[0012] A "transcription regulatory element" refers to nucleotide sequences of a gene involved in regulation of genetic transcription including a promoter, plus response elements, activator and enhancer sequences for binding of transcription factors to aid RNA polymerase binding and promote expression, and operator or silencer sequences to which repressor proteins bind to block RNA polymerase attachment and prevent expression. The term "liver specific transcription regulatory element" refers to a regulatory element that modulates gene expression specifically in the liver tissue. Examples of liver specific regulatory elements include, but are not limited to, the mouse thyretin promoter (mTTR), the endogenous human factor VIII promoter (F8), human alpha-1-antitrypsin promoter (hAAT) and active fragments thereof, human albumin minimal promoter, and mouse albumin promoter. Enhancers derived from liver specific transcription factor binding sites are also contemplated, such as EBP, DBP, HNF1, HNF3, HNF4, HNF6, with Enh1.~~.~~

[0012a] In one aspect, there is provided an adeno-associated virus (AAV) Factor VIII (FVIII) vector comprising a nucleic acid comprising an AAV2 5' inverted terminal repeat (ITR), a liver-specific transcription regulatory region, a codon-optimized functionally active FVIII coding region, optionally one or more introns, a polyadenylation sequence, and an AAV2 3' ITR.

[0013] In one ~~embodiment~~example, the AAV vector of the ~~invention~~ disclosure comprises a nucleic acid encoding functionally active FVIII having the B domain replaced by the 14 amino acid SQ sequence, i.e., encoding FVIII SQ. The SQ sequence is disclosed in Ward et al., Blood, 117:798-807, 2011, and McIntosh et al., Blood 121:3335-3344, 2013. The FVIII coding region sequence is a codon-optimized sequence (see Nathwani et al., US Pat. App. Pub. No. 2013/0024960A1, published January 24, 2013, which is incorporated herein by reference in its entirety, and McIntosh et al., Blood 121:3335-3344, 2013). This sequence is referred herein as the "UCL SQ FVIII."

[0014] In ~~a first aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 1, which is depicted schematically in Figure 2A, and comprises the nucleic acid sequence set forth in SEQ ID NO: 1.

[0015] In ~~a second aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 1S, which is depicted schematically in Figure 2B, and comprises the nucleic acid sequence set forth in SEQ ID NO: 2.

[0016] In ~~a third aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 2S, which is depicted schematically in Figure 2C, and comprises the nucleic acid sequence set forth in SEQ ID NO: 3.

[0017] In ~~a fourth aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 3S, which is depicted schematically in Figure 2D, and comprises the nucleic acid sequence set forth in SEQ ID NO: 4.

[0018] In another ~~embodiment~~example, the AAV vector of the ~~invention~~ disclosure comprises a nucleic acid encoding FVIII lacking the entire B domain, including the SQ sequence, and the a3 domain, which is located just N-terminal to the light chain or C domain. The FVIII coding region sequence is a codon-optimized sequence (see Nathwani et al., US Pat. App. Pub. No. 2013/0024960A1, published January 24, 2013, which is incorporated herein by reference in its entirety, and McIntosh et al., Blood 121:3335-3344, 2013).

[0019] In ~~a first aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 4, which is depicted schematically in Figure 3A, and comprises the nucleic acid sequence set forth in SEQ ID NO: 5.

[0020] In ~~a second aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 5, which is depicted schematically in Figure 3B, and comprises the nucleic acid sequence set forth in SEQ ID NO: 6.

[0021] In ~~a third aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 6, which is depicted schematically in Figure 3C, and comprises the nucleic acid sequence set forth inSEQ ID NO: 7.

[0022] In ~~a fourth aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Proto 7, which is depicted schematically in Figure 3D, and comprises the nucleic acid sequence set forth in SEQ ID NO: 8.

[0023] In another ~~embodiment~~example, the AAV vector of the ~~invention~~ disclosure comprises a nucleic acid comprising an AAV2 5' inverted terminal repeat (ITR), a liver-specific transcription regulatory region, a codon-optimized functionally active FVIII coding region, optionally one or more introns, a polyadenylation sequence, and an AAV2 3' ITR. In ~~a preferred embodiment~~one example, the liver-specific transcription regulatory region comprises a shortened ApoE enhancer sequence, a 186 base human alpha anti-trypsin (hAAT) proximal promoter, including 42 bases of the 5' untranslated region (UTR), and one or more enhancers selected from the group consisting of (i) a 34 base human ApoE/C1 enhancer, (ii) a 32 base human AAT promoter distal X region and (iii) 80 additional bases of distal element of the human AAT proximal promoter; and a codon-optimized functionally active FVIII coding regions encodes the FVIII SQ variant. In another

~~preferred embodiment~~example, the liver specific transcription regulatory region comprises a a1 micro-globulin enhancer sequence and the 186 base human alpha anti-trypsin (AAT) proximal promoter.

[0024] In ~~a first aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Construct 100ATG comprising the nucleic acid sequence forth in SEQ ID NO: 9.

[0025] In ~~a second aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Construct 100ATG bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO: 10.

[0026] In one example~~a third aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100ATG short bGH polyA sequence set forth in SEQ ID NO: 11.

[0027] In ~~a fourth aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Construct 103ATG comprising the nucleic acid sequence forth in SEQ ID NO: 12.

[0028] In ~~a fifth aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Construct 103ATG short bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO: 13.

[0029] In ~~a sixth aspect~~one example, the AAV vector of the ~~invention~~ disclosure comprises Construct 105ATG bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO: 14.

[0030] In one example~~a seventh aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct DC172ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO: 15.

[0031] In one example~~an eighth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct DC172ATG FVIII hAAT comprising the nucleic acid sequence set forth in SEQ ID NO: 16.

[0032] In one example~~a ninth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct DC172 2xHCR ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO: 17.

[0033] In one example~~a tenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct DC172 2xHCR ATG FVIII hAAT comprising the nucleic acid sequence set forth in SEQ ID NO: 18.

[0034] In one example~~an eleventh aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 2x SerpinA hAAT ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO: 19.

[0035] In one example~~a twelfth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 2x SerpinA hAAT ATG FVIII 2x μ-globulin enhancer comprising the nucleic acid sequence set forth in SEQ ID NO: 20.

[0036] In one example~~a thirteenth aspect~~, the AAV vector of the ~~invention~~ disclosure Construct 100ATG short polyA 2x μ-globulin enhancer comprising the nucleic acid sequence set forth in SEQ ID NO: 21.

[0037] In one example~~a fourteenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct Factor VIII-BMN001 comprising the nucleic acid sequence set forth in SEQ ID NO: 22.

[0038] In one example~~a fifteenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct Factor VIII-BMN002 sequence set forth in SEQ ID NO: 23.

[0039] In one example~~a sixteenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 99 comprising the nucleic acid sequence set forth in SEQ ID NO: 24.

[0040] In one example~~a seventeenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100 comprising the nucleic acid sequence set forth in SEQ ID NO: 25.

[0041] In one example~~an eighteenth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100 reverse orientation comprising the nucleic acid sequence set forth in SEQ ID NO: 26.

[0042] In one example~~a nineteenth aspect~~, the AAV vector of the ~~invention~~ disclosure Construct 100AT comprising the nucleic acid sequence set forth in SEQ ID NO: 27.

[0043] In one example~~a twentieth aspect~~, the AAV vector of the ~~invention~~ disclosure Construct 100AT 2x MG comprising the nucleic acid sequence set forth in SEQ ID NO: 28.

[0044] In one example~~a twenty-first aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100AT 2x MG bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO: 29.

[0045] In one example~~a twenty-second aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100AT 2x MG (reverse) bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO: 30.

[0046] In one example~~a twenty-third aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100 bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO: 31.

[0047] In one example~~a twenty-fourth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 100-400 comprising the nucleic acid sequence set forth in SEQ ID NO: 32.

[0048] In one example~~a twenty-fifth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 101 comprising the nucleic acid sequence set forth in SEQ ID NO: 33.

[0049] In one example~~a twenty-sixth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 102 sequence comprising the nucleic acid sequence set forth in SEQ ID NO: 34.

[0050] In one example~~a twenty-seventh aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 103 comprising the nucleic acid sequence set forth in SEQ ID NO: 35.

[0051] In one example~~a twenty-ninth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 103 reverse orientation comprising the nucleic acid sequence set forth in SEQ ID NO: 36.

[0052] In one example~~a thirtieth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 103AT comprising the nucleic acid sequence set forth in SEQ ID NO: 37.

[0053] In one example~~a thirty-first aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 103AT 2xMG comprising the nucleic acid sequence set forth in SEQ ID NO: 38.

[0054] In one example~~a thirty-second aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 103AT 2xMG bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO: 39.

[0055] In one example~~a thirty-third aspect~~, the AAV vector of the ~~invention~~ disclosure comprises the Construct 103 bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO: 40.

[0056] In one example~~a thirty-fourth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 104 comprising the nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 41.

[0057] In one example~~a thirty-fifth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 105 comprising the nucleic acid sequence set forth in SEQ ID NO: 42.

[0058] In one example~~a thirty-sixth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 106 comprising the nucleic acid sequence set forth in SEQ ID NO: 43.

[0059] In one example~~a thirty-seventh aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 106AT comprising the nucleic acid sequence set forth in SEQ ID NO: 44.

[0060] In one example~~a thirty-eighth aspect~~, the AAV vector of the ~~invention~~ disclosure comprises Construct 2x SerpinA hAAT comprising the nucleic acid sequence set forth in SEQ ID NO: 45.

[0061] In yet other ~~embodiments~~examples, the present ~~invention~~ disclosure is directed to vector constructs encoding a functional Factor VIII polypeptide, wherein said constructs comprise one or more of the individual elements of the above described constructs and combinations thereof, in one or

more different orientation(s). The present ~~invention~~ disclosure is also directed to the above described constructs in an opposite orientation.

[0062] The AAV vectors of the ~~invention~~ disclosure in single strand is less than about 7.0 kb in length, or is less than 6.5kb in length, or is less than 6.4 kb in length, or is less than 6.3 kb in length, or is less than 6.2 kb in length, or is less than 6.0 kb in length, or is less than 5.8 kb in length, or is less than 5.6 kb in length, or is less than 5.5 kb in length, or is less than 5.4 kb in length, or is less than 5.4 kb in length, or is less than 5.2 kb in length or is less than 5.0 kb in length. The AAV vectors of the invention in single strand ranges from about 5.0 kb to about 6.5 kb in length, or ranges from about 4.8 kb to about 5.2 k in length, or 4.8 kb to 5.3 kb in length, or ranges from about 4.9 kb to about 5.5 kb in length, or about 4.8 kb to about 6.0 kb in length, or about 5.0 kb to 6.2 kb in length or about 5.1 kb to about 6.3 kb in length ,or about 5.2 kb to about 6.4 kb in length, or about 5.5 kb to about 6.5 kb in length.

[0062a] In another aspect, there is provided a method of producing a recombinant adeno-associated virus (AAV) particle comprising A) culturing a cell that has been transfected with a AAV vector as described herein; and B) recovering recombinant AAV particle from the supernatant of the transfected cell.

[0063] In another ~~embodiment~~example, the ~~invention~~ disclosure provides for methods of producing a recombinant adeno-associated virus (AAV) particle comprising any of the AAV vectors of the invention. The methods comprise the steps of culturing a cell that has been transfected with any of the AAV vectors of the invention and recovering recombinant AAV from the supernatant of the transfected cell.

[0064] The cells of the ~~invention~~ disclosure are any cell type are susceptible to baculovirus infection, including insect cells such as High Five, Sf9, Se301, SeIZD2109, SeUCR1, Sf9, Sf900+, Sf21, BTI-TN-5B1-4, MG-1, Tn368, HzAm1, BM-N, Ha2302, Hz2E5 and Ao38. Preferred mammalian cells used can be HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT 1080, A549, Cos-7, ARPE-19 and MRC-5 cells, and including mammalian cells such as HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT 1080, A549, Cos-7, ARPE-19 and MRC-5 cells.

[0064a] In yet another aspect, there is provided a viral particle comprising the viral vector as described herein.

[0064b] In yet another aspect, there is provided a cell comprising the viral vector as described herein.

[0065] The ~~invention~~ disclosure also provides for a viral particle comprising any of the AAV vectors of the invention or any viral particle produced by the forgoing methods of the invention.

[0066] An "AAV virion" or "AAV viral particle" or "AAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically

referred to as an "AAV vector particle" or simply an "AAV vector". Thus, production of AAV vector particle necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

[0067] The invention also provides for cells comprising any of the AAV vectors of the invention, and viral particles produced by these cells of the invention.

[0068] In another ~~embodiment~~example, the ~~invention~~ disclosure provides for methods of treating a patient suffering from hemophilia A comprising administering to the patient an effective amount of any of the AAV vectors of the ~~invention~~disclosure, or a viral particle of the invention or a viral particles produced by a method of the ~~invention~~disclosure.

[0068a] In yet another aspect, there is provided use of an AAV FVIII vector as described herein for preparation of a medicament for treatment of hemophilia A.

[0069] In a further ~~embodiment~~example, the ~~invention~~ disclosure provides for a use of any of the AAV vectors of the ~~invention~~ disclosure for preparation of a medicament for the treatment of hemophilia A. In one ~~aspect~~example, the medicament comprises an amount of AAV vector that expresses human FVIII in an amount effective to treat hemophilia A.

[0069a] In yet another aspect, there is provided a composition comprising an AAV FVIII vector as described herein for the treatment of hemophilia A.

[0070] In another ~~embodiment~~example, the ~~invention~~ disclosure provides for a composition comprising any of the AAV vectors of the ~~invention~~ disclosure for the treatment of hemophilia A. In one ~~aspect~~example, the composition comprises an amount of AAV vector that expresses human FVIII in an amount effective to treat hemophilia A.

[0071] In another ~~embodiment~~example, the AAV vectors of the invention are used to produce AAV viral particles that are useful to treat a patient suffering from Hemophilia A.

DESCRIPTION OF DRAWINGS

[0072] Figure 1 provides a schematic of the UCL SQ vector. From left to right, the UCL SQ vector comprises the AAV2 5' ITR, wild-type AAV2 viral sequence, the 34 base human ApoE/C1 enhancer, the 32 base human AAT promoter distal X region, the 186 base human AAT promoter, including 42 bases of 5' UTR sequence, the codon-optimized human FVIII SQ sequence (see Nathwani et al., US Pat. App. Pub. No. 2013/0024960A1, published January 24,

2013, which is incorporated herein by reference in its entirety, and McIntosh et al., Blood 121:3335-3344, 2013), the 49 bases synthetic polyadenylation sequence, wild-type AAV2 viral sequence, and the AAV2 3'ITR. The UCL SQ vector is 5081 bases in length.

4. A viral particle comprising the viral vector of claim 1 or 2.

5. A cell comprising the viral vector of claim 1 or 2.

~~6. A method of treating a patient suffering from hemophilia A comprising administering to the patient an effective amount of an AAV FVIII vector of claim 1 or 2.~~

~~7.~~6. Use of an AAV FVIII vector of claim 1 or 2 for preparation of a medicament for treatment of hemophilia A.

~~8.~~7. A composition comprising an AAV FVIII vector of claim 1 or 2 for the treatment of hemophilia A.