GENE THERAPY FOR THE TREATMENT OF GALACTOSEMIA

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Serial No. 62/725,225, August 30, 2018, the contents of which are hereby incorporated by reference herein.

BACKGROUND

[0002] The present disclosure relates generally to the field of gene therapy and in particular, for the treatment of Galactosemia.

[0003] Currently there are no treatment options for Galactosemia patients other than dietary restriction of galactose containing food. Even with strict adherence to a galactose free diet, many patients suffer from long-term mental and physical deficits due to small amounts of galactose found in foods and galactose produced endogenously. Thus a need exists in the art for an effective treatment. This disclosure satisfies this need and provides related advantages as well.

SUMMARY

[0004] The disclosed gene therapy provides a sustained, long-term treatment option for Galactosemia patients by efficiently delivering the GALT gene to their cells, including the brain, to provide a sustained expression of functional GALT protein and alleviation of disease symptoms.

[0005] To achieve this therapy, provided herein is a polynucleotide or a recombinant adeno-associated viral ("AAV") vector comprising, or alternatively consisting essentially of, or yet further consisting of a polynucleotide sequence encoding galactose-1-phosphate uridyl transferase ("GALT"). In one example, the polynucleotide sequence encoding the GALT comprises, or consists essentially of, or yet further consist of, a nucleotide sequence at least 85% identical to SEQ ID NO: 1. In one example, the sequence is at least 85 % identical to SEQ ID NO: 1 with the provisio that at least one or more of the nucleotides that have been modified from the wild type sequence are not modified from SEQ ID NO: 1 (see **FIG. 4**). In another example, the polynucleotide sequence encoding GALT comprises, or consists essentially of, or yet further consist of, the nucleotide sequence set forth in SEQ ID NO: 1. In

another example, the polynucleotide sequence encodes an amino acid sequence of SEQ ID NO:4 or an equivalent thereof.

[0005a] In one aspect, provided here in a recombinant AAV vector comprising a polynucleotide encoding a polypeptide having an amino acid sequence with at least 85% identity to SEQ ID NO:4 and having GALT activity, or a complement thereof, wherein said polynucleotide has a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO: 1 or 13, and wherein the polynucleotide is operably linked to a promoter and is flanked by inverted terminal repeat ("ITR") sequences.

[0006] In one example, the vector is a self-complementary vector or a single stranded DNA (ssDNA) vector.

[0007] In one example, the recombinant adeno-associated viral ("AAV") comprises, or consists essentially of, or yet furher consists of, a AAV, such as a scAAV or a ssAAV flanked by two Inverted Terminal Repeats (ITRs). These ITRs form hairpins at the end of the sequence to serve as primers to initiate synthesis of the second strand before subsequent steps of infection can begin. The second strand synthesis is considered to be one of several blocks to efficient infection. Additional advantages of scAAV include increased and prolonged transgene expression in vitro and in vivo. Thus, in one example, the AAV further comprises two ITRs.

[0008] Non-limiting examples of recombinant AAV backbones to create the vector include AAV vector serotypes from the group of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV PHP.B, or AAV rh74. In a further example, the vector backbone is an AAV9 serotype, an rh74 serotype, or a modified AAVrh74 serotype. Also provided is a polynucleotide encoding a modified AAVrh74 VP1 capsid protein comprising one or more modifications selected from the group of a substitution of isoleucine for asparagine at amino acid position 502, and an optional substitution of tryptophan to arginine at amino acid 505 of the VP1 of AAVrh74, and an insertion of the peptide YIG or YIGSR at amino acid position 591 of the VP1 of AAVrh74, or an equivalent thereof, or a polynucleotide that is at least 85% identical with the proviso that one or more, or two or more, or three or more, or four or more modifications are identical to the amino acids in the modifice AAVrh74 VP1 capsid protein and the complements of these polynucleotides. Also provided is modified AAVrh74 VP1 capsid protein comprising one or more modifications selected from the group of a substitution of isoleucinefor

asparagine at amino acid position 502, and an optional substation of tryptophan to arginine at amino acid 505 of the VP1 of AAVrh74, and an insertion of the peptide YIG at amino acid position 591 of the VP1 of AAVrh74, or an equivalent thereof, or a polynucleotide that is at least 85% identical with the proviso that one or more, or two or more, or three or more, or four or modifications are identical to the amino acids in the modifice AAVrh74 VP1 capsid protein.

[0009] The polynucleotide sequence encoding GALT is optionally operably linked to a promoter, a tissue-specific control element, or a constitutive promoter. Non-limiting examples of constitutive promoters include, for example, a Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), a cytomegalovirus (CMV) promoter, an SV40 promoter, a dihydrofolate reductase promoter, a β-actin promoter, a phosphoglycerol kinase (PGK) promoter, or an EF1 promoter. In a particular example, the β-actin promoter is a chicken β-actin ("CBA") promoter. In another example, the promoter is selected from a CMV promoter, an EF1a promoter, an SV40 promoter, a PGK1 (human or mouse) promoter, a P5 promoter, a Ubc promoter, a human beta actin promoter, a CAG promoter, a TRE promoter, a UAS promoter, an Ac5 promoter, a polyhedrin promoter, a CaMKIIa promoter, a Gal1 promoter, a TEF1, a GDS promoter, an ADH1 promoter, a CaMV35S promoter, a Ubi promoter, an H1 promoter, a U6 promoter, or an Alpha-1-antitrypsin promoter.

[0010] In a further example, the polynucleotide or recombinant AAV further comprises a polynucleotide encoding an enhancer element. Non-limiting examples include a CMV enhancer, a WPRE and a RSV enhancer.

[0011] In a particular example, the recombinant polynucleotide or AAV vector comprises, or alternatively consists essentially of, or yet further consists of a nucleotide sequence at least 85% identical to SEQ ID NO: 2 or SEQ ID NO: 3. In one example, the sequence is at least 85 % identical to SEQ ID NO: 2 or 3 with the provisio that at least one or more of the nucleotides that have been modified from the wild type GALT (see **FIG. 4**) sequence are not modified from SEQ ID NO: 2 or 3. In a further example, the recombinant AAV vector, comprises, or alternatively consists essentially of, or yet further consists nucleotide sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 3.

[0012] The recombinant polynucleotide or AAV vector as described herein can further comprise a detectable or purification marker. The polynucleotides or vectors can be

contained as compositions comprising the polynucleotides or the vectors and a carrier, such as a preservative or pharmaceutically acceptable carrier.

[0013] Also provided is a cell or a viral particle which comprises the polynucleotides and/or AAV vectors as described herein. The cells can be prokaryotic or eukaryotic and can include packaging cell lines. The cells and viral particles can be contained as compositions comprising the vectors and a carrier, such as a preservative or pharmaceutically acceptable carrier.

[0013a] In one aspect, provided herein is a recombinant AAV viral particle comprising the recombinant AAV vector as described herein and an AAV capsid protein.

[0014] The polynucleotides and vectors are useful in therapeutic or other methods and are contained within wildtype or modified capsid proteins. Thus, provided herein are AAV viral particles comprising a recombinant polynucleotide and/or AAV vector as disclosed herein, as well as compositions containing the polynucleotide and/or viral particles for use in the methods as disclosed herein. Further provided are methods to prepare vectors, polynucleotides and viral particles containing them.. In one aspect, a method is provided for introducing a functional GALT enzyme into a cell, comprising contacting the cell with a recombinant polynucleotide and/or AAV vector or a capsid or viral particle containing the same, or a composition as described herein. The contacting can be *ex vivo* or *in vivo*.

[0014a] In one aspect, provided herein is a composition comprising the recombinant AAV viral particle as described herein, and a pharmaceutically acceptable carrier.

[0015] Also provided is a method for treating galactosemia in a subject in need thereof, comprising or alternatively consisting essentially of, or yet further consisting of administering to the subject an effective amount of the recombinant polynucleotide and/or AAV vector, the viral particle comprising the recombinant polynucleotide and/or AAV vector, or a composition as described herein.

[0016] Also provided is a method of increasing galactose metabolism in a subject that may, in one example, be suffering from galactosemia the method comprising or alternatively consisting essentially of, or yet further consisting of administering to the subject the recombinant polynucleotide and/or AAV vector, the viral particle, or a composition as described herein. In one example, the amount delivered is an effective amount as determined by the treating professional.

[0017] Yet further provided is a method of reducing a disease condition or symptom associated with galactosemia in a subject suffering from galactosemia comprising, or alternatively consisting essentially of, or yet further consisting of administering to the subject the recombinant polynucleotide and/or AAV vector, the viral particle, or a composition as described herein, wherein the disease condition comprises one or more of jaundice, hepatosplenomegaly, hepatocellular insufficiency, hypoglycemia, renal tubular dysfunction, muscle hypotonia, sepsis, cataract, ataxia, tremor, decreased bone density, fertility in adult females, impaired motor functions and growth restriction in the GalT-deficient, or primary ovarian insufficiency. In one example, the method further comprises identifying a subject as suffering from galactosemia, and then treating the subject identified by the method. In one example, the amount delivered is an effective amount as determined by the treating professional.

[0017a] In one aspect provided herein is a use of a therapeutically effective amount of the recombinant AAV viral particle as described herein in the manufacture of a medical for treating galactosemia in a mammalian subject in need thereof, wherein the medicament is to be administered to the subject.

[0017b] In one aspect, provided herein is a use of an amount of the recombinant AAV viral particle as described herein effective to increase galactose metabolism in the manufacture of a medicament for increasing galactose metabolism in a mammalian subject suffering from galactosemia, wherein the medicament is to be administered to the subject.

[0017c] In one aspect, provided herein is a use of a therapeutically effective amount of the recombinant AAV viral particle as described herein in the manufacture of a medicament for reducing a disease condition in a mammalian subject suffering from galactosemia, wherein the disease condition is one or more of jaundice, hepatosplenomegaly, hepatocellular insufficiency, hypoglycemia, renal tubular dysfunction, muscle hypotonia, sepsis, cataract, ataxia, tremor, decreased bone density, and primary ovarian insufficiency, wherein the medicament is to be administered to the subject.

[0018] In each of these methods, the galactosemia to be treated is a type 1 galactosemia, a type 2 galactosemia, or a type 3 galactosemia. In a particular example, the galactosemia is the type 1 galactosemia.

[0019] The recombinant polynucleotide and/or AAV vector, the viral particle, or a composition is administered by intramuscular injection or intravenous injection.

Alternatively, the recombinant polynucleotide and/or AAV vector, the polynucleotide and/or viral particle, or a composition is administered systemically. Yet further, the recombinant polynucleotide and/or AAV vector, the viral particle, or a composition is parentally administration by injection, infusion or implantation. In a further example, the polynucleotide, the recombinant AAV vector, the viral particle, or a composition is administered by intramuscular injection or intravenous injection and then subsequently systemically.

[0020] A kit is also provided by this disclosure, the kit comprising the recombinant polynucleotide and/or AAV vector, the viral particle, and/or the composition as described herein. The kits can optionally contain instructions for making and using the polynucleotides, vectors, viral particles or compositions.

[0020a] In one aspect, provided herein is a cell comprising an AAV helper plasmid, an AAV Ad accessory plasmid and an AAV construct plasmid, the AAV helper plasmid comprising *rep* and *cap*, and the AAV construct plasmid comprising the recombinant AAV vector as described herein.

[0020b] In one aspect, provided herein is a method of producing a recombinant AAV viral particle, said method comprising culturing the cell as described herein under conditions appropriate for production of the recombinant AAV viral particle.

[0020c] In one aspect, provided herein is the recombinant AAV viral particle as described herein for use in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIGS. 1A-1C** show the maps of three exemplary vectors: pAAV-CB-GALT-WPRE-kan (**FIG. 1A**), pAAV-CB-hGALT (**FIG. 1B**), and pAAV-CB-hGALT-WPREv2 (**FIG. 1C**).

[0022] **FIG. 2** shows bio-distribution of the pAAV-CB-GALT-WPRE-kan vector (**FIG 1A**).

[0023] **FIG. 3** shows expression of human GALT protein expressed using the vector of the pAAV-CB-GALT-WPRE-Kan vector (**FIG 1A**).

[0024] **FIG. 4** is a sequence alignment of the codon optimized recombinant polynucleotide encoding GALT as described herein as compared to corresponding wild-type sequence.

[0025] **FIG. 5** shows AAV9 vector bio-distribution. C57Bl/6J (WT) or GalT $^{-/-}$ (CG) female mice were injected intravenously with 1E+12 viral genomes (Vg) of AAV9/lucEYFP

reporter virus and 1 week later tissues were harvested and assayed for vector genome copies by qPCR.

[0026] FIG. 6 shows protein expression**.** Plasmid pAAV-CB-GALT-WPRE or pAAV- CB-lucEYFP reporter plasmid was transfected into HEK293 cells and two days later assayed for GALT expression by Western blot analysis. GALT transfected cell lysate (30 or 3 µg), LucEYFP cell lysate (138 µg), HepG2 endogenous GALT protein control (135 µg), purified

What Is Claimed Is:

1. A recombinant AAV vector comprising a polynucleotide encoding a polypeptide having an amino acid sequence with at least 85% identity to SEQ ID NO:4 and having GALT activity, or a complement thereof, wherein said polynucleotide has a nucleotide sequence at least 85% identical to the nucleotide sequence of SEQ ID NO: 1 or 13, and wherein the polynucleotide is operably linked to a promoter and is flanked by inverted terminal repeat ("ITR") sequences.

2. The recombinant AAV vector of claim 1, wherein the polynucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 1.

3. The recombinant AAV vector of claim 1, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 13.

4. The recombinant AAV vector of any one of claims 1 to 3, wherein the ITR sequences comprise an AAV2 ITR.

5. The recombinant AAV vector of any one of claims 1 to 4, wherein one ITR sequence is a self-complementary AAV ("scAAV") ITR sequence.

6. The recombinant AAV vector of any one of claims 1 to 5, wherein the promoter comprises a Rous sarcoma virus ("RSV") LTR promoter (optionally with the RSV enhancer), a cytomegalovirus ("CMV") promoter, a simian vacuolating virus 40 ("SV40") promoter, a dihydrofolate reductase promoter, a β-actin promoter, a phosphoglycerol kinase ("PGK") promoter, a U6 promoter, a chicken β-actin ("CBA"), promoter, a CMV early enhancer/chicken β-actin/rabbit β-globin splice acceptor ("CAG") promoter or an elongation factor 1 ("EF1") promoter.

7. The recombinant AAV vector of any of claims 1 to 6, wherein the polynucleotide encodes a polypeptide having an amino acid sequence of SEQ ID NO: 4.

8. The recombinant AAV vector of claim 1, which comprises the nucleotide sequence set forth in SEQ ID NO: 2 or SEQ ID NO:3.

9. A recombinant AAV viral particle comprising the recombinant AAV vector of any one of claims 1 to 8 and an AAV capsid protein.

10. The recombinant AAV viral particle of claim 9, wherein the capsid protein is an AAV1 capsid protein, an AAV2 capsid protein, an AAV3 capsid protein, an AAV4 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, an AAV7 capsid protein, an AAV8 capsid protein, an AAV9 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid protein, an AAV13 capsid protein, or an rh74 capsid protein.

11. A composition comprising the recombinant AAV viral particle of any one of claims 9 or 10, and a pharmaceutically acceptable carrier.

12. A method for introducing a functional GALT enzyme into a cell, the method comprising contacting the cell with the recombinant AAV vector of any one of claims 1 to 8, or the recombinant AAV viral particle of claims 9 or 10.

13. Use of a therapeutically effective amount of the recombinant AAV viral particle of claims 9 or 10 in the manufacture of a medical for treating galactosemia in a mammalian subject in need thereof, wherein the medicament is to be administered to the subject.

14. Use of an amount of the recombinant AAV viral particle of claims 9 or 10 effective to increase galactose metabolism in the manufacture of a medicament for increasing galactose metabolism in a mammalian subject suffering from galactosemia, wherein the medicament is to be administered to the subject.

15. Use of a therapeutically effective amount of the recombinant AAV viral particle of claims 9 or 10 in the manufacture of a medicament for reducing a disease condition in a mammalian subject suffering from galactosemia, wherein the disease condition is one or more of jaundice, hepatosplenomegaly, hepatocellular insufficiency, hypoglycemia, renal tubular dysfunction, muscle hypotonia, sepsis, cataract, ataxia, tremor, decreased bone density, and primary ovarian insufficiency, wherein the medicament is to be administered to the subject.

16. The use of any one of claims 13 to 15, wherein the galactosemia is a type 1 galactosemia.

17. The use of any of claims 13 to 16, wherein the subject is a human.

18. The use of claim 17, wherein the human is a newborn.

19. The use of claim 18, wherein the newborn lacks expression of a functional GALT gene.

20. The use of any one of claims 13 to 19, wherein the medicament is to be administered by intramuscular injection, localized delivery to the liver, intrathecal injection, or intravenous injection.

21. A cell comprising an AAV helper plasmid, an AAV Ad accessory plasmid and an AAV construct plasmid, the AAV helper plasmid comprising *rep* and *cap*, and the AAV construct plasmid comprising the recombinant AAV vector of any of claims 1 to 8.

22. A method of producing a recombinant AAV viral particle, said method comprising culturing the cell of claim 21 under conditions appropriate for production of the recombinant AAV viral particle.

23. The recombinant AAV viral particle of claims 9 or 10 for use in therapy.