FUSION PROTEINS COMPRISING SULFOGLUCOSAMINE SULFOHYDROLASE ENZYMES AND METHODS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 63/091,800, filed October 14, 2020. The entire content of the application referenced above is hereby incorporated by reference herein.

BACKGROUND

Sanfilippo syndrome, or MPS III, is a rare, neurodegenerative disorder that results from certain defects in lysosomal function. The most common type of Sanfilippo syndrome is type A, which is caused by genetic mutations in the *SGSH* gene. Insufficient N-sulfoglucosamine sulfohydrolase (SGSH) activity leads to accumulation of heparan sulfate-derived oligosaccharides and to lysosomal dysfunction in multiple organs and tissues, particularly the brain and spinal cord. Treatments for Sanfilippo syndrome remain largely supportive; while the deficient enzyme may be administered intravenously, it has little effect on the brain due to difficulties in delivering the recombinant enzyme across the blood-brain barrier (BBB). Accordingly, there is a need for more effective therapies that treat both the peripheral and central nervous system (CNS) symptoms of Sanfilippo syndrome A.

SUMMARY

Thus, provided herein is a specific enzyme replacement therapy, which has the capability of crossing the BBB and treating both the peripheral and CNS manifestations of Sanfilippo syndrome A. In particular, certain examples provide a protein comprising: (a) a first Fc polypeptide linked to a first N-sulfoglucosamine sulfohydrolase (SGSH) amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof; and (b) a second Fc polypeptide linked to a second SGSH amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof, wherein the second Fc polypeptide comprises a sequence having at least 80% identity to SEQ ID NO: 37, and having Ala at position 389, according to EU numbering. In some examples, the second Fc polypeptide comprises at the following positions, according to EU numbering: Glu at position 380; Ala at position 389; and Asn at position 390. In some examples, the second Fc polypeptide

comprises at the following positions, according to EU numbering: Glu at position 380; Tyr at position 384; Thr at position 386; Glu at position 387; Trp at position 388; Ala at position 389; Asn at position 390; Thr at position 413; Glu at position 415; Glu at position 416; and Phe at position 421. In certain examples, the second Fc polypeptide specifically binds to a transferrin receptor (TfR) or is capable of specifically binding to a TfR. In one aspect, the present disclosure refers to a protein comprising: a. a first Fc polypeptide linked to a first N-sulfoglucosamine sulfohydrolase (SGSH) amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof; and b. a second Fc polypeptide linked to a second SGSH amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof, wherein the second Fc polypeptide is capable of specifically binding to a transferrin receptor (TfR); and wherein the second Fc polypeptide comprises a sequence having at least 80% identity to SEQ ID NO: 37 and has Ala at position 389, according to EU numbering. In certain examples, the second Fc polypeptide binds to the apical domain of the TfR. In certain examples, the binding of the protein to the TfR does not substantially inhibit binding of transferrin to the TfR. In certain examples, the protein binds to a TfR with an affinity of from about 100 nM to about 500 nM, or optionally from about 150 nM to about 400 nM. In certain examples, the protein is capable of being transported across the blood-brain barrier of a subject.

In certain examples, the first SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS:58-60. In certain examples, the first SGSH amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS:58-60.

In certain examples, the second SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS:58-60. In certain examples, the second SGSH amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS:58-60.

In certain examples, the first Fc polypeptide is linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In certain examples, the second Fc polypeptide is linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In certain examples, the polypeptide linker is a flexible polypeptide linker. In certain examples, the flexible polypeptide linker is a glycine-rich linker. In certain examples, the polypeptide linker is GS (SEQ ID NO:7), G_4S (SEQ ID NO:8) or $(G_4S)_2$ (SEQ ID NO:9).

In certain examples, the N-terminus of the first Fc polypeptide is linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof. In certain examples, the C-terminus of the first Fc polypeptide is linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof. In certain examples, the N-terminus of the second Fc polypeptide is linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or a

catalytically active fragment thereof. In certain examples, the C-terminus of the second Fc polypeptide is linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof. In certain examples, the N-terminus of the first Fc polypeptide is linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof; and the N-terminus of the second Fc polypeptide is linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof. In certain examples, the C-terminus of the first Fc polypeptide is linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof; and the C-terminus of the second Fc polypeptide is linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof.

In certain examples, the first Fc polypeptide and the second Fc polypeptide each contain modifications that promote heterodimerization. In certain examples, one of the Fc polypeptides has a T366W substitution and the other Fc polypeptide has T366S, L368A, and Y407V substitutions, according to EU numbering.

In certain examples, the first Fc polypeptide contains the T366S, L368A, and Y407V substitutions and the second Fc polypeptide contains the T366W substitution. In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 12-19 and 28-31; and the second Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 34-41 and 54-57.

In certain examples, the first Fc polypeptide contains the T366W substitution and the second Fc polypeptide contains the T366S, L368A, and Y407V substitutions. In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 24-27; and the second Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 48-53.

In certain examples, the first Fc polypeptide and/or the second Fc polypeptide comprises a native FcRn binding site.

In certain examples, the first Fc polypeptide and the second Fc polypeptide do not have effector function. In certain examples, the first Fc polypeptide and/or the second Fc polypeptide includes a modification that reduces effector function. In certain examples, the

modification that reduces effector function is the substitutions of Ala at position 234 and Ala at position 235, according to EU numbering.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 14-19 and 26-31. In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 14, 15, 28, and 29. In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS:18, 19, 30, and 31. In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 61-88, and 117-118. In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 61-68, 73-76, 81-84, and 117-118.

In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 36-41 and 50-57. In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 36, 37, 54, and 55. In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS:40 41, 56, and 57. In certain examples, the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 89-116, and 119-120. In certain examples, the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS: 89-96, 101-104, 109-112, and 119-120.

In certain examples, the first Fc polypeptide and/or the second Fc polypeptide comprises amino acid changes relative to the native Fc sequence that extend serum half-life. In certain examples, the amino acid changes comprise substitutions of Tyr at position 252, Thr at position 254, and Glu at position 256, according to EU numbering. In certain examples, the amino acid changes comprise substitutions of Leu at position 428 and Ser at position 434,

according to EU numbering. In certain examples, the amino acid changes comprise a substitution of Ser or Ala at position 434, according to EU numbering.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 61-68, 73-76, and 81-84; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 89-96, 101-104, and 109-112.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 61-64; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 89-92. In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 63 or 64; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 91 or 92.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 75 or 76; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 103 or 104.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 83 or 84; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 111 or 112.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 65-68; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of any one of SEQ ID NOS: 93-96. In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 67 or 68; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NOS: 95 or 96.

In certain examples, the first Fc polypeptide linked to the first SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NO: 118; and the second Fc polypeptide linked to the second SGSH amino acid sequence comprises an amino acid sequence of SEQ ID NO: 120.

In certain examples, uptake of the SGSH amino acid sequence into the brain is at least ten-fold greater as compared to the uptake of the SGSH amino acid sequence in the absence of the first Fc polypeptide and the second Fc polypeptide or as compared to the uptake of the SGSH enzyme without the modifications to the second Fc polypeptide that result in TfR binding.

In certain examples, the first Fc polypeptide is not modified to bind to a blood-brain barrier (BBB) receptor and the second Fc polypeptide is modified to specifically bind to a TfR.

In certain examples, the protein does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

Certain examples also provide, a polypeptide comprising a Fc polypeptide linked to an SGSH amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof, wherein the Fc polypeptide i) comprises a sequence having at least 90% identity to SEQ ID NO: 37; ii) has one or more modifications that promote its heterodimerization to another Fc polypeptide; and iii) has Ala at position 389, according to EU numbering. In some examples, the Fc polypeptide comprises at the following positions, according to EU numbering: Glu at position 380; Ala at position 389; and Asn at position 390. In some examples, the Fc polypeptide comprises at the following positions, according to EU numbering: Glu at position 380; Tyr at position 384; Thr at position 386; Glu at position 387; Trp at position 388; Ala at position 389; Asn at position 390; Thr at position 413; Glu at position 415; Glu at position 416; and Phe at position 421. In certain examples, the Fc polypeptide specifically binds to a transferrin receptor (TfR) or is capable of specifically binding to a TfR. In one aspect, the present disclosure refers to a polypeptide comprising a Fc polypeptide linked to an SGSH amino acid sequence, an SGSH variant amino acid sequence, or a catalytically active fragment thereof, wherein the Fc polypeptide i) is capable of specifically binding to a transferrin receptor (TfR); ii) comprises a sequence having at least 90% identity to SEQ ID NO: 37; iii) has one or more modifications that promote its heterodimerization to another Fc polypeptide; and iv) has Ala at position 389, according to EU numbering. In certain examples, the Fc polypeptide is linked to the SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In certain examples, the polypeptide is a fusion polypeptide comprising from N- to C-terminus: the SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment; a polypeptide linker; and the Fc polypeptide. In certain examples, the polypeptide is a fusion polypeptide comprising from N- to C-terminus: the Fc polypeptide; a polypeptide linker; and the SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment.

In certain examples, the Fc polypeptide contains T366S, L368A, and Y407V substitutions, according to EU numbering. In certain examples, the polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS:97-100, 105-108, and 113-116.

In certain examples, the Fc polypeptide contains a T366W substitution. In certain examples, the polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95% or 100% identity to any one of SEQ ID NOS:89-96, 101-104, 109-112, and 119-120.

In one aspect, the present disclosure refers to a protein comprising the polypeptide as described herein and the other Fc polypeptide. In certain examples, a protein comprises the Fc polypeptide, wherein the Fc polypeptide is dimerized to the other Fc polypeptide. Thus, certain examples provide a protein comprising a Fc polypeptide as described herein and the other Fc polypeptide.

In one aspect, the present disclosure refers to a polynucleotide comprising a nucleic acid sequence encoding a polypeptide as described herein. In one aspect, the present disclosure refers to a vector comprising a polynucleotide as described herein. In one aspect, the present disclosure refers to a host cell comprising a polynucleotide as described herein or a vector as described herein. In certain examples, such a host cell further comprises a polynucleotide comprising a nucleic acid sequence encoding the other Fc polypeptide.

Provided herein is a method of making a protein or polypeptide as described herein.

In one aspect, the present disclosure refers to a method for producing a polypeptide comprising an Fc polypeptide that is linked to an SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment, comprising culturing a host cell under conditions in which the polypeptide encoded by a polynucleotide as described herein is expressed.

Certain examples provide a pair of polynucleotides comprising a first nucleic acid sequence encoding a first Fc polypeptide linked to a first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof; and a second nucleic acid sequence encoding a second Fc polypeptide linked to a second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof. In one aspect, the present disclosure refers to a pair of polynucleotides comprising a first nucleic acid sequence encoding the first Fc polypeptide linked to the first SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment; and a second nucleic acid sequence encoding the second Fc polypeptide linked to the second SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment, as described herein. In one aspect, the present disclosure refers to one or more vectors comprising a pair of polynucleotides as described herein. In one aspect, the present disclosure refers to a host cell comprising a pair of polynucleotides as described herein, or one or more vectors as described herein.

Certain examples also provide a method for producing a protein comprising a first Fc polypeptide linked to a first SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof, and a second Fc polypeptide linked to a second SGSH amino acid sequence, SGSH variant amino acid sequence, or a catalytically active fragment thereof, comprising culturing a host cell under conditions in which a pair of polynucleotides as described herein are expressed. In one aspect, the present disclosure refers to a method for producing a protein comprising a first Fc polypeptide linked to a first SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment, and a second Fc polypeptide linked to a second SGSH amino acid sequence, SGSH variant amino acid sequence, or catalytically active fragment, comprising culturing a host cell under conditions in which the pair of polynucleotides as described herein is expressed.

Certain examples provide a pharmaceutical composition comprising a protein or polypeptide as described herein and a pharmaceutically acceptable carrier and/or excipient. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the protein as described herein or the polypeptide as described herein and a pharmaceutically acceptable excipient.

Certain examples provide a method of treating Sanfilippo syndrome A, the method comprising administering a protein as described herein or a polypeptide as described herein to a patient in need thereof.

In one aspect, the present disclosure refers to a protein as described herein or a polypeptide as described herein for use in treating Sanfilippo syndrome A in a patient in need thereof.

In one aspect, the present disclosure refers to use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for treating Sanfilippo syndrome A in a patient in need thereof.

Certain examples provide a method of decreasing the accumulation of a toxic metabolic product in a patient having Sanfilippo syndrome A, the method comprising administering a protein as described herein or a polypeptide as described herein to the patient.

Certain examples provide a protein as described herein or a polypeptide as described herein for use in decreasing the accumulation of a toxic metabolic product in a patient having Sanfilippo syndrome A. In one aspect, the present disclosure refers to a protein as described herein or a polypeptide as described herein for use in decreasing the accumulation of a toxic metabolic product in a patient having Sanfilippo syndrome A, wherein the toxic metabolic product comprises heparan sulfate-derived oligosaccharides.

Certain examples provide the use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having Sanfilippo syndrome A. In one aspect, the present disclosure refers to use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having Sanfilippo syndrome A, wherein the toxic metabolic product comprises heparan sulfate-derived oligosaccharides.

In certain examples, the toxic metabolic product comprises heparan sulfate-derived oligosaccharides.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C. Illustration of exemplary ETV:SGSH fusion proteins having varying linker lengths between the SGSH enzyme and the Fc polypeptide hinge region.

Figure 2. fGly content of SGSH-Fc and ETV:SGSH fusion proteins as determined by LCMS.

Figure 3. *In vitro* evaluation of enzymatic activity of SGSH-Fc and ETV:SGSH fusion proteins.

Figure 4. Evaluation of cellular activity of SGSH-Fc fusion proteins in fibroblasts from MPSIIIA patients and healthy controls using a ^{35}S pulse-chase assay.

Figure 5. Serum concentration of SGSH-Fc and ETV:SGSH fusion proteins.