(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 May 2013 (16.05.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/071168 A1

(51) International Patent Classification:
C07K 14/755 (2006.01) *C12Q 1/37* (2006.01)

(21) International Application Number:
PCT/US2012/064526

(22) International Filing Date:
9 November 2012 (09.11.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/558,927 11 November 2011 (11.11.2011) US

(71) Applicant: **GEN-PROBE GTI DIAGNOSTICS, INC., C/O GEN-PROBE INCORPORATED** [US/US]; 10210 Genetic Center Drive, San Diego, California 92121-4362 (US).

(72) Inventors: **VISENTIN, Gian Paolo**; 1623 E Sunset Dr. Apt. 205, Waukesha, Wisconsin 53189 (US). **CHANCE, Suzette C.**; 3207 Holy Hill Road, Richfield, Wisconsin 53076 (US). **WUITSCHICK, Elizabeth**; 1308 N 63rd St., Wauwatosa, Wisconsin 53213 (US).

(74) Agent: **LANDES, Jeffrey, E.**; Gen-Probe Incorporated, 10210 Genetic Center Drive, San Diego, California 92121-4362 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

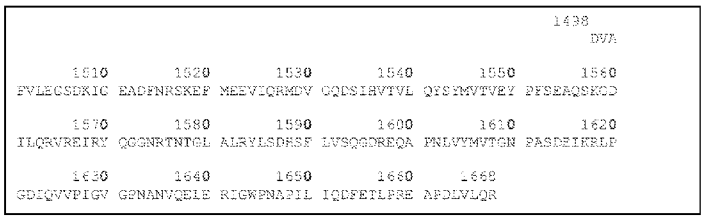
Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: POLYPEPTIDE SUBSTRATE FOR THE DETECTION OF VON WILLEBRAND FACTOR CLEAVING PROTEASE ADAMTS13

FIGURE 1



(57) **Abstract**: In a first aspect, there is provided an isolated polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) that is from 45 to 70 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and (iii) the amino acids corresponding to Q1624 to R1641 of SEQ ID NO: 2 are deleted. In another aspect, there is provided an ADAMTS13 polypeptide substrate that is from 50 to 75 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; (iii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and (iv) the amino acids corresponding to G1631 to R1641 of SEQ ID NO: 2 are deleted.

WO 2013/071168 A1

POLYPEPTIDE SUBSTRATE FOR THE DETECTION OF VON WILLEBRAND FACTOR CLEAVING PROTEASE ADAMTS13

CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 61/558,927, filed on November 11, 2011, the entire contents of which is hereby incorporated by reference.

BACKGROUND

[0002] von Willebrand factor (VWF) is a large multimeric plasma glycoprotein crucial in the maintenance of hemostasis by functioning as both an antihemophilic factor carrier and a platelet-vessel wall mediator in the blood coagulation system, mainly by mediating tethering and adhesion of circulating platelets at sites of vascular injury. Mutations in this gene or deficiencies in this protein result in von Willebrand's disease (VWD).

[0003] VWF is expressed by endothelial cells and megakaryocytes. It is synthesized as 250-kDa monomers, which undergo intracellular processing, glycosylation, multimerization and propeptide removal that leads to formation of mature VWF multimers.

[0004] VWF multimeric size is modulated by the plasma metallopeptidase ADAMTS13 (a disintegrin and metallopeptidase with thrombospondin type I motif, member 13, a “cleaving protease”), which cleaves at a single site in the VWF A2 domain (AA1498-1665; UniProtKB/Swiss-Pro database; Accession: P04275. SEQ ID NO:2) between Y1605 and M1606.

[0005] ADAMTS13 is a protease that is activated in the presence of barium and other metal ions. ADAMTS13 has been demonstrated to degrade full-length multimeric vWF into multimers of smaller size and into lower molecular weight polypeptides or peptides. For this reason, the ADAMTS13 protease has been termed vWF-cleaving protease or the "ATS protease". The activity of the protease has been demonstrated to be reduced in patients with Thrombotic Thrombocytopenia Purpura (TTP).

[0006] Severe deficiency of the protease has been described in patients with chronic relapsing TTP, a deficiency that may be inherited or acquired as a result of an autoimmune mechanism.

[0007] In the past, assays for the presence or absence of ADAMTS13 utilized a cumbersome technique in which plasma from a patient is incubated with exogenous multimeric vWF in the presence of barium chloride on the surface of a membrane floating on a buffer containing 1.5 molar urea. More recently an alternative method has been developed by Kokame *et al.* (Kokame, K., Y. Nobe, Y. Kokubo, A. Okayama, and T. Miyata. 2005. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br.J.Haematol.* 129:93-100. See also Wu JJ, Fujikawa K, McMullen BA, Chung DW. Characterization of a core binding site for ADAMTS13 in the A2 domain of von Willebrand factor. *Proc Natl Acad Sci* U S A. 2006; 103: 18470-4.). Kokame's method utilizes a polypeptide substrate for ADAMTS13 activity, wherein the substrate is 73 amino acid residues in the A2 domain of VWF, called VWF73. FRETS-VWF73 is within this domain and the 73-amino-acid polypeptide sequence corresponds to the region from D1596 to R1668 of VWF (see SEQ ID NO:6 herein), Q1599 and N1610 when substituted with A2pr(Nma) and A2pr(Dnp) respectively. Several assays have been developed using SEQ ID NO:6. VWF73-based ADAMTS13 assays have the potential to contribute to improved clinical treatments.

[0008] However, the de novo synthesis of SEQ ID NO:6 is difficult and the FRETS-VWF73 substrate works near the UV spectrum. The signal that is generated therefore suffers from heavy contribution of autofluorescence which can be exacerbated by the fact that the assay is homogeneous, i.e. is performed in a single step without washing away the plasma, one of the major contributors to the autofluorescence noise. Because of its susceptibility to autofluorescence, an assay based on the FRETS-VWF73 substrate is very sensitive to dust microparticles, potentially resulting in poor replicates and aberrant results. Furthermore, FRETS-VWF73 substrate assays typically result in a non-linear calibration curve which can result in low accuracy below 10% of ADAMTS13 activity. This is problematic since the resolution of ADAMTS13 activity at between 0-10% is important to clinicians to confirm the diagnosis of TTP and to monitor and fine tune the therapeutic intervention (such as plasma

exchange). Further, ADAMTS13 activity assays using a SEQ ID NO:6 polypeptide suffer from poor sensitivity.

[0010] As a result, there is a need in the art for an improved ADAMTS13 polypeptide substrate. The present invention seeks to address this need.

ASPECTS AND EMBODIMENTS OF THE INVENTION

[0009] In a first aspect, there is provided an isolated polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) that is from 45 to 70 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and (iii) the amino acids corresponding to Q1624 to R1641 of SEQ ID NO: 2 are deleted.

[00010] In a second aspect, there is provided an ADAMTS13 polypeptide substrate that is from 50 to 75 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; (iii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and (iv) the amino acids corresponding to G1631 to R1641 of SEQ ID NO: 2 are deleted.

[00011] Suitably, the amino acid at the N-terminus of said polypeptide substrate corresponds to D1596 of SEQ ID NO: 2.

[00012] Suitably, the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2.

[00013] Suitably, the polypeptide is a synthetic polypeptide that comprises a detectable label.

[00014] Suitably, the detectable label is a fluorophore and a quencher.

[00015] Suitably, the attachment site for the fluorophore is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or wherein the attachment site for the quencher is at the amino acid corresponding to position 1599 of SEQ ID NO: 2 or wherein attachment site for the quencher is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or wherein the attachment site for the fluorophore is at the amino acid corresponding to position 1599 of SEQ ID NO: 2.

[00016] Suitably, the ADAMTS13 polypeptide substrate comprises, consists or consists essentially of the sequence set forth in SEQ ID NO: 7.

[00017] Suitably, the ADAMTS13 polypeptide substrate comprises, consists or consists essentially of the sequence set forth in SEQ ID NO: 1.

[00018] Suitably, the ADAMTS13 polypeptide substrate is lyophilized.

[00019] In a further aspect, there is provided a method for cleaving the ADAMTS13 polypeptide substrate, comprising contacting said ADAMTS13 polypeptide substrate with an ADAMTS13 protease.

[00020] In a further aspect, there is provided a method for measuring ADAMTS13 activity in a sample comprising the use of the ADAMTS13 polypeptide substrate.

[00021] Suitably, the method comprises the steps of: (a) providing a sample comprising, or suspected of comprising, an ADAMTS13; (b) contacting said sample with the ADAMTS13 polypeptide substrate; and (c) determining the fragmentation of the ADAMTS13 polypeptide substrate, wherein the fragmentation of the ADAMTS13 polypeptide substrate is optionally compared to one or more controls and/or calibrators in order to arrive at a measurement of ADAMTS13 activity.

[00022] Suitably, the cleavage of the ADAMTS13 polypeptide substrate is measured by monitoring the change in fluorescence.

[00023] Suitably, the sample at step (a) is a plasma sample or is derived from a plasma sample.

[00024] Suitably, the ADAMTS13 polypeptide substrate is in solution during contacting step (b). Suitably, the ADAMTS13 polypeptide substrate is in solution when cleaved by a protease. Suitably, the ADAMTS13 polypeptide substrate is in solution when cleaved by an ADAMTS13 protease.

[00025] Suitably, the ADAMTS13 polypeptide substrate is attached to a solid support during contacting step (b). Suitably, the ADAMTS13 polypeptide substrate is attached to a solid support when cleaved by a protease. Suitably, the ADAMTS13 polypeptide substrate is attached to a solid support when cleaved by an ADAMTS13 protease. Suitably, the ADAMTS13 polypeptide substrate is attached to a well during contacting step (b). Suitably, the ADAMTS13 polypeptide substrate is attached to two or more wells of a microwell strip during contacting step (b). Suitably, the ADAMTS13 polypeptide substrate is attached to a bead during contacting step (b).

[00026] Suitably, step (d) is a quantitative determination of the fragmentation of the ADAMTS13 polypeptide substrate.

[00027] In a further aspect, there is provided a kit for in vitro testing of ADAMTS13 activity in a subject, comprising the ADAMTS13 polypeptide substrate, one or more calibrators containing a known concentration of ADAMTS13 activity and/or one or more positive controls for ADAMTS13 activity optionally together with a specimen diluent and/or a substrate buffer.

[00028] In a further aspect, there is provided the use of the ADAMTS13 polypeptide substrate for measuring the activity of ADAMTS13 protease in a sample.

[00029] The ADAMTS13 polypeptide substrates that are described herein have a number of advantages.

[00030] By way of example, the polypeptide substrate can be reliably synthesised. When the polypeptide substrate is synthesized by chemical synthesis it can be produced at lower cost as compared to recombinant synthesis and 73-mer synthesis.

[00031] By way of further example, a linear calibration curve can be achieved along with higher resolution, sensitivity and precision as compared to the existing ADAMTS13 activity-based assays.

[00032] By way of further example, reduced signal-to-noise ratio in the ADAMTS13 assay can be obtained.

[00033] By way of further example, faster reaction time (15 minutes or less reaction time vs. the 30 minutes required by the FRETS-VWF73-based assay) in the ADAMTS13 assay can be obtained.

[00034] By way of further example, when detectable labels are used, excitation and emission occurs at the most widely used wavelengths which makes detection simpler.

[00035] By way of further example, a higher dynamic range of the assay can be achieved resulting in the ability to precisely determine ADAMTS13 in the range of about 0-20% activity, a range that cannot be efficiently resolved in the existing activity-based assay. Thus, improvements in the differential diagnosis of TTP from other disorders including hemolytic uremic syndrome (HUS), which present similar clinical symptoms, can be achieved. Improvements in the prognostic management of TTP can also be achieved.

BRIEF DESCRIPTION OF THE DRAWINGS

[00036] FIG. 1 shows the protein sequence of a portion of VWF (AA1498-1668) encompassing the A2 domain (AA1498-1665).

[00037] FIG. 2 shows the amino acid sequence of SEQ ID NO:1.

[00038] FIG. 3 displays a series of calibration curves obtained with the prior art FRETS-VWF73.

[00039] FIG. 4 displays a series of calibration curves obtained with Applicants synthetic 62 (sixty two) amino acids in length polypeptide sequence designated as "GTI_FRET4" SEQ ID NO: 1.

[00040] FIG. 5 displays a series of calibration curves obtained with Applicants synthetic 55 (fifty five) amino acids in length polypeptide sequence designated as "GTI_FRET5" SEQ ID NO: 7 Showing a change in fluorescence with time.

DETAILED DESCRIPTION

[00041] Definitions

[00042] In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention. The technical terms and expressions used within the scope of this application are generally to be given the meaning commonly applied to them in the art. All of the following term definitions apply to the complete content of this application. The word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The terms "essentially", "about", "approximately" and the like in the context of a given numerate value or range refers to a value or range that is within 20 %, within 10 %, or within 5 % of the given value or range. Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

[00043] As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, amplification products, fragments generated by any of ligation, scission, endonuclease activity, and exonuclease activity, genomic DNA, recombinant vectors and

chemically synthesized molecules. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides, or analogs of naturally-occurring nucleotides (e.g., alpha-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Nucleic acids can be either single stranded or double stranded.

[00044] The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

[00045] The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

[00046] An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic nucleic acid of an organism. For example, a nucleic acid molecule that has been separated from the genomic nucleic acid of a cell is an isolated nucleic acid molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete nucleic acid molecule of a chromosome from that species.

[00047] A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

[00048] A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups, fluorescent detection moieties and/or linkers. These non-peptidic components may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell.

Proteins are defined herein in terms of their amino acid backbone structures; non-peptidic components are generally not specified when generally referring to the amino acid sequence, but may be present nonetheless.

[00049] A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

[00050] An "isolated polypeptide" or "isolated peptide" is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide or isolated peptide contains the polypeptide or peptide in a highly purified form, i.e., at least 80% pure, at least 90% pure, at least 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide or peptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide or peptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms. As was described above, the term "at least 80% pure" is inclusive of all whole or partial numbers from 80% purity to 100% purity. This same applies to "at least 90% pure" and "at least 95% pure." The term "greater than 95% pure" means 95.01% to 100% purity, as described above, and including all whole and partial numbers there between.

[00051] The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides or peptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide or peptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide or peptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide or peptide.

[00052] The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

[00053] A "detectable label" is a molecule or atom which can be conjugated, attached to or incorporated into a polypeptide to produce a molecule useful for diagnosis. The label can be any type of label which, when attached to or incorporated into a polypeptide renders the polypeptide detectable. A detectable label may have one or more of the following characteristics: fluorescence, color, radiosensitivity, or photosensitivity. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties such as a fluorescent resonance energy transfer (FRET) donor and/or acceptor

[00054] The term "affinity tag" is used herein to denote a polypeptide or peptide segment that can be attached to a second polypeptide or peptide to provide for purification or detection of the second polypeptide or peptide or provide sites for attachment of the second polypeptide or peptide to a substrate. In principal, any polypeptide or peptide for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075 (1985); Nilsson et al., Methods Enzymol. 198:3 (1991)), glutathione S transferase (Smith and Johnson, Gene 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952 (1985)), substance P, FLAG peptide (Hopp et al., Biotechnology 6:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

[00055] The term "substantially similar" when used to describe polypeptide or peptide sequences or polynucleotide sequences herein means that the two sequences share at least 70% or 75% identity over a corresponding range. More preferably, that percent identity is at least 80% identity, more preferably still at least 85%, more preferably still at least 90% identity, more preferably still at least 95% identity and most preferably at least 96%, 97%, 98% or 99% identity. Differences in identity can be due to additions, deletions or

substitutions of residues in a first sequences compared to a second sequences. Those ordinarily skilled in the art will readily calculate percent identity between a polypeptide or peptide sequence or a polynucleotide sequences and a reference sequence. For example, the % identity of two polynucleotide sequences may be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (Nucl. Acids Res. 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). Typical default parameters for the GAP program include: (1) a unary comparison matrix (comprising a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14:6745, 1986, as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Various programs known to persons skilled in the art of sequence comparison can be alternatively utilized.

[00056] As is used herein, the terms "at least 70% identical" or “at least 70% identity” means that a polypeptide or peptide sequence or a polynucleotide sequence shares 70%-100% sequence identity with a reference sequence. This range of identity is inclusive of all whole numbers (e.g., 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) or partial numbers (e.g., 72.15, 87.27%, 92.83%, 98.11% - to two significant figures) embraced within the recited range numbers, therefore forming a part of this description. For example, an amino acid sequence with 200 residues that share 85% identity with a reference sequence would have 170 identical residues and 30 non-identical residues. Similarly, an amino acid sequence with 235 residues may have 200 residues that are identical to a reference sequence, thus the amino acid sequence will be 85.11% identical to the reference sequence. Similarly, the terms “at least 80%,” “at least 90%,” “at least 95%” and “at least 99%” and the like are inclusive of all whole or partial numbers within the recited range. As is used herein, the terms “greater than 95% identical” or “greater than 95% identity” means that a sequence shares 95.01%-100% sequence identity with a reference sequence. This range is all inclusive. Differences in identity can be due to additions, deletions or substitutions of residues in a first sequences compared to a second sequence.

[00057] The term "sample" as used herein includes a biological fluid such as blood, plasma or tissue of a subject. The sample may be obtained or obtainable from a human – such as a human subject - suspected of having a disorder associated with ADAMTS13.

[00058] Detailed description of the invention

[00059] One embodiment relates to an ADAMTS13 polypeptide substrate. Suitably, the ADAMTS13 polypeptide substrate is from 45 to 75 amino acids in length – such as from 45 to 72 amino acids in length or from 45 to 70 amino acids in length or from 50 to 75 amino acids in length. More suitably, the ADAMTS13 polypeptide substrate is from 45 to 65 amino acids in length, from 50 to 65 amino acids in length, from 50 to 60 amino acids in length, from 51 to 59 amino acids in length, from 52 to 58 amino acids in length, from 53 to 57 amino acids in length, from 54 to 56 amino acids in length, from 50 to 70 amino acids in length, from 55 to 70 amino acids in length, from 55 to 65 amino acids in length, from 60 to 65 amino acids in length, from 61 to 64 amino acids in length or from 61 to 63 amino acids in length. In one embodiment, the ADAMTS13 polypeptide substrate is from 55 to 62 amino acids in length. In one embodiment, the ADAMTS13 polypeptide substrate is 55 amino acids in length. In one embodiment, the ADAMTS13 polypeptide substrate is 62 amino acids in length. In one embodiment, the ADAMTS13 polypeptide substrate is 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 or 75 amino acids in length and contains a feature as described herein.

[00060] The ADAMTS13 polypeptide substrate is an isolated chimeric or mutant amino acid construct encompassing portions of the VWF A2 domain.

[00061] In one aspect, the isolated polypeptide substrate is from 45 to 70 amino acids in length and has an amino acid sequence that is substantially similar to part of the VWF A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and (iii) the amino acids corresponding to Q1624 to I1642 of SEQ ID NO: 2 are deleted.

[00062] In another aspect, the isolated polypeptide substrate is from 50 to 75 amino acids in length and has an amino acid sequence that is substantially similar to part of the VWF A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K; and (ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and (iii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and (iv) the amino acids corresponding to G1631 to R1641 of SEQ ID NO: 2 are deleted.

[00063] Suitably, the amino acid at the N-terminus of said polypeptide substrate corresponds to D1596 of SEQ ID NO: 2. Suitably, the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2. Suitably, the amino acid at the N-terminus of said polypeptide substrate corresponds to D1596 of SEQ ID NO: 2 and the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2.

[00064] SEQ ID NO:2 corresponds to a fragment of the A2 domain of VWF from Homo Sapiens; Accession number P04275-1 (UniProtKB/Swiss-Pro); UPI0001BBE42F (UniParc); IPI00023014.2 (International Protein Index).

[00065] In one embodiment, the ADAMTS13 polypeptide substrate comprises, consists or consists essentially of the sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 1 or a sequence that has substantial identity thereto. Isomers thereof are also contemplated. According to a further embodiment, the ADAMTS13 polypeptide substrate may comprise one or more further amino acids at the N-terminus or the C-terminus or the N-terminus and the C-terminus of the polypeptide substrate.

[00066] Cleavage products of the SEQ ID NO: 1 or SEQ ID NO: 7 polypeptide substrate are also disclosed, particularly those cleavage products generated following fragmentation with ADAMTS13. In particular, C-terminal fragments are disclosed. Thus, in a further aspect there is provided an isolated polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) that is or is at least 52

(fifty two) amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and (ii) the amino acids corresponding to Q1624 to R1641 of SEQ ID NO: 2 are deleted. Suitably, the amino acid at the N-terminus of said polypeptide substrate corresponds to M1606 of SEQ ID NO: 2. Suitably, the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2. Suitably, said polypeptide is a synthetic polypeptide that comprises at least one portion of a detectable label. Suitably, at least one portion of the detectable label is a fluorophore or a quencher. Suitably, the attachment site for the fluorophore or the quencher is at the amino acid corresponding to position 1610 of SEQ ID NO: 2. In another aspect, there is provided an isolated polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) that is or is at least 45 (forty five) amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications: (i) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; (ii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and (iii) the amino acids corresponding to G1631 to R1641 of SEQ ID NO: 2 are deleted. Suitably, the amino acid at the N-terminus of said polypeptide substrate corresponds to M1606 of SEQ ID NO: 2. Suitably, the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2. Suitably, said polypeptide is a synthetic polypeptide that comprises at least one portion of a detectable label. Suitably, the at least one portion of the detectable label is a fluorophore or a quencher. Suitably, the attachment site for the fluorophore or the quencher is at the amino acid corresponding to position 1610 of SEQ ID NO: 2.

[00067] Isolated nucleotide sequences encoding the polypeptide substrates described herein are also disclosed. In addition, functional fragments of VWF genes are disclosed. Within the context of this disclosure, a "functional fragment" or "fragment" of a VWF gene refers to a nucleic acid molecule that encodes a portion of a VWF polypeptide which is a domain described herein or at least specifically interacts with ADAMTS13 as a substrate for the cleavage activity of ADAMTS13. A functional fragment of the VWF gene need not

encode a polypeptide that contains each contiguous amino acid residue of the portion of VWF to which the functional fragment corresponds. In other words, the function fragment of VWF can align to a portion of native VWF and can include one or more of an insertion, a deletion or a substitution, so long as the functional fragment is a substrate to ADAMTS13 cleavage activity.

[00068] VWF is a large multimeric plasma glycoprotein crucial in the maintenance of hemostasis by functioning as both an antihemophilic factor carrier and a platelet-vessel wall mediator in the blood coagulation system, mainly by mediating tethering and adhesion of circulating platelets at sites of vascular injury. Mutations in this gene or deficiencies in this protein result in von Willebrand's disease (VWD).

[00069] VWF is expressed by endothelial cells and megakaryocytes. It is synthesized as 250-kDa monomers, which undergo intracellular processing, glycosylation, multimerization and propeptide removal that leads to formation of mature VWF multimers.

[00070] VWF multimeric size is modulated by the plasma metallopeptidase ADAMTS13 (a disintegrin and metallopeptidase with thrombospondin type I motif, member 13), which cleaves at a single site in the VWF A 2 domain (AA1498-1665; UniProtKB/Swiss-Pro database; Accession: P04275; FIG. 1) between Y1605 and M1606 (FIG. 2).

[00071] As described herein, a synthetic 55 (fifty five) amino acids (AA) in length polypeptide sequence designated as "GTI_FRET5" SEQ ID NO: 7 is disclosed, optionally modified with the insertion of a detectable label – such as a quencher and a fluorophore, that when recognized and cleaved by ADAMTS13 emits fluorescence. A synthetic 62 (sixty two) amino acids (AA) in length polypeptide sequence designated as "GTI_FRET4" SEQ ID NO: 1 is also disclosed, optionally modified with the insertion of a detectable label – such as a quencher and a fluorophore, that when recognized and cleaved by ADAMTS13 emits fluorescence.

[00072] Suitably, the polypeptide(s) are prepared using chemical synthesis techniques that are known in the art. The synthesis may utilize solid- or liquid-phase peptide synthesis. When modification of amino acid residues is required, modified amino acids can be introduced into a peptide synthesizer as appropriate.

[00073] It is also possible to produce the polypeptide substrates by recombinant procedures. Production of polypeptides by recombinant procedures can be carried out by methods well known to those skilled in the art, such as methods described by Sambrook, J., E. F. Fritsch, and T. Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

[00074] Suitably, the polypeptide(s) can be lyophilized polypeptide(s). Lyophilization can be carried out according to procedures known to those skilled in the art, such as methods described in U.S. Pat. No. 5,556,771 and references therein.

[00075] The activity of ADAMTS13 in a subject can be measured using the polypeptide substrate for ADAMTS13. For example, the polypeptide substrate can be contacted with a sample from a subject – such as plasma - and the resultant polypeptide fragments of the polypeptide substrate are analysed. Various methods in the art can be used to analyse the resultant polypeptide fragments including the use of SDS-polyacrylamide gel electrophoresis. The proteins are stained using, for example, Coomassie Blue or silver staining or the like and the fragments produced are analysed. Alternatively, it may be possible to carry out Western blotting following the SDS-PAGE. Suitably, the results are compared with a control sample and/or a calibrator sample. The control sample may be or may be derived from a subject who is known to have 'normal' activity of ADAMTS13, such that a diagnosis of abnormal activity can be made.

[00076] Although the detectable label may be directly attached to an amino acid residue of a polypeptide, a detectable label may also be indirectly attached, for example, by being complexed with a chelating group that is attached (for example, linked via a covalent bond or indirectly linked) to an amino acid residue of the polypeptide. In a particular embodiment, the "detectable label" is any type of label that only substantially releases a detectable signal once the polypeptide substrate is cleaved. Thus, the detectable label may

comprise a fluorescent resonance energy transfer (FRET) donor and/or acceptor. In one embodiment, the polypeptide substrate is modified by the incorporation or insertion of at least one quencher and at least one fluorophore, so that when recognized and cleaved by ADAMTS13 emits fluorescence. Suitably the substrate is a synthetic polypeptide (in contrast to a recombinant polypeptide) since this allows the direct incorporation of a quencher(s) and a fluorophore(s) therein. In the uncleaved substrate, fluorescence resonance energy transfer between the quencher and the fluorophore leads to low (for example, substantially no) fluorescence. Upon cleavage of the substrate by ADAMTS13, the quencher and fluorophore are separated which results in a detectable increase in fluorescence which can be measured.

[00077] Thus, in one embodiment, the polypeptide substrate includes a detectable label that allows the fragmentation of the polypeptide substrate to be measured directly. In one particular embodiment, the detectable label is a fluorophore and a quencher, wherein the quenching of the fluorophore is diminished as fragmentation occurs. Accordingly, fragmentation of the ADAMTS13 polypeptide substrate results in an increase in fluorescent signal. The cleavage of the substrate is detected by reading the fluorescence that results when the substrate is cleaved. According to the this embodiment of the invention, the skilled person will recognize that the polypeptide substrate will need to be synthesised by chemical synthesis techniques since recombinant approaches do not typically allow the incorporation of detectable labels therein.

[00078] The attachment site for the fluorophore and the quencher will typically be within the polypeptide substrate. Suitably, the fluorophore and the quencher will be separated from each other in such a manner that fluorescence from the fluorophore is substantially quenched when the polypeptide substrate is intact and fluorescence from the fluorophore is not quenched once the polypeptide substrate is cleaved. In one embodiment, the fluorophore and the quencher are separated by 8, 9, 10, 11 or 12 amino acids, suitably, the fluorophore and the quencher are separated by 9, 10, or 11 amino acids, more suitably, the fluorophore and the quencher are separated by 10 amino acids. In one embodiment, the attachment site for the fluorophore is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or the attachment site for the quencher is at the amino acid corresponding to position 1599 of SEQ ID NO: 2. It also contemplated that the positions of the fluorophore

and quencher are reversed such that the quencher is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or the attachment site for the fluorophore is at the amino acid corresponding to position 1599 of SEQ ID NO: 2.

[00079] Another aspect relates to a method for measuring the activity of ADAMTS13 in a sample, which comprises contacting the polypeptide substrate described herein with a sample from a subject and analyzing the fragmentation products thereof.

[00080] There is also disclosed a kit or a diagnostic composition for in vitro testing of the ADAMTS13 activity in a subject (for example, a decrease or deficiency of ADAMTS13 activity) and therefore the presence of TTP or the predisposition to TTP, or for making a definitive diagnosis of TTP and a discrimination between TTP and HUS. Mild or moderately decreased levels of ADAMTS13 activity have also been associated with other disease states and conditions (see, for example, Kokame et al. Blood (2004) 103, 607; and Kokame et al. Br. J. Haematol (2005) 129, 93). The kit or the composition comprises a polypeptide substrate for ADAMTS13 as described herein. Typically, the kit will also include a one or more positive controls and/or one or more calibrators. Typically, the kit will also include a specimen diluent and/or a substrate buffer (for example, a buffer solution whose pH corresponds to a pH range of 5.8 to 6.7 that is suitable for in vitro testing of the proposed polypeptide substrates.). A set of instructions may also be provided. Methods for carrying out the in vitro testing of the ADAMTS13 activity in a subject are known in the art (*see e.g.*, Miyata, T., K. Kokame, F. Banno, Y. Shin, and M. Akiyama. 2007. ADAMTS13 assays and ADAMTS13-deficient mice. *Curr.Opin.Hematol.* 14:277-283). Numerous vendors sell kits for detecting and/or determining the activity of ADAMTS-13 (*see e.g.*, FRETS-VWF73 (Peptides International, U.S.A., Cat# SFR-3224-s), TECHNOZYM® ADAMTS-13 INH ELISA (Kordia, Netherlands, Cat# TC 5450401), Human ADAMTS13 ELISA Kit and ADAMTS13 Antibody Agarose Immobilized (both available from Bethyl Laboratories, U.S.A., Cat#s E88-500 and S300-391) and IMUBIND® ADAMTS13 ELISA (American Diagnostica, GmbH, Germany, Cat# 813). Methods for collecting, transporting and processing blood specimens for coagulation testing and general performance of coagulation assays are known in the art (see for example, Approved Guideline H21-A4 NCCLS, Volume 23, Number 35, December 2003; Br.J.Haematol. 129:93-100 and Proc Natl Acad Sci U S A.

2006; 103: 18470-4.). The kit can also include an activator of ADAMTS13 – such as divalent metal ions.

[00081] The polypeptide substrate may have a tag sequence attached at the N-terminus and/or at the C-terminus thereof. The tag sequence may be useful in the detection, quantification, or separation of cleaved products. Also, the tag sequence may be useful for immobilizing the polypeptide substrate onto a solid phase. Thus, the present invention also encompasses polypeptide substrates which are immobilized onto a solid phase using such tag sequences. The tag sequence can include, but are not limited to, proteins (for example, glutathione transferase, luciferase, beta-galactosidase), peptides (for example, His tags), coupling agents (for example, carbodiimide reagents), various kinds of labels (for example, radioactive labels, chromophores, and enzymes).

[00082] In further embodiments, the present invention relates to use of the polypeptide substrate for producing the diagnostic composition or the kit as described above.

[00083] The disclosure is further described in the Examples below, which are provided to describe the invention in further detail. These examples, which set forth a preferred mode presently contemplated for carrying out the invention, are intended to illustrate and not to limit the invention.

[00084] EXAMPLES

[00085] Example 1: Evaluation of SEQ ID NO:1 polypeptide substrate (GTI_FRET4) as an ADAMTS13 substrate

[00086] Purpose:

[00087] The purpose of this experiment was to evaluate the polypeptide of SEQ ID NO: 1 (GTI_FRET4; 62AA; MW 7855.9; polypeptide purity 95.5%) for use as an ADAMTS13 substrate in a second generation ADAMTS13 assay.

[00088] Synopsis of the Procedure:

[00089] The procedure in this example was performed substantially as described in Kokame, K., Y. Nobe, Y. Kokubo, A. Okayama, and T. Miyata. 2005. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br.J.Haematol. 129:93-100*, but using SEQ ID NO: 1 in place of the 73 amino acid substrate described therein. The FRETS-VWF73 substrate solution was dissolved in 25% dimethyl sulphoxide/water to prepare the 100 microM stock solution. The GTI_FRET4 SEQ ID NO: 1 was dissolved in 100% DMSO. Both substrates were diluted to equal concentrations using ATS-13 substrate buffer (Gen-Probe GTI Diagnostics, Inc., U.S.A., Cat# ATS-13).

[00090] Plasma samples were diluted according to the ATS-13 Direction Insert using ATS-13 specimen diluent (Gen-Probe GTI Diagnostics, Inc., U.S.A., Cat# ATS-13). The diluted plasma samples were mixed with the diluted substrate and the fluorescence was read at 0, 5, 10, 15, 20, 30, 45 minutes using a Biotek FLX800 at the appropriate excitation and emission wavelengths for each substrate. The fluorescence values are reported in Table 1.

[00091] The fluorophore-quencher pair in the SEQ ID NO: 1 polypeptide substrate is FAM-5/TQ_2™ (Ex 485±20; Em 528±20; AAT Bioquest, Inc. Sunnyvale, CA U.S.A.). The fluorophore and quencher pair of FRETS-VWF73 (Nma/Dnp) described in Kokame has been substituted with FAM-5 and TQ_2 respectively in GTI_FRET4 SEQ ID NO: 1. Furthermore, in the SEQ ID NO: 1 polypeptide the position of the fluorophore (FAM-5) and quencher (TQ_2) has been swapped relative to the position of the fluorophore and quencher of the FRETS-VWF73 construct. Therefore, for GTI_FRET4 SEQ ID NO: 1, attachment of the fluorophore (FAM-5) occurs by substituting asparagine with cystine at position 15. The quencher (TQ_2) was attached by substituting glutamine with lysine at position 4 (Figure 2 and Table 4).

[00092] Results:

[00093] The results of this experiment demonstrate that by using SEQ ID NO: 1 polypeptide substrate, as compared to the prior art FRETS-VWF73 substrate, a larger dynamic range is obtained. In this experiment, at 30 minutes there was approximately 34,000 Relative Fluorescence Units (RFU) difference between Calibrator A (equivalent to 0% of ADAMTS13 activity) and Calibrator E (equivalent to 100% of ADAMTS13 activity)

compared to approximately 1500 to 2000 RFU difference for the FRETS-VWF73 substrate. See Table 1 and FIG. 3 and FIG. 4 for the change in fluorescence observed at all time points.

[00094] The calibration curves result in a linear trend line (see FIG. 4) compared to the FRETS-VWF73 assay which produces a calibration curve requiring a polynomial trend line (see FIG. 3). The calibration curve for the SEQ ID NO: 1 polypeptide substrate continued to be linear up through 45 minutes.

[00095] The % Normal (%N) activity (see Table 2) is calculated using the linear trend lines observed from the calibration curve at each time point. The %N ADAMTS13 activity calculated for each sample plateaus at 30 minutes and shows comparable results to FRETS-VWF73 after only 15 minutes.

[00096] Example 2: Direct comparison of SEQ ID NO: 1 polypeptide substrate (GTI_FRET4) and the prior art FRETS-VWF73 polypeptide substrate (Peptides International; Louisville, KY)

[00097] Purpose:

[00098] The purpose of this experiment is to compare the SEQ ID NO: 1 polypeptide substrate to FRETS-VWF73 (SEQ ID NO: 6).

[00099] Synopsis of the Procedure:

[000100] For this experiment, substrate concentration and fluorescence reader settings determined on the previous experiment are used. The specimens tested (listed in Table 3), include a panel of proficiency samples prepared for use with ATS-13 (Gen-Probe GTI Diagnostics, Inc., U.S.A., Cat# ATS-13) which included samples with normal or deficient ADAMTS13 activity levels. In addition, six Factor Assay ConTrol plasma were used (2 FACT, 2 A-FACT and 2 B-FACT, from George King Biomedical Inc., Kansas, USA). The assays for the SEQ ID NO: 1 substrate and for the prior art FRETS-VWF73 assay were performed generally as according to the procedure described in Example 1. Substrate is prepared according to the conditions used for initial testing of the substrate, which prepared the molar amount of SEQ ID NO: 1 polypeptide substrate used in the assay to be equivalent

to the molar amount of FRETS-VWF73 used in the prior art. The ELISA assays were read at 0, 5, 10, 15, 20, 30, 45 minutes.

[000101] Results

[000102] The results of these experiments confirm that the SEQ ID NO: 1 polypeptide substrate provides a much larger dynamic range compared to the FRETS-VWF73 substrate. At 30 minutes the difference between Calibrator A and E is approximately 35000 RFU compared to 2500 RFU observed for FRETS-VWF73 (FIG.s 3-4). The larger dynamic range would result in better sensitivity for samples with low ADAMTS13 activity. Moreover, when used at the same concentration as the FRETS-VWF73 substrate, the reaction time is faster. Consistent %N activity values are observed by the 15 minute reading (Table 2). The calibration curves are linear which would eliminate complicated analysis of results for the user.

[000103] Example 3: Evaluation of the cleavage of SEQ ID NO: 7 polypeptide substrate (GTI_FRET5).

[000104] The purpose of this experiment is to compare the SEQ ID NO: 7 polypeptide substrate with the SEQ ID NO: 1 polypeptide substrate.

[000105] Synopsis of the Procedure:

[000106] Testing of substrate for cleavability by ADAMTS13 is determined as is generally described in Example 1.

[000107] Results:

[000108] The change in fluorescence with time is shown in Figure 5 and demonstrates that cleavage of the SEQ ID NO: 7 polypeptide substrate occurs.

[000109] Example 4: Evaluation of the solubility of the SEQ ID NO: 7 polypeptide substrate and assay analysis.

[000110] The purpose of this experiment is to evaluate the solubility of the SEQ ID NO: 7 polypeptide substrate and to compare its performance with the SEQ ID NO:1 polypeptide substrate.

[000111] 250 .micro.L of working solution is prepared as above. The solution is vortexed vigorously and appears to be in solution. The solution is centrifuged at ~12,000g for about 2 minutes. After centrifugation, a very small pink pellet is noted at the bottom of the tube. This suggests that at least some amount of the material precipitates. Another tube is prepared as above substituting water for the substrate buffer. This tube is also centrifuged. Once again a pink pellet is observed in the bottom of the tube. The pellet observed in the water solution is noticeably larger than the pellet observed in the substrate buffer solution. This suggests that the polypeptide is less soluble in water than in the buffer.

The purpose of this experiment is to compare the SEQ ID NO: 7 GTI_FRET5 polypeptide substrate to GTI_FRET4 (SEQ ID NO: 1).Testing of substrate for cleavability by ADAMTS13 is determined as is generally described in Example 1 but using GTI_FRET5 instead of FRETS-VWF73.

[000112] The assay is read at 0, 5, 10, 15, 20, 30, 45, 60, and 90 minutes.

[000113] Results:

[000114] The data from this experiment demonstrates that the SEQ ID NO: 7 polypeptide substrate is not completely soluble in the working solution as prepared. However, the resulting calibration curve that is obtained is linear (see FIG. 5) and cleavage of the substrate occurs. A comparison of the activity obtained using SEQ ID NO:1 polypeptide substrate or SEQ ID NO:7 polypeptide substrate at 30 minutes post addition of substrate is shown in Table 5. The calibration curve for the SEQ ID NO: 7 polypeptide substrate continued to be linear up through 60 minutes.

[000115] Any publication cited or described herein provides relevant information disclosed prior to the filing date of the present application. Statements herein are not to be construed as an admission that the inventors are not entitled to antedate such disclosures. All publications mentioned in the above specification are herein incorporated by reference.

Various modifications and variations of the disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled the art are intended to be within the scope of the following claims.

TABLE 1

Change in fluorescence observed using the SEQ ID NO: 1 peptide substrate

Increase in Fluorescence Values at Each Time Point (X minute - 0 minute Reading)						
Sample ID	**15 min**	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Calibrator A	676	843	921	1,166	1,013	959
Calibrator B	1,765	3,000	4,164	5,358	6,468	7,581
Calibrator C	6,178	11,701	16,807	21,705	26,685	32,209
Calibrator D	10,887	20,626	29,206	37,289	44,543	53,613
Calibrator E	18,718	35,085	49,418	61,068	70,603	82,999
Positive Control High	9,146	17,281	24,859	31,857	39,838	47,473
Positive Control Low	2,182	3,902	5,511	7,169	8,789	10,370
VF	1,292	2,436	3,552	4,690	5,784	7,260
UAMS041609	3,876	7,931	11,692	15,886	19,867	24,709
MON110707	17,881	33,432	46,848	58,747	68,370	78,371

TABLE 2

Calculated %N activity using the linear trend line obtained for each time point using the SEQ ID NO: 1 peptide substrate

	Assigned/Expected %Normal ADAMTS13 Activity Values Based on FRETS-VWF73 Substrate	**% Normal ADAMTS13 Values Calculated Using GTI_FRET4 Substrate**					
Incubation Time	30 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes	75 Minutes	90 Minutes
Sample ID							
Calibrator A	0	1	1	0	0	0	0
Calibrator B	9	7	7	7	7	6	6
Calibrator C	34	32	33	33	34	35	36
Calibrator D	55	58	59	59	60	61	62
Calibrator E	102	101	101	100	100	98	98
Positive Control High	36-54	48	49	50	51	54	55
Positive Control Low	6-17	10	10	10	10	10	9
VF	~20	5	6	6	6	5	6
UAMS041609	~35	19	22	23	24	26	27
MON110707	~90-100	97	96	95	96	95	92

TABLE 3

Materials used performing the examples

Material	Manufacturer (Cat. No.) or Associated Date	Lot No.
ATS-13 Calibrators/Controls	GTI	CA-CE020410, PCH020410, PCL020410
Substrate buffer (SBA)	GTI	SBA011810
Specimen diluent (SDA)	GTI	SDA011810
Substrate (SA)	GTI	SA112509
Plate	GTI	ATS-011410
DMSO	Sigma (D8418)	038K07101
Normal Pooled Plasma	GTI	NPP032206
ATS-13 Proficiency Samples 1-5	GTI	020910-ATS
A-FACT plasma	George King BioMedical (A-FACT)	1284
A-FACT plasma	George King BioMedical (A-FACT)	900
B-FACT plasma	George King BioMedical (B-FACT)	1114
B-FACT plasma	George King BioMedical (B-FACT)	1266
FACT plasma	George King BioMedical (FACT)	1223
FACT plasma	George King BioMedical (FACT)	222e1
VF	04/09/01	03/08/10
BCM2	07/22/08	07/22/08
UAMS041609	04/16/09	04/16/09
ATS AC and AB CNTL	05/14/209	2051008

TABLE 4

Amino acid sequences

SEQ ID NO:	Sequence. N-terminus to C-Terminus.	note
1	DREKAPNLVYMVTGCPASDEIKRLPGDIQVVPIEVIGWPNAPILIQDFETLP REAPDLVLQR	GTI_FRET4
2	MIPARFAGVLLALALILPGTLCAEGTRGRSSTARCSLFGSDFVNTFDGSMYS FAGYCSYLLAGGCQKRSFSIIGDFQNGKRVSLSVYLGEFFDIHLFVNGTVTQ GDQRVSMPYASKGLYLETEAGYYKLSGEAYGFVARIDGSGNFQVLLSDRYFN KTCGLCGNFNIFAEDDFMTQEGTLTSDPYDFANSWALSSGEQWCERASPPSS SCNISSGEMQKGLWEQCQLLKSTSVFARCHPLVDPEPFVALCEKTLCECAGG LECACPALLEYARTCAQEGMVLYGWTDHSACSPVCPAGMEYRQCVSPCARTC QSLHINEMCQERCVDGCSCPEGQLLDEGLCVESTECPCVHSGKRYPPGTSLS RDCNTCICRNSQWICSNEECPGECLVTGQSHFKSFDNRYFTFSGICQYLLAR DCQDHSFSIVIETVQCADDRDAVCTRSVTVRLPGLHNSLVKLKHGAGVAMDG QDVQLPLLKGDLRIQHTVTASVRLSYGEDLQMDWDGRGRLLVKLSPVYAGKT CGLCGNYNGNQGDDFLTPSGLAEPRVEDFGNAWKLHGDCQDLQKQHSDPCAL NPRMTRFSEEACAVLTSPTFEACHRAVSPLPYLRNCRYDVCSCSDGRECLCG ALASYAAACAGRGVRVAWREPGRCELNCPKGQVYLQCGTPCNLTCRSLSYPD EECNEACLEGCFCPPGLYMDERGDCVPKAQCPCYYDGEIFQPEDIFSDHHTM CYCEDGFMHCTMSGVPGSLLPDAVLSSPLSHRSKRSLSCRPPMVKLVCPADN LRAEGLECTKTCQNYDLECMSMGCVSGCLCPPGMVRHENRCVALERCPCFHQ GKEYAPGETVKIGCNTCVCQDRKWNCTDHVCDATCSTIGMAHYLTFDGLKYL FPGECQYVLVQDYCGSNPGTFRILVGNKGCSHPSVKCKKRVTILVEGGEIEL FDGEVNVKRPMKDETHFEVVESGRYIILLLGKALSVVWDRHLSISVVLKQTY QEKVCGLCGNFDGIQNNDLTSSNLQVEEDPVDFGNSWKVSSQCADTRKVPLD SSPATCHNNIMKQTMVDSSCRILTSDVFQDCNKLVDPEPYLDVCIYDTCSCE SIGDCACFCDTIAAYAHVCAQHGKVVTWRTATLCPQSCEERNLRENGYECEW RYNSCAPACQVTCQHPEPLACPVQCVEGCHAHCPPGKILDELLQTCVDPEDC PVCEVAGRRFASGKKVTLNPSDPEHCQICHCDVVNLTCEACQEPGGLVVPPT DAPVSPTTLYVEDISEPPLHDFYCSRLLDLVFLLDGSSRLSEAEFEVLKAFV VDMMERLRISQKWVRVAVVEYHDGSHAYIGLKDRKRPSELRRIASQVKYAGS QVASTSEVLKYTLFQIFSKIDRPEASRITLLLMASQEPQRMSRNFVRYVQGL KKKKVIVIPVGIGPHANLKQIRLIEKQAPENKAFVLSSVDELEQQRDEIVSY LCDLAPEAPPPTLPPDMAQVTVGPGLLGVSTLGPKRNSMVLDVAFVLEGSDK IGEADFNRSKEFMEEVIQRMDVGQDSIHVTVLQYSYMVTVEYPFSEAQSKGD	1-22 Signal Peptide; 23-763 von willebrand antigen II; 764-2813 vwf.

SEQ ID NO:	Sequence. N-terminus to C-Terminus.	note
	ILQRVREIRYQGGNRTNTGLALRYLSDHSFLVSQGDREQAPNLVYMVTGNPA SDEIKRLPGDIQVVPIGVGPNANVQELERIGWPNAPILIQDFETLPREAPDL VLQRCCSGEGLQIPTLSPAPDCSQPLDVILLLDGSSSFPASYFDEMKSFAKA FISKANIGPRLTQVSVLQYGSITTIDVPWNVVPEKAHLLSLVDVMQREGGPS QIGDALGFAVRYLTSEMHGARPGASKAVVILVTDVSVDSVDAAADAARSNRV TVFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDLPTMVTLGNSFLHKLC SGFVRICMDEDGNEKRPGDVWTLPDQCHTVTCQPDGQTLLKSHRVNCDRGLR PSCPNSQSPVKVEETCGCRWTCPCVCTGSSTRHIVTFDGQNFKLTGSCSYVL FQNKEQDLEVILHNGACSPGARQGCMKSIEVKHSALSVELHSDMEVTVNGRL VSVPYVGGNMEVNVYGAIMHEVRFNHLGHIFTFTPQNNEFQLQLSPKTFASK TYGLCGICDENGANDFMLRDGTVTTDWKTLVQEWTVQRPGQTCQPILEEQCL VPDSSHCQVLLLPLFAECHKVLAPATFYAICQQDSCHQEQVCEVIASYAHLC RTNGVCVDWRTPDFCAMSCPPSLVYNHCEHGCPRHCDGNVSSCGDHPSEGCF CPPDKVMLEGSCVPEEACTQCIGEDGVQHQFLEAWVPDHQPCQICTCLSGRK VNCTTQPCPTAKAPTCGLCEVARLRQNADQCCPEYECVCDPVSCDLPPVPHC ERGLQPTLTNPGECRPNFTCACRKEECKRVSPPSCPPHRLPTLRKTQCCDEY ECACNCVNSTVSCPLGYLASTATNDCGCTTTTCLPDKVCVHRSTIYPVGQFW EEGCDVCTCTDMEDAVMGLRVAQCSQKPCEDSCRSGFTYVLHEGECCGRCLP SACEVVTGSPRGDSQSSWKSVGSQWASPENPCLINECVRVKEEVFIQQRNVS CPQLEVPVCPSGFQLSCKTSACCPSCRCERMEACMLNGTVIGPGKTVMIDVC TTCRCMVQVGVISGFKLECRKTTCNPCPLGYKEENNTGECCGRCLPTACTIQ LRGGQIMTLKRDETLQDGCDTHFCKVNERGEYFWEKRVTGCPPFDEHKCLAE GGKIMKIPGTCCDTCEEPECNDITARLQYVKVGSCKSEVEVDIHYCQGKCAS KAMYSIDINDVQDQCSCCSPTRTEPMQVALHCTNGSVVYHEVLNAMECKCSP RKCSK	

SEQ ID NO:	Sequence. N-terminus to C-Terminus.	note
3	MHQRHPRARCPPLCVAGILACGFLLGCWGPSHFQQSCLQALEPQAVSSYLSP GAPLKGRPPSPGFQRQRQRQRRAAGGILHLELLVAVGPDVFQAHQEDTERYV LTNLNIGAELLRDPSLGAQFRVHLVKMVILTEPEGAPNITANLTSSLLSVCG WSQTINPEDDTDPGHADLVLYITRFDLELPDGNRQVRGVTQLGGACSPTWSC LITEDTGFDLGVTIAHEIGHSFGLEHDGAPGSGCGPSGHVMASDGAAPRAGL AWSPCSRRQLLSLLSAGRARCVWDPPRPQPGSAGHPPDAQPGLYYSANEQCR VAFGPKAVACTFAREHLDMCQALSCHTDPLDQSSCSRLLVPLLDGTECGVEK WCSKGRCRSLVELTPIAAVHGRWSSWGPRSPCSRSCGGGVVTRRRQCNNPRP AFGGRACVGADLQAEMCNTQACEKTQLEFMSQQCARTDGQPLRSSPGGASFY HWGAAVPHSQGDALCRHMCRAIGESFIMKRGDSFLDGTRCMPSGPREDGTLS LCVSGSCRTFGCDGRMDSQQVWDRCQVCGGDNSTCSPRKGSFTAGRAREYVT FLTVTPNLTSVYIANHRPLFTHLAVRIGGRYVVAGKMSISPNTTYPSLLEDG RVEYRVALTEDRLPRLEEIRIWGPLQEDADIQVYRRYGEEYGNLTRPDITFT YFQPKPRQAWVWAAVRGPCSVSCGAGLRWVNYSCLDQARKELVETVQCQGSQ QPPAWPEACVLEPCPPYWAVGDFGPCSASCGGGLRERPVRCVEAQGSLLKTL PPARCRAGAQQPAVALETCNPQPCPARWEVSEPSSCTSAGGAGLALENETCV PGADGLEAPVTEGPGSVDEKLPAPEPCVGMSCPPGWGHLDATSAGEKAPSPW GSIRTGAQAAHVWTPAAGSCSVSCGRGLMELRFLCMDSALRVPVQEELCGLA SKPGSRREVCQAVPCPARWQYKLAACSVSCGRGVVRRILYCARAHGEDDGEE ILLDTQCQGLPRPEPQEACSLEPCPPRWKVMSLGPCSASCGLGTARRSVACV QLDQGQDVEVDEAACAALVRPEASVPCLIADCTYRWHVGTWMECSVSCGDGI QRRRDTCLGPQAQAPVPADFCQHLPKPVTVRGCWAGPCVGQGTPSLVPHEEA AAPGRTTATPAGASLEWSQARGLLFSPAPQPRRLLPGPQENSVQSSACGRQH LEPTGTIDMRGPGQADCAVAIGRPLGEVVTLRVLESSLNCSAGDMLLLWGRL TWRKMCRKLLDMTFSSKTNTLVVRQRCGRPGGGVLLRYGSQLAPETFYRECD MQLFGPWGEIVSPSLSPATSNAGGCRLFINVAPHARIAIHALATNMGAGTEG ANASYILIRDTHSLRTTAFHGQQVLYWESESSQAEMEFSEGFLKAQASLRGQ YWTLQSWVPEMQDPQSWKGKEGT	ADAMTS13 Isoform 1. 1-29 signal Peptide; 30-74 propeptide; 75-1427 ADAMTS13 chain.

SEQ ID NO:	Sequence. N-terminus to C-Terminus.	note
4	MHQRHPRARCPPLCVAGILACGFLLGCWGPSHFQQSCLQALEPQAVSSYLSP GAPLKGRPPSPGFQRQRQRQRRAAGGILHLELLVAVGPDVFQAHQEDTERYV LTNLNIGAELLRDPSLGAQFRVHLVKMVILTEPEGAPNITANLTSSLLSVCG WSQTINPEDDTDPGHADLVLYITRFDLELPDGNRQVRGVTQLGGACSPTWSC LITEDTGFDLGVTIAHEIGHSFGLEHDGAPGSGCGPSGHVMASDGAAPRAGL AWSPCSRRQLLSLLSAGRARCVWDPPRPQPGSAGHPPDAQPGLYYSANEQCR VAFGPKAVACTFAREHLDMCQALSCHTDPLDQSSCSRLLVPLLDGTECGVEK WCSKGRCRSLVELTPIAAVHGRWSSWGPRSPCSRSCGGGVVTRRRQCNNPRP AFGGRACVGADLQAEMCNTQACEKTQLEFMSQQCARTDGQPLRSSPGGASFY HWGAAVPHSQGDALCRHMCRAIGESFIMKRGDSFLDGTRCMPSGPREDGTLS LCVSGSCRTFGCDGRMDSQQVWDRCQVCGGDNSTCSPRKGSFTAGRAREYVT FLTVTPNLTSVYIANHRPLFTHLAVRIGGRYVVAGKMSISPNTTYPSLLEDG RVEYRVALTEDRLPRLEEIRIWGPLQEDADIQVYRRYGEEYGNLTRPDITFT YFQPKPRQAWVWAAVRGPCSVSCGAGLRWVNYSCLDQARKELVETVQCQGSQ QPPAWPEACVLEPCPPYWAVGDFGPCSASCGGGLRERPVRCVEAQGSLLKTL PPARCRAGAQQPAVALETCNPQPCPARWEVSEPSSCTSAGGAGLALENETCV PGADGLEAPVTEGPGSVDEKLPAPEPCVGMSCPPGWGHLDATSAGEKAPSPW GSIRTGAQAAHVWTPAAGSCSVSCGRGLMELRFLCMDSALRVPVQEELCGLA SKPGSRREVCQAVPCPARWQYKLAACSVSCGRGVVRRILYCARAHGEDDGEE ILLDTQCQGLPRPEPQEACSLEPCPPRWKVMSLGPCSASCGLGTARRSVACV QLDQGQDVEVDEAACAALVRPEASVPCLIADCTYRWHVGTWMECSVSCGDGI QRRRDTCLGPQAQAPVPADFCQHLPKPVTVRGCWAGPCVGQGACGRQHLEPT GTIDMRGPGQADCAVAIGRPLGEVVTLRVLESSLNCSAGDMLLLWGRLTWRK MCRKLLDMTFSSKTNTLVVRQRCGRPGGGVLLRYGSQLAPETFYRECDMQLF GPWGEIVSPSLSPATSNAGGCRLFINVAPHARIAIHALATNMGAGTEGANAS YILIRDTHSLRTTAFHGQQVLYWESESSQAEMEFSEGFLKAQASLRGQYWTL QSWVPEMQDPQSWKGKEGT	ADAMTS13 Isoform 2.

SEQ ID NO:	Sequence. N-terminus to C-Terminus.	note
5	MHQRHPRARCPPLCVAGILACGFLLGCWGPSHFQQSCLQALEPQAVSSYLSP GAPLKGRPPSPGFQRQRQRQRRAAGGILHLELLVAVGPDVFQAHQEDTERYV LTNLNIGAELLRDPSLGAQFRVHLVKMVILTEPEGAPNITANLTSSLLSVCG WSQTINPEDDTDPGHADLVLYITRFDLELPDGNRQVRGVTQLGGACSPTWSC LITEDTGFDLGVTIAHEIGHSFGLEHDGAPGSGCGPSGHVMASDGAAPRAGL AWSPCSRRQLLSLLSANEQCRVAFGPKAVACTFAREHLDMCQALSCHTDPLD QSSCSRLLVPLLDGTECGVEKWCSKGRCRSLVELTPIAAVHGRWSSWGPRSP CSRSCGGGVVTRRRQCNNPRPAFGGRACVGADLQAEMCNTQACEKTQLEFMS QQCARTDGQPLRSSPGGASFYHWGAAVPHSQGDALCRHMCRAIGESFIMKRG DSFLDGTRCMPSGPREDGTLSLCVSGSCRTFGCDGRMDSQQVWDRCQVCGGD NSTCSPRKGSFTAGRAREYVTFLTVTPNLTSVYIANHRPLFTHLAVRIGGRY VVAGKMSISPNTTYPSLLEDGRVEYRVALTEDRLPRLEEIRIWGPLQEDADI QVYRRYGEEYGNLTRPDITFTYFQPKPRQAWVWAAVRGPCSVSCGAGLRWVN YSCLDQARKELVETVQCQGSQQPPAWPEACVLEPCPPYWAVGDFGPCSASCG GGLRERPVRCVEAQGSLLKTLPPARCRAGAQQPAVALETCNPQPCPARWEVS EPSSCTSAGGAGLALENETCVPGADGLEAPVTEGPGSVDEKLPAPEPCVGMS CPPGWGHLDATSAGEKAPSPWGSIRTGAQAAHVWTPAAGSCSVSCGRGLMEL RFLCMDSALRVPVQEELCGLASKPGSRREVCQAVPCPARWQYKLAACSVSCG RGVVRRILYCARAHGEDDGEEILLDTQCQGLPRPEPQEACSLEPCPPRWKVM SLGPCSASCGLGTARRSVACVQLDQGQDVEVDEAACAALVRPEASVPCLIAD CTYRWHVGTWMECSVSCGDGIQRRRDTCLGPQAQAPVPADFCQHLPKPVTVR GCWAGPCVGQGACGRQHLEPTGTIDMRGPGQADCAVAIGRPLGEVVTLRVLE SSLNCSAGDMLLLWGRLTWRKMCRKLLDMTFSSKTNTLVVRQRCGRPGGGVL LRYGSQLAPETFYRECDMQLFGPWGEIVSPSLSPATSNAGGCRLFINVAPHA RIAIHALATNMGAGTEGANASYILIRDTHSLRTTAFHGQQVLYWESESSQAE MEFSEGFLKAQASLRGQYWTLQSWVPEMQDPQSWKGKEGT	ADAMTS13 Isoform 3.
6	DREQAPNLVYMVTGNPASDEIKRLPGDIQVVPIGVGPNANVQELERIGWPNA PILIQDFETLPREAPDLVLQR	FRETS-VWF73
7	DREAPNLVYMVTGCPASDEIKRLPGDIIGWPNAPILIQDFETLPREAPDLVL QR	GTI_FRET5

TABLE 5

A comparison of the activity obtained using SEQ ID NO:1 polypeptide substrate versus SEQ ID NO:7 polypeptide substrate at 30 minutes post addition of substrate

%Normal ADAMTS13 Activity: 30 Minute Incubation Time		
Sample ID	**GTI_FRET5 Substrate**	**GTI_FRET4 Substrate**
Calibrator A	3	4
Calibrator B	8	9
Calibrator C	30	29
Calibrator D	64	61
Calibrator E	108	110
Positive Control High	46	50
Positive Control Low	13	14
90 (ATS13-1)	65	92
72 (ATS13-2)	52	72
50 (ATS13-3)	40	47
22 (ATS13-4)	18	21
5 (ATS13-5)	6	7
UAMS041609	14	31
BCM2	22	19
VF040901	10	10
CNTL	<Calibrator A	<Calibrator A
NPP032206	84	>Calibrator E
NPP032206 HI	12	9
NPP032206 mixed	<Calibrator A	57
BCM2 HI	15	12
BCM2 mixed	37	51
CNTL HI	<Calibrator A	<Calibrator A
CNTL mixed	<Calibrator A	4
NPP032206 at 37C	77	106
A-FACT lot 1284	<Calibrator A	10
A-FACT lot 900	<Calibrator A	10
B-FACT lot 1114	28	45
B-FACT lot 1266	23	46
FACT lot 1223	82	107
FACT lot 222e1	85	111

CLAIMS

1. An isolated polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) that is from 45 to 70 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications:

(i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K;

(ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C; and

(iii) the amino acids corresponding to Q1624 to R1641 of SEQ ID NO: 2 are deleted.

2. An ADAMTS13 polypeptide substrate that is from 50 to 75 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications:

(i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K;

(ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C;

(iii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and

(iv) the amino acids corresponding to G1631 to R1641 of SEQ ID NO: 2 are deleted.

3. The ADAMTS13 polypeptide substrate according to claim 1 or claim 2, wherein the amino acid at the N-terminus of said polypeptide substrate corresponds to D1596 of SEQ ID NO: 2.

4. The ADAMTS13 polypeptide substrate according to any of claims 1 to 3, wherein the amino acid at the C-terminus of said polypeptide substrate corresponds to R1668 of SEQ ID NO: 2.

5. The ADAMTS13 polypeptide substrate according to any of the preceding claims, wherein said polypeptide is a synthetic polypeptide that comprises a detectable label.

6. The ADAMTS13 polypeptide substrate according to claim 5, wherein the detectable label is a fluorophore and a quencher.

7. The ADAMTS13 polypeptide substrate according to claim 6, wherein the attachment site for the fluorophore is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or wherein the attachment site for the quencher is at the amino acid corresponding to position 1599 of SEQ ID NO: 2 or wherein attachment site for the quencher is at the amino acid corresponding to position 1610 of SEQ ID NO: 2 and/or wherein the attachment site for the flurophore is at the amino acid corresponding to position 1599 of SEQ ID NO: 2.

8. The ADAMTS13 polypeptide substrate according to any of claims 1 and 3 to 7, comprising, consisting or consisting essentially of the sequence set forth in SEQ ID NO: 7.

9. The ADAMTS13 polypeptide substrate according to any of claims 2 to 7, comprising, consisting or consisting essentially of the sequence set forth in SEQ ID NO: 1.

10. A lyophilized polypeptide substrate, wherein the substrate is from 45 to 70 amino acids in length and has an amino acid sequence that is substantially similar to part of the von Willebrand factor A2 domain sequence set forth in SEQ ID NO:2, with one or more of the following modifications:

(i) the amino acid corresponding to position 1599 of SEQ ID NO: 2 is mutated from Q to K;

(ii) the amino acid corresponding to position 1610 of SEQ ID NO: 2 is mutated from N to C;

(iii) the amino acid corresponding to position 1629 of SEQ ID NO: 2 is mutated from G to E; and

(iv) the amino acids corresponding to Q1624 to R1641 of SEQ ID NO: 2 are deleted.

11. A lyophilized polypeptide substrate for a disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 13 (ADAMTS13) according to any one of claims 1 to 9.

12. A method for cleaving the ADAMTS13 polypeptide substrate according to any of claims 1 to 11, comprising contacting said ADAMTS13 polypeptide substrate with an ADAMTS13 protease.

13. A method for measuring ADAMTS13 activity in a sample comprising the use of the ADAMTS13 polypeptide substrate according to any of claims 1 to 11.

14. The method according to claim 13, comprising the steps of:

(a) providing a sample comprising, or suspected of comprising, an ADAMTS13;

(b) contacting said sample with the ADAMTS13 polypeptide substrate according to any of claims 1 to 11; and

(c) determining the fragmentation of the ADAMTS13 polypeptide substrate,

wherein the fragmentation of the ADAMTS13 polypeptide substrate is optionally compared to one or more controls and/or calibrators in order to arrive at a measurement of ADAMTS13 activity.

15. The method according to claim 14, wherein the cleavage of the ADAMTS13 polypeptide substrate is measured by monitoring the change in fluorescence.

16. The method according to claim 14, wherein the ADAMTS13 polypeptide substrate is in solution in step (b).

17. The method according to claim 14, wherein the ADAMTS13 polypeptide substrate attached to a solid support.

18. A method for the quantitative measurement of ADAMTS13 protease activity, comprising the steps of:

(a) providing a plasma sample comprising, or suspected of comprising, an ADAMTS13;

(b) contacting said sample with the ADAMTS13 polypeptide substrate according to any of claims 1 to 11; and

(c) determining the fragmentation of the ADAMTS13 polypeptide substrate,

wherein the fragmentation of the ADAMTS13 polypeptide substrate is optionally compared to one or more controls and/or calibrators in order to arrive at a measurement of ADAMTS13 activity.

19. The method according to claim 18, wherein the ADAMTS13 polypeptide substrate is in solution in step (b).

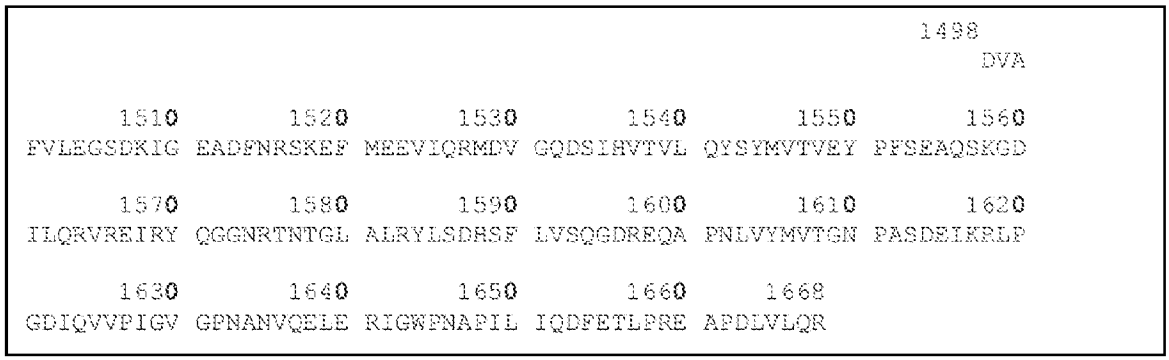
20. The method according to claim 18, wherein the ADAMTS13 polypeptide substrate attached to a solid support.

21. A kit for *in vitro* testing of ADAMTS13 activity in a subject, comprising the ADAMTS13 polypeptide substrate according to any of claims 1 to 11, one or more calibrators containing a known concentration of ADAMTS13 activity and/or one or more positive controls for ADAMTS13 activity optionally together with a specimen diluent and/or a substrate buffer.

22. Use of the ADAMTS13 polypeptide substrate according to any of claims 1 to 11 for measuring the activity of ADAMTS13 in a sample.

1/5

FIGURE 1


1498
DVA
1510 1520 1530 1540 1550 1560
FVLEGSDKIG EADFNRSKEF MEEVIQRMDV GQDSIHVTVL QYSYMVTVEY PFSEAQSKGD
1570 1580 1590 1600 1610 1620
ILQRVREIRY QGGNRTNTGL ALRYLSDHSF LVSQGDREQA PNLVYMVTGN PASDEIKRLP
1630 1640 1650 1660 1668
GDIQVVPIGV GPNANVQELE RIGWPNAPIL IQDFETLPRE APDLVLQR

2/5

FIGURE 2

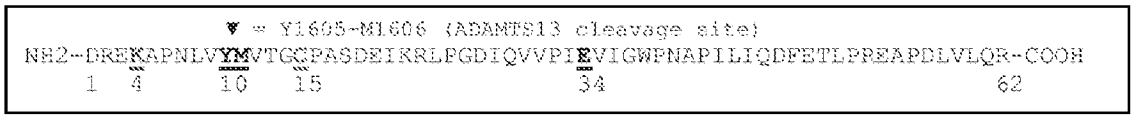

▼ = Y1605-M1606 (ADAMTS13 cleavage site)
NH2-DREKAPNLVYMVTGQPASDEIKRLPGDIQVVPIEVIGWPNAPILIQDFETLPREAPDLVLQR-COOH
1 4 10 15 34 62

FIGURE 3

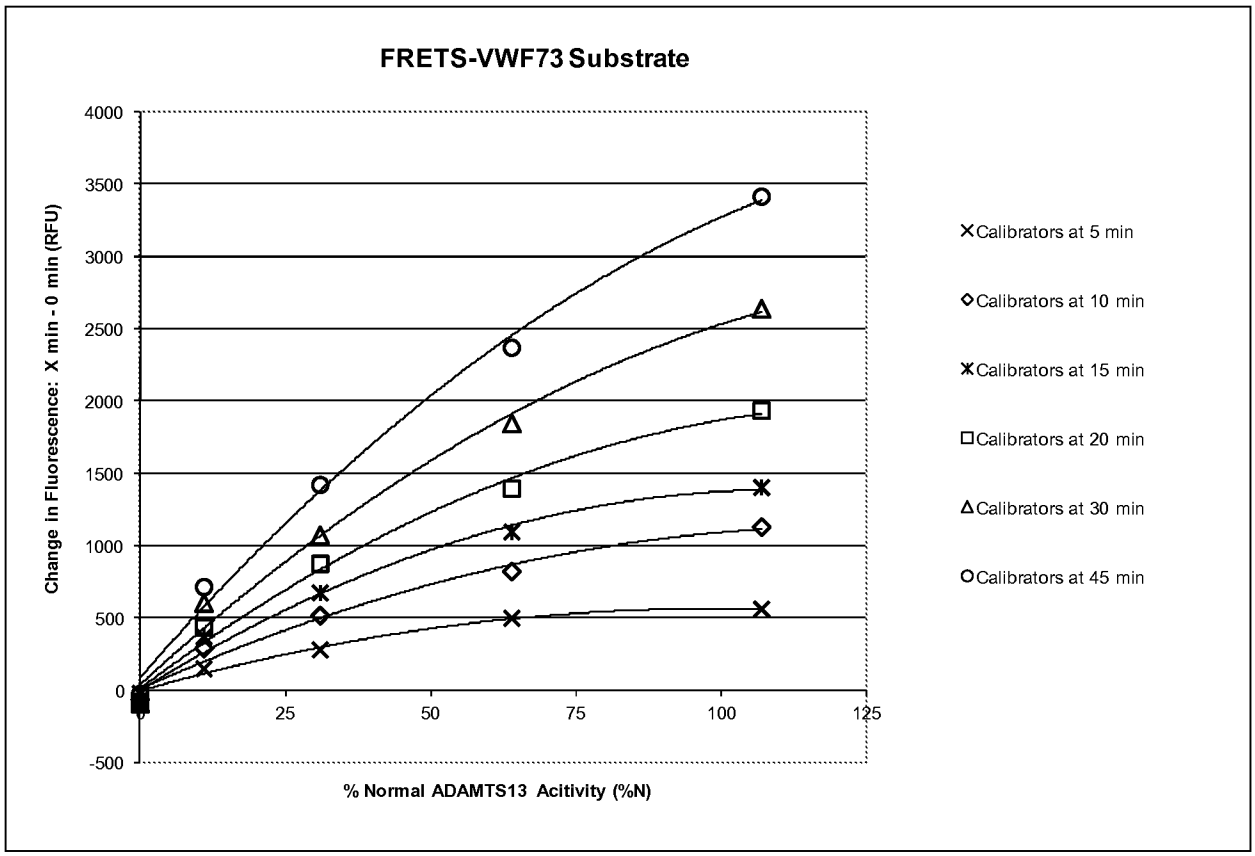

FRETS-VWF73 Substrate
Change in Fluorescence: X min - 0 min (RFU)
4000
3500
3000
2500
2000
1500
1000
500
0
-500
25
50
75
100
125
% Normal ADAMTS13 Acitivity (%N)
Calibrators at 5 min
Calibrators at 10 min
Calibrators at 15 min
Calibrators at 20 min
Calibrators at 30 min
Calibrators at 45 min

FIGURE 4

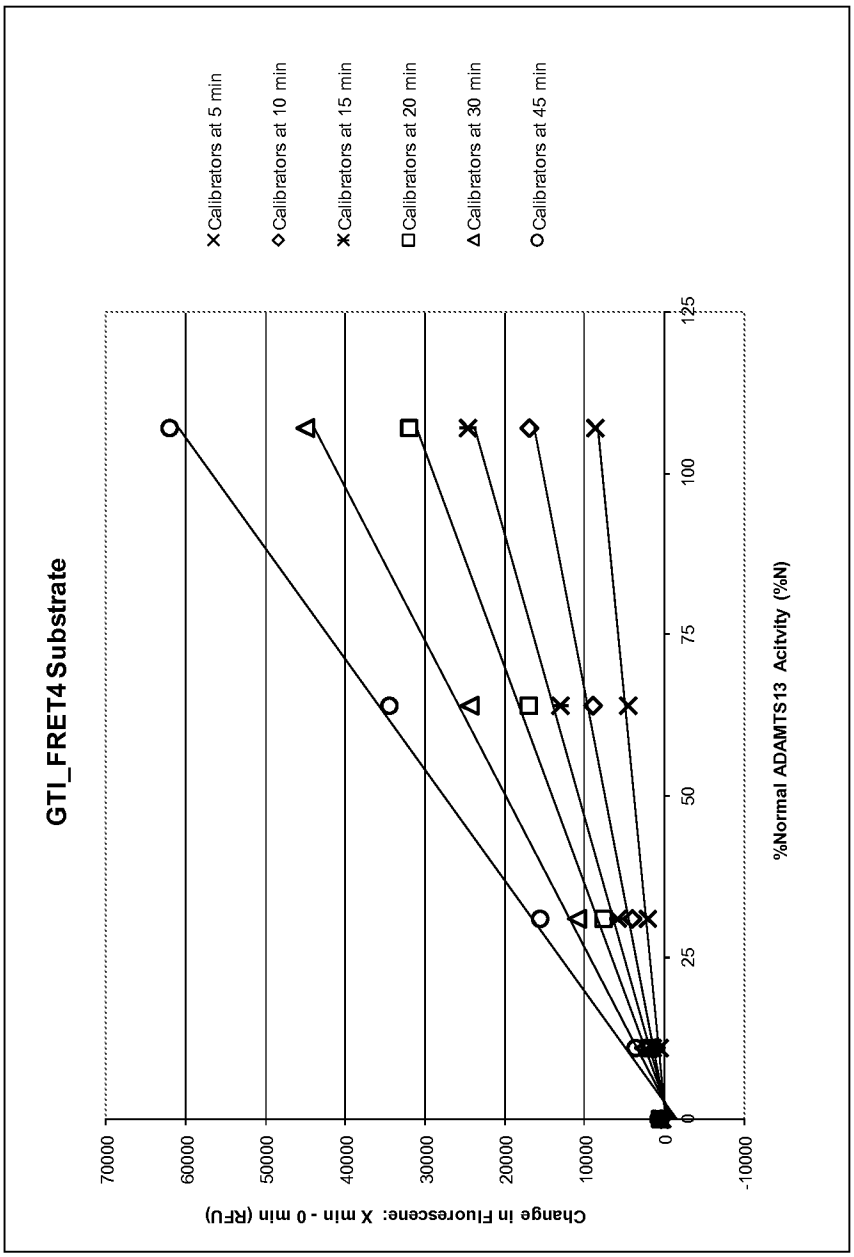
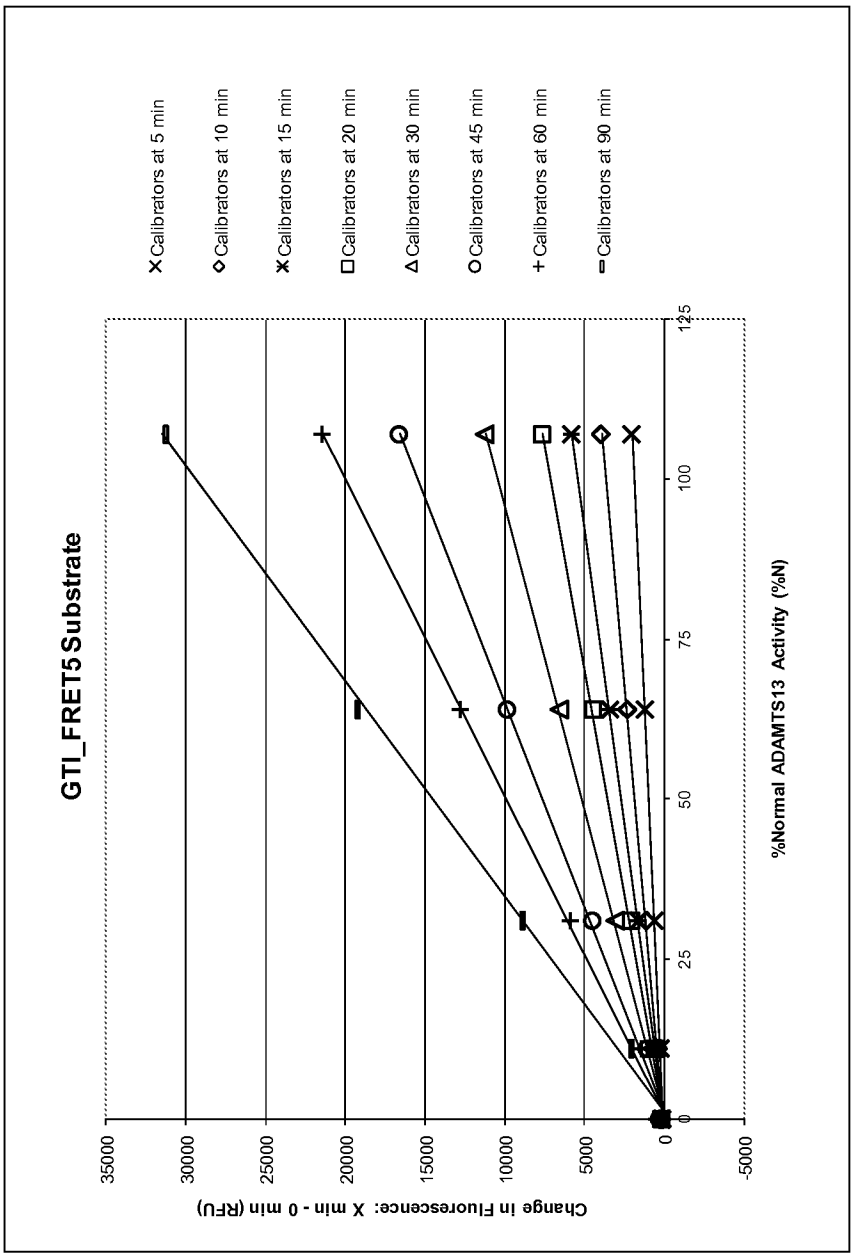

GTI_FRET4 Substrate
Calibrators at 5 min
Calibrators at 10 min
Calibrators at 15 min
Calibrators at 20 min
Calibrators at 30 min
Calibrators at 45 min
Change in Fluorescene: X min - 0 min (RFU)
70000
60000
50000
40000
30000
20000
10000
0
-10000
0
25
50
75
100
125
%Normal ADAMTS13 Acitvity (%N)

FIGURE 5


GTI_FRET5 Substrate
Calibrators at 5 min
Calibrators at 10 min
Calibrators at 15 min
Calibrators at 20 min
Calibrators at 30 min
Calibrators at 45 min
Calibrators at 60 min
Calibrators at 90 min
Change in Fluorescence: X min - 0 min (RFU)
35000
30000
25000
20000
15000
10000
5000
0
-5000
%Normal ADAMTS13 Activity (%N)
125
100
75
50
25
0

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/064526

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/755 C12Q1/37
ADD.

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)
C07K C12Q

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)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOICHI KOKAME ET AL: "FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay", BRITISH JOURNAL OF HAEMATOLOGY, vol. 129, no. 1, 14 March 2005 (2005-03-14), pages 93-100, XP055052565, ISSN: 0007-1048, DOI: 10.1111/j.1365-2141.2005.05420.x abstract page 94, left-hand column, paragraph 2 page 98, left-hand column, paragraph 4 - right-hand column, line 2 figure 1 ----- -/--	1-22

[X] 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
7 February 2013	15/02/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Tudor, Mark

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/064526

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KANNAYAKANAHALLI M DAYANANDA ET AL: "-derived von Willebrand factor-A2 domain fluorescence/Frster resonance energy transfer proteins that quantify ADAMTS13 activity", ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS INC, NEW YORK, vol. 410, no. 2, 6 December 2010 (2010-12-06), pages 206-213, XP028146262, ISSN: 0003-2697, DOI: 10.1016/J.AB.2010.12.005 [retrieved on 2010-12-10] abstract page 206, right-hand column, paragraph 3 - page 207, left-hand column, paragraph 1 page 208, right-hand column, paragraph 4 - page 209, left-hand column, line 4 figure 1A -----	1-22