COMPOSITIONS AND METHODS FOR TREATING SLC26A4-ASSOCIATED HEARING LOSS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/024,466, filed May 13, 2020, the contents of which is hereby incorporated in its entirety.

BACKGROUND

[1] Hearing loss can be conductive (arising from the ear canal or middle ear), sensorineural (arising from the inner ear or auditory nerve), or mixed. Most forms of nonsyndromic deafness are associated with permanent hearing loss caused by damage to structures in the inner ear (sensorineural deafness), although some forms may involve changes in the middle ear (conductive hearing loss). The great majority of human sensorineural hearing loss is caused by abnormalities in the hair cells of the organ of Corti in the cochlea (poor hair cell function). The hair cells may be abnormal at birth, or may be damaged during the lifetime of an individual (e.g., as a result of noise trauma or infection).

SUMMARY

[2] The present disclosure provides the recognition that diseases or conditions associated with hearing loss can be treated via, e.g., the replacement or addition of certain gene products. The present disclosure further provides that gene products involved in the development, function, and/or maintenance of inner ear cells can be useful for treatment of diseases or conditions associated with hair cell and/or supporting cell loss. The present disclosure thus provides for the administration of compositions that result in expression of gene products involved in the development, function, and/or maintenance of inner ear cells including supporting cells and hair cells, and/or the use of such compositions in the treatment of hearing loss, or diseases or conditions associated with hearing loss. In one aspect, the present disclosure provides an AAV particle comprising a construct, wherein the construct comprising a coding sequence is operably linked to a promoter, wherein the coding sequence encodes an SLC26A4 gene. In another aspect, the present disclosure provides a composition comprising the AAV particle as disclosed herein. In another aspect, the present disclosure provides a composition comprising the AAV particle as disclosed herein for use in therapy, wherein the composition is to be introduced into the inner ear of a subject. In another aspect, the present disclosure provides the use of a composition comprising the AAV particle as disclosed herein in the manufacture of a medicament for treating hearing loss in a subject suffering from or at risk of hearing loss. In another aspect, the present disclosure provides a kit comprising a composition that comprises an AAV particle as disclosed herein, or a composition as disclosed herein. In some ~~embodiment~~examples, a gene product can be encoded by an *SLC26A4* gene or a characteristic portion thereof. In some ~~embodiment~~examples, a gene product can be pendrin protein or a characteristic portion thereof.

[3] The present disclosure further provides that AAV particles can be useful for administration of compositions that result in expression of gene products involved in the development, function, and/or maintenance of inner ear cells, and/or the treatment of hearing

loss, or diseases or conditions associated with hearing loss. As described herein, AAV particles comprise (i) a AAV polynucleotide construct (e.g., a recombinant AAV polynucleotide construct), and (ii) a capsid comprising capsid proteins. In some ~~embodiment~~examples, an AAV polynucleotide construct comprises an *SLC26A4* gene or a characteristic portion thereof.

[4] The present disclosure further provides compositions comprising polynucleotide constructs comprising an *SLC26A4* gene or a characteristic portion thereof. In some ~~embodiment~~examples, a construct may further include regulatory elements operably attached to a coding sequence. In certain ~~embodiment~~examples, included regulatory elements facilitate tissue specific expression at physiologically suitable levels.

[5] The present disclosure also provides a genetically modified mouse whose genome comprises a modified *Slc26a4* gene. In some ~~embodiment~~examples, the genetically modified mouse comprises a modified *Slc26a4* gene that encodes a polypeptide according to SEQ ID NO: 57. In some ~~embodiment~~examples, the genetically modified mouse is of a mouse strain suitable for use in audiological analysis experiments. In some ~~embodiment~~examples, the genetically modified mouse is of a mouse strain suitable for use in coordination analysis experiments. In some ~~embodiment~~examples, the genetically modified mouse is not of CBA/CaJ or CBA/J strain. In some ~~embodiment~~examples, the genetically modified mouse suitable for use in audiological analysis experiments is of FVB strain. In some ~~embodiment~~examples, the genetically modified mouse suitable for use in audiological analysis experiments is of FVB, 129/Sv−+p+Tyr−c+Mgf−SIJ/J, A/HeJ, AKR/J, BALB/cByJ, BALB/cJ, BDP/J, BXSB/MpJ, C3H/HeJ, C3H/HeOuJ, C3HeB/FeJ, C57BL/10J, C57BL/10SnJ, C57BL/6ByJ, CASA/RK, CAST/Ei, CBA/J, CZECH II/Ei, DBA/2HaSmn, FVB/NJ, HRS/J hrl+, MOLD/Rk, MOLF/Ei, MOLG/Dn, NON/LtJ, NZB/B1NJ, NZO/NIJ, NZW/LacJ, PERA/camEi, PERC/Ei, PL/J, RBA/Dn, RBF/DnJ, RF/J, RHJ/Le hrrh−J/+, RIIIS/J, SEC/1ReJ, SENCARC/PtJ, SF/CamEi, SHR/GnEi, SJL/J, SM/J, SPRET/Ei, ST/bJ, or SWR/J strain. In some ~~embodiment~~examples, a genetically modified mouse is treated with AAV particles, constructs, or compositions described herein.

[6] Also provided herein are methods of administering constructs and compositions described herein. In certain ~~embodiment~~examples, administration involves surgical intervention and the delivery of rAAV particles comprising therapeutic constructs. In certain ~~embodiment~~examples, AAV particles may be delivered to the inner ear of a subject in need thereof by surgical introduction through the round window membrane. In some ~~embodiment~~examples, a purpose of an intervention is to

treat hearing loss in a subject. In some ~~embodiment~~examples, efficacy of an intervention is determined through established tests, and measurements are compared to known control measurements.

DEFINITIONS

[7] The scope of the present disclosure is defined by the claims appended hereto and is not limited by certain embodiments described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described embodiments, or otherwise within the scope of the claims. In general, terms used herein are in accordance with their understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[8] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[9] The articles "a" and "an," as used herein, should be understood to include the plural referents unless clearly indicated to the contrary. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. In some embodiments, exactly one member of a group is present in, employed in, or otherwise relevant to a given product or process. In some embodiments, more than one, or all group members are present in, employed in, or otherwise relevant to a given product or process. It is to be understood that the present disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists (e.g., in Markush group or similar format), it is to be understood

WHAT IS CLAIMED IS:

1. An AAV particle comprising a construct, wherein the construct comprising a coding sequence is operably linked to a promoter, wherein the coding sequence encodes an SLC26A4 genea pendrin protein.

2. The construct of claim 1, wherein the coding sequence is an SLC26A4 gene.

32. The AAV particleconstruct of claim 21, wherein the SLC26A4 gene is a primate SLC26A4 gene.

43. The AAV particle construct of claim 2 1 or 32, wherein the SLC26A4 gene is a human SLC26A4 gene.

5. The construct of claim 4, wherein the human SLC26A4 gene comprises a nucleic acid sequence according to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

6. The construct of claim 4 or 5, wherein the human SLC26A4 gene comprises a nucleic acid sequence according to SEQ ID NO: 1.

7. The construct of claim 1, wherein the pendrin protein is a primate pendrin protein.

8. The construct of claim 1 or 7, wherein the pendrin protein is a human pendrin protein.

9. The construct of claim 8, wherein the pendrin protein comprises an amino acid sequence according to SEQ ID NO: 6.

104. The AAV particleconstruct of any one of clams 1-93, wherein the promoter is an inducible promoter, a constitutive promoter, or a tissue-specific promoter.

~~115~~. The AAV particle ~~construct~~ of any one of claims 1-~~104~~, wherein the promotor is an inner ear cell-specific promoter.

~~126~~. The AAV particle ~~construct~~ of claim ~~115~~, wherein the inner ear cell-specific promoter is a GJB2 promoter, a GJB6 promoter, a SLC26A4 promoter, a TECTA promoter, a DFNA5 promoter, a COCH promoter, a NDP promoter, a SYN1 promoter, a GFAP promoter, a PLP promoter, a TAK1 promoter, a SOX21 promoter, a SOX2 promoter, a FGFR3 promoter, a PROX1 promoter, a GLAST1 promoter, a LGR5 promoter, a HES1 promoter, a HES5 promoter, a NOTCH1 promoter, a JAG1 promoter, a CDKN1A promoter, a CDKN1B promoter, a SOX10 promoter, a P75 promoter, a CD44 promoter, a HEY2 promoter, a LFNG promoter, or a S100b promoter.

~~137~~. The AAV particle ~~construct~~ of any one of claims 1-~~104~~, wherein the promoter is a CAG promoter, a CBA promoter, a CMV promoter, or a CB7 promoter.

~~14. The construct of claim 13, wherein the promoter comprises a nucleic acid sequence according to SEQ ID NO: 43.~~

~~158~~. The AAV particle~~construct~~ of any one of claims 1-~~147~~, further comprising two AAV inverted terminal repeats (ITRs), wherein the two AAV ITRs flank the coding sequence and promoter.

~~169~~. The AAV particle~~construct~~ of claim ~~158~~, wherein the two AAV ITRs are or are derived from AAV2 ITRs.

~~17. The construct of claim 15, wherein the two AAV ITRs comprise:~~

~~(i) a 5′ ITR comprising a nucleic acid sequence according to SEQ ID NO: 10 and a 3′ ITR comprising a nucleic acid sequence according to SEQ ID NO: 11; or~~

~~(ii) a 5′ ITR comprising a nucleic acid sequence according to SEQ ID NO: 12 and a 3′ ITR comprising a nucleic acid sequence according to SEQ ID NO: 13.~~

~~18. The construct of claim 1, wherein the construct comprises a nucleic acid sequence according to SEQ ID NO: 39.~~

~~19. The construct of claim 1, wherein the construct comprises a nucleic acid sequence according to SEQ ID NO: 40.~~

~~20. An AAV particle comprising the construct of any one of claims 1-19.~~

~~21~~10. The AAV particle of any one of claims 1-9 ~~20~~, further comprising an AAV capsid, wherein the AAV capsid is or is derived from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV-rh8, AAV-rh10, AAV-rh39, AAV-rh43 or AAV Anc80 capsid.

~~22~~11. The AAV particle of claim ~~21~~10, wherein the AAV capsid is an AAV Anc80 capsid.

~~23. A composition comprising the construct of any one of claims 1-19.~~

~~24~~12. A composition comprising the AAV particle of any one of claims ~~20~~1-~~22~~11.

~~25. The composition of claim 23 or 24, wherein the composition is a pharmaceutical composition.~~

~~26~~13. The composition of claim ~~25~~12, further comprising a pharmaceutically acceptable carrier.

~~27. An ex vivo cell comprising a composition of any one of claims 23-26.~~

~~28. A method comprising, transfecting an ex vivo cell with:~~

~~(i) a construct of any one of claims 15-19; and~~

~~(ii) one or more helper plasmids collectively comprising an AAV Rep gene, AAV Cap gene, AAV VA gene, AAV E2a gene, and AAV E4 gene.~~

~~29. A method comprising:~~

~~introducing a composition of claim 25 or 26 into the inner ear of a subject.~~

~~30~~14. ~~A method of treatment comprising:~~

~~introducing a composition of claim 25 or 26~~ A composition comprising the AAV particle of any one of claims 1-11 for use in therapy, wherein the composition is to be introduced into the inner ear of a subject. ~~.~~

~~31~~15. The composition for use~~method~~ of claim ~~29 or 30~~14, wherein the composition ~~of claim 25 or 26~~ is to be introduced into the cochlea of the subject.

~~32~~16. The composition for use ~~method~~ of claim ~~29-31~~15, wherein the composition ~~of claim 25 or 26~~ is to be introduced via a round window membrane injection.

~~33. The method of any one of claims 29-32, further comprising measuring a hearing level of the subject.~~

~~34. The method of claim 33, wherein a hearing level is measured by performing an auditory brainstem response (ABR) test.~~

~~35. The method of claim 33 or 34, further comprising comparing the hearing level of the subject to a reference hearing level.~~

~~36. The method of claim 35, wherein the reference hearing level is a published or historical reference hearing level.~~

~~37. The method of claim 35, wherein the hearing level of the subject is measured after the composition of claim 25 or 26 is introduced, and the reference hearing level is a hearing level of the subject that was measured before the composition of claim 25 or 26 was introduced.~~

~~38. The method of any one of claims 29-37, further comprising measuring a level of pendrin protein in the subject.~~

~~39. The method of claim 38, wherein the level of pendrin protein is measured in the inner ear of the subject.~~

~~40. The method of claim 38 or 39, wherein the level of pendrin protein is measured in the cochlea of the subject.~~

~~41. The method of any one of claims 38-40, further comprising comparing the level of pendrin protein in the subject to a reference pendrin protein level.~~

~~42. The method of claim 41, wherein the reference pendrin protein level is a published or historical pendrin protein level.~~

~~43. The method of claim 41, wherein the level of pendrin protein in the subject is measured after the composition of claim 25 or 26 is introduced, and the reference pendrin protein level is a pendrin protein level of the subject that was measured before the composition of claim 25 or 26 was introduced.~~

~~44~~17. Use of ~~a construct of any one of claims 1-19, an AAV particle of any one of claims 20-22, or~~ a composition comprising the AAV particle of any one of claims 1-11 ~~of any one of claims 23-27 for the~~in the manufacture of a medicament for ~~treatment of~~treating hearing loss in a subject suffering from or at risk of hearing loss.~~.~~

18. The use of claim 17, wherein the composition is to be introduced into the inner ear of a subject.

19. The use of claim 18, wherein the composition is to be introduced into the cochlea of the subject.

20. The use of claim 19, wherein the composition is to be introduced via a round window membrane injection.

~~45. Use of a construct of any one of claims 1-19, an AAV particle of any one of claims 20-22, or a composition of any one of claims 23-27 in the manufacture of a medicament for the treatment of hearing loss.~~

~~46. A construct of any one of claims 1-19, an AAV particle of any one of claims 20-22, or a composition of any one of claims 23-27 for use as a medicament.~~

~~47. A construct of any one of claims 1-19, an AAV particle of any one of claims 20-22, or a composition of any one of claims 23-27 for use in the treatment of hearing loss.~~

~~48. A genetically modified mouse whose genome comprises a modified *Slc26a4* gene encoding polypeptide according to SEQ ID NO: 57, and wherein the genetically modified mouse is a genetically modified version of a mouse strain suitable for use in audiological analysis experiments.~~

~~49. The genetically modified mouse of claim 48, wherein the mouse strain suitable for use in audiological analysis experiments is not CBA/CaJ or CBA/J.~~

~~50. The genetically modified mouse of claim 48, wherein the mouse strain suitable for use in audiological analysis experiments is FVB, 129/Sv +p+Tyr+c+Mgf SIJ/J, A/HeJ, AKR/J, BALB/cByJ, BALB/cJ, BDP/J, BXSB/MpJ, C3H/HeJ, C3H/HeOuJ, C3HeB/FeJ, C57BL/10J, C57BL/10SnJ, C57BL/6ByJ, CASA/RK, CAST/Ei, CBA/J, CZECH II/Ei, DBA/2HaSmn, FVB/NJ, HRS/J hrl+, MOLD/Rk, MOLF/Ei, MOLG/Dn, NON/LtJ, NZB/B1NJ, NZO/NIJ, NZW/LacJ, PERA/camEi, PERC/Ei, PL/J, RBA/Dn, RBF/DnJ, RF/J, RHJ/Le hrrh J/+, RIIIS/J, SEC/1ReJ, SENCARC/PtJ, SF/CamEi, SHR/GnEi, SJL/J, SM/J, SPRET/Ei, ST/bJ, or SWR/J strain.~~

~~51. A method comprising,~~

~~injection of a composition according to any one of claims 1-19, an AAV particle according to any one of claims 20-22, or a composition according to any one of claims 23-26~~

~~through a perforation in a round window membrane in a mouse according to any one of claims 48-50.~~

~~52. A method of treating hearing loss comprising,~~

~~introducing a composition according to any one of claims 1-19, an AAV particle according to any one of claims 20-22, or a composition according to any one of claims 23-26 into the inner ear of a subject.~~

~~53. The method of claim 52, wherein the composition is introduced via a round window membrane injection.~~

~~54~~21. The use of claim 17~~method of claim 52 or 53~~, wherein the hearing loss is associated with a mutation in a SLC26A4 gene.

~~55~~22. The use of claim 17~~method of any one of claims 52-54~~, wherein the hearing loss and treatment of hearing loss are characterized as a function of ABR and/or Distortion Product Otoacoustic Emissions (DPOAE) measurements recorded prior to receiving any treatment and compared to ABR and/or DPOAE measurements after treatment.

~~56~~23. A kit comprising a composition that comprises ~~a construct of any one of claims 1-19, a composition that comprises~~ an AAV particle of any one of claims ~~20-22~~1-11, or a composition of any one of claims ~~23-27~~12-13.

~~57. The kit of claim 56, wherein the composition is pre-loaded in a device.~~

~~58. The kit of claim 57, wherein the device is a microcatheter.~~

~~59. The kit of claim 58, wherein the microcatheter is shaped such that it can enter the middle ear cavity via the external auditory canal and contact the end of the microcatheter with the RWM.~~

~~60. The kit of claim 57 or 58, wherein a distal end of the microcatheter is comprised of at least one microneedle with diameter of between 10 and 1,000 microns.~~

~~61. The kit of claim 56, further comprising a device.~~

~~62. The kit of claim 61, wherein the device is a device described in FIGS. 15-18 or a device as described herein.~~

~~63. The kit of claim 62, wherein the device comprises a needle comprising a bent portion and an angled tip.~~