

[0009] Somatic gene therapy holds great promise for the treatment of hemophilia A because it would remedy the underlying under-expression functional Factor VIII activity (e.g., due to missense or nonsense mutations), rather than provide a one-time dose of Factor VIII activity to the individual. Because of this difference in the mechanism of action, as compared to Factor VIII replacement therapy, one-time administration of a Factor VIII gene therapy vector may provide an individual with Factor VIII for several years, reducing the cost of treatment and eliminating the need for continued patient compliance.

[0010] Coagulation Factor IX (FIX) gene therapy has been used effectively to treat individuals with hemophilia B, a related blood coagulation condition characterized by diminished Factor IX activity (Manno C.S., et al., Nat Med., 12(3):342-47 (2006)). However, Factor VIII gene therapy presents several unique challenges. For example, the full-length, wild-type Factor VIII polypeptide (2351 amino acids; UniProt accession number P00451) is five times larger than the full-length, wild-type Factor IX polypeptide (461 amino acids; UniProt accession number P00740). As such, the coding sequence of wild-type Factor VIII is 7053 base pairs, which is too large to be packaged in conventional AAV gene therapy vectors. Further, reported recombinant expression of B-domain deleted variants of Factor VIII (BDD-FVIII) has been poor. As such, several groups have attempted to alter the codon usage of BDD-FVIII constructs, with limited success.

## SUMMARY

[0011] Accordingly, there is a need for Factor VIII variants whose coding sequences are more efficiently packaged into, and delivered via, gene therapy vectors. There is also a need for synthetic, codon-altered nucleic acids which express Factor VIII more efficiently. Such Factor VIII variants and codon-altered nucleic acids allow for improved treatment of Factor VIII deficiencies (e.g., hemophilia A). The above deficiencies and other problems associated with the treatment of Factor VIII deficiencies (e.g., hemophilia A) are reduced or eliminated by the disclosed codon-altered Factor VIII variants.

[0012] In accordance with some embodiments, the present disclosure provides nucleic acids encoding Factor VIII variants that have high sequence identity to the disclosed codon-altered sequences of the Factor VIII heavy chain (e.g., CS01-HC-NA, CS04-HC-NA, or CS23-HC-NA)

and light chain (CS01-LC-NA, CS04-LC-NA, or CS23-LC-NA). In some embodiments, these nucleic acids further include a sequence encoding a linker sequence that replaces the native Factor VIII B-domain (e.g., a linker sequences comprising a furin cleavage site), between the sequences coding for the Factor VIII heavy and light chains.

[0013] In one embodiment, the disclosure provides a polynucleotide including a nucleotide sequence encoding a Factor VIII polypeptide. The Factor VIII polypeptide includes a light chain, a heavy chain, and a polypeptide linker joining the C-terminus of the heavy chain to the N-terminus of the light chain. The heavy chain of the Factor VIII polypeptide is encoded by a first nucleotide sequence having at least 95% identity to CS04-HC-NA (SEQ ID NO: 3). The light chain of the Factor FVIII polypeptide is encoded by a second nucleotide sequence having at least 95% identity to CS04-LC-NA (SEQ ID NO: 4). The polypeptide linker comprises a furin cleavage site.

[0014] In one embodiment of the polynucleotides described above, the polypeptide linker is encoded by a third nucleotide sequence having at least 95% identity to BDLO04 (SEQ ID NO: 6).

[0015] In one embodiment, the disclosure provides a polynucleotide including a nucleotide sequence encoding a Factor VIII polypeptide. The Factor VIII polypeptide includes a light chain, a heavy chain, and a polypeptide linker joining the C-terminus of the heavy chain to the N-terminus of the light chain. The heavy chain of the Factor VIII polypeptide is encoded by a first nucleotide sequence having at least 95% identity to CS01-HC-NA (SEQ ID NO: 24). The light chain of the Factor FVIII polypeptide is encoded by a second nucleotide sequence having at least 95% identity to CS01-LC-NA (SEQ ID NO: 25). The polypeptide linker comprises a furin cleavage site.

[0016] In one embodiment of the polynucleotides described above, the polypeptide linker is encoded by a third nucleotide sequence having at least 95% identity to BDLO01 (SEQ ID NO: 5).

[0017] In one embodiment, the disclosure provides a polynucleotide including a nucleotide sequence encoding a Factor VIII polypeptide. The Factor VIII polypeptide includes a light chain, a heavy

[0026] In one embodiment of the polynucleotides described above, the first nucleotide sequence encoding the heavy chain of the Factor VIII polypeptide is CS01-HC-NA (SEQ ID NO: 24), and the second nucleotide sequence encoding the light chain of the Factor FVIII polypeptide is CS01-LC-NA (SEQ ID NO: 25).

[0027] In one embodiment of the polynucleotides described above, the first nucleotide sequence encoding the heavy chain of the Factor VIII polypeptide is CS23-HC-NA (SEQ ID NO: 22), and the second nucleotide sequence encoding the light chain of the Factor FVIII polypeptide is CS23-LC-NA (SEQ ID NO: 23).

[0028] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS04-FL-NA, wherein the polynucleotide encodes a Factor VIII polypeptide.

[0029] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS01-FL-NA, wherein the polynucleotide encodes a Factor VIII polypeptide.

[0030] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS23-FL-NA, wherein the polynucleotide encodes a Factor VIII polypeptide.

[0031] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 96% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0032] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 97% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0033] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 98% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0034] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 99% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0035] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 99.5% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0036] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 99.9% identity to the respective full-length polynucleotide sequence (e.g., CS04-FL-NA (SEQ ID NO: 1), CS01-FL-NA (SEQ ID NO: 13), or CS23-FL-NA (SEQ ID NO: 20)).

[0037] In one embodiment of the polynucleotides described above, the nucleotide sequence is CS04-FL-NA (SEQ ID NO: 1).

[0037a] In a first aspect, there is provided a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1, wherein the polynucleotide encodes a Factor VIII polypeptide.

[0038] In one embodiment of the polynucleotides described above, the nucleotide sequence is CS01-FL-NA (SEQ ID NO: 13).

[0039] In one embodiment of the polynucleotides described above, the nucleotide sequence is CS23-FL-NA (SEQ ID NO: 20).

[0040] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 95% identity to CS04-FL-AA (SEQ ID NO: 2).

[0041] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 96% identity to CS04-FL-AA (SEQ ID NO: 2).

[0042] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 97% identity to CS04-FL-AA (SEQ ID NO: 2).

[0043] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 98% identity to CS04-FL-AA (SEQ ID NO: 2).

[0044] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 99% identity to CS04-FL-AA (SEQ ID NO: 2).

[0045] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 99.5% identity to CS04-FL-AA (SEQ ID NO: 2).

[0046] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising an amino acid sequence having at least 99.9% identity to CS04-FL-AA (SEQ ID NO: 2).

[0047] In one embodiment of the polynucleotides described above, the polynucleotide encodes a Factor VIII polypeptide comprising the amino acid sequence of CS04-FL-AA (SEQ ID NO: 2).

[0048] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS04-SC1-NA (SEQ ID NO: 9), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0049] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS04-SC2-NA (SEQ ID NO: 11), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0050] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS01-SC1-NA (SEQ ID NO: 26), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0051] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS01-SC2-NA (SEQ ID NO: 27), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0052] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS23-SC1-NA (SEQ ID NO: 28), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0053] In one embodiment, the disclosure provides a polynucleotide comprising a nucleotide sequence having at least 95% identity to CS23-SC2-NA (SEQ ID NO: 29), wherein the polynucleotide encodes a single-chain Factor VIII polypeptide.

[0054] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 96% identity to the respective full-length polynucleotide sequence (e.g., CS04-SC1-NA (SEQ ID NO: 9), CS04-SC2-NA (SEQ ID NO: 11), CS01-SC1-NA (SEQ ID NO: 26), CS01-SC2-NA (SEQ ID NO: 27), CS23-SC1-NA (SEQ ID NO: 28), or CS23-SC2-NA (SEQ ID NO: 29)).

[0055] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 97% identity to the respective full-length polynucleotide sequence (e.g., CS04-SC1-NA (SEQ ID NO: 9), CS04-SC2-NA (SEQ ID NO: 11), CS01-SC1-NA (SEQ ID NO: 26), CS01-SC2-NA (SEQ ID NO: 27), CS23-SC1-NA (SEQ ID NO: 28), or CS23-SC2-NA (SEQ ID NO: 29)).

[0056] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 98% identity to the respective full-length polynucleotide sequence (e.g., CS04-SC1-NA (SEQ ID NO: 9), CS04-SC2-NA (SEQ ID NO: 11), CS01-SC1-NA (SEQ ID NO: 26), CS01-SC2-NA (SEQ ID NO: 27), CS23-SC1-NA (SEQ ID NO: 28), or CS23-SC2-NA (SEQ ID NO: 29)).

[0057] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 99% identity to the respective full-length polynucleotide sequence (e.g., CS04-SC1-NA (SEQ ID NO: 9), CS04-SC2-NA (SEQ ID NO: 11), CS01-SC1-NA (SEQ ID NO: 26), CS01-SC2-NA (SEQ ID NO: 27), CS23-SC1-NA (SEQ ID NO: 28), or CS23-SC2-NA (SEQ ID NO: 29)).

[0058] In one embodiment of the polynucleotides described above, the nucleotide sequence has at least 99.5% identity to the respective full-length polynucleotide sequence (e.g., CS04-SC1-NA (SEQ ID NO: 9), CS04-SC2-NA (SEQ ID NO: 11), CS01-SC1-NA (SEQ ID NO: 26), CS01-SC2-NA (SEQ ID NO: 27), CS23-SC1-NA (SEQ ID NO: 28), or CS23-SC2-NA (SEQ ID NO: 29)).

NA, CS04-HC-NA, CS04-LC-NA, CS23-FL-NA, CS23-HC-NA, CS23-LC-NA, CS01-SC1-NA, CS04-SC1-NA, CS23-SC1-NA, CS01-SC2-NA, CS04-SC2-NA, and CS23-SC2-NA.

[0074] In one embodiment of the polynucleotides described above, the encoded Factor VIII polypeptide comprises a glycosylation polypeptide positioned between two consecutive amino acids.

[0075] In one embodiment of the polynucleotides described above, the polynucleotide also includes a promoter element operably linked to the polynucleotide encoding the Factor VIII polypeptide.

[0076] In one embodiment of the polynucleotides described above, the polynucleotide also includes an enhancer element operably linked to the polynucleotide encoding the Factor VIII polypeptide.

[0077] In one embodiment of the polynucleotides described above, the polynucleotide also includes a polyadenylation element operably linked to the polynucleotide encoding the Factor VIII polypeptide.

[0078] In one embodiment of the polynucleotides described above, the polynucleotide also includes an intron operatively linked to the nucleotide sequence encoding the Factor VIII polypeptide.

[0079] In one embodiment of the polynucleotides described above, the intron is positioned between a promoter element and the translation initiation site (e.g., the first coding ATG) of the nucleotide sequence encoding a Factor VIII polypeptide.

[0080] In another embodiment, the disclosure provides a mammalian gene therapy vector including a polynucleotide as described above.

[0081] In one embodiment of the mammalian gene therapy vector described above, the mammalian gene therapy vector is an adeno-associated virus (AAV) vector.

[0082] In one embodiment of the mammalian gene therapy vector described above, the AAV vector is an AAV-8 vector.

[0082a] In a second aspect, there is provided an adeno-associated virus (AAV) vector comprising any of the polynucleotide disclosed herein.

[0082b] In a third aspect, there is provided an adeno-associated virus (AAV) particle comprising any of the polynucleotide disclosed herein.

[0082c] In a fourth aspect, there is provided a host cell infected an adeno-associated virus (AAV) particle comprising any of the polynucleotide disclosed herein.

[0082d] In a fifth aspect, there is provided a method for producing an adeno-associated virus (AAV) particle comprising introducing any of the polynucleotide disclosed herein into a mammalian host cell, wherein the polynucleotide is competent for replication in the mammalian host cell.

[0083] In another embodiment, the disclosure provides a method for treating hemophilia A including administering, to a patient in need thereof, a mammalian gene therapy vector as described above.

[0084] In another embodiment, the disclosure provides a mammalian gene therapy vector as described above for treating hemophilia A.

[0085] In another embodiment, the disclosure provides the use of a mammalian gene therapy vector as described above for the manufacture of a medicament for treating hemophilia A.

[0085a] In a sixth aspect, there is provided an adeno-associated virus (AAV) particle according to the third aspect for use in the treatment of hemophilia A.

[0085b] In a seventh aspect, there is provided an in vitro method for transducing a host cell comprising contacting the host cell with an adeno-associated virus (AAV) particle according to the third aspect.

[0085c] In an eighth aspect, there is provided use of an adeno-associated virus (AAV) particle according to third aspect in the manufacture of a medicament for treating hemophilia A.

## **BRIEF DESCRIPTION OF DRAWINGS**

[0086] Figure 1 shows schematic illustrations of the wild-type and ReFacto-type human Factor VIII protein constructs.

[0087] Figures 2A and 2B show the CS04 codon-altered nucleotide sequence (SEQ ID NO: 1) encoding a Factor VIII variant in accordance with some embodiments (“CS04-FL-NA” for full-length coding sequence).

[0088] Figure 3 shows the Factor VIII variant amino acid sequence (SEQ ID NO: 2) encoded by the CS04 codon-altered nucleotide sequence in accordance with some embodiments (“CS04-FL-AA” for full-length amino acid sequence).

[0089] Figure 4 shows the portion of the CS04 codon-altered nucleotide sequence (SEQ ID NO: 3) encoding the heavy chain of a Factor VIII variant in accordance with some embodiments (“CS04-HC-NA”).

[0090] Figure 5 shows the portion of the CS04 codon-altered nucleotide sequence (SEQ ID NO: 4) encoding the light chain of a Factor VIII variant in accordance with some embodiments (“CS04-LC-NA”).

[0091] Figure 6 shows exemplary coding sequences (SEQ ID NOS: 5-7) for B-domain substituted linkers in accordance with some embodiments. BDLO01 (SEQ ID NO: 5), BDLO04 (SEQ ID NO: 6), and BDLO23 (SEQ ID NO: 7) are the respective portions of the CS01, CS04, and CS23 codon-altered nucleotide sequences that encode a B-domain substituted linker, respectively.

#### **WHAT IS CLAIMED IS:**

1. A polynucleotide comprising the nucleotide sequence of SEQ ID NO:1, wherein the polynucleotide encodes a Factor VIII polypeptide.
2. The polynucleotide of claim 1, further comprising a promoter element operably linked to the polynucleotide encoding the Factor VIII polypeptide.
3. The polynucleotide of claim 2, wherein the promoter element is a liver-specific promoter sequence upstream of the nucleotide sequence encoding the Factor VIII polypeptide.
4. The polynucleotide of claim 3, further comprising an intron sequence positioned between the liver-specific promoter sequence and the nucleotide sequence encoding the Factor VIII polypeptide.
5. An adeno-associated virus (AAV) vector comprising a polynucleotide according to any one of claims 1 to 4.
6. An adeno-associated virus (AAV) particle comprising a polynucleotide according to any one of claims 1 to 4.
7. A host cell infected with an adeno-associated virus (AAV) particle comprising a polynucleotide according to any one of claims 1 to 4.
8. A method for producing an adeno-associated virus (AAV) particle comprising introducing a polynucleotide according to any one of claims 1 to 4 into a mammalian host cell, wherein the polynucleotide is competent for replication in the mammalian host cell.
9. An adeno-associated virus (AAV) particle according to claim 6 for use in the treatment of hemophilia A.

10. An in vitro method for transducing a host cell comprising contacting the host cell with an adeno-associated virus (AAV) particle according to claim 6.

11. Use of an adeno-associated virus (AAV) particle according to claim 6 in the manufacture of a medicament for treating hemophilia A.