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

[0008] In certain examples, the present disclosure provides variants of ADAMTS13. In certain 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 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 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 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 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 examples, the amino acid change is from a Q to a D, E, K, H, L, N, P, or R. In certain examples, the amino acid change is from a Q to an R. In certain 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 a single amino acid substitution is at amino acid Q97 as denoted in or an equivalent amino acid position in a human wildtypeADAMTS13, wherein the single amino acid change is from a Q to an R.

[0009] In certain 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 examples, the present disclosure provides pharmaceutical compositions comprising at least one ADAMTS13 variant and a pharmaceutically acceptable carrier or excipient. In certain examples, the pharmaceutical composition comprises a combination of at least one ADAMTS13 variant and at

In certain 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 examples, the relative abundance, percentage, and/or ratio is based on intensities in extracted ion chromatograms. In certain 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 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 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 examples, the ADAMTS13 variant and/or wildtype is recombinant. In certain examples, the wildtype ADAMTS13 variant and/or wildtype is plasma derived.

[00014] In one 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 a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered 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 a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered to the subject in need thereof, 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 a therapeutically effective amount of the ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered to the subject in need thereof, 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), comprising a therapeutically effective amount of the ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered to the subject in need thereof, or

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

(F) Treating or preventing a blood clotting disorder associated with cardiovascular disease in a subject, comprising a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered to the subject in need thereof, 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 a therapeutically effective amount of an ADAMTS13 variant or pharmaceutical composition as disclosed herein to be administered to the subject in need thereof.

[00015] In certain 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

[00029] In one 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, the present disclosure provides a vector comprising a nucleic acid molecule encoding for the recombinant ADAMTS13 variant as disclosed herein. In one 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 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, the present disclosure provides a host cell comprising the vector as disclosed herein or stably integrated with a nucleic acid molecule encoding the ADAMTS13 variant as disclosed herein In one 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 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 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 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 examples, the CHO cell is a CHO DBX-11 or CHOZN cell line. In certain examples, the CHOZN cell is a CHO DBX-11 cell line. In certain 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 to a non-variant ADAMTS13 in a sample, 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 the 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).

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant ADAMTS13 variant, wherein the ADAMTS13 variant comprises a single amino acid substitution at amino acid Q97 as denoted in SEQ ID NO: 1, or an equivalent amino acid position in a human wildtype ADAMTS13, wherein the single amino acid change is from a Q to an R.

2. The recombinant ADAMTS13 variant of claim 1, wherein the ADAMTS13 variant comprises at least amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 2.

3. The recombinant ADAMTS13 variant of claim 1, wherein the ADAMTS13 variant comprises the amino acid sequence of SEQ ID NO: 2.

4. The recombinant ADAMTS13 variant of claim 2, wherein the ADAMTS13 variant consists essentially of the amino acid sequence of SEQ ID NO: 2.

5. The recombinant ADAMTS13 variant of claim 2, wherein the ADAMTS13 variant consists of the amino acid sequence of SEQ ID NO: 2.

6. The recombinant ADAMTS13 variant of claim 2, wherein the ADAMTS13 variant consists essentially of amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 2.

7. The recombinant ADAMTS13 variant of claim 2, wherein the ADAMTS13 variant consists of amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 2.

8. The recombinant ADAMTS13 variant of claim 1, wherein the ADAMTS13 variant lacks the signal peptide and/or propeptide.

9. A pharmaceutical composition comprising at least one ADAMTS13 variant of any one of claims 1-8 and a pharmaceutically acceptable carrier or excipient.

10. The pharmaceutical composition of claim 9, further comprising an ADAMTS13 protein.

11. The pharmaceutical composition of claim 10, wherein the ADAMTS13 protein comprises at least amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 80% sequence identity thereof.

12. The pharmaceutical composition of claim 11, wherein the ADAMTS13 protein comprises the amino acid sequence of SEQ ID NO: 1.

13. The pharmaceutical composition of claim 11, wherein the ADAMTS13 protein consists essentially of amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 1.

14. The pharmaceutical composition of claim 11, wherein the ADAMTS13 protein consists of amino acids 75-1427 of the amino acid sequence of SEQ ID NO: 1.

15. The pharmaceutical composition of claim 11, wherein the ADAMTS13 protein lacks the signal peptide and/or propeptide.

16. The pharmaceutical composition of any one of claims 11-15, wherein the ADAMTS13 protein is recombinantly produced.

17. The pharmaceutical composition of any one of claims 11-15, wherein the ADAMTS13 protein is plasma derived.

18. The pharmaceutical composition of any one of claims 11-17, wherein the ratio of ADAMTS13 variant to ADAMTS13 protein is 4:1 to 1:4, 1:1 to 3:1, 1:1 or 3:2.

19. The pharmaceutical composition of any one of claims 11-17, wherein the ADAMTS13 variant constitutes between 40% to 90% or between 52% to 72% or between 47% to 84% of total amount of all ADAMTS13 proteins and variants in the composition.

20. The pharmaceutical composition of claim 18 or 19, wherein the ratio of ADAMTS13 variant to ADAMTS13 protein or the percentages of ADAMTS13 variant and ADAMTS13 protein

(i) is as determined by peptide mapping method, or

(ii) is as determined by HPLC analysis of tryptic peptides separated by liquid chromatography followed by mass spectrometry analysis.

21. The pharmaceutical composition of any one of claims 18-20, wherein the ratio of ADAMTS13 variant to ADAMTS13 protein or the percentages of ADAMTS13 variant and ADAMTS13 protein is as determined based on intensities in extracted ion chromatograms, and/or wherein the ratio of ADAMTS13 variant to ADAMTS13 protein or the percentages of ADAMTS13 variant and ADAMTS13 protein is as 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, 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, 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).

22. The pharmaceutical composition of claim 21, wherein the tryptic peptide measured for the ADAMTS13 protein is AAGGILHLELLVAVGPDVFQAHQEDTER (SEQ ID NO: 6).

23. The pharmaceutical composition of claim 18 or claim 19, wherein the ratio of ADAMTS13 variant to ADAMTS13 protein or the percentages of ADAMTS13 variant and ADAMTS13 protein is as determined based on total weight of ADAMTS13 variant in relation to a sum total weight of all ADAMTS13 proteins and variants in the composition.

24. Use of the ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 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 a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-8or pharmaceutical composition of any one of claims 9-23to be administered 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 a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 to be administered to the subject in need thereof, 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 therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 to be administered to the subject in need thereof, 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), comprising a therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims9-23 to be administered to the subject in need thereof, or

(E) Treating a cerebral infarction in a subject by recanalization of an occluded blood vessel in the subject, comprising a therapeutically effective amount of the ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 to be administered to the subject in need thereof, thereby recanalizing the occluded blood vessel, or

(F) Treating or preventing a blood clotting disorder associated with cardiovascular disease in a subject, comprising a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 to be administered to the subject in need thereof, 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 a therapeutically effective amount of an ADAMTS13 variant of any one of claims 1-8 or pharmaceutical composition of any one of claims 9-23 to be administered to the subject in need thereof.

25. The use of claim 24 wherein the subject is a human.

26. The use of claim 24 or claim 25, or the pharmaceutical composition of any one of claims 9-23, wherein the composition is lyophilized, wherein optionally the composition is reconstituted with a pharmaceutically acceptable vehicle suitable for injection prior to administration.

27. The use of claim 24 or claim 25, or the pharmaceutical composition of any one of claims 9-23, wherein the composition is in a stable aqueous solution ready for administration.

28. A vector comprising a nucleic acid molecule encoding for the recombinant ADAMTS13 variant of any one of claims 1-8.

29. A host cell comprising the vector of claim 28 or or stably integrated with a nucleic acid molecule encoding the ADAMTS13 variant of any one of claims 1-8.

30. A host cell line comprising cells modified to express the ADAMTS13 variant of any one of claims 1-8 and at least one ADAMTS13 protein.

31. The host cell line of claim 29 or claim 30, wherein

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

(ii) the ADAMTS13 variant and the ADAMTS13 protein are expressed in the same cell, and/or

(iii) the cell is a CHO, COS, HEK 293, BHK, SK-Hep, or HepG2 cell, wherein optionally the CHO cell is a CHO DBX-11 or CHOZN cell line, wherein further optionally the CHOZN cell is a CHO DBX-11 cell line or a CHOZN glutamine synthetase (GS) -/- cell line.

32. A method for detecting an ADAMTS13 variant of any one of claims 1-8, 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).

33. A method of determining the ratio or percentage of an ADAMTS13 variant to a non-variant ADAMTS13 in a sample 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 the 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.