However, public health concerns regarding the risk of viruses or other blood-borne contaminants have limited the usefulness of porcine factor VIII purified from porcine blood. A recombinant porcine factor VIII variant has therefore been developed, which is designated “OBI-1” and described e.g. in WO 01/68109. OBI-1 is a partially B-domain deleted porcine FVIII. This molecule is presently in clinical development.

B-domain deleted factor VIII variants are known to keep the procoagulant and cofactor activity of factor VIII. In addition to this, Mertens et al. (British Journal of Haematology 1993, 85, 133-142) describe recombinant human factor VIII variants lacking the B-domain and the heavy-chain sequence spanning from Lysine 713 to Arginine 740.

Many hemophiliacs require daily replacement of factor VIII to prevent bleeding and the resulting deforming hemophilic arthropathy. In view of this, there is a need for a more potent factor VIII molecule.

SUMMARY

The present disclosure refers to an isolated and recombinant, fully or partially B-domain deleted porcine factor VIII (FVIII) variant, the FVIII variant being devoid of an amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7.

In one aspect, the present disclosure refers to an isolated and recombinant, fully or partially B-domain deleted porcine factor VIII (FVIII) variant, the FVIII variant comprising a sequence being at least 90% identical to SEQ ID NO: 1, and being devoid of the amino acid sequence corresponding to amino acids 714 to 740 of SEQ ID NO: 7.

In a one example, the disclosure relates to an isolated, recombinant, fully or partially B-domain deleted factor VIII (FVIII) variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7. This 27 amino acid sequence, NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:10), may be partially or completely deleted.

In one example, disclosure relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In another example, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another example, 27, 26, or 25 amino acids are deleted. In another example, the 27 amino acids corresponding to DIGDYYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10) are deleted.

Provided herein is an isolated and recombinant, fully or partially B-domain deleted factor VIII (FVIII) variant, the FVIII variant being devoid of an amino acid sequence corresponding to amino acids 716 to 742 of human factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 8.

In one example, the disclosure relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of human factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 8, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In another example, 27, 26, 25, 24, 23, 22, 21 or

20 amino acids are deleted. In another example, 27, 26, or 25 amino acids are deleted. In another example, the 27 amino acids corresponding to NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11) are deleted.

In another example, the disclosure relates to an isolated, recombinant, fully or partially B-domain deleted porcine FVIII variant being completely devoid of the 27 amino acids corresponding to DIGDYYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10).

In another example, the disclosure relates to an isolated, recombinant, fully or partially B-domain deleted human FVIII variant being completely devoid of the 27 amino acids corresponding to NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11).

In another example, the disclosure relates to an isolated, recombinant, fully or partially B-domain deleted canine FVIII variant being completely devoid of the 27 amino acids corresponding to NIDDYYEDTYEDIPTPLLNENNVIKPR (SEQ ID NO:12).

In one aspect, there is provided a polynucleotide encoding an isolated and recombinant, fully or partially B-domain deleted porcine factor VIII (FVIII) variant, the FVIII variant comprising a sequence being at least 90% identical to SEQ ID NO: 1, and being devoid of an amino acid sequence corresponding to amino acids 714 to 740 of SEQ ID NO: 7.

In one aspect, there is provided an expression vector comprising a polynucleotide as defined herein.

In one aspect, there is provided a mammalian cell comprising an expression vector as defined herein.

In one aspect, there is provided a method for producing a FVIII variant, comprising the steps of:

a. Culturing a mammalian cell as defined herein; and

b. Isolating from the mammalian cell the FVIII variant.

In one aspect, there is provided a pharmaceutical composition comprising a FVIII variant as defined herein.

In one example, the disclosure relates to a method of treating a patient suffering from hemophilia comprising administering a

CLAIMS

1. An isolated and recombinant, fully or partially B-domain deleted porcine factor VIII (FVIII) variant, the FVIII variant comprising a sequence being at least 90% identical to SEQ ID NO: 1, and being devoid of the amino acid sequence corresponding to amino acids 714 to 740 of SEQ ID NO: 7.

2. The FVIII variant according to claim 1, wherein said variant shares at least 95% identity to SEQ ID NO:1.

3. The FVIII variant according to claim 2, wherein said variant shares at least 99.5% identity to SEQ ID NO: 1.

4. The FVIII variant according to claim 3, wherein said variant is SEQ ID NO:1.

5. The FVIII variant according to claim 1, wherein the FVIII variant is partially B-domain deleted and the remaining portion of the B-domain is SEQ ID NO: 4.

6. A pharmaceutical composition comprising a factor VIII variant according to claim 1.

7. Use of FVIII variant according to claim 1 in the manufacture of a medicament for treating a patient suffering from hemophilia.

8. The use according to claim 7, wherein said hemophilia is hemophilia A.

9. The use according to claim 7, wherein said hemophilia is acquired hemophilia.

10. The use according to claim 7, wherein said patient has human FVIII antibodies.

11. The use according to claim 7, wherein the factor VIII variant is to be administered in an amount of no more than 200 µg/dose.

12. A polynucleotide encoding an isolated and recombinant, fully or partially B-domain deleted porcine factor VIII (FVIII) variant, the FVIII variant comprising a sequence being at least 90% identical to SEQ ID NO: 1, and being devoid of an amino acid sequence corresponding to amino acids 714 to 740 of SEQ ID NO: 7.

13. An expression vector comprising a polynucleotide according to claim 12.

14. A mammalian cell comprising an expression vector according to claim 13.

15. A method for producing a FVIII variant, comprising the steps of:

a. Culturing a mammalian cell according to claim 14; and

b. Isolating from the mammalian cell the FVIII variant.

16. The method according to claim 15, further comprising the step of c. Formulating the factor VIII variant together with appropriate excipients into a pharmaceutical composition.

17. The polynucleotide of claim 12, wherein the FVIII variant shares at least 95% identity to SEQ ID NO: 1.

18. The polynucleotide of claim 12, wherein the FVIII variant shares at least 99.5% identity to SEQ ID NO: 1.

19. The polynucleotide of claim 18, wherein the FVIII variant is SEQ ID NO: 1.

20. The polynucleotide of claim 12, wherein the FVIII variant is partially B-domain deleted and the remaining portion of the B-domain is selected from a sequence consisting of SEQ ID NO: 4.

21. An expression vector comprising a polynucleotide according to claim 20.

22. An expression vector comprising a polynucleotide according to claim 17.

23. An expression vector comprising a polynucleotide according to claim 18.

24. An expression vector comprising a polynucleotide according to claim 19.

25. A mammalian cell comprising an expression vector according to claim 21.

26. A mammalian cell comprising an expression vector according to claim 22.

27. A mammalian cell comprising an expression vector according to claim 23.

28. A mammalian cell comprising an expression vector according to claim 24.

29. A method for producing a FVIII variant, comprising the steps of:

a. Culturing a mammalian cell according to claim 26; and

b. Isolating from the mammalian cell the FVIII variant.

30. A method for producing a FVIII variant, comprising the steps of:

a. Culturing a mammalian cell according to claim 28; and

b. Isolating from the mammalian cell the FVIII variant.