccharides such as saccharose and maltose, sugar alcohols such as mannitol and sorbitol, neutral salts such as sodium chloride, amino acids such as glycine, nonionic surfactants such as polyethylene glycol, a polyoxyethylene-polyoxypropylene copolymer (Pluronic), and polyoxyethylene sorbitan fatty acid ester (Tween), and human albumin. It is preferred that any of these stabilizers on the order of 1 to 10 w/v% should be added to the preparation.

[0024] The pharmaceutical composition of the present invention can be administered in an effective amount by, for example, intravenous injection, intramuscular injection, or subcutaneous injection and is administered in a single dosage or several dosages. The dosage varies depending on the symptom, age, body weight, and so on, of a patient and is preferably 0.001 mg to 100 mg per dosage.

[0025] The present specification encompasses contents described in the specification and/or drawings of JP Patent Application No. 2003-071979 that serves as a basis for the priority of the present application.

Brief Description of the Drawings

[0026]

Figure 1 is a diagram showing a method of producing a C-terminus deletion mutant for determining an epitope for an antibody;

Figure 2 is a photograph in which the expression and abundance of the prepared C-terminus deletion mutant has been confirmed by Western blotting under non-reducing conditions using an anti-FLAG antibody;

Figure 3 is a photograph in which a region recognized by purified IgG derived from an acquired TTP patient 003 has been confirmed by Western blotting under non-reducing conditions;

Figure 4 is a photograph in which a region recognized by purified IgG derived from an acquired TTP patient 004 has been confirmed by Western blotting under non-reducing conditions;

Figure 5 is a photograph in which a region recognized by purified IgG derived from an acquired TTP patient 009 has been confirmed by Western blotting under non-reducing conditions; and

Figure 6 is a photograph in which a more precise region recognized by purified IgG derived from the acquired TTP patients has been confirmed.

Best Mode for Carrying Out the Invention

[0027] Although the present invention will be described hereinafter in detail with reference to Examples, the present invention is not intended to be limited to these Examples by any means.

Examples

Preparation Example 1

(Preparation of C-terminus deletion mutant of ADAMTS-13)

[0028] Expression vectors for full-length and mutant (sequentially lacking domains from the C terminus) (Full1427, and T1135X, W1016X, W897X, W808X, W746X, W688X, T581X, Q449X, W387X, and P285X; each numerical value represents the number of amino acid residues from Met encoded by an initiation codon ATG to a stop codon, and X represents a stop codon) genes described in the previous patent application (JP Patent Application No. 2002-279924) were utilized to perform transfection using Hela cells in the procedures below. Each mutant location in a full-length sequence is shown in Figure 1.

[0029] At first, 24 hours before transfection, 1-3x10^5 cells per 35-mm dish were seeded. On the following day, 10 μl of the Polyamine Transfection Reagent TransIT (manufactured by TAKARA) per 2 μg of each of the expression vectors was added to 200 μl of a serum-free medium such as Opti-MEM to prepare a complex with DNA according to the instructions attached to the reagent. The complex was then added dropwise to the above-described various cells

prepared in advance and was incubated for 6 hours. Seventy-two hours after the incubation, the medium was collected. The detection of each appropriately concentrated mutant was conducted Western blotting using an anti-FLAG-M2 antibody (manufactured by Kodak) and staining with an anti-mouse IgG-alkaline phosphatase enzyme-labeled antibody system (the result of confirming the expression of the mutants is shown in Figure 2).

Example 1

(Analysis of epitope for antibody in acquired TTP patient using Western blotting)

[0030] IgG fractions (antibody concentration: 2 to 5 mg/ml) were prepared from plasma samples from acquired TTP patients using a protein A column according to a standard method, and diluted 200-fold to perform Western blotting. An IgG-alkaline phosphatase-labeled antibody was used as a secondary antibody in a filter, and the filter was stained with a BCIP/NBT substrate to visualize bands (Figures 3 to 5). The region thereby determined which is recognized by the antibody was confirmed to be located on the C-terminus side from the Q449X, because a region up to the W688X shows reactivity and a region at the Q449X shows no reactivity with all of the antibody fractions from the three patients,.

Example 2

(Analysis of precise epitope for antibody in acquired TTP patient using Western blotting based on principle of competitive inhibition)

[0031] For narrowing down the more precise location of the epitope recognized by the neutralizing antibody of the present invention, supernatants from the W688X mutant and the full-length wild-type ADAMTS-13 were subjected to electrophoresis and transferred to a PVDF membrane. The PVDF membrane was utilized in a competitive inhibition system using primary antibody reaction solutions obtained by previously preincubating the above-described antibodies from the patients with a concentrated supernatant having the considerable overexpression of the Q449X mutant, the W688X mutant, or the full-length wild-type ADAMTS-13. As a result, bands positive for the full-length wild type ADAMTS-13 for all of the samples were confirmed to disappear by the W688X mutant (Figure 6).
[0032] This has suggested that all of the antibodies from the three patients recognize a region located on the N-terminus side from the W688X.
[0033] From the results shown in Examples 1 and 2, a major neutralizing region for the autoantibodies used from the three patients was confirmed to be a region located on the terminus side of the Q449X and on the N-terminus side from the W688X, that is, a region from a Cys-rich region to a spacer region which is between the Q449X and the X688X.
[0034] All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

Industrial Applicability

[0035] The findings brought about by the present invention demonstrate that a polypeptide of this invention shows specific immunoreactivity with an anti-ADAMTS-13 antibody. Therefore, the use of the polypeptide allows the rapid detection of the amount of the anti-ADAMTS-13 antibody, diagnosis for diseases associated with variations in the amount of a protease of the present invention, or the neutralization of the binding or inhibitory activity of the anti-ADAMTS-13 antibody. Thus, the polypeptide provided by the present invention provides a wide variety of applications including the detection of the anti-ADAMTS-13 antibody and other applications.
[0036] The present invention exhibits such remarkable action or effect and can be said to be an invention of great significance which contributes to every field.

SEQUENCE LISTING

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<120>Composition comprising peptide fragment(s) recognized by antibody agai
nst von Willebrand Factor cleaving protease

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35 40 45

Val Ser Ser Tyr Leu Ser Pro Gly Ala Pro Leu Lys Gly Arg Pro

10 50 55 60

Pro Ser Pro Gly Phe Gln Arg Gln Arg Gln Arg Gln Arg Arg Ala

15 65 70 75

Ala Gly Gly Ile Leu His Leu Glu Leu Leu Val Ala Val Gly Pro

80 85 90

20 Asp Val Phe Gln Ala His Gln Glu Asp Thr Glu Arg Tyr Val Leu

95 100 105

Thr Asn Leu Asn Ile Gly Ala Glu Leu Leu Arg Asp Pro Ser Leu

25 110 115 120

Gly Ala Gln Phe Arg Val His Leu Val Lys Met Val Ile Leu Thr

30 125 130 135

Glu Pro Glu Gly Ala Pro Asn Ile Thr Ala Asn Leu Thr Ser Ser

140 145 150

35 Leu Leu Ser Val Cys Gly Trp Ser Gln Thr Ile Asn Pro Glu Asp

155 160 165

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170 175 180

Phe Asp Leu Glu Leu Pro Asp Gly Asn Arg Gln Val Arg Gly Val

45 185 190 195

Thr Gln Leu Gly Gly Ala Cys Ser Pro Thr Trp Ser Cys Leu Ile

200 205 210

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845 850 855

55 Pro Glu Pro Cys Val Gly Met Ser Cys Pro Pro Gly Trp Gly His

860 865 870

5 Leu Asp Ala Thr Ser Ala Gly Glu Lys Ala Pro Ser Pro Trp Gly

875 880 885

Ser Ile Arg Thr Gly Ala Gln Ala Ala His Val Trp Thr Pro Ala

10 890 895 900

Ala Gly Ser Cys Ser Val Ser Cys Gly Arg Gly Leu Met Glu Leu

15 905 910 915

Arg Phe Leu Cys Met Asp Ser Ala Leu Arg Val Pro Val Gln Glu

920 925 930

20 Glu Leu Cys Gly Leu Ala Ser Lys Pro Gly Ser Arg Arg Glu Val

935 940 945

Cys Gln Ala Val Pro Cys Pro Ala Arg Trp Gln Tyr Lys Leu Ala

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Ala Cys Ser Val Ser Cys Gly Arg Gly Val Val Arg Arg Ile Leu

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Claims

1. A polypeptide comprising a neutralizing epitope region in von Willebrand factor-specific cleaving protease (hereinafter, also referred to as vWFCP or ADAMTS-13), which is recognized by an antibody against the protease, or a peptide fragment derived from the polypeptide.

2. The polypeptide or the peptide fragment derived from the polypeptide according to claim 1, wherein the neutralizing epitope region is located in a region from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1.

3. A polypeptide comprising an amino acid sequence from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1, or a peptide fragment derived from the polypeptide.

4. A polypeptide comprising an amino acid sequence consisting of an amino acid sequence from position 449 to position 687 in an amino acid sequence shown in SEQ ID No. 1, where one or several amino acids are deleted, substituted, or added, the polypeptide being recognized by an antibody against von Willebrand factor-specific cleaving protease, or a peptide fragment derived from the polypeptide.

5. An antibody capable of binding to a polypeptide or a peptide fragment derived from the polypeptide according to any one of clams 1 to 4.

6. The antibody according to claim 5, which is present in blood of a patient positive for an anti-ADAMTS-13 antibody.

7. The antibody according to claim 5 or 6, which is present in blood of a patient with non-familial thrombocytopenic purpura (hereinafter, also referred to as TTP).

8. A reagent for antibody measurement comprising a polypeptide having a complete sequence composing ADAMTS-13, or a polypeptide or a peptide fragment derived from the polypeptide according to any one of claims 1 to 4.

9. The reagent for antibody measurement according to claim 8, wherein an autoantibody in a TTP patient is an object

to be detected.

10. A pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody comprising, as an active ingredient, a polypeptide or a peptide fragment derived from the polypeptide according to any one of claims I to 4.

11. The pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody according to claim 10, wherein the pharmaceutical composition comprises, as an active ingredient, a polypeptide or a peptide fragment derived from the polypeptide composed of a polypeptide or a peptide fragment derived from the polypeptide according to any one of claims 1 to 4, which lacks reactivity with an anti-ADAMTS-13 antibody by modification such as molecular substitution, deletion, or insertion.

12. The pharmaceutical composition for treating a patient positive for an anti-ADAMTS-13 antibody according to claim 10 or 11, wherein the pharmaceutical composition is administered to the patient to thereby neutralize the antibody.

13. A composition comprising a ligand specific to an anti-ADAMTS-13 antibody for treating a patient positive for an anti-ADAMTS-13 antibody, comprising, as an active ingredient, a polypeptide or a peptide fragment derived from the polypeptide according to any one of claims 1 to 4, which is bound with a carrier and brought into contact with plasma from the patient to be used for removing the anti-ADAMTS-13 antibody from the plasma from the patient.

14. A method of producing blood or plasma free from an anti-ADAMTS 13 antibody by bringing a carrier bound with a polypeptide or a peptide fragment derived from the polypeptide according to any one of claims 1 to 4 into contact with blood or plasma from a patient positive for an anti-ADAMTS-13 antibody and binding an anti-ADAMTS-13 antibody in the blood or the plasma to the ligand to remove the anti-ADAMTS-13 antibody from the blood or the plasma.

Fig. 1

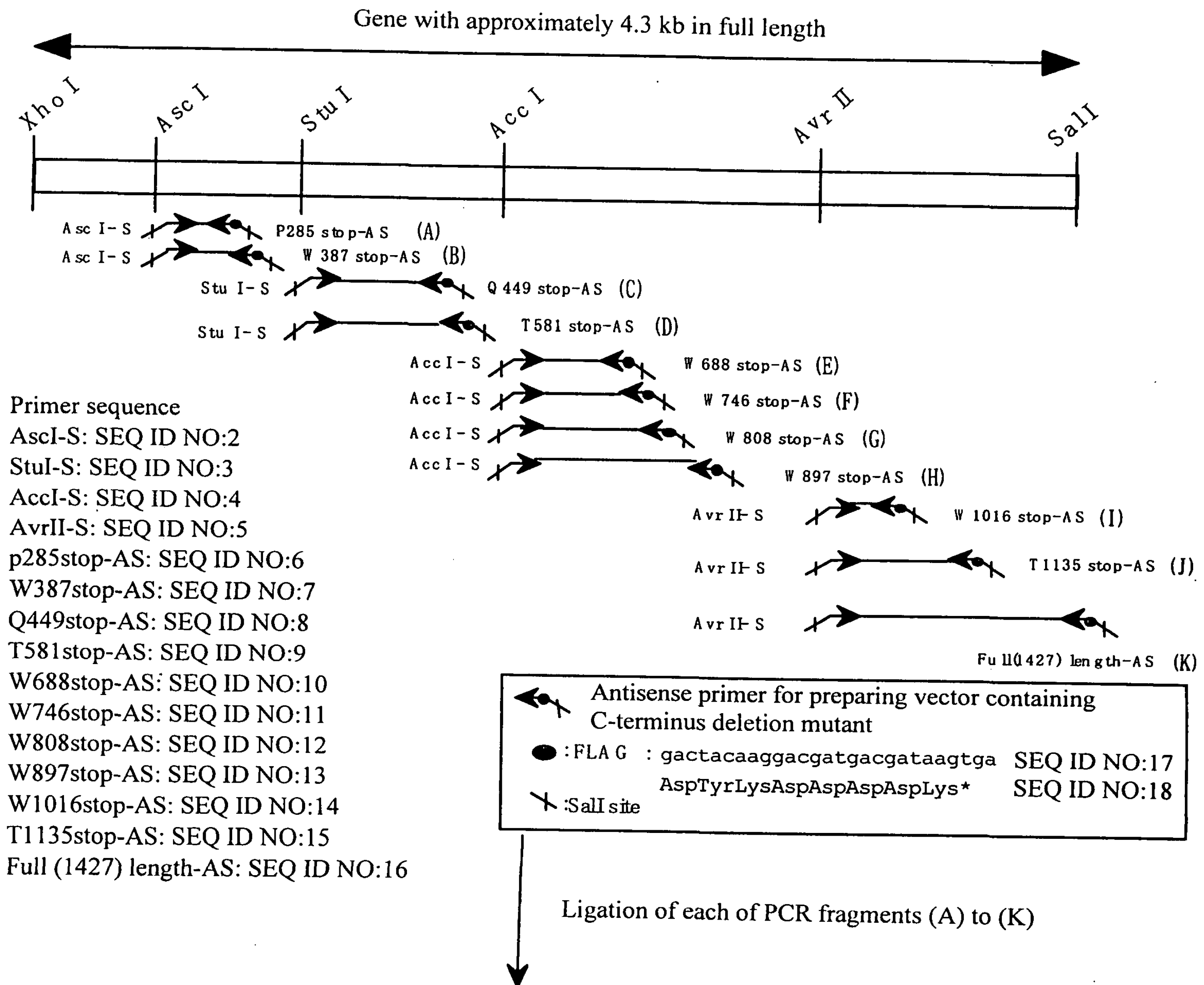

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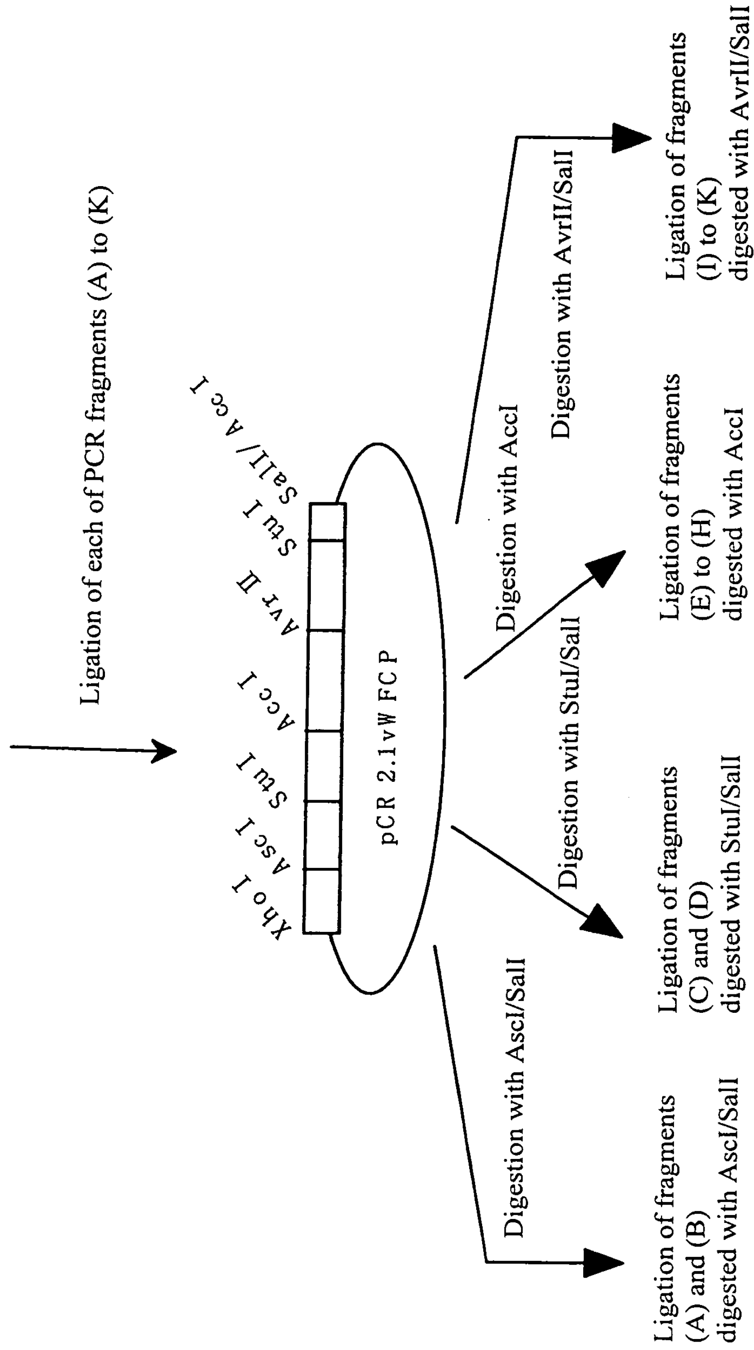
Fig. 1



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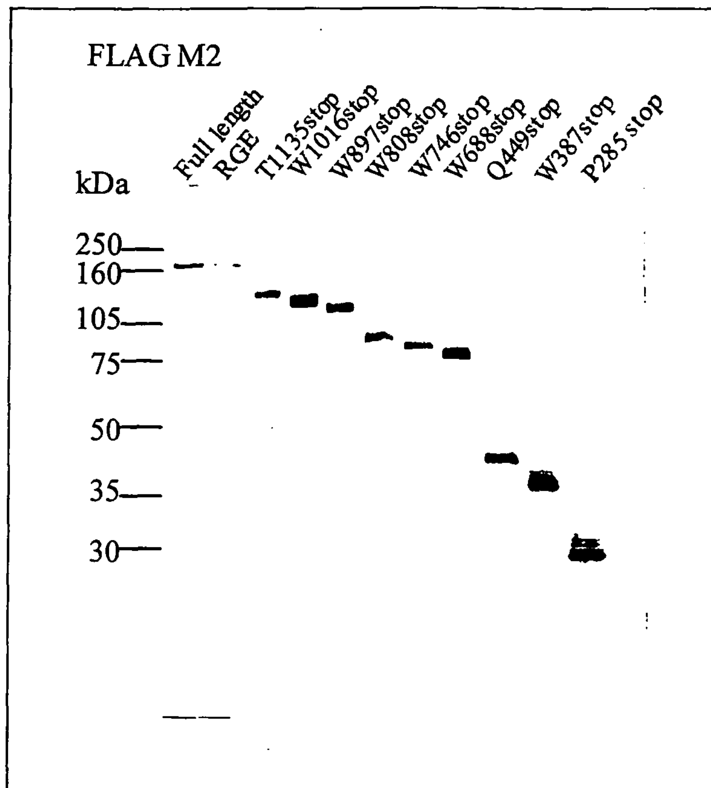

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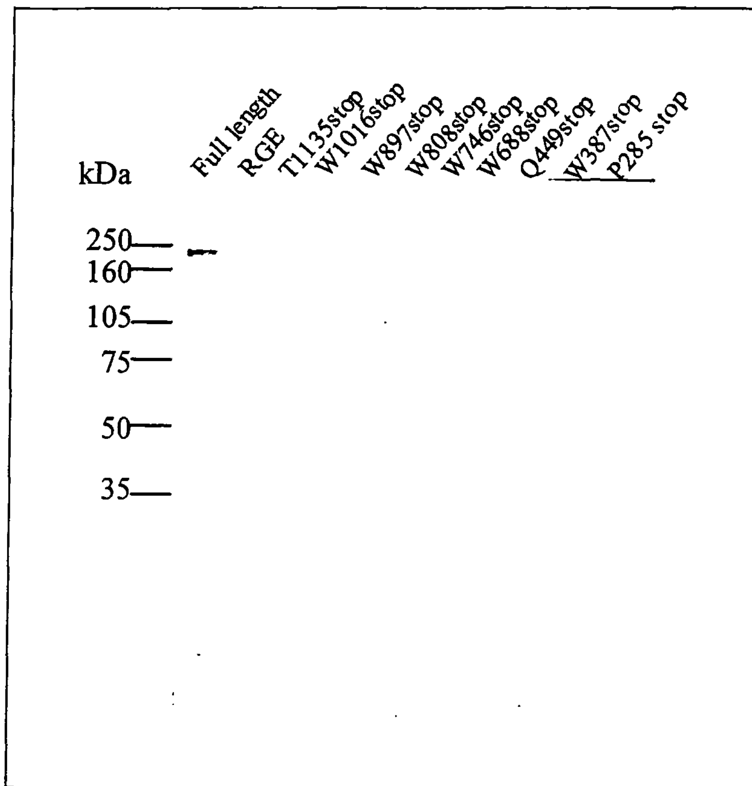

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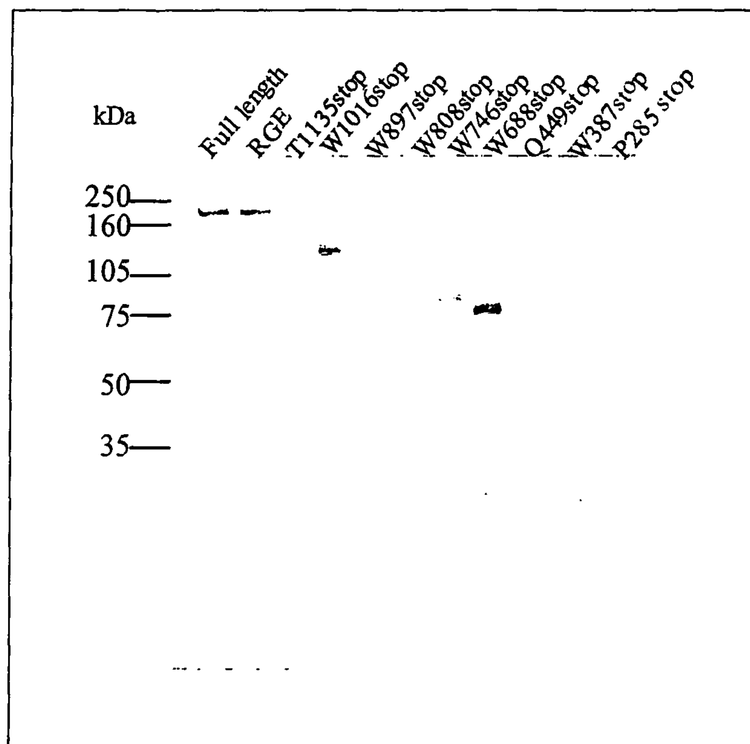

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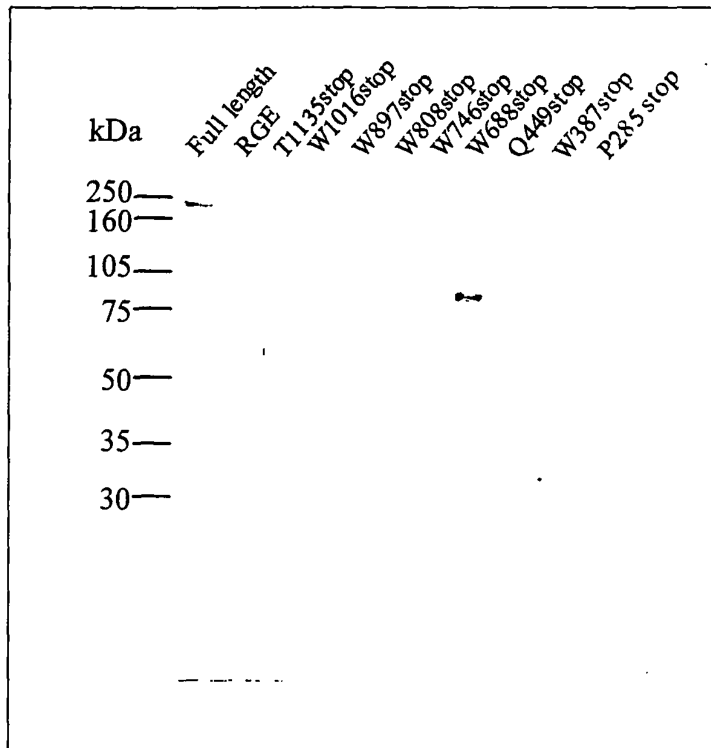

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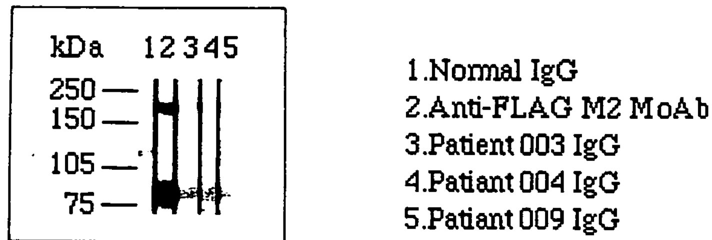

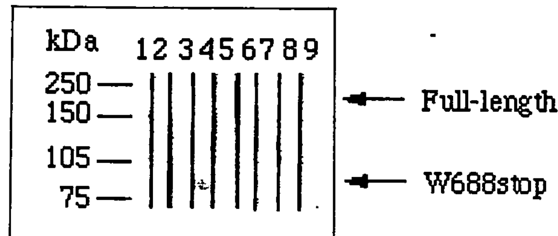

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2004/003602

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C07K16/40, A61K39/00, A61P7/04, C12N9/64, G01N33/53, G01N33/564

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C07K16/40, A61K39/00, A61P7/04, C12N9/64, G01N33/53, G01N33/564

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
SwissProt/PIR/GeneSeq, Genbank/EMBL/DDBJ/GeneSeq, BIOSIS, MEDLINE, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PLAOMAUER, B. et al., Epitope Mapping of Anti-ADAMTS-13 Antibodies in Patients with Acquired TTP., Blood, 16 November, 2003 (16.11.03), Vol.102(11), p.540a	1-14
X/Y1	PLAIMAUER, B. et al., Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13)., Blood, 15 November, 2002 (15.11.02), Vol.100(10), pages 3626 to 3632	1,2/3-14
X/Y1	GERRITSEN, HE. et al., Partial amino acid sequence of purified von Willebrand factor-cleaving protease., Blood, 15 September, 2001 (15.09.01), Vol.98(6), pages 1654 to 1661	1,2/3-14

☒ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April, 2004 (19.04.04)	11 May, 2004 (11.05.04)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2004/003602

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y2	HUANG, CC. et al., Epitope mapping of factor VIII inhibitor an tibodies of Chinese origin., Br J.Haematol., 2001 June, Vol.113(4), pages 915 to 924	1-14
Y2	NARDI, MA. et al., GPIIIa-(49-66) is a major pathophysiologically relevant antigenic determinant for anti-platelet GPIIIa of HIV-1-related immunologic thrombocytopenia., Proc.Natl.Acad.Sci.USA, 08 July, 1997 (08.07.97), Vol.94(14), pages 7589 to 7594	1-14
Y2	JP 10-500705 A (President and Fellows of Harvard College), 20 January, 1998 (20.01.98), & CA 2189738 A & EP 759771 A & WO 96/27387 A	1-14
A	KOKAME, K. et al., Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity., Proc.Natl.Acad.Sci.USA., 03 September, 2002 (03.09.02), Vol.99(18), pages 11902 to 11907	1-14
A	CAL, S. et al., Cloning, expression analysis, and structural characterization of seven novel human ADAMTSs, a family of metalloproteinases with disintegrin and thrombospondin-1 domains., Gene., 23 January, 2002 (23.01.02), Vol.283(1-2), pages 49 to 62	1-14

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2004/003602

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material
      - [X] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [ ] in written format
      - [X] in computer readable form

   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [X] filed together with the international application in computer readable form
      - [ ] furnished subsequently to this Authority for the purposes of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet (1)) (January 2004)

patsnap

专利名称(译)	构建体包含针对血管性血友病因子特异性切割酶的抗体的识别结构域		
公开(公告)号	EP1609804A1	公开(公告)日	2005-12-28
申请号	EP2004721377	申请日	2004-03-17
申请(专利权)人(译)	法律基础，化学及血清疗法研究所		
当前申请(专利权)人(译)	法律基础，化学及血清疗法研究所		
[标]发明人	SOEJIMA KENJI THE CHEMO SERO THERAPEUTIC R I NAKAGAKI TOMOHIRO CHEMO SERO THER R I MATSUMOTO MASANORI FUJIMURA YOSHIHIRO		
发明人	SOEJIMA, KENJI, THE CHEMO-SERO-THERAPEUTIC R. I. NAKAGAKI, TOMOHIRO, CHEMO-SERO-THER. R. I. MATSUMOTO, MASANORI FUJIMURA, YOSHIHIRO		
IPC分类号	A61P7/04 C07K16/40 C12N9/64 A61K39/00 G01N33/53 G01N33/564		
CPC分类号	C12N9/6489 A61K2039/505 C07K16/40		
优先权	2003071979 2003-03-17 JP		
其他公开文献	EP1609804B1 EP1609804A4		
外部链接	Espacenet		

摘要(译)

本发明提供抗体识别的表位（下文中，也称为抗ADAMTS-13抗体）针对血管性血友病因子特异性的裂解蛋白酶（下文中，也称为ADAMTS-13）（下文中，也称为抗体）。作为vWF）和包含表位区域的多肽。本发明还提供了位于组成ADAMTS-13的氨基酸序列中第449位至第687位的区域的多肽，其被抗ADAMTS-13抗体识别，或者衍生自该多肽的肽片段。


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nst von Willebrand Factor cleaving protease

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