(inhibitors) formation in response to protein therapy that occurs in 25-30% of severe (<1% FVIII) HA patients.

[0007] The invention provides improved Factor VIII variants useful for treatment in patients in need thereof, such as a patient with HA.

Summary

[0008] In accordance with the invention, codon-optimized Factor VIII (FVIII) encoding nucleic acid variants distinct from wild-type nucleic acid encoding wild-type FVIII are provided. Such codon-optimized FVIII encoding nucleic acid variants encode FVIII protein, optionally without a B domain, such as FVIII protein (e.g., human FVIII protein) without a B domain (see, e.g., Fig. 1). Such codon-optimized FVIII encoding nucleic acid variants exhibit increased expression, 4-5 fold for a particular codon-optimized sequence (CO/CO3), when transferred into a cell (see, e.g., Fig. 6B), leading to increased FVIII protein secretion and therefore activity (see, e.g., Fig. 7). Such codon-optimized FVIII encoding nucleic acid variants also are packaged more efficiently in different AAV vector serotypes, leading to increased production of AAV vectors across AAV serotypes (see, e.g., Tables 3-5).

[0009] Surprisingly, these features dramatically increase FVIII expression, as compared to expression of wild-type nucleic acid encoding wild-type FVIII, or expression of wild type FVIII without B domain, and also increase packaging efficiency into an AAV vector, leading to higher vector yields. Codon-optimized FVIII encoding sequences may exhibit a reduction in rearrangement events that impact the integrity of the FVIII transgene after AAV packaging that is observed with wild type FVIII and other codon-optimized hFVIII transgenes, e.g., CO/CO3 is superior to either of CO1 and CO2. In addition, non-codon optimized and codon-optimized FVIII variants that have a single amino acid change or small deletion (1-4 amino acids) at a PACE-furin cleavage site can exhibit increased secretion *in vivo* (non-codon optimized Fig. 3B, and codon-optimized Fig. 6B), and specific activity of the FVIII protein. Invention FVIII encoding nucleic acid variants, that encode FVIII with or without B-domain, and/or with or without mutated PACE-furin cleavage recognition site can increase expression of FVIII, increase production of AAV vector, as well as provide increased efficacy in the context of gene transfer by increased circulating levels of FVIII and achieving hemostasis with lower FVIII thereby reducing the effective dose required for beneficial therapeutic outcomes.

[0009a] In one embodiment, there is provided a Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said nucleic acid variant exhibits greater expression when compared to wild type FVIII expression, wherein said nucleic acid variant has at least 83 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

[0009b] In one embodiment, there is provided a Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said nucleic acid variant comprises a B domain deletion and exhibits greater expression when compared to wild type FVIII comprising a B domain deletion expression, wherein said nucleic acid variant has at least 83 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

[0009c] In one embodiment, there is provided the use of the nucleic acid variant as described herein, the expression vector as described herein, or the virus or AAV vector as described herein in the manufacture of a medicament for treating a mammal with hemophilia.

[0009d] In one embodiment, there is provided the use of the nucleic acid variant as described herein, the expression vector as described herein, or the virus or AAV vector as described herein in the manufacture of a medicament for treating a mammal with hemophilia, wherein the medicament is to be delivered or transferred into a mammalian cell.

[0010] In one embodiment, a Factor VIII (FVIII) encoding nucleic acid variant exhibits greater expression when compared to wild type FVIII expression. In another embodiment, a Factor VIII (FVIII) encoding nucleic acid variant comprises a B domain deletion and exhibits greater expression when compared to wild type FVIII comprising a B domain deletion

WHAT IS CLAIMED IS:

1. A Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said nucleic acid variant exhibits greater expression when compared to wild type FVIII expression, wherein said nucleic acid variant has at least 83 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

2. A Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said nucleic acid variant comprises a B domain deletion and exhibits greater expression when compared to wild type FVIII comprising a B domain deletion expression, wherein said nucleic acid variant has at least 83 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

3. The variant FVIII as claimed in claim 1 or 2, wherein said nucleic acid variant is more efficiently packaged into an adenovirus-associated virus (AAV) vector.

4. The variant FVIII as claimed in any of claims 1-3, wherein said FVIII encoded by said nucleic acid variant exhibits greater biological activity when compared to wild type FVIII or when compared to wild type FVIII comprising a B domain deletion.

5. The variant FVIII as claimed in any of claims 1-3, wherein said biological activity is determined by a clotting assay or reduced bleeding in a FVIII assay or FVIII deficiency model.

6. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has 2-5, 5-10, 10-20, 20-50, 50-100, 100-250, 250-500, 500-750 or 750-850 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

7. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has greater than 85% CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

8. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has all CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.

9. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has one or more AAG lysine codons compared to AAA lysine codons in wild type FVIII.

10. The variant FVIII as claimed in claim 1, wherein said nucleic acid variant has between about 50-59%, or 50-56%, or 50-53% GC content.

11. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant is at least 75% identical to wild type human FVIII nucleic acid or wild type human FVIII nucleic acid comprising a B domain deletion.

12. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant is human FVIII, and/or wherein said wild type FVIII or wild type FVIII comprising a B domain deletion is human FVIII.

13 The variant FVIII as claimed in claim 1, wherein said nucleic acid variant comprises any of SEQ ID NOs:1-7 and 9.

14. The variant FVIII as claimed in any of claims 1-13, wherein 1, 2, 3 or all 4 codons encoding a PACE/furin cleavage site is/are deleted.

15. The variant FVIII as claimed in any of claims 1-13, wherein 1, 2, 3 or all 4 codons encoding a PACE/furin cleavage site set forth as HHQR or RHQR from positions 1645-1648 is/are deleted.

16. An expression vector comprising the variant FVIII of any of claims 1-15.

17. The expression vector of claim 16, selected from the group consisting of an adenovirus-associated virus (AAV) vector, a retroviral vector, an adenoviral vector, a plasmid, or a lentiviral vector.

18. The expression vector of claim 17, wherein said AAV vector comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotype.

19. The expression vector of claim 16, further comprising an intron, an expression control element, one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs) and/or a filler polynucleotide sequence.

20. The expression vector of claim 19, wherein the intron is within or flanks the variant FVIII, or wherein the expression control element is operably linked to the variant FVIII, or wherein the AAV ITR(s) flanks the 5' or 3' terminus of the variant FVIII, or wherein the filler polynucleotide sequence flanks the 5' or 3'terminus of the variant FVIII.

21. The expression vector of claim 19, wherein the expression control element comprises a constitutive or regulatable control element, or a tissue-specific expression control element or promoter.

22. The expression vector of claim 19, wherein the expression control element comprises an element that confers expression in liver.

23. The expression vector of claim 19, wherein the expression control element comprises a TTR promoter or mutant TTR promoter.

24. The expression vector of claim 23, wherein the mutant TTR promoter comprises SEQ ID NO:8.

25. The expression vector of claim 19, wherein the ITR comprises one or more ITRs of any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotypes, or a combination thereof.

26. A host cell expressing the FVIII encoded by said nucleic acid variant of any of claims 1-15.

27. A host cell comprising the nucleic acid variant of any of claims 1-15 or the expression vector of any of claims 16-25.

28. A virus vector comprising the nucleic acid variant of any of claims 1-15 or the expression vector of any of claims 16-25.

29. An AAV vector comprising the nucleic acid variant of any of claims 1-15 or the expression vector of any of claims 16-25.

30. The AAV vector of claim 29, wherein the AAV vector comprises a VP1, VP2 and/or VP3 capsid sequence having 75% or more sequence identity to AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 VP1, VP2 and/or VP3 sequences.

31. The AAV vector of claim 29, wherein the AAV vector comprises a VP1, VP2 and/or VP3 capsid sequence selected from any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 and AAV-2i8 AAV serotypes.

32. A pharmaceutical composition comprising the nucleic acid variant of any of claims 1-15, the expression vector of any of claims 16-25, or the virus or AAV vector of any of claims 29-31 in a biologically compatible carrier or excipient, optionally including empty capsid AAV.

33. The nucleic acid variant of any of claims 1-15, the expression vector of any of claims 16-25, or the virus or AAV vector of any of claims 29-31 encapsulated in a liposome or mixed with phospholipids or micelles.

34. Use of the nucleic acid variant of any of claims 1-15, the expression vector of any of claims 16-25, or the virus or AAV vector of any of claims 29-31in the manufacture of a medicament for treating a mammal with hemophilia, wherein the medicament is to be delivered or transferred into a mammalian cell.

35. The use according to claim 34, wherein said mammalian cell is in culture or is present in the mammal.

36. Use of the nucleic acid variant of any of claims 1-15, the expression vector of any of claims 16-25, or the virus or AAV vector of any of claims 29-31 in the manufacture of a medicament for treating a mammal with hemophilia.