It has been found that three linker regions in the distal domains of ADAMTS13 are important for flexibility and enable the interaction between the proximal and the T8-CUB2 domains during the inactive state (Deforche et al., 2015). More recent research performed binding and functional studies on a panel of truncated ADAMTS13 variants to develop a model for the conformational activation of structurally quiescent ADAMTS13 by VWF (South *et al*., 2017). Results indicated that both the isolated CUB1 and CUB2 domains in ADAMTS13 bind to the spacer domain exosite of a truncated ADAMTS13 variant. However, only the CUB1 domain inhibited proteolytic activity of the truncated ADAMTS13 variant. The combination of findings from this study support an ADAMTS13 activation model in which VWF D4-CK engages the TSP8-CUB2 domains, inducing the conformational change that disrupts the CUB1-spacer domain interaction and thereby activates ADAMTS13. This study therefore suggests that the most important domains for ADAMTS13 conformational activation are the two CUB domains and the spacer region.

ADAMTS13 variants have previously been generated with amino acid substitutions in the spacer region in an effort to prevent the need for conformational activation and overcome the problems with wild type ADAMTS13 therapy. The gain of function (GoF) ADAMTS13 variant (R568K/F592Y/R660K/Y661F/ Y665F), was shown to have disrupted autoinhibition and enhanced proteolytic activity (Jian *et al*., 2012). Furthermore, this GoF variant restored cerebral blood flow at a lower dose than wild type ADAMTS-13 and retained some ability to recanalize vessels when administration was delayed by 1 h in a murine stroke model (South *et al*., 2018). However, the reduction in dose requirement demonstrated by the GoF mutant may not be sufficient to fully overcome the problem associated with wildtype ADAMTS13. Additionally, the efficacy of the GoF mutant is reduced when administration is delayed. The development of variants with a greater reduction in dose requirement and an improved efficacy after delayed administration could lead to a more clinically significant therapy.

The present disclosure has been devised in light of the above considerations. The inventors have identified that the linker 3 region of the ADAMTS13 protein is key to the conformational activation mechanism and have produced a number of improved ADAMTS13 variants with amino acid substitutions in the linker 3 region for the first time.

Summary

In a first aspect, the present disclosure provides an ADAMTS13 variant having an amino acid sequence comprising one or more amino acid substitutions in the region corresponding to SEQ ID NO:48 relative to the amino acid sequence of wildtype human ADAMTS13.

In some examples, the ADAMTS13 variant comprises substitution at one or more of the following positions relative to the amino acid sequence of wildtype human ADAMTS13: A1144, A1145, A1146, P1147, P1154, P1171, P1173, P1175, P1180, and P1182.

In some examples, an ADAMTS13 variant comprises substitution of an alanine residue with a valine, isoleucine, lysine, leucine, or methionine residue relative to the amino acid sequence of wildtype human ADAMTS13. In some examples, an ADAMTS13 variant comprises substitution of an alanine residue

with a valine, isoleucine, or lysine residue relative to the amino acid sequence of wildtype human ADAMTS13.

In some examples, an ADAMTS13 variant comprises substitution of a proline residue with a valine, isoleucine, lysine, leucine, or methionine residue relative to the amino acid sequence of wildtype human ADAMTS13. In some examples, an ADAMTS13 variant comprises substitution of a proline residue with a valine, isoleucine, or lysine residue relative to the amino acid sequence of wildtype human ADAMTS13.

In some examples, the ADAMTS13 variant comprises an amino acid sequence according to SEQ ID NO:50 or 156.

In some examples, the ADAMTS13 variant comprises substitution to valine, isoleucine, or lysine at one or more of the following positions relative to the amino acid sequence of wildtype human ADAMTS13: A1144, A1145, A1146, P1147, P1154, P1171, P1173, P1175, P1180, and P1182.

In some examples, the ADAMTS13 variant comprises:
(i) the amino acid sequence of SEQ ID NO:51, 52, 53, 54, 55, 56, 57, 58, 59, or 60; or
(ii) the amino acid sequence of SEQ ID NO:136, 137, 138, 139, 140, 141, 142, 143, 144, or 145; or
(iii) the amino acid sequence of SEQ ID NO:146, 147, 148, 149, 150, 151, 152, 153, 154, or 155.

In some examples, the ADAMTS13 variant comprises: (i) the amino acid sequence of SEQ ID NO:51; or (ii) the amino acid sequence of SEQ ID NO:52; or (iii) the amino acid sequence of SEQ ID NO:53; or (iv) the amino acid sequence of SEQ ID NO:54; or (v) the amino acid sequence of SEQ ID NO:55; or (vi) the amino acid sequence of SEQ ID NO:56; or (vii) the amino acid sequence of SEQ ID NO:57; or (viii) the amino acid sequence of SEQ ID NO:58; or (ix) the amino acid sequence of SEQ ID NO:59; or (x) the amino acid sequence of SEQ ID NO:60.

In some examples, the ADAMTS13 variant comprises: (i) the amino acid sequence of SEQ ID NO:136; or (ii) the amino acid sequence of SEQ ID NO:137; or (iii) the amino acid sequence of SEQ ID NO:138; or (iv) the amino acid sequence of SEQ ID NO:139; or (v) the amino acid sequence of SEQ ID NO:140; or (vi) the amino acid sequence of SEQ ID NO:141; or (vii) the amino acid sequence of SEQ ID NO:142; or (viii) the amino acid sequence of SEQ ID NO:143; or (ix) the amino acid sequence of SEQ ID NO:144; or (x) the amino acid sequence of SEQ ID NO:145.

In some examples, the ADAMTS13 variant comprises: (i) the amino acid sequence of SEQ ID NO:146; or (ii) the amino acid sequence of SEQ ID NO:147; or (iii) the amino acid sequence of SEQ ID NO:148; or (iv) the amino acid sequence of SEQ ID NO:149; or (v) the amino acid sequence of SEQ ID NO:150; or (vi) the amino acid sequence of SEQ ID NO:151; or (vii) the amino acid sequence of SEQ ID NO:152; or (viii) the amino acid sequence of SEQ ID NO:153; or (ix) the amino acid sequence of SEQ ID NO:154; or (x) the amino acid sequence of SEQ ID NO:155.

In some examples, the ADAMTS13 variant comprises substitution to valine, isoleucine, or lysine at one or both of positions P1180 and/or P1182 relative to the amino acid sequence of wildtype human ADAMTS13.

In some examples, the ADAMTS13 variant comprises the amino acid sequence of SEQ ID NO:59, 60, 144, 145, 154, or 155.

In some examples, the ADAMTS13 variant comprises or consists of: (i) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:61; or (ii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:62; or (iii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:63; or (iv) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:64; or (v) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:65; or (vi) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:66; or (vii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:67; or (viii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:68; or (ix) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:69; or (x) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:70.

In some examples, the ADAMTS13 variant displays increased proteolytic activity as compared to wildtype human ADAMTS13.

In another aspect, the present disclosure also provides a nucleic acid encoding an ADAMTS13 variant according to the present disclosure.

In another aspect, the present disclosure also provides an expression vector, comprising a nucleic acid according to the present disclosure.

In another aspect, the present disclosure also provides a cell comprising an ADAMTS13 variant, a nucleic acid, or an expression vector according to the present disclosure.

In yet another aspect, the present disclosure also provides a method for producing an ADAMTS13 variant, comprising culturing a cell comprising a nucleic acid or expression vector according to the present disclosure, under conditions suitable for expression of an ADAMTS13 variant by the cell.

In another aspect, the present disclosure also provides a pharmaceutical composition comprising an ADAMTS13 variant, a nucleic acid, an expression vector, or a cell according to the present disclosure, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

The present disclosure also provides an ADAMTS13 variant, a nucleic acid, an expression vector, a cell, or a composition according to the present disclosure, for use in a method of medical treatment or prophylaxis.

In another aspect, the present disclosure provides an ADAMTS13 variant as disclosed herein, a nucleic acid as disclosed herein, an expression vector as disclosed herein, a cell according as disclosed herein, or a composition as disclosed herein, for use in therapy.

The present disclosure also provides an ADAMTS13 variant, a nucleic acid, an expression vector, a cell, or a composition according to the present disclosure, for use in a method of treatment or prevention of a disease or condition characterised by one or more of: an increased level and/or activity of VWF, or of a complex comprising VWF; a reduced level of ADAMTS13; a reduced level of ADAMTS13 proteolytic activity; thrombosis; and inflammation.

The present disclosure also provides the use of an ADAMTS13 variant, a nucleic acid, an expression vector, a cell, or a composition according to the present disclosure in the manufacture of a medicament for use in a method of treatment or prevention of a disease or condition characterised by one or more of: an increased level and/or activity of VWF, or of a complex comprising VWF; a reduced level of ADAMTS13; a reduced level of ADAMTS13 proteolytic activity; thrombosis; and inflammation.

The present disclosure also provides a method of treating or preventing a disease or condition characterised by one or more of: an increased level and/or activity of VWF, or of a complex comprising VWF; a reduced level of ADAMTS13; a reduced level of ADAMTS13 proteolytic activity; thrombosis; and inflammation, comprising administering to a subject a therapeutically or prophylactically effective amount of an ADAMTS13 variant, a nucleic acid, an expression vector, a cell, or a composition according to the present disclosure.

In some examples, the disease or condition is selected from: a disease/condition characterised by thrombosis, a disease/condition characterised by inflammation, thrombotic thrombocytopenic purpura (TTP), ischaemic stroke, haemorrhagic stroke, subarachnoid haemorrhage (SAH), intracerebral haemorrhage (ICH), chronic thromboembolic pulmonary hypotension (CTEPH), myocardial infarction (MI), ST-elevation myocardial infarction (STEMI), unstable angina (UA), ischemia, reperfusion, deep venous thrombosis, pulmonary embolism, intravascular coagulation (DIC), hemolytic-uremic syndrome (HUS), cerebral infarction, systemic lupus erythematosus (SLE), disease cause by infection with a SARSr-CoV (e.g. SARS-CoV-2; e.g. COVID-19), acute respiratory distress syndrome (ARDS), pneumonia, kidney damage, nephropathy, microvascular diseases, dementia, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, and bacterial diarrhoea.

In another aspect, the present disclosure provides use of an ADAMTS13 variant as disclosed herein, a nucleic acid as disclosed herein, an expression vector as disclosed herein, a cell as disclosed herein, or a composition as disclosed herein, in the manufacture of a medicament for treating a disease or condition, wherein the disease or condition is selected from: a disease/condition characterised by thrombosis, a disease/condition characterised by inflammation, thrombotic thrombocytopenic purpura (TTP), ischaemic stroke, haemorrhagic stroke, subarachnoid haemorrhage (SAH), intracerebral haemorrhage (ICH), chronic thromboembolic pulmonary hypotension (CTEPH), myocardial infarction (MI), ST-elevation myocardial infarction (STEMI), unstable angina (UA), ischemia, reperfusion, deep venous thrombosis, pulmonary embolism, intravascular coagulation (DIC), hemolytic-uremic syndrome (HUS), cerebral infarction, systemic lupus erythematosus (SLE), disease cause by infection with a SARSr-CoV (e.g. SARS-CoV-2; e.g. COVID-19), acute respiratory distress syndrome (ARDS), pneumonia, kidney damage, nephropathy, microvascular diseases, dementia, Crohn's disease, inflammatory bowel disease, ulcerative colitis, and bacterial diarrhoea.

In another aspect, the present disclosure also provides a method of cleaving VWF, comprising contacting VWF or a complex comprising VWF with an ADAMTS13 variant according to the present disclosure.

The present disclosure includes the combination of the aspects and preferred features described herein except where such combinations are clearly impermissible or expressly avoided.

Claims:

1. An ADAMTS13 variant having an amino acid sequence comprising one or more amino acid substitutions in the region corresponding to SEQ ID NO:48 relative to the amino acid sequence of wildtype human ADAMTS13.

2. The ADAMTS13 variant according to claim 1, wherein the ADAMTS13 variant comprises substitution at one or more of the following positions relative to the amino acid sequence of wildtype human ADAMTS13: A1144, A1145, A1146, P1147, P1154, P1171, P1173, P1175, P1180, and P1182.

3. The ADAMTS13 variant according to claim 1 or claim 2, wherein the ADAMTS13 variant comprises an amino acid sequence according to SEQ ID NO:50 or 156.

4. The ADAMTS13 variant according to any one of claims 1 to 3, wherein the ADAMTS13 variant comprises:
(i) substitution to valine, isoleucine, or lysine at one or more of the following positions relative to the amino acid sequence of wildtype human ADAMTS13: A1144, A1145, A1146, P1147, P1154, P1171, P1173, P1175, P1180, and P1182; or
(ii) the amino acid sequence of SEQ ID NO:51, 52, 53, 54, 55, 56, 57, 58, 59, or 60; or
(iii) the amino acid sequence of SEQ ID NO:136, 137, 138, 139, 140, 141, 142, 143, 144, or 145; or
(iv) the amino acid sequence of SEQ ID NO:146, 147, 148, 149, 150, 151, 152, 153, 154, or 155.

5. The ADAMTS13 variant according to any one of claims 1 to 4, wherein the ADAMTS13 variant comprises:
(i) substitution to valine, isoleucine, or lysine at one or both of positions P1180 and/or P1182 relative to the amino acid sequence of wildtype human ADAMTS13; or
(ii) the amino acid sequence of SEQ ID NO:59, 60, 144, 145, 154, or 155.

6. The ADAMTS13 variant according to any one of claims 1 to 5, wherein the ADAMTS13 variant comprises or consists of: (i) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:61; or (ii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:62; or (iii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:63; or (iv) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:64; or (v) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:65; or (vi) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:66; or (vii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:67; or (viii) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:68; or (ix) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:69; or (x) an amino acid sequence having at least 60% sequence identity to the amino acid sequence of SEQ ID NO:70.

7. The ADAMTS13 variant according to any one of claims 1 to 6, wherein the ADAMTS13 variant displays increased proteolytic activity as compared to wildtype human ADAMTS13.

8. A nucleic acid encoding an ADAMTS13 variant according to any one of claims 1 to 7.

9. An expression vector, comprising a nucleic acid according to claim 8.

10. A cell comprising an ADAMTS13 variant according to any one of claims 1 to 7, a nucleic acid according to claim 8, or an expression vector according to claim 9.

11. A method for producing an ADAMTS13 variant, comprising culturing a cell comprising a nucleic acid according to claim 8, or an expression vector according to claim 9, under conditions suitable for expression of an ADAMTS13 variant by the cell.

12. A pharmaceutical composition comprising an ADAMTS13 variant according to any one of claims 1 to 7, a nucleic acid according to claim 8, an expression vector according to claim 9, or a cell according to claim 10, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

13. An ADAMTS13 variant according to any one of claims 1 to 7, a nucleic acid according to claim 8, an expression vector according to claim 9, a cell according to claim 10, or a composition according to claim 12, for use in therapy.

14. Use of an ADAMTS13 variant according to any one of claims 1 to 7, a nucleic acid according to claim 8, an expression vector according to claim 9, a cell according to claim 10, or a composition according to claim 12, in the manufacture of a medicament for treating a disease or condition, wherein the disease or condition is selected from: a disease/condition characterised by thrombosis, a disease/condition characterised by inflammation, thrombotic thrombocytopenic purpura (TTP), ischaemic stroke, haemorrhagic stroke, subarachnoid haemorrhage (SAH), intracerebral haemorrhage (ICH), chronic thromboembolic pulmonary hypotension (CTEPH), myocardial infarction (MI), ST-elevation myocardial infarction (STEMI), unstable angina (UA), ischemia, reperfusion, deep venous thrombosis, pulmonary embolism, intravascular coagulation (DIC), hemolytic-uremic syndrome (HUS), cerebral infarction, systemic lupus erythematosus (SLE), disease cause by infection with a SARSr-CoV (e.g. SARS-CoV-2; e.g. COVID-19), acute respiratory distress syndrome (ARDS), pneumonia, kidney damage, nephropathy, microvascular diseases, dementia, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, and bacterial diarrhoea.

15. The use of claim 14, wherein the disease or condition is characterized by one or more of: an increased level and/or activity of VWF, or of a complex comprising VWF a reduced level of ADAMTS13; a reduced of ADAMTS13; or a reduced level of ADAMTS13 proteolytic activity.

16. A method of cleaving VWF, comprising contacting VWF or a complex comprising VWF with an ADAMTS13 variant according to any one of claims 1 to 7.