Dystonia and seizures are common. Dysarthria and dysphagia eventually become disabling, making oral feeding impossible; death usually occurs in the late second or third decade from aspiration pneumonia. Adults are more likely to present with dementia or psychiatric symptoms.

[0005] 2-hydroxypropyl-ß-cyclodextrin (HPBCD) has been shown to reduce the cholesterol and lipid accumulation and prolongs survival in NPC1 animal models. However, there are no therapies for NPC1 approved by the Food and Drug Administration (FDA). Accordingly, there is an urgent need for compositions and methods for treating, curing, and/or preventing NPC1.

SUMMARY

[0006] Provided herein are gene therapy compositions and methods for treating, preventing, and/or curing NPC1. More specifically, the disclosure provides Adeno-associated virus (AAV) vectors and nucleic acids (including nucleic acids comprising AAV transfer cassettes) for treating, preventing, and/or curing NPC1.

[0006a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises, from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene that encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0006b] In another aspect, the present disclosure refers to an adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0006c] In another aspect, the present disclosure refers to a composition comprising the AAV vector as disclosed herein.

[0006d] In another aspect, the present disclosure refers to a cell comprising the AAV vector as disclosed herein.

[0006e] In another aspect, the present disclosure refers to the AAV vector as disclosed herein, the composition as disclosed herein, or the cell as disclosed herein for use in therapy.

[0006f] In yet another aspect, the present disclosure refers to the use of the AAV vector as disclosed herein, the composition as disclosed herein, or the cell as disclosed herein in the manufacture of a medicament for treating Neimann-Pick Disease Type C in a subject.

[0007] In some examples, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises, from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene that encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0008] In some examples, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0009] In some examples, the transfer cassette comprises an intronic sequence. In some examples, the intronic sequence comprises the sequence of SEQ ID NO: 10.

In some examples, the intronic sequence may be located between the promoter and the transgene.

[0010] In some examples, the 5' ITR comprises the sequence of SEQ ID NO: 3003. In some examples, the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0011] In some examples, the promoter is the CBA promoter. In some examples, the promoter comprises the sequence of SEQ ID NO: 3005.

[0012] In some examples, the NPC1 protein is the human NPC1 protein. In some examples, the NPC1 protein comprises the sequence of SEQ ID NO: 3001. In some examples, the transgene comprises the sequence of SEQ ID NO: 3002.

[0013] In some examples, the polyadenylation signal is the SV40 polyadenylation signal. In some examples, the polyadenylation signal comprises the sequence of SEQ ID NO: 3012.

[0014] In some examples, the transfer cassette comprises an enhancer.

[0015] In some examples, the transfer cassette comprises the sequence of SEQ ID NO: 3014. In some examples, the transfer cassette comprises the sequence of any one of SEQ ID NO: 3015-3019.

[0016] Also provided herein are compositions comprising an AAV vector of the disclosure. Also provided herein are cells comprising an AAV vector of the disclosure.

[0017] Also provided here in are methods for treating a subject in need thereof comprising administering to the subject an effective amount of an AAV vector, a nucleic acid, a composition, or a cell of the disclosure. In some examples, the subject has Neimann-Pick Disease Type C. In some examples, the subject is a human subject

[0018] These and other examples are described in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1A-1C**. Bubble plots showing analysis of library diversity, directed evolution and enrichment of novel antigenic footprints. Parental (FIG. 1A) and evolved libraries from a first round (FIG. 1B) and a second round (FIG. 1C) of evolution were subjected to high-throughput sequencing using the Illumina MiSeq platform. Following analysis with a custom Perl script, enriched amino acid sequences were plotted. Each bubble represents a distinct capsid protein subunit amino acid sequence with the radius of the bubble

CLAIMS

What is claimed is:

1. An adeno-associated virus (AAV) vector comprising:

(i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180; and

(ii) a nucleic acid encapsidated by the protein capsid;

wherein the nucleic acid comprises a transfer cassette;

wherein the transfer cassette comprises, from 5' to 3':

a 5' inverted terminal repeat (ITR);

a promoter;

a transgene that encodes the NPC1 protein;

a polyadenylation signal; and

a 3' ITR.

2. The AAV vector of claim 1, wherein the transfer cassette comprises an intronic sequence.

3. The AAV vector of claim 2, wherein the intronic sequence comprises the sequence of SEQ ID NO: 10.

4. The AAV vector of claim 1, wherein the 5' ITR comprises the sequence of SEQ ID NO: 3003.

5. The AAV vector of claim 1, wherein the 3' ITR comprises the sequence of SEQ ID NO: 3004.

6. The AAV vector of claim 1, wherein the promoter is the CBA promoter.

7. The AAV vector of claim 1, wherein the promoter comprises the sequence of SEQ ID NO: 3005.

8. The AAV vector of claim 1, wherein the NPC1 protein is the human NPC1 protein.

9. The AAV vector of claim 1, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 3001.

10. The AAV vector of claim 1, wherein the transgene comprises the sequence of SEQ ID NO: 3002.

11. The AAV vector of claim 1, wherein the polyadenylation signal is the SV40 polyadenylation signal.

12. The AAV vector of claim 1, wherein the polyadenylation signal comprises the sequence of SEQ ID NO: 3012.

13. The AAV vector of claim 1, wherein the cassette comprises an enhancer.

14. The AAV vector of claim 1, wherein the AAV transfer cassette comprises the sequence of SEQ ID NO: 3014

15. The AAV vector of claim 1, wherein the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3015-3019.

16. An adeno-associated virus (AAV) vector comprising:

(i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180; and

(ii) a nucleic acid encapsidated by the protein capsid;

wherein the nucleic acid comprises a transfer cassette;

wherein the transfer cassette comprises from 5’ to 3’:

a 5’ inverted terminal repeat (ITR);

a promoter;
a transgene which encodes the NPC1 protein;
a polyadenylation signal; and
a 3' ITR.

17. A composition comprising the AAV vector of any one of claims 1-16.

18. A cell comprising the AAV vector of any one of claims 1-16.

19. The AAV vector of any one of claims 1-16, the composition of claim 17, or the cell of claim 18 for use in therapy.

20. Use of the AAV vector of any one of claims 1-16, the composition of claim 17, or the cell of claim 18 in the manufacture of a medicament for treating Neimann-Pick Disease Type C in a subject.

21. The use of claim 20, wherein the subject is a human subject.