decade from aspiration pneumonia. Adults are more likely to present with dementia or psychiatric symptoms.

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

Described herein are AAV transfer cassettes, nucleic acids and plasmids used in the production of recombinant adeno-associated viral (rAAV) vectors for the delivery of nucleic acids (e.g., nucleic acids comprising transgenes). The disclosed cassettes, nucleic acids and plasmids comprise sequences that may be used to express one or more transgenes having therapeutic efficacy in the amelioration, treatment and/or prevention of one or more diseases or disorders, such as NPC1.

In one aspect, the present disclosure refers to an Adeno-Associated Virus (AAV) transfer cassette comprising, from 5' to 3': a 5' inverted terminal repeat (ITR); a chicken beta-actin promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the transfer cassette comprises an intronic sequence; and wherein the transgene encodes the NPC1 protein.

In another aspect, the present disclosure refers to a plasmid comprising the AAV transfer cassette as disclosed herein.

In another aspect, the present disclosure refers to a nucleic acid comprising, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the nucleic acid comprises an intronic sequence; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 1.

In another aspect, the present disclosure refers to a cell comprising the AAV transfer cassette as disclosed herein, the plasmid as disclosed herein, or the nucleic acid as disclosed herein.

In another aspect, the present disclosure refers to a recombinant AAV vector comprising a protein capsid and a nucleic acid encapsidated by the protein capsid:

wherein the nucleic acid comprises the AAV transfer cassette as disclosed herein.

In another aspect, the present disclosure refers to a method of producing a recombinant AAV vector, the method comprising contacting an AAV producer cell with the AAV transfer cassette as disclosed herein, the plasmid as disclosed herein, or the nucleic acid as disclosed herein.

In another aspect, the present disclosure refers to a recombinant AAV vector produced by the method as disclosed herein.

In another aspect, the present disclosure refers to a composition comprising the AAV transfer cassette as disclosed herein, the plasmid as disclosed herein, the nucleic acid as disclosed herein, the cell as disclosed herein, or the recombinant AAV vector as disclosed herein.

In yet another aspect, the present disclosure refers to the use of the AAV transfer cassette as disclosed herein, the plasmid as disclosed herein, the nucleic acid as disclosed herien, the cell as disclosed herein, or the recombinant AAV vector as disclosed herein in the manufacture of a medicament for treating a subject in need thereof.

In some ~~embodiment~~examples, the disclosure provides an adeno-associated virus (AAV) transfer cassette comprising a 5' inverted terminal repeat (ITR), a promoter, a transgene, a polyadenylation signal, and a 3' ITR, wherein the transfer cassette comprises an intronic sequence. In some ~~embodiment~~examples, the intronic sequence is located between the promoter and the transgene. In some ~~embodiment~~examples, the transgene encodes the NPC1 protein. In some ~~embodiment~~examples, the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 14-19.

In some ~~embodiment~~examples, the disclosure provides a recombinant AAV vector comprising a protein capsid and a nucleic acid encapsidated by the protein capsid, wherein the nucleic acid comprises an transfer cassette comprising, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR. In some ~~embodiment~~examples, the transfer cassette comprises an intronic sequence, such as an intronic sequence located between the promoter and the transgene. In some ~~embodiment~~examples, the transgene encodes the NPC1 protein. In some ~~embodiment~~examples, the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 14-19.

In some ~~embodiment~~examples, at least one of the 5' ITR and the 3' ITR is about 110 to about 160 nucleotides in length. In some ~~embodiment~~examples, the 5' ITR is the same length as the 3' ITR. In some ~~embodiment~~examples, the 5' ITR and the 3' ITR have different lengths. At least one of the 5' ITR and the 3' ITR may be isolated or derived from, for example, the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV. In some ~~embodiment~~examples, the 5' ITR comprises the sequence of SEQ ID NO: 3, or a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some ~~embodiment~~examples, the 3' ITR comprises the sequence of SEQ ID NO: 4, or a sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

In some ~~embodiment~~examples, the promoter is a constitutive promoter. In some ~~embodiment~~examples, the promoter is an inducible promoter. In some ~~embodiment~~examples, the promoter is a tissue-specific promoter. In some ~~embodiment~~examples, the promoter may be selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, the HSVTK promoter, the CMV promoter, the SV40 early promoter, the SV40 late promoter, the metallothionein promoter, the murine mammary tumor virus (MMTV) promoter, the Rous sarcoma virus (RSV) promoter, the polyhedrin promoter, the chicken β–actin (CBA) promoter, the EF-1 alpha promoter, the EF-1 short promoter, the dihydrofolate reductase (DHFR) promoter, and the phosphoglycerol kinase (PGK) promoter. In some ~~embodiment~~examples, the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, and the HSVTK promoter. In some ~~embodiment~~examples, the promoter comprises a sequence at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to any one of SEQ ID NO: 5-8.

In some ~~embodiment~~examples, the intronic sequence is a chimeric sequence or a hybrid sequence. In some ~~embodiment~~examples, the intronic sequence comprises sequences isolated or derived from SV40. In some ~~embodiment~~examples, the intronic sequence comprises the sequence of any one of SEQ ID NO: 10-11.

The NPC1 protein may be, for example, the human NPC1 protein. In some ~~embodiment~~examples, the NPC1 protein has a sequence that is at least 90%, at least 95%, at

least 95%, at least 97%, at least 98%, or at least 99% identical to the sequence of the human NPC1 protein. In some ~~embodiment~~examples, the NPC1 protein comprises the sequence of SEQ ID NO: 1, or a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some ~~embodiment~~examples, the NPC1 protein comprises the sequence of SEQ ID NO: 20, or a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some ~~embodiment~~examples, the NPC1 protein has a sequence as shown in UniProt Accession No. O15118, incorporated herein by reference in its entirety.

In some ~~embodiment~~examples, the transgene comprises a sequence of SEQ ID NO: 2, or a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

In some ~~embodiment~~examples, the polyadenylation signal is selected from simian virus 40 (SV40), rabbit beta globin (rBG), α-globin, β-globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) and bovine growth hormone (bGH). In some ~~embodiment~~examples, the polyadenylation signal is the SV40 polyadenylation signal. In some ~~embodiment~~examples, the polyadenylation signal is the rBG polyadenylation signal. In some ~~embodiment~~examples, the polyadenylation signal comprises the sequence of SEQ ID NO: 12 or SEQ ID NO: 13. In some ~~embodiment~~examples, the polyadenylation signal comprises a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 12 or 13.

In some ~~embodiment~~examples, the AAV transfer cassette further comprises an enhancer. The enhancer may be, for example, the CMV enhancer. In some ~~embodiment~~examples, the enhancer comprises the sequence of SEQ ID NO: 9, or a sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

Also provided herein is a nucleic acid comprising an AAV transfer cassette of the disclosure. Also provided herein is a plasmid or bacmid comprising an AAV transfer cassette of the disclosure.

Also provided herein is a recombinant AAV vector comprising a protein capsid and a nucleic acid encapsidated by the protein capsid, wherein the nucleic acid comprises an AAV transfer cassette of the disclosure.

Also provided is a cell comprising the AAV transfer cassette of the disclosure.

Also provided is a method of producing a recombinant AAV vector, the method comprising contacting an AAV producer cell with an AAV transfer cassette or plasmid/bacmid of the disclosure. Also provided is a recombinant AAV vector produced by this method. The recombinant AAV vector may be of a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV and Bovine AAV. The recombinant AAV vector may comprise a protein capsid comprising a capsid protein subunit, wherein the capsid protein subunit comprises one or more mutations compared to a capsid protein subunit of a wildtype AAV.

Also provided are compositions comprising an AAV transfer cassette, a nucleic acid (e.g., a plasmid or a bacmid), a cell, or a recombinant AAV vector of the disclosure.

Also provided is a method for treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of an AAV transfer cassette, a nucleic acid (e.g., a plasmid), a cell, or a recombinant AAV vector of the disclosure. In some ~~embodiment~~example**s**, the subject is a human subject. In some ~~embodiment~~example**s**, the subject has NPC1.

These and other ~~embodiment~~example**s** are addressed in more detail in the detailed description set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a graph that shows lysosomal phenotype, as determined by measuring LysoTracker® accumulation, in wildtype U2OS cells, NPC1-defincient ($NPC1^{-/-}$) U2OS cells, and $NPC1^{-/-}$ cells transduced with AAV2-hNPC at a Multiplicity of Infection (MOI) of either 5×10^3 or 10×10^3. Statistical significance determined using one-way ANOVA. Error bars represent standard error of the mean (SEM).

FIG. 1B is a graph that shows cholesterol accumulation, as determined using filipin staining, in wildtype U2OS cells, NPC1-defincient ($NPC1^{-/-}$) U2OS cells, and $NPC1^{-/-}$ cells transduced with AAV2-hNPC at a Multiplicity of Infection (MOI) of either 5×10^3 or 10×10^3. Statistical significance determined using one-way ANOVA. Error bars represent SEM.

CLAIMS

What is claimed is:

1. An Adeno-Associated Virus (AAV) transfer cassette comprising, from 5' to 3':
a 5' inverted terminal repeat (ITR);
a chicken beta-actin promoter;
a transgene;
a polyadenylation signal; and
a 3' ITR;
wherein the transfer cassette comprises an intronic sequence; and
wherein the transgene encodes the NPC1 protein.

2. The AAV transfer cassette of claim 1, wherein the 5' ITR comprises the sequence of SEQ ID NO: 3.

3. The AAV transfer cassette of claim 1, wherein the 3' ITR comprises the sequence of SEQ ID NO: 4.

4. The AAV transfer cassette of claim 1, wherein the promoter comprises the sequence of SEQ ID NO: 5.

5. The AAV transfer cassette of claim 1, wherein the intronic sequence is an SV40 intron.

6. The AAV transfer cassette of claim 1, wherein the intronic sequence comprises the sequence of SEQ ID NO: 10.

7. The AAV transfer cassette of claim 1, wherein the NPC1 protein is the human NPC1 protein.

8. The AAV transfer cassette of claim 1, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 1.

9. The AAV transfer cassette of claim 1, wherein the transgene comprises the sequence of SEQ ID NO: 2.

10. The AAV transfer cassette of claim 1, wherein the polyadenylation signal is the SV40 polyadenylation signal.

11. The AAV transfer cassette of claim 1, wherein the polyadenylation signal comprises the sequence of SEQ ID NO: 12.

12. The AAV transfer cassette of claim 1, wherein the cassette comprises an enhancer.

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

14. The AAV transfer cassette of claim 1, wherein the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 15-19.

15. A plasmid comprising the AAV transfer cassette of any one of claims 1-14.

16. A nucleic acid comprising, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the nucleic acid comprises an intronic sequence; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 1.

17. A cell comprising the AAV transfer cassette of any one of claims 1-14, the plasmid of claim 15, or the nucleic acid of claim 16.

18. A recombinant AAV vector comprising a protein capsid and a nucleic acid encapsidated by the protein capsid:

wherein the nucleic acid comprises the AAV transfer cassette of any one of claims 1-14.

19. The recombinant AAV vector of claim 18, wherein the protein capsid comprises a capsid protein subunit that is from an AAV of any one of the following serotypes: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.

20. The recombinant AAV vector of claim 18, wherein the protein capsid comprises a capsid protein subunit that has one or more amino acid mutations relative to a capsid protein subunit of any one of the following AAV serotypes: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.

21. A method of producing a recombinant AAV vector, the method comprising contacting an AAV producer cell with the AAV transfer cassette of any one of claims 1-14, the plasmid of claim 15, or the nucleic acid of claim 16.

22. A recombinant AAV vector produced by the method of claim 21.

23. A composition comprising the AAV transfer cassette of any one of claims 1-14, the plasmid of claim 15, the nucleic acid of claim 16, the cell of claim 17, or the recombinant AAV vector of any one of claims 18-20 or 22.

24. Use of the AAV transfer cassette of any one of claims 1-14, the plasmid of claim 15, the nucleic acid of claim 16, the cell of claim 17, or the recombinant AAV vector of any one of claims 18-20 or 22 in the manufacture of a medicament ~~A method~~ for treating a subject in need thereof ~~comprising administering to the subject an effective amount of the AAV transfer cassette of any one of claims 1-14, the plasmid of claim 15, the nucleic~~

~~acid of claim 16, the cell of claim 17, or the recombinant AAV vector of any one of claims 18-20 or 22~~.

25. The ~~method~~ use of claim 24, wherein the subject has NPC1.

26. The ~~method~~ use of claim 24, wherein the subject is a human subject.