[0004] C1EI is a serine protease inhibitor, which directly or indirectly inhibits several proteases associated with HAE attacks. It is a major inhibitor of several complement proteases such as C1r and C1s and contact proteases, including factor XIIa and kallikrein, and a minor inhibitor of fibrinolytic proteases such as plasmin and factor XIa. In patients with HAE, uninhibited activation of the contact pathway due to insufficient levels of functional C1-INH results in unregulated cleavage of high molecular weight kininogen by kallikrein, leading to generation of excessive free bradykinin, a potent vasoactive peptide that increases capillary permeability and edema. See, e.g., Riedl M. "Recombinant human C1 esterase inhibitor in the management of hereditary angioedema." Clin Drug Investig. 2015;35(7):407–417.

SUMMARY

[0004a] In one aspect, the present disclosure provides for a recombinant vector construct comprising a nucleic acid sequence that encodes a functional C1 esterase inhibitor (C1EI) operably linked to a heterologous liver-specific transcription regulatory region, optionally a nucleic acid sequence comprising SEQ ID NO: 1, 10, 11, 12, 13, 59 or 60. In another aspect, the present disclosure provides for an rAAV particle comprising the vector construct as disclosed herein and an AAV capsid.

[0004b] In another aspect, the present disclosure provides for a method of producing an rAAV particle comprising the steps of (a) providing a cell permissive for AAV replication with one or more nucleic acid constructs comprising: (i) a recombinant vector construct comprising (1) at least one AAV ITR, (2) a heterologous liver-specific transcription regulatory region, and (3) a nucleic acid encoding a functional C1EI, (ii) a nucleotide sequence encoding one or more AAV Rep proteins which is operably linked to a promoter that is capable of driving expression of the Rep protein(s) in the cell; and (iii) a nucleotide sequence encoding one or more AAV capsid proteins which is operably linked to a promoter that is capable of driving expression of the capsid protein(s) in the cell; (b) culturing the cell under conditions permitting expression of the Rep and the capsid proteins; and optionally (c) recovering the AAV particle. In yet another aspect, the present disclosure provides for a population of rAAV particles produced by the method as disclosed herein, optionally enriched for particles comprising full length or nearly full-length vector genomes by steps that reduce the number of empty capsids.

[0004c] In another aspect, the present disclosure provides for a pharmaceutical composition comprising the vector construct as disclosed herein, or the rAAV particle as disclosed herein, or the population of rAAV particles as disclosed herein in an aqueous suspension with a sterile pharmaceutically acceptable excipient.

[0004d] In another aspect, the present disclosure provides for use of the vector construct as disclosed herein or the rAAV particle as disclosed herein, the population of rAAV particles as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating hereditary angioedema in a mammal, or treating or preventing any symptom thereof.

[0004e] In another aspect, the present disclosure provides for use of an rAAV particle comprising a vector construct that comprises a nucleic acid sequence that encodes a functional C1EI, optionally linked to a heterologous liver-specific transcription regulatory element in the manufacture of a medicament for treating hereditary angioedema in a mammal, or treating or preventing any symptom thereof.

[0004f] In yet another aspect, the present disclosure provides for use of the vector construct as disclosed herein, the rAAV particle as disclosed herein, the population of rAAV particles as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for a) increasing the level of functional C1EI in the blood of a mammal; or b) treating a deficiency in functional C1EI in a mammal; wherein the medicament is to be administered at an amount effective to increase the level of functional C1EI in the blood of a mammal, optionally wherein the amount is effective to increase the level of functional C1EI to at least about 0.4 IU/ml, or 1 IU/ml or higher, or about 16 mg/dL or higher.

[0004g] In a further aspect, the present disclosure provides for use of the vector construct as disclosed herein, rAAV particle as disclosed herein, or the pharmaceutical as disclosed herein in the manufacture of a medicament for treating HAE or for treating a deficiency in functional C1EI or for increasing blood levels of functional C1EI in a mammal.

[0005] The examples described herein relate to a vector construct, a recombinant replication deficient AAV particle, cells, and pharmaceutical compositions for delivering functional human C1 esterase inhibitor (C1EI) to a subject in need thereof, particularly a subject with hereditary angioedema, or a deficiency in functional C1EI. The examples described herein also relate to the use of such AAV particles or such vector constructs to deliver a gene encoding human C1EI to liver cells of patients (human subjects) diagnosed with hereditary angioedema, or a deficiency in functional C1EI.

[0006] In one example, the examples described herein provide a vector construct comprising a nucleic acid sequence that encodes a functional C1 esterase inhibitor (C1EI). In one or more examples, the functional C1EI comprises an amino acid sequence at least 90%, 95% or 98% identical to amino acids 23-500 of SEQ ID NO: 2 (a human C1EI, or "hC1EI"). In some examples, the nucleic acid sequence encoding the functional C1EI is a wild-type sequence, of which SEQ ID NO: 1 is one example, or is codon optimized, or is a variant. Alternative codon optimized human C1EI-encoding sequences are set out in SEQ ID NOs: 10-13, 59 or 60. In some examples, the nucleic acid sequence encoding the functional C1EI is comprises a nucleotide sequence having at least 90% homology to at least 100, 200, 300, 400, or 500 consecutive bases of SEQ ID NO: 1 or 10-13 or 59-60, and which encodes functional human C1 esterase inhibitor (hC1EI) at least 95% identical to amino acids 23-500 of SEQ ID NO: 2. The coding sequence for hC1EI is, in some examples, codon optimized for expression in humans. In one example, the codon optimized hC1EI nucleic acid comprises a

reduced CpG di-nucleotide content. In a specific example, the CpG di-nucleotide content is less than 25.

[0007] In one or more examples, the nucleic acid sequence encoding C1EI is operably linked to one or more heterologous expression control elements. Preferably, expression of the hC1EI-encoding transgene is controlled by liver-specific expression control elements. Thus, in such examples, in the vector constructs described herein, the nucleic acid sequence encoding C1EI is operably linked to a heterologous liver-specific transcription regulatory region. In some examples, in the vector constructs described herein, the expression control elements include one or more of the following: a promoter and/or enhancer; optionally an intron; and a polyadenylation (polyA) signal. Such elements are further described herein.

[0008] The liver-specific transcription regulatory region may comprise one or more liver-specific expression control elements. In one or more examples, the liver-specific transcription regulatory region is a synthetic promoter sequence comprising portions of a human alpha-1-antitrypsin (hAAT) promoter, a hepatic control region (HCR) enhancer, and/or an apolipoprotein E (ApoE) enhancer. In some examples, the liver-specific transcription regulatory region comprises (a) a shortened ApoE enhancer sequence at least 90% identical to SEQ ID NO: 16; (b) an alpha anti-trypsin (hAAT) proximal promoter sequence at least 90% identical to SEQ ID NO: 3, (c) one or more enhancers selected from the group consisting of (i) an ApoE/HCR enhancer at least 90% identical to SEQ ID NO: 4, (ii) an AAT promoter distal X region , and (iii) an AAT promoter distal region. In an example, the sequence of the liver-specific transcription regulatory region comprises a nucleotide sequence at least 80%, 85%, 90% or 95% identical to SEQ ID NO: 5. In some examples, the liver-specific transcription regulatory region comprises (a) an α-microglobulin enhancer sequence at least 90% identical to SEQ ID NO: 17, and/or (b) an alpha anti-trypsin (AAT) proximal promoter at least 90% identical to SEQ ID NO: 3.

[0009] In some examples, the vector construct comprises one or more introns. In some examples, the intron also enhances expression of the C1EI-encoding nucleic acid, and optionally enhances expression in the liver. In one or more examples, the intron is a composite hAAT/hemoglobin intron sequence. In an example t, the intron comprises a nucleotide sequence at least 80%, 85%, 90% or 95% identical to SEQ ID NO: 6, or a

nucleotide sequence at least 80%, 85%, 90% or 95% identical to SEQ ID NO: 61. In some examples, the nucleic acid sequence that encodes the C1EI comprises the intron.

[0010] In some examples, the vector construct comprises a polyadenylation signal, optionally a bovine growth hormone (bGH) polyA signal (e.g., SEQ ID NO: 19) or preferably a human growth hormone (hGH) polyA signal (e.g., SEQ ID NO: 7).

[0011] The vector construct is preferably a recombinant AAV vector construct. In some examples, the vector construct comprises (a) one or both of (i) an AAV 5' inverted terminal repeat (ITR) and (ii) an AAV3' ITR; (b) a promoter and/or enhancer, e.g. a liver-specific transcription regulatory region; and (c) a nucleic acid sequence encoding a functionally active human C1 esterase inhibitor protein, or a fragment thereof. In some examples, the vector construct comprises (a) an AAV 5' inverted terminal repeat (ITR) sequence (e.g., SEQ ID NO: 54); (b) a promoter and/or enhancer, e.g. a liver-specific transcription regulatory region; (c) a nucleic acid sequence encoding a functionally active human C1 esterase inhibitor protein; and (d) an AAV 3' ITR (e.g., SEQ ID NO: 55). The AAV 5' ITR and/or AAV 3' ITR may be from a heterologous AAV pseudotype (which may or may not be modified as known in the art). In some examples, the 5' ITR and 3' ITR sequences are derived from AAV2. In one or more examples, the vector construct is an AAV vector genome about 3 kb to about 5 kb in size, or about 2.7 kb to about 4 kb in size. In one or more examples, the vector construct is an AAV vector genome about 2.7 kb to about 3.3 kb in size, or about 3.7kb to about 4.1 kb in size, e.g., SEQ ID NOs 57 and 58.

[0012] In some examples, the vector construct comprises a nucleotide sequence at least 80%, 85%, 90% or 95% identical to any one of SEQ ID NOs: 9, 20-36 or 57-58.

[0013] In another example, provided herein is a recombinant adeno-associated virus (rAAV) particle comprising an AAV capsid and the vector construct as described in one or more of the examples herein. In some examples, the recombinant AAV (rAAV) particle used for delivering the C1EI-encoding gene ("rAAV.SERPIN G1" or "AAV-SERPIN G1") has tropism for the liver. In such examples, the rAAV comprises an AAV capsid with liver tropism, for example, an AAV5 capsid at least 90% identical to SEQ ID NO: 46, or a simian AAV capsid, optionally a baboon-derived AAV capsid, or a variant thereof, that exhibits liver tropism. In one or more examples, the AAV capsid is a capsid for which preexisting humoral immunity is

similar to AAV5, or reduced compared to AAV5, e.g., when evaluated by IVIG neutralization *in vitro*.

[0014] In another example, provided herein are methods for the production of a AAV particle, useful as a gene delivery vector, the method comprising the steps of: (1) providing an insect cell comprising one or more nucleic acid constructs (a) comprising a vector construct as described herein comprising a nucleic acid as described herein that is flanked by two AAV ITR nucleotide sequences; (b) a nucleotide sequence encoding one or more AAV Rep proteins which is operably linked to a promoter that is capable of driving expression of the Rep protein(s) in an insect cell (c) a nucleotide sequence encoding one or more AAV capsid proteins which is operably linked to a promoter that is capable of driving expression of the capsid protein(s) in the insect cell; wherein (b) and (c) are in the same expression cassette or in two different expression cassettes; and (d) optionally genes encoding AAP and MAAP contained in the VP2/3; (2) culturing the insect cell defined in (1) under conditions conducive to the expression of the Rep and the capsid proteins; and, optionally (3) recovering the AAV particle.

[0015] In yet another example, provided herein are pharmaceutical compositions comprising the vector construct described herein or the rAAV particle described herein, and a sterile pharmaceutically acceptable diluent, excipient or carrier.

[0016] In a further example, provided herein are methods of delivering a C1EI gene to a mammalian subject. Such methods include methods of expressing C1EI in a mammalian subject comprising administering to the subject a composition comprising the vector construct described herein, the rAAV particle described herein, or the pharmaceutical composition described herein, thereby expressing the encoded C1EI protein in the subject. Preferably, in such methods, the mammal is a human and the C1EI is functional human C1EI as described herein. Such methods include a method of expressing C1EI in the liver of a mammal by administering an amount of the vector construct, rAAV particle or pharmaceutical composition effective to increase the level of C1EI expression in the liver of the mammal. Such methods also include a method of increasing the level of functional C1EI in the blood of a mammal by administering an amount of the vector construct, rAAV particle or pharmaceutical composition effective to increase the level of functional C1EI in the blood of a mammal. Such methods also include a method of treating a deficiency in functional C1EI in a mammal by administering an amount of the vector construct, rAAV particle or pharmaceutical composition effective to increase the level of functional C1EI

in the blood of a mammal. In some examples, the amount of the vector construct, rAAV particle or pharmaceutical composition is effective to increase the level of functional C1EI in blood to about 0.4 IU/ml or 1 IU/ml or higher, or the level of C1EI to about 16 mg/dL or higher.

[0017] Such methods also include a method of treating hereditary angioedema in a mammal, or treating or preventing any symptom thereof, comprising administering a therapeutically effective amount of the vector construct, rAAV particle or pharmaceutical composition. In one or more examples, such methods reduce the frequency or severity of submucosal or subcutaneous edema in the mammal, acute HAE attacks, or amount of on-demand therapy administered to treat the acute HAE attacks.

[0018] In any of the methods described herein, the rAAV particle is delivered at a dose of about 1 x 10^{12} to about 1 x 10^{14} vg/kg or 1 x 10^{15} vg/kg, or alternatively about 2 x 10^{12} to about 6 x 10^{13} vg/kg, or alternatively about 6 x 10^{13} vg/kg to about 1 x 10^{15} vg/kg.

[0019] in an aqueous suspension. In any of the methods described herein, the administration of the vector construct, rAAV particle, or pharmaceutical composition may further comprise administration of prophylactic or therapeutic corticosteroid treatment, and/or may further include administration of a second therapy for treating HAE. In any of the methods herein, prior to administration of an AAV particle to a patient as described above, the prospective patient may be assessed for the presence of anti-AAV capsid antibodies or anti-AAV neutralizing antibodies that are capable of blocking cell transduction or otherwise reduce the overall efficiency of the treatment.

[0020] Other examples will be evident to one skilled in the art upon reading the present specification.

BRIEF DESCRIPTION OF DRAWINGS

[0021] Figures 1A-1C depict schematics of the organization of a variety of vector constructs.

[0022] Figure 2 depicts Serpin G1 ELISA results from transient transfection of HepG2 cells.

[0023] Figures 3 A&B depict human C1EI levels in the blood of mice administered AAV particles comprising the vector construct ApoE/HCR-hAAT.hhI.SERPIN G1.hGH (HAE15) (Figure 3A) in Example 2. Two different AAV capsids were exemplified, an AAV5 type capsid (greater than 90% identical to SEQ ID NO: 46) and a baboon-derived AAV capsid AAVBba49 (greater than 90% identical to SEQ ID NO: 56). Figure 3B Illustrates the Serpin G1 ELISA

CLAIMS

1. A recombinant vector construct comprising a nucleic acid sequence that encodes a functional C1 esterase inhibitor (C1EI) operably linked to a heterologous liver-specific transcription regulatory region, optionally a nucleic acid sequence comprising SEQ ID NO: 1, 10, 11, 12, 13, 59 or 60.

2. The vector construct of claim 1, wherein the mammal is a human and the C1EI is human C1EI.

3. The vector construct of claim 1 or 2, wherein the functional C1EI comprises an amino acid sequence at least 95% identical to amino acids 23-500 of SEQ ID NO: 2.

4. The vector construct of any of the preceding claims, wherein the liver-specific transcription regulatory region comprises a synthetic promoter sequence comprising portions of an hAAT promoter and an HCR enhancer/ApoE enhancer, optionally
a) wherein the liver-specific transcription regulatory region comprises
(a1) a shortened ApoE enhancer sequence at least 90% identical to SEQ ID NO: 16;
(a2) an alpha anti-trypsin (hAAT) proximal promoter sequence at least 90% identical to SEQ ID NO: 3; and/or
(a3) one or more enhancers selected from the group consisting of (i) an ApoE/HCR enhancer at least 90% identical to SEQ ID NO: 4;

b) wherein the liver-specific transcription regulatory region comprises (b1) an α-microglobulin enhancer sequence at least 90% identical to SEQ ID NO: 17, and (b2) an alpha anti-trypsin (AAT) proximal promoter at least 90% identical to SEQ ID NO: 3; or

c) wherein the liver-specific transcription regulatory region comprises a nucleotide sequence at least 80% identical to SEQ ID NO: 5.

5. The vector construct of any of the preceding claims further comprising:

a) a polyadenylation signal, optionally wherein the polyadenylation signal is a human growth hormone polyadenylation signal or functional fragment thereof; and/or

b) an intron, optionally
i) wherein the intron is a composite hAAT/hemoglobin intron sequence; or
ii) wherein the intron comprises a nucleotide sequence at least 80% identical to any of SEQ ID NOs: 6 or 61-69; and/or
iii) wherein the nucleic acid sequence that encodes the functional C1 esterase inhibitor (C1EI) comprises the intron.

6. The vector construct of any of the preceding claims further comprising an AAV 5' ITR and/or AAV3' ITR from AAV2.

7. The vector construct of claim 1 that comprises a nucleotide sequence at least 80% identical to any one of SEQ ID NOs: 9, 20-36, 57 or 58.

8. The vector construct of any of the preceding claims that is an rAAV vector construct about 2.7 kb to about 4 kb, or about 4 kb to about 5 kb in size.

9. An rAAV particle comprising the vector construct of any of the preceding claims and an AAV capsid.

10. The rAAV particle of claim 9 that comprises:
a) an AAV5 type capsid;
b) a simian AAV capsid; or
c) a baboon-derived AAV capsid.

11. The rAAV of any of claims 9 or 10, wherein the rAAV particle comprises an AAV capsid with liver tropism.

12. A method of producing an rAAV particle comprising the steps of
(a) providing a cell permissive for AAV replication with one or more nucleic acid constructs comprising: (i) a recombinant vector construct comprising (1) at least one AAV ITR, (2) a heterologous liver-specific transcription regulatory region, and (3) a nucleic acid encoding a functional C1EI, (ii) a nucleotide sequence encoding one or more AAV Rep proteins which is operably linked to a promoter that is capable of driving expression of the Rep protein(s) in the cell; and (iii) a nucleotide sequence encoding one or more AAV capsid proteins which is operably linked to a promoter that is capable of driving expression of the capsid protein(s) in the cell;
(b) culturing the cell under conditions permitting expression of the Rep and the capsid proteins; and optionally
(c) recovering the AAV particle.

13. The method of claim 12, wherein the cell:
a) is an insect cell;
b) is a mammalian cell; or
c) is provided with a recombinant vector construct of any of claims 1-8.

14. A population of rAAV particles produced by the method of any one of claims 12 or 13, optionally enriched for particles comprising full length or nearly full-length vector genomes by steps that reduce the number of empty capsids.

15. A pharmaceutical composition comprising the vector construct of any of claims 1-8 or the rAAV particle of any of claims 9-11 or the population of rAAV particles of claim 14 in an aqueous suspension with a sterile pharmaceutically acceptable excipient.

16. Use of the vector construct of any of claims 1-8 or the rAAV particle of any of claims 9-11, the population of rAAV particles of claim 14, or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating hereditary angioedema in a mammal, or treating or preventing any symptom thereof.

17. Use of an rAAV particle comprising a vector construct that comprises a nucleic acid sequence that encodes a functional C1EI, optionally linked to a heterologous liver-specific transcription regulatory element in the manufacture of a medicament for treating hereditary angioedema in a mammal, or treating or preventing any symptom thereof.

18. The use of claim 17 wherein:
a) the C1EI is a functional human C1EI that comprises an amino acid sequence at least 95% identical to amino acids 23-500 of SEQ ID NO: 2; and/or
b) the medicament reduces the frequency or severity of submucosal or subcutaneous edema in the mammal.

19. Use of the vector construct of any of claims 1-8, the rAAV particle of any of claims 9-11, the population of rAAV particles of claim 14, or the pharmaceutical composition of claim 15 in the manufacture of a medicament for:
a) increasing the level of functional C1EI in the blood of a mammal; or
b) treating a deficiency in functional C1EI in a mammal;
wherein the medicament is to be administered at an amount effective to increase the level of functional C1EI in the blood of a mammal, optionally wherein the amount is effective to increase the level of functional C1EI to at least about 0.4 IU/ml, or 1 IU/ml or higher, or about 16 mg/dL or higher.

20. The use of any of claims 16-19, wherein:
a) the rAAV particle or vector construct is to be administered intravenously;
b) the mammal is to be concurrently administered corticosteroid therapy with the vector construct or rAAV particle or pharmaceutical composition; and/or
c) the mammal is to be administered the rAAV particle at a dose ranging from 1×10^{12} to 1×10^{15} vg/kg.

21. Use of the vector construct of any of claims 1-8, rAAV particle of any of claims 9-11, or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating HAE or

for treating a deficiency in functional C1EI or for increasing blood levels of functional C1EI in a mammal.