ADAMTS13 DTCS (residues 287-685) (Akiyama M., Takeda S., Kokame K., Takagi J., Miyata T. 2009 Crystal structures of the noncatalytic domains of ADAMTS13 reveal multiple discontinuous exosites for von Willebrand factor, Proceedings of the National Academy of Sciences 106: 19274-19279, which is incorporated herein by reference in its entirety for all purposes). Structural analyses indicate that ADAMTS family members share sequence conservation and structure similarity of MDTCS domains (Akiyama et al., 2009 *supra*; Mosyak L., Georgiadis K., Shane T., Svenson K. et al. 2008 Crystal structures of the two major aggrecan degrading enzymes, ADAMTS4 and ADAMTS5, Protein Science 17: 16-21, each of which is incorporated herein by reference in their entirety for all purposes). The VWF-proteolytic activity of ADAMTS13 is highly dependent on divalent cations, which has also been observed in other the metalloprotease domains in this ADAMTS family (Zheng et al., 2001 *supra*; Gardner M.D., Chion C.K., de Groot R., Shah A., Crawley J.T. et al. 2009 A functional calcium-binding site in the metalloprotease domain of ADAMTS13, Blood 113: 1149-1157, which is incorporated herein by reference in its entirety for all purposes). Moreover, ADAMTS13 activity undergoes allosteric regulation by binding to VWF and interactions between N-terminal MDTCS and C-terminal CUB domains (Muia J., Zhu J., Gupta G., Haberichter S.L., Friedman K.D. et al. 2014 Allosteric activation of ADAMTS13 by von Willebrand factor, Proceedings of the National Academy of Sciences 111: 18584-18589; South K., Luken B.M., Crawley J.T.B., Phillips R., Thomas M., et al. 2014 Conformational activation of ADAMTS13, Proceedings of the National Academy of Sciences 111: 18578-18583, each of which is incorporated herein by reference in their entirety for all purposes).

[0006] Described herein are variants of ADAMTS13, including the use of variants of ADAMTS13 in compositions and methods of treating a disease or condition associated with ADAMTS13 and VWF dysfunction.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

[0007] The disclosure includes variants of ADAMTS13. The disclosure also includes pharmaceutical compositions and/or uses of ADAMTS13 variants for the preparation of medicaments. The disclosure also includes a method for treating, ameliorating, and/or preventing a disease or disorder associated with an ADAMTS13 deficiency, high vWF levels,

and/or high vWF antigen levels with ADAMTS13 variants. Other related ~~aspects~~examples are also provided in the disclosure.

[0008] In certain ~~embodiments~~examples, the present disclosure provides variants of ADAMTS13. In certain ~~embodiments~~examples, the ADAMTS13 variant comprises at least one amino acid substitution as compared to (i.e., relative to) an ADAMTS13 protein amino acid (e.g., SEQ ID NO: 1). In certain ~~embodiments~~examples, the single amino acid substitution is within the catalytic domain of ADAMTS13 (e.g., amino acids 80 to 286 of SEQ ID NO: 1). In certain ~~embodiments~~examples, the single amino acid substitution is at least one of $I^{79}M$, $V^{88}M$, $H^{96}D$, $Q^{97}R$, $R^{102}C$, $S^{119}F$, $I^{178}T$, $R^{193}W$, $T^{196}I$, $S^{203}P$, $L^{232}Q$, $H^{234}Q$, $D^{235}H$, $A^{250}V$, $S^{263}C$, and/or $R^{268}P$ as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13. In certain ~~embodiments~~examples, the single amino acid substitution is not $I^{79}M$, $V^{88}M$, $H^{96}D$, $R^{102}C$, $S^{119}F$, $I^{178}T$, $R^{193}W$, $T^{196}I$, $S^{203}P$, $L^{232}Q$, $H^{234}Q$, $D^{235}H$, $A^{250}V$, $S^{263}C$, and/or $R^{268}P$ as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13. In certain ~~embodiments~~examples, the ADAMTS13 variant comprises a single amino acid substitution at Q^{97} as denoted in SEQ ID NO: 1, or the equivalent amino acid in an ADAMTS13. In certain ~~embodiments~~examples, the amino acid change is from a Q to a D, E, K, H, L, N, P, or R. In certain ~~embodiments~~examples, the amino acid change is from a Q to an R. In certain ~~embodiments~~examples, the ADAMTS13 variant is ADAMTS13 $Q^{97}R$ (SEQ ID NO: 2), or an amino acid sequence having at least 80% sequence identity thereof. In one aspect, the present disclosure refers to a recombinant ADAMTS13 variant, wherein the ADAMTS13 variant comprises an amino acid sequence with a single amino acid substitution as compared to an ADAMTS13 protein, wherein the single amino acid substitution is at amino acid Q97 as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13, wherein the single amino acid change is from a Q to an R.

[0009] In certain ~~embodiments~~examples, the present disclosure provides pharmaceutical compositions comprising at least one ADAMTS13 variant. In one aspect, the present disclosure refers to a pharmaceutical composition comprising at least one ADAMTS13 variant as disclosed herein and a pharmaceutically acceptable carrier or excipient. In certain ~~embodiments~~examples, the present disclosure provides pharmaceutical compositions comprising at least one ADAMTS13 variant and a pharmaceutically acceptable carrier or excipient. In certain ~~embodiments~~examples, the pharmaceutical composition comprises a combination of at least one ADAMTS13 variant and at

least one ADAMTS13 protein (e.g., wildtype). In certain ~~embodiments~~examples, the pharmaceutical composition comprises a combination of at least one ADAMTS13 variant and at least one ADAMTS13 protein (e.g., wildtype) and a pharmaceutically acceptable carrier or excipient. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to wildtype ADAMTS13 (e.g., SEQ ID NO: 1) is about 4:1 to about 1:4. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to ADAMTS13 wildtype is about 3:1. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to ADAMTS13 wildtype is about 2:1. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to

ADAMTS13 wildtype is about 1:1. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to ADAMTS13 wildtype is about 1:2. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to ADAMTS13 wildtype is about 2:3. In certain ~~embodiments~~examples, the ratio of ADAMTS13 variant to ADAMTS13 wildtype is about 3:2. In certain ~~embodiments~~examples, the ADAMTS13 variant comprises a single amino acid substitution at Q^{97} as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13. In certain ~~embodiments~~examples, the ADAMTS13 variant is ADAMTS13 $Q^{97}R$ (SEQ ID NO: 2), or an amino acid sequence having at least 80% sequence identity thereof. In certain ~~embodiments~~examples, the wildtype ADAMTS13 is human ADAMTS13 or a biologically active derivative or fragment thereof as described in U.S. Patent Application Publication No. 2011/0229455, which is incorporated herein by reference for all purposes. In one embodiment, the amino acid sequence of hADAMTS13 is that of GenBank accession number NP_620594. In certain ~~embodiments~~examples, the hADAMTS13 is SEQ ID NO: 1.

[00010] In certain ~~embodiments~~examples, the pharmaceutical composition comprises a combination of at least one ADAMTS13 variant and at least one ADAMTS13 protein (e.g., wildtype). In certain ~~embodiments~~examples, the pharmaceutical composition comprises a combination of at least one ADAMTS13 variant and at least one ADAMTS13 protein (e.g., wildtype) and a pharmaceutically acceptable carrier or excipient. In certain ~~embodiments~~examples, the ADAMTS13 variant constitutes between about 52% to about 72% or between about 47% to about 84% of total amount of all ADAMTS13 proteins and variants in the composition. In certain ~~embodiments~~examples, the ADAMTS13 variant comprises a single amino acid substitution at Q^{97} as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13. In certain ~~embodiments~~examples, the ADAMTS13 variant is ADAMTS13 $Q^{97}R$ (SEQ ID NO: 2), or an amino acid sequence having at least 80% sequence identity thereof. In certain ~~embodiments~~examples, the wildtype ADAMTS13 is human ADAMTS13 or a biologically active derivative or fragment thereof as described in U.S. Patent Application Publication No. 2011/0229455, which is incorporated herein by reference for all purposes. In one embodiment, the amino acid sequence of hADAMTS13 is that of GenBank accession number NP_620594. In certain ~~embodiments~~examples, the hADAMTS13 is SEQ ID NO: 1.

[00011] In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is determined by a peptide mapping method. In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is determined by a peptide mapping method as described in Example 3.

In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is determined by HPLC analysis of tryptic peptides separated by liquid chromatography followed by mass spectrometry analysis. In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is based on intensities in extracted ion chromatograms. In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is determined based on the peak area of tryptic peptides of the ADAMTS13 variant (e.g., $Q^{97}R$ ADAMTS13 variant) in relation to the sum of the peak areas of all ADAMTS13 proteins and variants in the composition (e.g., sum total of $Q^{97}R$ ADAMTS13 variant and Q^{97} ADAMTS13 protein). In certain ~~embodiments~~examples, the tryptic peptides of all ADAMTS13 proteins and variants in the composition being measured are specific to the at least one amino acid difference between the ADAMTS13 variant as compared to all other ADAMTS13 proteins and variants in the composition. For example, the tryptic peptide(s) that can be measured for the $Q^{97}R$ ADAMTS13 variant can be AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5) and the tryptic peptide measured for the Q^{97} ADAMTS13 protein can be AAGGILHLELLVAVGPDVFQAHQEDTER (SEQ ID NO: 6).

[00012] In certain ~~embodiments~~examples, the relative abundance, percentage, and/or ratio is determined based on total weight of ADAMTS13 variant in relation to the sum total weight of all ADAMTS13 proteins and variants in the composition.

[00013] In certain ~~embodiments~~examples, the ADAMTS13 variant and/or wildtype is recombinant. In certain ~~embodiments~~examples, the wildtype ADAMTS13 variant and/or wildtype is plasma derived.

[00014] In one ~~aspect~~example, the present disclosure provides a method for treating or preventing a blood clotting disorder in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including one comprising an ADAMTS13 protein. In another aspect, the present disclosure provides a use of the ADAMTS13 variant or pharmaceutical composition as disclosed herein in the manufacture of a medicament for

(A) Treating or preventing a blood clotting disorder in a subject suffering from or at risk of suffering from a blood clotting disorder, comprising administration of a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein to the subject in need thereof, wherein optionally the blood clotting disorder is inherited TTP, acquired TTP, infarction, cerebral

infarction, myocardial infarction, ischemic/reperfusion injury, deep vein thrombosis, or sepsis-related disseminated intravascular coagulation, or

(B) Treating or preventing a bleeding episode in a subject suffering from or at risk of suffering from a bleeding disorder, comprising administration to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein, wherein optionally the bleeding episode is associated with inherited TTP, acquired TTP, infarction, cerebral infarction, myocardial infarction, ischemic/reperfusion injury, deep vein thrombosis, or sepsis-related disseminated intravascular coagulation, or

(C) Treating or preventing a vaso-occlusive crisis in a subject suffering from sickle cell disease, comprising administration to the subject in need thereof a therapeutically effective amount of the ADAMTS13 variant or pharmaceutical composition as disclosed herein, or

(D) Treating or preventing lung injury in a subject suffering from or at risk of suffering from acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS), the use comprising administration to the subject in need thereof a therapeutically effective amount of the ADAMTS13 variant or pharmaceutical composition as disclosed herein, or

(E) Treating a cerebral infarction in a subject by recanalization of an occluded blood vessel in the subject, comprising administration to the subject in need thereof a therapeutically effective amount of the ADAMTS13 variant or pharmaceutical composition as disclosed herein, thereby recanalizing the occluded blood vessel, or

(F) Treating or preventing a blood clotting disorder associated with cardiovascular disease in a subject, comprising administration to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein, wherein optionally the blood clotting disorder associated with cardiovascular disease is associated with myocardial infarction, myocardial ischemia, deep vein thrombosis, peripheral vascular disease, stroke, transient ischemic attack, or medical device associated thrombosis, or

(G) Treating or preventing hematologic disease in a subject, comprising administration to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein.

[00015] In certain ~~embodiments~~examples of the methods provided herein, the clotting disorder is selected from the group consisting of inherited TTP (also referred to as congenital TTP, hereditary TTP, familial TTP and Upshaw-Schulman syndrome), acquired TTP (also referred to as immune-mediated TTP), infarction, cerebral infarction, myocardial infarction, ischemic/reperfusion injury, deep vein thrombosis, and sepsis-related disseminated intravascular

coagulation. In one ~~embodiment~~example of the methods provided herein, the clotting disorder is inherited TTP. In one ~~embodiment~~example of the methods provided herein, the clotting disorder is acquired TTP.

[00016] In one ~~aspect~~example, the present disclosure provides a method for treating a bleeding episode in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including one comprising an ADAMTS13 protein.

[00017] In certain ~~embodiments~~examples, the bleeding episode is associated with inherited TTP, acquired TTP, infarction, myocardial infarction, cerebral infarction, and/or ischemia reperfusion injury.

[00018] In one ~~aspect~~example, the present disclosure provides a method for treating or preventing a vaso-occlusive crisis in a subject suffering from sickle cell disease, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00019] In one ~~aspect~~example, the present disclosure provides a method for treating or preventing a vaso-occlusive crisis in a subject suffering from acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS), the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00020] In one ~~aspect~~example, the present disclosure provides a method for recanalization of an occluded blood vessel in a subject having a cerebral infarction, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00021] In one ~~aspect~~example, the present disclosure provides a method of improving the recovery of sensorimotor function in a subject that has experienced a cerebral infarction, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00022] In one ~~aspect~~example, the present disclosure provides a method for treating or preventing a blood clotting disorder associated with cardiovascular disease in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00023] In one ~~aspect~~example, the present disclosure provides a method for treating or preventing hematologic disease in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant or composition thereof, including a composition comprising an ADAMTS13 protein.

[00024] In certain ~~embodiments~~examples of the methods provided herein, the ADAMTS13 variant or composition, including a composition comprising ADAMTS13, is administered in a single bolus injection, monthly, every two weeks, weekly, twice a week, daily, every 12 hours, every 8 hours, every six hours, every four hours, every two hours, or every hour.

[00025] In certain ~~embodiments~~examples, the ADAMTS13 variant or composition, including a composition comprising ADAMTS13, is administered intravenously or subcutaneously.

[00026] In certain ~~embodiments~~examples, the ADAMTS13 variant(s) and/or ADAMTS13 protein (e.g., wildtype) is recombinant. In certain ~~embodiments~~examples, the ADAMTS13 variant(s) and/or ADAMTS13 protein is recombinantly produced by HEK293 cells. In certain ~~embodiments~~examples, the ADAMTS13 variant(s) and/or ADAMTS13 protein is recombinantly produced by CHO cells. In certain ~~embodiments~~examples, the ADAMTS13 variant(s) and/or ADAMTS13 protein is plasma derived.

[00027] In certain ~~embodiments~~examples, the subject is a mammal. In certain ~~embodiments~~examples, the subject is a human.

[00028] In certain ~~embodiments~~examples, the composition is a stable aqueous solution ready for administration. In certain ~~embodiments~~examples, the composition is lyophilized. In certain ~~embodiments~~examples, the composition is reconstituted with a pharmaceutically acceptable vehicle suitable for injection prior to administration.

[00029] In one ~~aspect~~example, the present disclosure provides a nucleic acid molecule encoding an ADAMTS13 variant as disclosed herein (e.g., SEQ ID NO: 4).

[00030] In one ~~aspect~~example, the present disclosure provides a vector comprising a nucleic acid molecule encoding an ADAMTS13 variant as disclosed herein (e.g., SEQ ID NO: 4). In certain ~~embodiments~~examples, the vector is an expression vector wherein the polynucleotide sequence encoding the ADAMTS13 variant is operably linked to a promoter that is capable of mediating expression of the ADAMTS13 variant in a host cell.

[00031] In one ~~aspect~~example, the present disclosure provides a host cell comprising a nucleic acid molecule encoding an ADAMTS13 variant as disclosed herein (e.g., SEQ ID NO: 4) or a vector as disclosed herein.

[00032] In one ~~aspect~~example, the present disclosure provides a host cell line comprising cells modified to express the ADAMTS13 variant as disclosed herein (e.g., SEQ ID NO: 2) and at least one ADAMTS13 protein (e.g., SEQ ID NO: 1). In certain ~~embodiments~~examples, the ADAMTS13 variant and the ADAMTS13 protein are expressed in different cells in the host cell line. the ADAMTS13 variant and the ADAMTS13 protein are expressed in the same cell in the host cell line.

[00033] In certain ~~embodiments~~examples, the host cell or host cell line is a CHO, COS, HEK 293, BHK, SK-Hep, or HepG2 cell or cell line. In certain ~~embodiments~~examples, the CHO cell is a CHO DBX-11 or CHOZN cell line. In certain ~~embodiments~~examples, the CHOZN cell is a CHO DBX-11 cell line. In certain ~~embodiments~~examples, the CHOZN cell is a CHOZN glutamine synthetase (GS) $^{-/-}$ cell line.

[00033A] In one aspect, the present disclosure provides a method for detecting an ADAMTS13 variant as disclosed herein, comprising:

a. subjecting a plasma sample to trypsin digestion to form a peptide mixture;

b. separating the peptide mixture by reversed phase chromatography; and

c. detecting the ADAMTS13 variant via detecting AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5).

[00033B] In another aspect, the present disclosure provides a method of determining the ratio or percentage of an ADAMTS13 variant of claims 1-3, comprising:

a. subjecting a plasma sample to trypsin digestion to form a peptide mixture;

b. separating the peptide mixture by reversed phase chromatography;

c. detecting AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5) to determine ADAMTS13 variant intensities;

d. detecting the presence of non-variant ADAMTS13 intensities; and

e. determining the ratio or percentage of the ADAMTS13 variant intensities as compared to the non-variant ADAMTS13 intensities.

DESCRIPTION OF THE DRAWINGS

[00034] The priority application file contains at least one drawing executed in color, which is accessible via United States Patent and Trademark Office's Patent Examination Research Dataset (PAIR).

[00035] Figure 1A-1C shows the alignment between wildtype ADAMTS13 (SEQ ID NO: 1) and ADAMTS13 $Q^{97}R$ variant (SEQ ID NO: 2).

[00036] Figure 2A-2C shows the alignment between wildtype ADAMTS13 (SEQ ID NO: 1) and wildtype gorilla ADAMTS13 (SEQ ID NO: 3).

[00037] Figure 3 is a boxplot of the specific activity (U/mg Ag) of the plasma derived ADAMTS13 (pdADAMTS13) and a combination of wildtype and $Q^{97}R$ variant recombinant ADAMTS13 (rADAMTS13). The pdADAMTS data examines at least 80 samples from different donors and the rADAMTS13 combination examines 35 different batches of rADAMTS13, wherein the abundance of variant is 52 to 72%.

[00038] Figure 4 shows a three-dimensional model of ADAMTS13, including the location of Q^{97}, which is exposed on the M proteas domain, and located in a short two residue turn in a stable helix-turn-helix structure.

[00039] Figure 5 is a UV chromatogram of a composition comprising a combination of wildtype ADAMTS13 and the $Q^{97}R$ variant. The insert shows that the tryptic peptides representing the two variants are well separated.

[00040] Figure 6 is a total ion chromatogram and extracted ion chromatograms of tryptic peptides from native variant and $Q^{97}R$ variant in a composition. The tryptic peptide of the native variant eluted before the correctly cleaved tryptic peptide of the $Q^{97}R$ variant. The miss-cleaved peptide of the $Q^{97}R$ variant eluted before the two other peptides. The area of the peaks represents the relative abundance of the peptides, and the variants, respectively. Figure 6 discloses SEQ ID NOS 115, 6, and 7, respectively, in order of appearance.

[00041] Figure 7 is a scatter plot of the specific activity (U/mg Ag) of rADAMTS13 compositions comprising different ratios of $Q^{97}R$ rADAMTS13 variant to wildtype rADAMTS13. A total of 35 different rADAMTS13 compositions were tested.

[00042] Figure 8 is a scatter plot of the VWF cleavage of rADAMTS13 compositions comprising different ratios of $Q^{97}R$ rADAMTS13 variant to wildtype rADAMTS13. A total of 35 different rADAMTS13 compositions were tested.

[00043] Figure 9 is a flamingo fluorescent stained CDC gel of $Q^{97}R$ rADAMTS13 and the $Q^{97}R$ rADAMTS13 variant. The Flamingo fluorescence staining represents the total protein amount. The gel image shows the following band pattern for both ADAMTS13 samples: one

Example 6: Glycosylation Analysis of Q^{97} ADAMTS13 and $Q^{97}R$ ADAMTS13

[000356] This example uses several analytical methods to determine the degree of glycosylation and the principle nature of the ADAMTS13 glycosylation pattern.

[000357] Both Q^{97} ADAMTS13 and $Q^{97}R$ ADAMTS13 were expressed together in a CHO DBX-11 host cell line (i.e., one cell line produces both ADAMTS13 proteins together).

[000358] For the tryptic peptide mapping, samples of purified ADAMTS13 were reduced with di-thio-threitol (DTT) and the free sulfhydryl groups were blocked with iodoacetamide. Reagents were removed by dialysis and rADAMTS13 was collected and lyophilized. Sequencing grade trypsin or LysC was added and allowed to react for 2 hours followed by a second addition of enzyme and incubation for 18 hours. In addition, N-glycans were removed with PNGase F to obtain more complete sequence coverage. The resulting peptide mixture was separated by reversed phase chromatography. Eluting peptides were detected by on-line UV detection at 214 nm and in addition detection by on-line mass spectrometry only for tryptic peptide mixture. The peptides covering the amino acid sequence of ADAMTS13 are listed in Table 9.

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T001	1-28	AAGGILHLELLVAVGPDVFQAHQEDTER (SEQ ID NO: 6)	--	67.0	2984.5437	2984.5354	2.8
T002	29-42	YVLTNLNIGAELLR (SEQ ID NO: 8)	--	60.1	1587.9023	1587.8984	2.5
T002	29-42	YVLTNLNIGAELLR (SEQ ID NO: 8)	--	60.9	1587.8956	1587.8984	-1.8
T003	43-51	DPSLGAQFR (SEQ ID NO: 9)	--	34.4	989.4910	989.4930	-2.0
T004	52-56	VHLVK (SEQ ID NO: 10)	--	24.9	594.3858	594.3853	0.8
T005*	57-106	MVILTEPEGAPNITANLTSSLLSVCGWSQTINPEDDTDPGHADLVLYITR (SEQ ID NO: 11)	Carbamidomethyl C(1), Deamidation N(2)	75.5	5425.5991	5425.6222	4.3
T006	107-116	FDLELPDGNR (SEQ ID NO: 12)	--	44.9	1174.5627	1174.5618	0.8

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
1006	107-116	FDLELPDGNR (SEQ ID NO: 12)	--	45.3	1174.5631	1174.5618	1.1
T007	117-119	QVR	--	10.2	401.23	401.23	1.7
T008*	120-183	GVTQLGGACSPTWSCLITEDTGFDLGVTIAHEIGHSFGLEHDGAPGSGCGPSGHVMASDGAAPR (SEQ ID NO: 13)	Carbamidomethyl C(3)	75.0	6460.8896	6460.9272	-5.8
T009*	184-193	AGLAWSPCSR (SEQ ID NO: 14)	Carbamidomethyl C(1)	37.1	1103.5157	1103.5182	-2.3
T010-011	194-204	RQLLSLLSAGR (SEQ ID NO: 15)	--	49.8	1212.7236	1212.7302	-5.4
T011	195-204	QLLSLLSAGR (SEQ ID NO: 16)	--	51.5	1056.6265	1056.6292	-2.6
T012	205-206	AR	--	n.d.	n.d.	245.14	n.d.
T013-014*	207-238	CVWDPPRPQPGSAGHPPDAQPGLYYSANEQCR (SEQ ID NO: 17)	Carbamidomethyl C(2)	42.2	3606.6201	3606.6157	1.2
T015	239-244	VAFGPK (SEQ ID NO: 18)	--	27.2	617.3556	617.3537	3.1
T016*	245-252	AVACTFAR (SEQ ID NO: 19)	Carbamidomethyl C(1)	27.2	894.4427	894.4382	5.0
T016*	245-252	AVACTFAR (SEQ ID NO: 19)	Carbamidomethyl C(1)	62.6	894.4373	894.4382	-1.0
T017*	253-275	EHLDMCQALSCHTDPLDQSSCSR (SEQ ID NO: 20)	Carbamidomethyl C(3)	42.4	2746.1299	2746.1204	3.5
T018*	276-290	LLVPLLDGTECGVEK (SEQ ID NO: 21)	Carbamidomethyl C(1)	55.0	1641.8633	1641.8647	-0.9
T018*	276-290	LLVPLLDGTECGVEK (SEQ ID NO: 21)	Carbamidomethyl C(1)	53.3	1641.8685	1641.8647	2.3

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T019*	291-294	WCSK (SEQ ID NO: 114)	Carbamidomethyl C(1)	14.7	579.2490	579.2476	2.4
T020	295-296	GR	--	n.d.	n.d.	231.1331	n.d.
T021	297-298	CR	--	n.d.	n.d.	334.1423	n.d.
T022	299-312	SLVELTPIAAVHGR (SEQ ID NO: 22)	--	49.0	1461.8273	1461.8303	-2.1
T022	299-312	SLVELTPIAAVHGR (SEQ ID NO: 22)	--	48.6	1461.8248	1461.8303	-3.8
T023	313-319	WSSWGPR (SEQ ID NO: 23)	--	35.6	874.4049	874.4086	-4.2
T023*	313-319	WSSWGPR (SEQ ID NO: 23)	C-Mannosylation W(1)	30.7	1036.4602	1036.4614	-1.2
T024*	320-324	SPCSR (SEQ ID NO: 24)	Carbamidomethyl C(1)	7.8	605.2609	605.2592	2.8
T025*	325-333	SCGGGVVTR (SEQ ID NO: 25)	Carbamidomethyl C(1), O-Fucosylation Glc-Fuc	17.7	1199.5342	1199.5339	0.3
T025*	325-333	SCGGGVVTR (SEQ ID NO: 25)	Carbamidomethyl C(1)	17.7	891.4241	891.4233	0.9
T025*	325-333	SCGGGVVTR (SEQ ID NO: 25)	Carbamidomethyl C(1), O-Fucosylation Glc-Fuc ST(1)	18.3	1199.5288	1199.5339	-4.3
T026	334-334	R	--	n.d.	n.d.	174.1117	n.d.
T027-028*	335-341	RQCNNPR (SEQ ID NO: 26)	Carbamidomethyl C(1)	9.9	943.4395	943.4407	-1.3

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T028*	336-341	QCNNPR (SEQ ID NO: 27)	Carbamidomethyl C(1)	8.6	787.3404	787.3395	1.1
T029	342-347	PAFGGR (SEQ ID NO: 28)	--	21.0	603.3132	603.3129	0.5
T030*	348-366	ACVGADLQAEMCNTQACEK (SEQ ID NO: 29)	Carbamidomethyl C(3)	36.9	2154.8833	2154.8804	1.3
T031*	367-378	TQLEFMSQQCAR (SEQ ID NO: 30)	Carbamidomethyl C(1)	37.5	1497.6735	1497.6705	2.0
T032	379-385	TDGQPLR (SEQ ID NO: 31)	--	18.2	785.4031	785.4031	0.0
T033*	386-410	SSPGGASFYHWGAAVPHSQGDALCR (SEQ ID NO: 32)	Carbamidomethyl C(1)	45.2	2614.1919	2614.1772	5.6
T034*	411-414	HMCR (SEQ ID NO: 33)	Carbamidomethyl C(1)	11.3	602.2433	602.2418	2.5
T035	415-423	AIGESFIMK (SEQ ID NO: 34)	--	42.4	994.5152	994.5157	-0.5
T036-037	424-433	RGDSFLDGTR (SEQ ID NO: 35)	--	32.8	1122.5411	1122.5417	-0.5
T036-037	424-433	RGDSFLDGTR (SEQ ID NO: 35)	--	30.7	1122.5444	1122.5417	2.4
T037	425-433	GDSFLDGTR (SEQ ID NO: 36)	--	32.8	966.4382	966.4407	-2.6
T037	425-433	GDSFLDGTR (SEQ ID NO: 36)	--	30.6	966.4375	966.4407	-3.3
T038*	434-440	CMPSGPR (SEQ ID NO: 37)	Carbamidomethyl C(1)	20.3	803.3391	803.3419	-3.5
T039*	441-454	EDGTLSLCVSGSCR (SEQ ID NO: 38)	Carbamidomethyl C(2)	35.9	1539.6646	1539.6658	-0.8
T039*	441-454	EDGTLSLCVSGSCR (SEQ ID NO: 38)	Carbamidomethyl C(2)	35.5	1539.6672	1539.6658	0.9
T040*	455-461	TFGCDGR (SEQ ID NO: 39)	Carbamidomethyl C(1)	19.4	811.3272	811.3283	-1.4
T041	462-470	MDSQQVWDR (SEQ ID NO: 40)	--	31.2	1163.5045	1163.5029	1.4

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T042*	471-484	CQVCGGDNSTCSPR (SEQ ID NO: 41)	Carbamidomethyl C(3), N-Glycosylation biFucSA N(1)	16.2	3656.3515	3656.3428	-2.4
T043-044	485-492	KGSFTAGR (SEQ ID NO: 42)	--	19.8	822.4337	822.4348	-1.3
T044	486-492	GSFTAGR (SEQ ID NO: 43)	--	19.8	694.3402	694.3398	0.6
T045	493-494	AR	--	n.d.	n.d.	245.1488	n.d.
T046*	495-515	EYVTFLTVTPNLTSVYIANHR (SEQ ID NO: 44)	N-Glycosylation biFucSA2 N(1)	56.7	4788.1118	4788.0942	3.7
T047	516-524	PLFTHLAVR (SEQ ID NO: 45)	--	45.6	1052.6162	1052.6130	3.0
T048	525-528	IGGR (SEQ ID NO: 46)	--	7.0	401.2378	401.2386	-2.0
T048	525-528	IGGR (SEQ ID NO: 46)	--	19.3	401.2385	401.2386	-0.2
T048	525-528	IGGR (SEQ ID NO: 46)	--	7.2	401.2373	401.2386	-3.2
T049	529-534	YVVAGK (SEQ ID NO: 47)	--	19.9	635.3650	635.3643	1.1
T050*	535-551	MSISPNTTYPSLLEDGR (SEQ ID NO: 48)	N-Glycosylation Man4 N(1)	44.1	3744.5572	3744.5327	-6.5
T051	552-555	VERY (SEQ ID NO: 49)	--	15.4	565.2887	565.2860	4.8
T052	556-562	VALTEDR (SEQ ID NO: 50)	--	23.4	802.4168	802.4185	-2.1
T053	563-565	LPR	--	16.2	384.2485	384.2485	0.0
T053	563-565	LPR	--	18.2	384.2488	384.2485	0.8

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T054	566-570	LEEIR (SEQ ID NO: 51)	--	24.4	658.3666	658.3650	2.4
T055	571-585	IWGPLQEDADIQVYR (SEQ ID NO: 52)	--	51.6	1801.8981	1801.8999	-1.0
T056	586-586	R	--	n.d.	n.d.	174.1117	n.d.
T057-058*	587-607	YGEEYGNLTRPDITFTYFQPK (SEQ ID NO: 53)	N-Glycosylation biFucSA2 N(1)	50.5	4889.0439	4889.0371	1.4
T058	597-607	PDITFTYFQPK (SEQ ID NO: 54)	--	49.8	1355.6742	1355.6761	-1.4
T059	608-609	PR	--	n.d.	n.d.	271.1644	n.d.
T060	610-618	QAWVWAAVR (SEQ ID NO: 55)	--	49.6	1085.5771	1085.5770	0.1
T061*	619-630	GPCSVSCGAGLR (SEQ ID NO: 56)	Carbamidomethyl C(2), O-Fucosylation Glc-Fuc ST(1)	26.9	1527.6554	1527.6545	0.6
T061*	619-630	GPCSVSCGAGLR (SEQ ID NO: 56)	Carbamidomethyl C(2)	26.9	1219.5457	1219.5438	1.6
T062*	631-641	WVNYSCLDQAR (SEQ ID NO: 57)	Carbamidomethyl C(1)	38.6	1410.6318	1410.6350	-2.3
T062*	631-641	WVNYSCLDQAR (SEQ ID NO: 57)	N-Glycosylation biFucSA2 N(1), Carbamidomethyl C(1)	35.2	3761.4629	3761.4653	-0.6
T062*	631-641	WVNYSCLDQAR (SEQ ID NO: 57)	N-Glycosylation triFucSA3 N(1), Carbamidomethyl C(1)	34.9	4417.7368	4417.6930	-9.9

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T063-064*	642-689	KELVETVQCQGSQQPPAWPEACVLEPCPPYWAVGDFGPCSASCGGGLR (SEQ ID NO: 58)	Carbamidomethyl C(5), O-Fucosylation Glc-Fuc ST(1)	60.2	5639.4849	5639.5088	-4.2
T064*	643-689	ELVETVQCQGSQQPPAWPEACVLEPCPPYWAVGDFGPCSASCGGGLR (SEQ ID NO: 59)	Carbamidomethyl C(5), O-Fucosylation Glc-Fuc ST(1)	61.0	5511.4258	5511.4141	2.1
T065-066	690-694	ERPVR (SEQ ID NO: 60)	--	13.3	655.3758	655.3765	-1.1
T067*	695-704	CVEAQGSLLK (SEQ ID NO: 61)	Carbamidomethyl C(1)	32.2	1103.5646	1103.5646	0.0
T067-068*	695-710	CVEAQGSLLKTLPPAR (SEQ ID NO: 62)	Carbamidomethyl C(1)	46.2	1738.9391	1738.9399	-0.5
T068	705-710	TLPPAR (SEQ ID NO: 63)	--	20.8	653.3878	653.3860	2.8
T068	705-710	TLPPAR (SEQ ID NO: 63)	--	20.0	653.3823	653.3860	-5.7
T068	705-710	TLPPAR (SEQ ID NO: 63)	--	20.3	653.3851	653.3860	-1.4
T068	705-710	TLPPAR (SEQ ID NO: 63)	--	21.1	653.3869	653.3860	1.4
T068	705-710	TLPPAR (SEQ ID NO: 63)	--	20.4	653.3839	653.3860	-3.2
T068-069*	705-712	TLPPARCR (SEQ ID NO: 64)	Carbamidomethyl C(1)	47.4	969.5197	969.5178	2.0
T070*	713-733	AGAQQPAVALETCNPQPCPAR (SEQ ID NO: 65)	Carbamidomethyl C(2)	33.2	2235.0525	2235.0525	0.0

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T071*	734-778	WEVSEPSSCTSAGGAGLALENETCVPGADGLEAPVTEGPGSVDEK (SEQ ID NO: 66)	Carbamidomethyl C(2), O-Glycosylation NeuAc2-Hex-HexNAc ST(1), N-Glycosylation biFucSA2 N(1)	43.4	7814.2805	7814.1704	-14.1
T071*	734-778	WEVSEPSSCTSAGGAGLALENETCVPGADGLEAPVTEGPGSVDEK (SEQ ID NO: 66)	Carbamidomethyl C(2), O-Glycosylation NeuAc2-Hex-HexNAc ST(1), N-Glycosylation triFucSA3 N(1)	43.0	8470.2893	8470.3980	12.8
T071*	734-778	WEVSEPSSCTSAGGAGLALENETCVPGADGLEAPVTEGPGSVDEK (SEQ ID NO: 66)	Carbamidomethyl C(2), O-Glycosylation NeuAc2-Hex-HexNAc ST(1), Deamidation N(1)	46.9	5464.3141	5464.3240	1.8
T072*	779-805	LPAPEPCVGMSCPPGWGHLDATSAGEK (SEQ ID NO: 67)	Carbamidomethyl C(2), Oxidation M(1)	45.6	2836.2776	2836.2620	5.5
T072*	779-805	LPAPEPCVGMSCPPGWGHLDATSAGEC (SEQ ID NO: 68)	Carbamidomethyl C(2)	47.0	2820.2888	2820.2671	7.7
T072*	779-805	LPAPEPCVGMSCPPGWGHLDATSAGEK (SEQ ID NO: 67)	Carbamidomethyl C(2), O-Glycosylation NeuAc2- Hex-HexNAc ST(1)	44.4	3767.6038	3767.5901	3.6

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T072*	779-805	LPAPEPCVGMSCPPGWGHLDATSAGEK (SEQ ID NO: 67)	Carbamidomethyl C(2), Oxidation M(1), O-Glycosylation NeuAc2-Hex-HexNAc ST(1)	43.0	3783.5906	3783.5850	1.5
T073	806-814	APSPWGSIR (SEQ ID NO: 69)	--	38.7	969.50	969.50	-2.5
T074*	815-836	TGAQAAHVWTPAAGSCSVSCGR (SEQ ID NO: 70)	Carbamidomethyl C(2)	34.5	2230.0056	2230.0007	2.2
T074*	815-836	TGAQAAHVWTPAAGSCSVSCGR (SEQ ID NO: 70)	Carbamidomethyl C(2), O-Fucosylation Glc-	34.5	2538.1042	2538.1116	-2.9
T075	837-842	GLMELR (SEQ ID NO: 71)	--	37.7	717.3845	717.3843	0.3
T076*	843-851	FLCMDSALR (SEQ ID NO: 72)	Carbamidomethyl C(1)	44.1	1111.5166	1111.5155	1.0
T076*	843-851	FLCMDSALR (SEQ ID NO: 72)	Carbamidomethyl C(1)	39.5	1111.5233	1111.5155	7.0
T077*	852-864	VPVQEELCGLASK (SEQ ID NO: 73)	Carbamidomethyl C(1)	41.1	1428.7297	1428.7283	1.0
T078	865-868	PGSR (SEQ ID NO: 74)	--	5.1	415.2180	415.2179	-0.2
T079-080*	869-880	REVCQAVPCPAR (SEQ ID NO: 75)	Carbamidomethyl C(2)	27.4	1441.6904	1441.6919	-1.0
T080*	870-880	EVCQAVPCPAR (SEQ ID NO: 76)	Carbamidomethyl C(2)	26.7	1285.5908	1285.5908	0.0
T081	881-884	WQYK (SEQ ID NO: 77)	--	24.8	623.3077	623.3068	1.4

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T082*	885-894	LAACSVSCGR (SEQ ID NO: 78)	Carbamidomethyl C(2), O-Fucosylation Glc-Fuc ST(1)	22.3	1387.5975	1387.5959	1.2
T082*	885-894	LAACSVSCGR (SEQ ID NO: 78)	Carbamidomethyl C(2)	22.3	1079.4866	1079.4852	1.3
T083	895-898	GVVR (SEQ ID NO: 79)	--	14.1	429.2697	429.2700	-0.7
T084	899-899	R	--	n.d.	n.d.	174.1117	n.d.
T085*	900-905	ILYCAR (SEQ ID NO: 80)	Carbamidomethyl C(1)	27.3	794.4083	794.4109	-3.3
T086*	906-926	AHGEDDGEEILLDTQCQGLPR (SEQ ID NO: 81)	Carbamidomethyl C(1)	44.2	2352.0686	2352.0652	1.4
T086-087*	906-941	AHGEDDGEEILLDTQCQGLPRPEPQEA CSLEPCPPR (SEQ ID NO: 82)	Carbamidomethyl C(3)	47.6	4099.8262	4099.8311	-1.2
T087*	927-941	PEPQEACSLEPCPPR (SEQ ID NO: 83)	Carbamidomethyl C(2)	32.2	1765.7749	1765.7764	-0.8
T087*	927-941	PEPQEACSLEPCPPR (SEQ ID NO: 83)	Carbamidomethyl C(2)	46.3	1765.7804	1765.7764	2.3
T088	942-943	WK	--	13.9	332.1848	332.1848	0.0
T089*	944-960	VMSLGPCSASCGLGTAR (SEQ ID NO: 84)	Carbamidomethyl C(2), O-Fucosylation Glc-Fuc ST(1)	37.7	2030.8949	2030.8959	-0.5
T089*	944-960	VMSLGPCSASCGLGTAR (SEQ ID NO: 84)	Carbamidomethyl C(2)	37.7	1722.7849	1722.7852	-0.2

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T089*	944-960	VMSLGPCSASCGLGTAR (SEQ ID NO: 84)	Carbamidomethyl C(2), Oxidation M(1),O-Fucosylation Glc-Fuc ST(1)	32.7	2046.8887	2046.8907	-1.0
T090	961-961	R	--	n.d.	n.d.	174.1117	n.d.
T091-092*	962-1001	SVACVQLDQGQDVEVDEAACAALVRPEASVPCLIADCTYR (SEQ ID NO: 85)	Carbamidomethyl C(4)	56.7	4435.0591	4435.0552	0.9
T092*	987-1001	PEASVPCLIADCTYR (SEQ ID NO: 86)	Carbamidomethyl C(2)	45.2	1750.8051	1750.8019	1.8
T093*	1002-1020	WHVGTWMECSVSCGDGIQR (SEQ ID NO: 87)	Carbamidomethyl C(2), O-Fucosylation Glc-Fuc ST(1)	46.7	2572.0864	2572.0669	7.6
T093*	1002-1020	WHVGTWMECSVSCGDGIQR (SEQ ID NO: 87)	Carbamidomethyl C(2)	46.7	2263.9734	2263.9563	7.6
T093*	1002-1020	WHVGTWMECSVSCGDGIQR (SEQ ID NO: 87)	Carbamidomethyl C(2), O-Fucosylation Glc-Fuc ST(1)	46.4	2572.0776	2572.0669	4.2
T094	1021-	R	--	n.d.	n.d.	174.11	n.d.
T095-096*	1022-1044	RDTCLGPQAQAPVPADFCQHLPK (SEQ ID NO: 88)	Carbamidomethyl C(2)	44.3	2605.2664	2605.2529	5.2
T096*	1023-1044	DTCLGPQAQAPVPADFCQHLPK (SEQ ID NO: 89)	Carbamidomethyl C(2)	45.0	2449.1646	2449.1519	5.2
T096-097*	1023-1049	DTCLGPQAQAPVPADFCQHLPKPVTVR (SEQ ID NO: 90)	Carbamidomethyl C(2)	48.0	3001.4922	3001.4902	0.7

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T097	1045-1049	PVTVR (SEQ ID NO: 91)	--	18.4	570.3502	570.3489	2.3
T098-099*	1050-1091	GCWAGPCVGQGTPSLVPHEEAAAPGRTTATPAGASLEWSQAR (SEQ ID NO: 92)	Carbamidomethyl C(2), O-Glycosylation NeuAc2- Hex-HexNAc ST(3)	42.6	7129.9219	7129.9868	-9.1
T098-099*	1050-1091	GCWAGPCVGQGTPSLVPHEEAAAPGRTTATPAGASLEWSQAR (SEQ ID NO: 92)	Carbamidomethyl C(2), O-Glycosylation NeuAc-Hex-HexNAc ST(1), O-Glycosylation NeuAc2-Hex-HexNAc ST(2)	43.4	6838.9045	6838.8915	-1.9
T098-099*	1050-1091	GCWAGPCVGQGTPSLVPHEEAAAPGRTTATPAGASLEWSQAR (SEQ ID NO: 92)	Carbamidomethyl C(2), O-Glycosylation NeuAc2- Hex-HexNAc ST(4)	38.7	8077.2681	8077.3099	5.2
T098-099*	1050-1091	GCWAGPCVGQGTPSLVPHEEAAAPGRTTATPAGASLEWSQAR (SEQ ID NO: 92)	Carbamidomethyl C(2), O-Glycosylation NeuAc-Hex-HexNAc ST(1), O-Glycosylation NeuAc2-Hex-HexNAc ST(3)	39.4	7786.0997	7786.2145	14.7
T099	1076-1091	TTATPAGASLEWSQAR (SEQ ID NO: 93)	--	54.4	1645.7960	1645.8060	-6.1
T100	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	--	38.4	1181.6520	1181.6556	-3.0
T100	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	--	39.6	1181.6561	1181.6556	0.4

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T100*	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	O-Glycosylation NeuAc2-Hex-HexNAc ST(1)	38.4	2128.9768	2128.9788	-0.9
T100*	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	O-Glycosylation NeuAc-Hex-HexNAc ST(1)	39.8	1837.9018	1837.8833	-10.1
T100*	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	O-Glycosylation NeuAc-Hex-HexNAc ST(1)	39.6	1837.8752	1837.8833	4.4
T100*	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	O-Glycosylation Hex-HexNAc ST(1)	40.9	1546.8016	1546.7879	-8.9
T101	1103-1103	R	--	n.d.	n.d.	174.1117	n.d.
T102*	1104-1120	LLPGPQENSVQSSACGR (SEQ ID NO: 95)	Carbamidomethyl C(1)	31.0	1798.8619	1798.8632	-0.7
T103	1121-1132	QHLEPTGTIDMR (SEQ ID NO: 96)	--	34.5	1396.6764	1396.6769	-0.4
T104*	1133-1145	GPGQADCAVAIGR (SEQ ID NO: 97)	Carbamidomethyl C(1)	28.7	1270.6053	1270.6088	-2.8
T105	1146-1154	PLGEVVTLR (SEQ ID NO: 98)	--	41.2	982.5804	982.5811	-0.7
T105	1146-1154	PLGEVVTLR (SEQ ID NO: 98)	--	45.4	982.5829	982.5811	1.8
T106*	1155-1173	VLESSLNCSAGDMLLLWGR (SEQ ID NO: 99)	N-Glycosylation biFucSA2 N(1), Carbamidomethyl C(1)	57.6	4470.8794	4470.8696	2.2

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide[1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T106*	1155-1173	VLESSLNCSAGDMLLLWGR (SEQ ID NO: 99)	N-Glycosylation biFucSA N(1), Carbamidomethyl C(1)	57.9	4179.7741	4179.7744	0.1
T107	1174-1177	LTWR (SEQ ID NO: 100)	--	31.3	574.3236	574.3228	1.4
T108	1178-1178	K	--	n.d.	n.d.	146.1055	n.d.
T109*	1179-1181	MCR	Carbamidomethyl C(1)	9.3	465.1831	465.1829	0.4
T110-111	1182-1191	KLLDMTFSSK (SEQ ID NO: 101)	--	43.7	1168.6167	1168.6162	0.4
T111	1183-1191	LLDMTFSSK (SEQ ID NO: 102)	--	43.7	1040.5210	1040.5212	-0.2
T111*	1183-1191	LLDMTFSSK (SEQ ID NO: 102)	Oxidation M(1)	34.3	1056.5184	1056.5161	2.2
T112	1192-1198	TNTLVVR (SEQ ID NO: 103)	--	27.9	801.4710	801.4708	0.2
T113	1199-1200	QR	--	n.d.	n.d.	302.1703	n.d.
T114-115*	1201-1211	CGRPGGGVLLR (SEQ ID NO: 104)	Carbamidomethyl C(1)	34.0	1140.6196	1140.6187	0.8
T115	1204-1211	PGGGVLLR (SEQ ID NO: 105)	--	33.2	767.4647	767.4653	-0.8
T116	1212-1223	YGSQLAPETFYR (SEQ ID NO: 106)	--	40.6	1430.6863	1430.6830	2.3
T117*	1224-1252	ECDMQLFGPWGEIVSPSLSPATSNAGGCR (SEQ ID NO: 107)	Carbamidomethyl C(2)	62.4	3122.3948	3122.3896	1.7
T118	1253-1262	LFINVAPHAR (SEQ ID NO: 108)	--	42.9	1136.6484	1136.6454	2.6
T119*	1263-1287	IAIHALATNMGAGTEGANASYILIR (SEQ ID NO: 109)	N-Glycosylation biFucSA2 N(1)	50.6	4878.1577	4878.1519	1.2
T120	1288-1293	DTHSLR (SEQ ID NO: 110)	--	15.9	727.3631	727.3613	2.5

Table 9. Identified Signals of the Peptide Mapping of ADAMTS13 Sorted by Sequence

Peptide [1]	Range AA[1]	Peptide Sequence	Modification[2]	tR [min]	Monoisotopic		Mass accuracy [ppm]
					Exp. [Da]	Theor. [Da]	
T121	1294-1322	TTAFHGQQVLYWESESSQAEMEFSEGFLK (SEQ ID NO: 111)	--	62.5	3365.5249	3365.5186	1.9
T122	1323-1328	AQASLR (SEQ ID NO: 112)	--	16.6	644.3618	644.3605	2.0
T123	1329-1348	GQYWTLQSWVPEMQDPQSWK (SEQ ID NO: 113)	--	61.8	2493.1362	2493.1423	-2.4
T124	1349-1350	GK	--	n.d.	n.d.	203.1270	n.d.
T125	1351-1353	EGT	--	n.d.	n.d.	305.1223	n.d.

[1]numbering without signal- and propeptide;
[2] no modification on this peptide; n.d. not detected.

[000359] The overlay of tryptic peptide maps of several samples revealed high comparability of the samples as no major difference could be observed in the peak pattern. Thus, similar posttranslational modifications occurred across the samples.

[000360] The recombinant ADAMTS13 shows a C-mannosylation that is typical for the Thrombospondin type-1 (TSP-1) motif containing the sequence WXXW. This modification could be detected and was found approximately 30% modified and 70% unmodified.

[000361] The main N-glycan variants that were found by the peptide mapping were a biantennary, monosialyated fucosylated glycan and a biantennary, disialylated fucosylated glycan.

[000362] The seven O-fucosylations on the TSP-1 motifs were all found to be completely occupied by the disaccharide Fuc-Glc. Furthermore, several mucine type O-glycans with the structure HexNAc-Hex-NeuAc$_{0-2}$ could be identified. In Table 10 these O-glycosylations with their approximate occupancy are summarized. Peptide T071 was found to be completely modified with HexNAc-Hex-NeuAc$_2$. For peptide T100 the major part (88%) is also modified

with this structure and only minor amounts are not modified. For Peptide T072 60% were detected in the unmodified form and approximately 40% were found to be modified with the O-glycan HexNac-Hex-NeuAc2. The tryptic peptide T098-099 was detected with up to four O-glycans (30%) of the same type with different levels of sialic acid, around 70% were found bearing three O-glycans and no unmodified peptide could be detected.

Table 10. Identified O-Linked Glycosylation of ADAMTS13

Peptide	AA range	Peptide	Area%	Monoisotopic mass Experimental [Da]	Mass Accuracy [ppm]
T071	734-778	WEVSEPSSCTSAGGAGLALENETCVP GADGLEAPVTEGPGSVDEK (SEQ ID NO: 66)	n.d.	n.d.	n.d.
		+ HexNac-Hex-NeuAc2	100	5464.3141	1.8246
T072	779-805	LPAPEPCVGMSCPPGWGHLDATSAGEK (SEQ ID NO: 67)	29.6	2820.2764	-3.3388
		ox	28.9	2836.2649	-1.0582
		ox + HexNac-Hex-NeuAc2	10.6	3783.5791	1.5404
		+ HexNac-Hex-NeuAc2	30.8	3767.6209	-8.1980
T098-099	1050-1091	GCWAGPCVGQGTPSLVPHEEAAAPGR TTATPAGASLEWSQAR (SEQ ID NO: 92)	n.d.	n.d.	n.d.
		+4x HexNac-Hex-NeuAc2	14.4	8077.2681	5.1805
		+ HexNac-Hex-NeuAc + 3x HexNac-Hex-NeuAc2	18.6	7786.0997	14.7477
		+3x HexNac-Hex-NeuAc2	47.3	7130.0397	-7.4032
		+ HexNac-Hex-NeuAc + 2x HexNac-Hex-NeuAc2	19.7	6838.9045	-1.9011
T100	1092-1102	GLLFSPAPQPR (SEQ ID NO: 94)	0.2	1181.6600	-3.6853
		+ HexNac-Hex-NeuAc2	88.8	2128.9902	-5.4135
		+ HexNac-Hex-NeuAc	10.3	1837.8752	4.3847
		+ HexNac-Hex	0.7	1546.8016	-8.8944

*n.d. = not detected

[000363] ADAMTS13 glycosylation was also evaluated by monosaccharide analysis of the liberated, hydrolyzed rADAMTS13 glycans. The procedure involved acid hydrolysis, labeling using 2-AA and separation/detection/quantification by reversed phase HPLC, linked with fluorescence detection. rADAMTS13 samples were desalted by acetone precipitation. The

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant ADAMTS13 variant, wherein the ADAMTS13 variant comprises an amino acid sequence with ~~at least one~~ a single amino acid substitution as compared to an ADAMTS13 protein~~.~~

~~2. The recombinant ADAMTS13 variant of claim 1~~, wherein the single amino acid substitution is at amino acid Q97 as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13~~.~~

~~3. The recombinant ADAMTS13 variant of claim 2, wherein the single amino acid change is from a Q to a D, E, K, H, L, N, P, or R.~~

~~4. The recombinant ADAMTS13 variant of claim 2~~, wherein the single amino acid change is from a Q to an R.

~~5~~2. The recombinant ADAMTS13 variant of claim ~~4~~1, wherein the ADAMTS13 variant comprises the amino acid sequence of SEQ ID NO: 2~~, or an amino acid sequence having at least 80% sequence identity thereof.~~

~~6. The recombinant ADAMTS13 variant of claim 5~~, wherein optionally the ADAMTS13 consists essentially of the amino acid sequence of SEQ ID NO: 2, or~~.~~ ~~7. The recombinant ADAMTS13 variant of claim 5, wherein~~ the ADAMTS13 consists of the amino acid sequence of SEQ ID NO: 2.

~~8~~3. The recombinant ADAMTS13 variant of claim **Error! Reference source not found.**~~1~~, wherein the ADAMTS13 protein is a human ADAMTS13~~.~~, or ~~9. The recombinant ADAMTS13 variant of claim 1,~~ wherein the ADAMTS13 protein comprises the amino acid sequence of SEQ ID NO: 1.

~~10. The recombinant ADAMTS13 variant of any of the preceding claims, wherein at least one of the single amino acid substitutions is within the ADAMTS13 catalytic domain as compared to the ADAMTS13 protein.~~

~~11. The recombinant ADAMTS13 variant of any of the preceding claims, wherein the single amino acid substitution is not $I^{79}M$, $V^{88}M$, $H^{96}D$, $R^{102}C$, $S^{119}F$, $I^{178}T$, $R^{193}W$, $T^{196}I$, $S^{203}P$, $L^{232}Q$, $H^{234}Q$, $D^{235}H$, $A^{250}V$, $S^{263}C$, and/or $R^{268}P$ as denoted in SEQ ID NO: 1, or the equivalent amino acid position in an ADAMTS13.~~

~~12~~4. A pharmaceutical composition comprising at least one ADAMTS13 variant of any one of claims 1-~~11~~3 and a pharmaceutically acceptable carrier or excipient.

~~13~~5. The pharmaceutical composition of claim ~~12~~4, further comprising an ADAMTS13 protein~~.~~, ~~14. The pharmaceutical composition of claim 13,~~ wherein optionally the ADAMTS13 protein comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 80% sequence identity thereof.

~~15. The pharmaceutical composition of claim 13, wherein the ADAMTS13 protein consists of the amino acid sequence of SEQ ID NO: 1.~~

~~16~~6. The pharmaceutical composition of ~~any one of~~ claim~~s~~ ~~13-15~~5, wherein the ADAMTS13 protein is recombinantly produced.

~~17~~7. The pharmaceutical composition of ~~any one of~~ claim~~s~~ ~~13-15~~5, wherein the ADAMTS13 protein is plasma derived.

~~18~~8. The pharmaceutical composition of ~~any one of~~ claim~~s~~ ~~13-15~~5, wherein the ratio of ADAMTS13 variant to ADAMTS13 protein is about 4:1 to about 1:4, about 1:1 to about 3:1, about 1:1 or about 3:2.

~~19~~9. The pharmaceutical composition of any one of claims ~~13-18~~5-8, wherein the ~~-~~ADAMTS13 variant constitutes between about 40% to about 90% or between about 52% to about 72% or between about 47% to about 84% of total amount of all ADAMTS13 proteins and variants in the composition.

~~20~~10. The pharmaceutical composition of any one of claims ~~18 or claim 19~~8 or 9, wherein

(i) the ratio or percentage is determined by peptide mapping method~~.~~, or

~~21. The pharmaceutical composition of claim 18 or claim 19, wherein~~ (ii) the ratio or percentage is determined by HPLC analysis of tryptic peptides separated by liquid chromatography followed by mass spectrometry analysis.

~~22~~11. ~~-~~The pharmaceutical composition of any one of claims ~~18~~8-~~21~~10, wherein the ratio or percentage is determined based on intensities in extracted ion chromatograms~~.~~, and/or

~~23. The pharmaceutical composition of any one of claims 18-22,~~ wherein the ratio or percentage is determined based on the peak area of tryptic peptides of the ADAMTS13 variant in relation to the sum of the peak areas of all ADAMTS13 proteins and variants in the composition~~.~~

~~24. The pharmaceutical composition of claim 23~~, wherein optionally the tryptic peptides of all ADAMTS13 proteins and variants in the composition being measured are specific to the at least

one amino acid difference between the ADAMTS13 variant as compared to all other ADAMTS13 proteins and variants in the composition.

25. The pharmaceutical composition of claim 24, wherein further optionally the tryptic peptide(s) measured for the ADAMTS13 variant is AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5).

2612. The pharmaceutical composition of claim 24 or claim 2511, wherein the tryptic peptide measured for the ADAMTS13 protein is AAGGILHLELLVAVGPDVFQAHQEDTER (SEQ ID NO: 6).

2713. The pharmaceutical composition of any one of claims 18 or claim 198 or 9, wherein the ratio or percentage is determined based on total weight of ADAMTS13 variant in relation to the a sum total weight of all ADAMTS13 proteins and variants in the composition.

2814. Use of tThe ADAMTS13 variant of any one of claims 1-3 or pharmaceutical composition of any one of claims 4-13 in the manufacture of a medicament for use in

(A) A method for tTreating or preventing a blood clotting disorder in a subject suffering from or at risk of suffering from a blood clotting disorder, comprising administration of administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-11 3 or pharmaceutical composition of any one of claims 124-2713. to the subject in need thereof,

29. The method of claim 28, wherein optionally the blood clotting disorder is inherited TTP, acquired TTP, infarction, cerebral infarction, myocardial infarction, ischemic/reperfusion injury, deep vein thrombosis, or sepsis-related disseminated intravascular coagulation., or

30. (B) A method for Tttreating or preventing a bleeding episode in a subject in a subject suffering from or at risk of suffering from a bleeding disorder, comprising administration administering to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-11 3 or pharmaceutical composition of any one of claims 124-2713. ,

31. The method of claim 30wherein optionally the bleeding episode is associated with inherited TTP, acquired TTP, infarction, cerebral infarction, myocardial infarction, ischemic/reperfusion injury, deep vein thrombosis, or sepsis-related disseminated intravascular coagulation., or

32. (C) A method for tTreating or preventing a vaso-occlusive crisis in a subject suffering from sickle cell disease, comprising administrationadministering to the subject in

need thereof a therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-~~11~~ 3 or pharmaceutical composition of any one of claims ~~12~~4-~~27~~13~~.~~, or

~~33.~~ (D) ~~A method for t~~Treating or preventing lung injury in a subject suffering from or at risk of suffering from acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS), the ~~method~~ use comprising administration ~~administering~~ to the subject in need thereof a therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-~~11~~ 3 or pharmaceutical composition of any one of claims ~~12~~4-~~27~~13~~.~~, or

~~34.~~ (E) ~~A method for t~~Treating a cerebral infarction in a subject by recanalization of an occluded blood vessel in the subject, comprising administration ~~administering~~ to the subject in need thereof a therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-~~11~~ 3 or pharmaceutical composition of any one of claims ~~12~~4-~~27~~13, thereby recanalizing the occluded blood vessel~~.~~, or

~~35.~~ (F) ~~A method for t~~Treating or preventing a blood clotting disorder associated with cardiovascular disease in a subject, comprising administration~~administering~~ to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-~~11~~ 3 or pharmaceutical composition of any one of claims ~~12~~4-~~27~~13~~.~~

~~36. The method of claim 35~~, wherein optionally the blood clotting disorder associated with cardiovascular disease is associated with myocardial infarction, myocardial ischemia, deep vein thrombosis, peripheral vascular disease, stroke, transient ischemic attack, or medical device associated thrombosis~~.~~, or

~~37.~~ (G) ~~A method for t~~Treating or preventing hematologic disease in a subject, comprising administration ~~administering~~ to the subject in need thereof a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-~~11~~ 3 or pharmaceutical composition of any one of claims ~~12~~4-~~27~~13.

~~38. The method of any one of claims 28-37, wherein the ADAMTS13 variant and/or ADAMTS13 protein is recombinantly produced.~~

~~39. The method of any one of claims 28-38, wherein the subject is a mammal.~~

~~40~~15.The use of ADAMTS13 variant or pharmaceutical composition ~~for use~~ according to ~~The method of any one of~~ claim~~s~~ ~~28-38~~14, wherein the subject is a human.

~~41~~16.The use of pharmaceutical composition of any one of claims 4-13 or the ADAMTS13 variant or pharmaceutical composition ~~for use The method~~ according to any one of claims ~~28~~14-~~40~~15, wherein the composition is lyophilized~~.~~

~~42. The method of claim 41~~, wherein optionally the composition is reconstituted with a pharmaceutically acceptable vehicle suitable for injection prior to administration.

~~43~~17. The use of pharmaceutical composition of any one of claims 4-13 or the ADAMTS13 variant or pharmaceutical composition ~~for use~~ according to any one of~~The method of any one of~~ claims ~~28~~14-~~40~~16, wherein the composition is in a stable aqueous solution ready for administration.

~~44~~18. A nucleic acid molecule encoding the ADAMTS13 variant of any one of any one of claims 1-~~11~~3.

~~45~~19. A vector comprising the nucleic acid molecule of claim ~~44~~18~~.~~
~~46. The vector of claim 45~~, wherein optionally the vector is an expression vector wherein the polynucleotide sequence encoding the ADAMTS13 variant is operably linked to a promoter that is capable of mediating expression of the ADAMTS13 variant in a host cell.

~~47~~20. A host cell comprising the nucleic acid molecule of claim ~~44~~18~~.~~
~~48. A host cell comprising~~ or the vector of claim ~~45 or claim 46~~19.

~~49~~21. A host cell line comprising cells modified to express the ADAMTS13 variant of any one of claims 1-~~12~~ 3 and at least one ADAMTS13 protein.
~~50. The host cell line of claim 49, wherein the ADAMTS13 variant comprises the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80% sequence identity thereof~~
~~51. The host cell line of claim 49 or claim 50, wherein the amino acid sequence of the ADAMTS13 protein comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 80% sequence identity thereof.~~
~~52. The host cell line of any one of claims 49-51, wherein the amino acid sequence of the ADAMTS13 protein consists of the amino acid sequence of SEQ ID NO: 1.~~

~~53~~22. The host cell line of any one of claims ~~49~~20-~~53~~21, wherein

(i) the ADAMTS13 variant and the ADAMTS13 protein are expressed in different cells in the host cell line~~.~~, and/or

~~54. The host cell line of any one of claims 49-53, wherein~~ (ii) the ADAMTS13 variant and the ADAMTS13 protein are expressed in the same cell~~.~~, and/or

~~55. The host cell or host cell line of any one of claims 47-54, wherein~~ (iii) the cell is a CHO, COS, HEK 293, BHK, SK-Hep, or HepG2 cell~~.~~

~~56. The host cell or host cell line of claim 55~~, wherein optionally the CHO cell is a CHO DBX-11 or CHOZN cell line~~.~~

~~57. The host cell or host cell line of claim 56~~, wherein further optionally the CHOZN cell is a CHO DBX-11 cell line~~.~~

~~58. The host cell or host cell line of claim 56, wherein the CHOZN cell is~~ or a CHOZN glutamine synthetase (GS) -/- cell line.

23. A method for detecting an ADAMTS13 variant of any one of claims 1-3, comprising:

a. subjecting a plasma sample to trypsin digestion to form a peptide mixture;

b. separating the peptide mixture by reversed phase chromatography; and

c. detecting the ADAMTS13 variant via detecting AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5).

24. A method of determining the ratio or percentage of an ADAMTS13 variant of claims 1-3, comprising:

a. subjecting a plasma sample to trypsin digestion to form a peptide mixture;

b. separating the peptide mixture by reversed phase chromatography;

c. detecting AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) or a combination of AAGGILHLELLVAVGPDVFQAHR (SEQ ID NO: 115) and EDTER (SEQ ID NO: 5) to determine ADAMTS13 variant intensities;

d. detecting the presence of non-variant ADAMTS13 intensities; and

e. determining the ratio or percentage of the ADAMTS13 variant intensities as compared to the non-variant ADAMTS13 intensities.