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 ~~OF THE INVENTION~~

In one aspect, there is provided 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 a ~~first~~one ~~aspect~~embodiment, the ~~invention~~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 embodiment, ~~the invention~~ 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 embodiment, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another embodiment, 27, 26, or 25 amino acids are deleted. In another embodiment, the 27 amino acids corresponding to DIGDYYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10) are deleted.

In one aspect, there is provided 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 embodiment, the ~~invention~~ 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 embodiment, 27, 26, 25, 24, 23, 22, 21 or

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

In another embodiment, the ~~invention~~ 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 embodiment, the ~~invention~~ 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 embodiment, the ~~invention~~ 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).

~~A second~~In one aspect, ~~of the invention relates to~~there is provided a polynucleotide encoding a polypeptide as defined ~~in the first aspect and the embodiments described~~ herein.

In ~~a third~~one aspect, ~~the invention relates to~~there is provided an expression vector comprising a polynucleotide as defined ~~in the second aspect~~herein.

~~A fourth aspect of the invention relates to~~In one aspect, there is provided a mammalian cell comprising an expression vector as defined ~~in the third aspect~~ herein.

~~In a fifth aspect, the invention relates to~~In one aspect, there is provided a method for producing a FVIII ~~as defined in the first aspect~~variant, comprising the steps of:

a. Culturing a mammalian cell ~~according to the fourth aspect~~as defined herein; and

b. Isolating from the mammalian cell the FVIII variant.

~~A sixth aspect of the invention relates to~~In one aspect, there is provided a pharmaceutical composition comprising a FVIII variant as defined ~~in the first aspect~~herein.

In ~~a seventh aspect~~ one embodiment, the ~~invention~~ disclosure relates to a method of treating a patient suffering from hemophilia comprising administering a

therapeutically effective amount of FVIII variant ~~according to the first aspect of the invention~~as defined herein to a patient in need thereof, thereby treating the hemophilia in said patient.

In one aspect, there is provided a FVIII variant as described herein for use in medicine

In ~~an eighth aspect~~one aspect, ~~the invention relates to~~there is provided a FVIII variant ~~according to the first aspect~~as defined herein for use in treating hemophilia.

In one aspect, there is provided use of FVIII variant as described herein in the preparation of a medicament for treating hemophilia in a patient in need thereof.

CLAIMS

1. 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.

2. The FVIII variant according to claim 1, comprising a sequence being at least 90% identical to SEQ ID NO: 1, the variant being devoid of the 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.

3. The FVIII variant according to claim 2, wherein said variant shares at least 95, 96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:1.

4. The FVIII variant according to claim 3, wherein said variant shares 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO: 1.

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

6. 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.

7. The FVIII variant according to claim 6, comprising a sequence being at least 90% identical to SEQ ID NO: 1, the variant being devoid of the 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.

8. The FVIII variant according to claim 7, wherein said variant shares at least 95, 96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:2.

9. The FVIII variant according to claim 8, wherein said variant shares 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:2.

10. The FVIII variant according to claim 9, wherein said variant is SEQ ID NO:2.

11. The FVIII variant according to any one of claims 1-10, 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 or SEQ ID NO:5.

12. A polynucleotide encoding a polypeptide according any one of claims 1-10.

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. A pharmaceutical composition comprising a factor VIII variant according to any one of claims 1 to 10.

18. Use of a FVIII variant according to any one of claims 1 to 10 in the preparation of a medicament for treating hemophilia in a patient in need thereof.

19. The use according to claim 18, wherein said hemophilia is hemophilia A.

20. The use according to claim 18, wherein said hemophilia is acquired hemophilia.

21. The use according to any one of claims 18 to 20, wherein said patient has developed human FVIII antibodies.

22. The use according to any one of claims 18 to 21, wherein the factor VIII is administered in an amount of no more than 200 µg/dose or 150 µg/dose or 140 µg/dose.

23. A FVIII variant according to any one of claims 1 to 10 for use in medicine.

24. A FVIII variant according to any one of claims 1 to 10 for use in treating hemophilia.

25. The FVIII variant according to claim 24, wherein said hemophilia is hemophilia A.

26. The FVIII variant according to claim 24, wherein said hemophilia is acquired hemophilia.

27. The FVIII variant according to any one of claims 24 to 26, wherein said patient has developed human FVIII antibodies.

28. The FVIII variant according to any one of claims 24 to 27, wherein the factor VIII variant is administered in an amount of no more than 200 µg/dose or 150 µg/dose or 140 µg/dose.

CLAIMS

1. An isolated, 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 porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7.

2. The FVIII variant according to claim 1, comprising a sequence being at least 90% identical to SEQ ID NO: 1, the variant being devoid of the 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.

3. The FVIII variant according to claim 2, wherein said variant shares at least 95, 96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:1.

4. The FVIII variant according to claim 3, wherein said variant shares 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:1.

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

6. An isolated, 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.

7. The FVIII variant according to claim 6, comprising a sequence being at least 90% identical to SEQ ID NO: 1, the variant being devoid of the 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: 8.

8. The FVIII variant according to claim 7, wherein said variant shares at least 95, 96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:2.

9. The FVIII variant according to claim 8, wherein said variant shares 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:2.

10. The FVIII variant according to claim 9, wherein said variant is SEQ ID NO:2.

11. The FVIII variant according to any one of the preceding claims, 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 or SEQ ID NO:5.

12. A polynucleotide encoding a polypeptide according any one of the preceding claims.

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 according to any one of claims 1 to 10, 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. A pharmaceutical composition comprising a factor VIII variant according to any one of claims 1 to 10.

18. A method of treating a patient suffering from hemophilia comprising administering a therapeutically effective amount of FVIII variant according to any one of claims 1 to 10 to a patient in need thereof, thereby treating the hemophilia in said patient.

19. A FVIII variant according to any one of claims 1 to 10 for use in treating hemophilia.

20. The method or use according to claim 18 or 19, wherein said hemophilia is hemophilia A.

21. The method or use according to claim 18 or 19, wherein said hemophilia is acquired hemophilia.

22. The method or use according to any one of claims 18 to 21, wherein said hemophilia is treated in patients having developed human FVIII antibodies.

23. The method or use according to any one of claims 18 to 22, wherein the factor VIII is administered in an amount of no more than 200 µg/dose or 150 µg/dose or 140 µg/dose.