fiber neuropathy. Generally, the pain is either chronic or episodic. Episodic pain in Fabry disease, termed “Fabry crises,” typically begins in the extremities and radiates proximally, and may be triggered by exercise, illness, temperature changes, or other physical and emotional stresses. This neuropathic pain is also associated with a lack of temperature perception.

[0006] The specific treatment of Fabry disease that is currently approved is enzyme replacement therapy ("ERT") which involves treatment of patients with either of two versions of recombinant human α-GAL, agalsidase alfa, which is produced by cultured human cell lines; and agalsidase beta, which is produced by Chinese hamster ovary cells transduced with GLA gene. While ERT is effective in many cases, this treatment requires life-long intravenous administration of α-GAL every two weeks. ERT resolves symptoms associated with Fabry disease but is not curative and does not stop disease progression. For example, the two α-GAL products discussed above have not been shown to substantially reduce the risk of stroke, the myocardium responds slowly to treatment, and the elimination of lipid deposits from some cell types in the kidney is limited. The insufficient pharmacologic response is largely due to the short circulatory half-life of the enzyme and suboptimal cellular delivery. Thus, there remains a need for therapies for treating Fabry disease that can stop disease progression and potentially be curative.

SUMMARY

[0007] The present application discloses methods and compositions for the treatment and/or prevention of Fabry disease. The present disclosure provides, in part, a gene therapy approach using recombinant adeno associated viral vectors (rAAV) to mediate transfer and expression of the GLA gene. This application is based on the discovery that gene delivery vehicles such as a rAAV vector that has broad tissue tropism and utilizes a ubiquitous promoter to drive widespread gene expression results in sustained high levels of protein expression and robust protein exposure to a wide range of tissues and/or decrease in GB3 or lysoGb3 levels. This application is also based on the discovery that codon optimized or engineered variants of GLA delivered using a rAAV vector with broad or tissue specific tropism and utilizing a ubiquitous or tissue specific promoter result in an increase in α-GAL activity in vivo, and/or a decrease in lysoGb3 or GB3 levels in vivo. Additionally, this gene delivery approach to drive expression of GLA variants that encode α-GAL protein with increased half-life and improved

cellular uptake provides further increases in α-GAL exposure in key target tissues. Collectively, these discoveries allow for the delivery vehicles described herein to achieve broad tissue distribution of administered transgenes, and also allows better treatment outcomes. The delivery vehicles comprising the GLA sequences described herein are particularly useful for the treatment of Fabry disease.

[0008] Described herein are methods and compositions for effective delivery of *α* -- Galactosidase A (GLA) gene into cells of a subject in need thereof. The delivered GLA transgene results in expression of α-GAL protein. The present disclosure is based, in part, on the development of a recombinant adeno associated viral (rAAV) vector that comprises *α* - Galactosidase A (GLA) gene, among other things, and which demonstrates robust α-GAL protein expression once present in a cell. The present disclosure is based, at least in part, on the surprising discovery that a construct comprising a GLA gene under a ubiquitous promoter and packaged in a rAAV capsid with broad tissue tropism results in persistent α-GAL protein expression and robust tissue biodistribution in the kidney, heart, gastrointestinal tract, brain, and peripheral neurons even at a low dose. The broad distribution obtained using this vector allows for efficient delivery of α-GAL to tissues that are affected by Fabry disease, and consequently allows for a robust therapeutic result.

[0009] The rAAV vectors described herein can be used with either a GLA gene having a wild type sequence (SEQ ID NO: 3) or a GLA gene having a modified sequence described herein. Such modified GLA sequences include, for example, codon optimized GLA and/or engineered variants of GLA. The rAAV vectors described herein allows for substrate clearance of globotriaosylsphingosine (lysoGb3) and/or globotriaosylceramide (GB3) in various tissues.

[0010] As described in more detail below, the gene therapy system described herein results in overall improvement in health as evidenced by gain in body mass, improved kidney function, and neurological symptoms in Fabry disease mouse models and is further expected to elicit the same in humans. The methods and compositions provided herein can be used to achieve sustained expression of GLA in a wide variety of tissues that are affected in Fabry disease. Thus, the present application provides composition and methods that are highly effective in the treatment of Fabry disease and alleviation of associated symptoms.

[0010a] In one aspect, the present disclosure provides a recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

a. a 5' inverted terminal repeat (ITR);
b. a ubiquitous promoter;
c. a nucleotide sequence encoding α-GAL enzyme;
d. a poly A; and
e. a 3' ITR.

[0010b] In another aspect, the present disclosure provides a recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

a. a 5' inverted terminal repeat (ITR);
b. a ubiquitous promoter;
c. a nucleotide sequence encoding α-GAL enzyme;
d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE);
e. a poly A; and
f. a 3' ITR.

[0011] In some examples, a recombinant adeno-associated virus (rAAV) vector with broad tissue tropism is provided, said vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a

ubiquitous promoter; (c) a nucleotide sequence encoding α-GAL enzyme; (d) a poly A; and (e) a 3' ITR.

[0012] In some examples, a recombinant adeno-associated virus (rAAV) vector with broad tissue tropism is provided, said vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a ubiquitous promoter; (c) a nucleotide sequence encoding α-GAL enzyme; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); (e) a poly A; and (f) a 3' ITR.

[0013] Various kinds of AAV capsids with broad tissue tropism (the terms "broad tissue tropism" "wide-tropism" are used interchangeably herein) can be used in the rAAV vector described herein. For example, in some examples, the AAV capsid is a wide-tropism AAV capsid selected from an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, AAV8 capsid, AAV9 capsid, AAV11, 12,13, AAVhu.37, AAVrh.8, AAVrh.10, and AAVrh.39, AAV-DJ, or AAV-DJ/8.

[0014] Accordingly, in some examples, the AAV capsid with wide-tropism is AAV is AAV1. In some examples, the AAV capsid with wide-tropism is AAV is AAV2. In some examples, the AAV capsid with wide-tropism is AAV is AAV3. In some examples, the AAV capsid with wide-tropism is AAV is AAV4. In some examples, the AAV capsid with wide-tropism is AAV is AAV5. In some examples, the AAV capsid with wide-tropism is AAV is AAV6. In some examples, the AAV capsid with wide-tropism is AAV is AAV7. In some examples, the AAV capsid with wide-tropism is AAV is AAV8. In some examples, the AAV capsid with wide-tropism is AAV is AAV9.

[0015] Various kinds of capsids and associated tropism are described in *Curr Opin Vir.* 2016 December 21:75-80, the contents of which are incorporated herein by reference. By "broad tissue tropism" it is meant that the capsid is able to enable gene transfer to two or more than 2, 3, 4, 5, 6, 7, 8 or more tissue types. For example, in some examples, a capsid having broad tissue tropism enable gene transfer to one or more of the following tissues: liver, kidney, heart, gastrointestinal tract, and/or peripheral neurons of the subject.

[0016] In some examples, the ubiquitous promoter is selected from chicken β actin (CBA) promoter, CAG promoter, EF-1α promoter, PGK promoter, UBC promoter, LSE beta-glucuronidase (GUSB) promoter, or ubiquitous chromatin opening element (UCOE) promoter. In some examples, the ubiquitous promoter comprises CBh (CMV enhancer, Chicken beta-actin promoter, Chicken-beta actin-MVM hybrid intron). Accordingly, in some examples,

the ubiquitous promoter is a chicken β actin (CBA) promoter. In some examples, the ubiquitous promoter is an EF-1α promoter. In some examples, the EF-1α promoter is in combination with chimeric intron from chicken β-actin and rabbit β-globin genes. In some examples, the ubiquitous promoter is a UBC promoter. In some examples, the ubiquitous promoter is an LSE beta-glucuronidase (GUSB) promoter. In some examples, the ubiquitous promoter is a ubiquitous chromatin opening element (UCOE) promoter. (Powell SK, et al. Discov Med. 2015 Jan;19(102):49-57.)

[0017] In some examples, the ubiquitous promoter comprises a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron.

[0018] In some examples, the ubiquitous promoter comprises a shortened EF-1α promoter and one or more introns.

[0019] In some examples, the one or more introns are from chicken β-actin and/or rabbit β-globin genes.

[0020] In some examples, the AAV9 capsid is naturally occurring or modified.

[0021] In some examples, the WPRE sequence is optional or is modified.

[0022] In some examples, the WPRE sequence is WPRE mut6delATG.

[0023] Exemplary polyA sequences that may be included in the gene therapy vectors encompassed by the present disclosure include human growth hormone polyA (hGHpA), synthetic polyA (SPA), Simian virus 40 late poly A (SV 40pA) and bovine growth hormone (BGH) poly A. In a particular example, the poly A is bovine growth hormone (BGH) poly A.

[0024] In some examples, the nucleotide sequence encoding α-GAL enzyme is codon optimized.

[0025] In some examples, the nucleotide sequence encoding α-GAL enzyme is codon optimized for human cells.

[0026] In some examples, the α-GAL enzyme has an unmodified sequence.

[0027] In some examples, the α-GAL enzyme has a modified sequence.

[0028] In some examples, the nucleotide sequence encoding the α-GAL enzyme is engineered.

[0029] In some examples, the nucleotide sequence encoding the α-GAL enzyme is engineered and codon optimized.

[0030] In some examples, the modified sequence comprises one or more amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30).

[0031] In some examples, the modified sequence comprises between 1 and 25 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). For example, in some examples, the modified sequence comprises between 5 and 25 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 5 and 20 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 5 and 15 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 5 and 10 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 10 and 25 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 10 and 20 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 10 and 15 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30).

[0032] In some examples, the modified sequence comprises between 1 and 10 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). For example, in some examples, the modified sequence comprises between 1 and 9 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 8 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 7 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 6 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 5 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 4 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30). In some examples, the modified sequence comprises between 1 and 3 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30).

[0033] In some examples, the modified sequence comprises 10 amino acid substitutions in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30).

[0034] In some examples, a recombinant alpha galactosidase A is provided. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 30, or a functional fragment thereof. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 86% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 87% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 88% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 89% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 91% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 92% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 93% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 94% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 96% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 97% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 98% sequence identity to SEQ ID NO: 30. In some examples, the recombinant alpha galactosidase A comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 30.

[0035] In some examples, the recombinant alpha galactosidase A comprises at least one substitution or substitution in SEQ ID NO:30 at one or more positions selected from: T41/M70/ L75/ S78/E79/Y123/R193/S197/K237/F248/N247/ N278/ L286/A292/ H302/ Q333/ K314/ L347/M353 /S364/A368/S371/ K374/K393/ F396/ E398/W399 /R404/ M423.

[0036] Additional exemplary GLA transgene and α-GAL enzyme sequences can be found in PCT publication nos: PCT/US2021/019811, PCT/US2019/067493 and PCT/US2015/063329, each of which is incorporated by reference herein, in its entirety.

[0037] In some examples, the modified α-GAL enzyme is selected from one of SEQ ID Nos: 7-17, 33, 34, and 46-60.

[0038] In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 7. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 8. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 9. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 10. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 11. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 12. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 13. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 14. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 15. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 16. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 17. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 33. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 34. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 46. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 47. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 48. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 49. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 50. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 51. In some examples, the modified α-GAL enzyme comprises

amino acid sequence of SEQ ID NO: 52. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 53. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 54. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 55. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 56. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 57. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 58. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 59. In some examples, the modified α-GAL enzyme comprises amino acid sequence of SEQ ID NO: 60.

[0039] In some examples, the modified α-GAL enzyme has increased stability in comparison to wild-type α-GAL enzyme (SEQ ID NO: 30).

[0040] In some examples, the modified α-GLA enzyme has increased intracellular activity in comparison to wild-type α-GLA enzyme (SEQ ID NO: 30).

[0041] In some examples, the modified α-GLA enzyme has improved serum stability in comparison to wild-type α-GLA enzyme (SEQ ID NO: 30).

[0042] In some examples, the modified α-GLA enzyme has improved lysosomal stability in comparison to wild-type α-GLA enzyme (SEQ ID NO: 30).

[0043] In some examples, the modified α-GLA enzyme has increased specific catalytic activity in comparison to wild-type α-GLA enzyme (SEQ ID NO: 30).

[0044] In another aspect, the present disclosure provides a pharmaceutical composition comprising a recombinant adeno-associated viral vector (rAAV) as described herein for use in therapy. In some examples, a method of treating Fabry disease in a subject is provided, the method comprising administering to a subject in need thereof a recombinant adeno-associated viral vector (rAAV) as described herein.

[0045] In some aspects, a pharmaceutical composition is provided, the composition comprising the rAAV vector as described herein.

[0046] In some aspects, a cell is provided, the cell comprising the rAAV vector as described herein. The cell can be any kind of mammalian cell. For example, in some examples, the cell is a heart cell. In some examples, the cell is a kidney cell. In some examples, the cell is a liver. In some examples, the cell is a skeletal muscle cell. In some examples, the cell is a cell of the gastrointestinal tract. In some examples, the cell

is a cell of the brain, such as for example a neuron or a glial cell. In some examples, the cell is a peripheral neuron.

[0047] In another aspect, the present disclosure provides a use of a recombinant adeno-associated viral vector (rAAV) packaged in a capsid with broad tissue tropism in the manufacture of a pharmaceutical composition for treating Fabry disease, the vector comprising: a. a 5' inverted terminal repeat (ITR); b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; c.a nucleotide sequence encoding α-GAL enzyme; d. a poly A; and e. a 3' ITR, wherein the use comprises that the pharmaceutical composition is to be administered to a subject in need thereof. In some examples, a method of treating Fabry disease in a subject is provided, the method comprising administering to a subject in need thereof a recombinant adeno-associated viral vector (rAAV) packaged in a rAAV capsid having broad tissue tropism, the vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; (c) a nucleotide sequence encoding α-GAL enzyme; (d) a poly A; and (e) a 3' ITR.

[0048] In another aspect, the present disclosure provides a use of a recombinant adeno-associated viral vector (rAAV) packaged in a capsid with broad tissue tropism in the manufacture of a pharmaceutical composition for treating Fabry disease, the vector comprising: a. a 5' inverted terminal repeat (ITR); b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; c. a nucleotide sequence encoding α-GAL enzyme; d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); e. a poly A; and f. a 3' ITR, wherein the use comprises that the pharmaceutical composition is to be administered to a subject in need thereof. In some examples, a method of treating Fabry disease in a subject is provided, the method comprising administering to a subject in need thereof a recombinant adeno-associated viral vector (rAAV) packaged in a rAAV capsid having broad tissue tropism, the vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; (c) a nucleotide sequence encoding α-GAL enzyme; (d) a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); (e) a poly A; and (f) a 3' ITR.

[0049] In some examples, the AAV capsid is a wide-tropism AAV capsid selected from an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, AAV8 capsid, or AAV9 capsid. In some examples, the wide-tropism AAV capsid is an AAV1 capsid. In some examples, the wide-tropism AAV capsid is an AAV2 capsid. In some examples, the wide-tropism AAV capsid is an AAV3 capsid. In some examples, the wide-tropism AAV capsid is an AAV4 capsid. In some examples, the wide-tropism AAV capsid is an AAV5 capsid. In some examples, the wide-tropism AAV capsid is an AAV6 capsid. In some examples, the wide-tropism AAV capsid is an AAV7 capsid. In some examples, the wide-tropism AAV capsid is an AAV8 capsid. In some examples, the wide-tropism AAV capsid is an AAV9 capsid.

[0050] In some examples, the rAAV vector is administered by intravenous, subcutaneous, or transdermal administration. Accordingly, in some examples, the rAAV vector is administered intravenously to a subject in need thereof. In some examples, the rAAV vector is administered subcutaneously to a subject in need thereof. In some examples, the rAAV vector is administered transdermally to a subject in need thereof.

[0051] In some examples, the transdermal administration is by gene gun.

[0052] In some examples, the rAAV vector is episomal following administration.

[0053] In some examples, a rAAV described herein is administered to a subject in need thereof at a dose lower than a dose expected to be used with a AAV vector that targets the liver for expression of α-GAL. In other examples, a rAAV described herein, when administered at an equivalent dose as a liver targeted rAAV exhibits higher α-GAL serum and tissue exposure.

[0054] In some examples, the rAAV vector with broad tissue tropism and using a ubiquitous promoter achieves a therapeutic effect for treating Fabry disease at a lower dose than a rAAV vector comprising an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, or AAV8 capsid using a liver specific promoter. Accordingly, in some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV1 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV2 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV3 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV3 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV4 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV5 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV6 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV7 capsid with a liver specific promoter. In some examples, the rAAV vector with broad tissue tropism and a ubiquitous promoter achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV8 capsid with a liver specific promoter.

[0055] In some examples, a rAAV vector capable of expressing an α-GAL enzyme may comprise a tissue specific promoter, e.g., a liver specific promoter. Exemplary liver-specific promoters include, but are not limited to, for example, transthyretin promoter (TTR); thyroxine-binding globulin (TBG) promoter; hybrid liver-specific promoter (HLP), and alpha-1-antitrypsin (AAT) promoter.

[0056] In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 5 weeks, 10 weeks, 15 weeks, 26 weeks, 1 year, 5 years, 10 years, or 20 years. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 5 weeks. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 10 weeks. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 15 weeks. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 26 weeks. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 1 year. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 5 years. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 10 years. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 15 years. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 20 years. In some examples, following administration of the rAAV vector, the subject has detectable α-GAL in the serum for the life of the subject.

[0057] In some examples, expression of modified α-GAL enzyme provides 3, 10, 30, 100, 300 fold higher serum α-GAL levels compared to the expression of WT α-GAL. In some examples, expression of modified α-GAL enzyme provides, 3, 10, 30, 100, 300 fold higher intracellular enzyme levels compared to expression of WT α-GAL.

[0058] In some examples, the administration results in α-GAL enzyme exposure in one or more of liver, kidney, heart, gastrointestinal tract, brain, and/or peripheral neurons of the subject. Accordingly, in some examples, administration results in α-GAL enzyme exposure in the liver. In some examples, administration results in α-GAL enzyme exposure in the

kidney. In some examples, administration results in α-GAL enzyme exposure in the heart. In some examples, administration results in α-GAL enzyme exposure in the gastrointestinal tract and cells associated with the gastrointestinal tract. In some examples, administration results in α-GAL enzyme exposure in the brain. In some examples, administration results in α-GAL enzyme exposure in peripheral neurons.

[0059] In some examples, administration of rAAV vector results in a survival benefit to Fabry mouse/patient. In some examples, administration of the rAAV vector results in reduced levels of globotriaosylceramide (GB3) in one or more of liver, heart, kidney and gastrointestinal tract of the subject. Accordingly, in some examples, administration of the rAAV vector results in reduced levels of GB3 in the heart. In some examples, administration of the rAAV vector results in reduced levels of GB3 in the skeletal muscle. In some examples, administration of the rAAV vector results in reduced levels of GB3 in the kidney. In some examples, administration of the rAAV vector results in reduced levels of GB3 in the gastrointestinal tract. Levels of GB3 can be assessed by any means known in the art including for example by chromatographic methods, including for example liquid chromatography-tandem mass spectrometry.

[0060] In yet another aspect, the present disclosure provides a use of a rAAV vector packaged in an AAV9 capsid in the manufacture of a pharmaceutical composition for expressing α-GAL enzyme in a cell, said vector comprising: a. a 5' inverted terminal repeat (ITR); b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; c. a nucleotide sequence encoding α-GAL enzyme; d. a bovine growth hormone (BGH) poly A; and e. a 3' ITR. In some examples, the present disclosure encompasses a method of expressing α-GAL enzyme in a cell, the method comprising administering a rAAV vector packaged in an AAV9 capsid, said vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; (c) a nucleotide sequence encoding α-GAL enzyme; (d) a bovine growth hormone (BGH) poly A; and (e) a 3' ITR.

[0061] In yet another aspect, the present disclosure provides a use of a rAAV vector packaged in an AAV9 capsid in the manufacture of a pharmaceutical composition for expressing α-GAL enzyme in a cell, said vector comprising: a. a 5' inverted terminal repeat (ITR); b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin

promoter, and a rabbit beta globin intron; c. a nucleotide sequence encoding α-GAL enzyme; d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) having mut6delATG mutation; e. a bovine growth hormone (BGH) poly A; and f. a 3' ITR. In some examples, the present disclosure encompasses a method of expressing α-GAL enzyme in a cell, the method comprising administering a rAAV vector packaged in an AAV9 capsid, said vector comprising: (a) a 5' inverted terminal repeat (ITR); (b) a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron; (c) a nucleotide sequence encoding α-GAL enzyme; (d) optionally a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) having mut6delATG mutation; (e) a bovine growth hormone (BGH) poly A; and (f) a 3' ITR.

[0062] In some aspects, the present disclosure encompasses a recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid, said vector comprising a 5' inverted

terminal repeat (ITR); a liver specific promoter; a nucleotide sequence encoding α-GAL enzyme; a poly A; and a 3' ITR.

[0063] In some aspects, the present disclosure encompasses a recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid, said vector comprising a 5' inverted terminal repeat (ITR); a liver-specific promoter; a nucleotide sequence encoding α-GAL enzyme; a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE); a poly A; and a 3' ITR.

BRIEF DESCRIPTION OF FIGURES

[0064] **FIG. 1** is a vector diagram of exemplary rAAV9 comprising wild-type GAL under the control of a ubiquitous promoter (referred to generally herein as "rAAV9") as described herein.

[0065] **FIG. 2** is a graph that shows the level of alpha galactosidase in serum over a period of 12-weeks post injection with rAAV9 in a Fabry (GLAko) mouse model as described herein.

[0066] **FIG. 3A-FIG. 3M** are a series of graphs showing expression of alpha galactosidase in various tissues and reduction of GB3 in various tissues after administration of rAAV9-WT in Fabry GLAko mice. **FIG. 3A** shows expression of alpha galactosidase in liver, post administration of rAAV9 as compared to rAAV9 –null ("null" refers to a AAV9 that does not contain any transgene) and untreated control. **FIG. 3B** shows expression of alpha galactosidase in kidney, post administration of rAAV9-WT as compared to rAAV9-null and untreated control. **FIG. 3C** shows expression of alpha galactosidase in heart, post administration of rAAV9-WT as compared to and untreated control. **FIG. 3D** shows expression of alpha galactosidase in duodenum, post administration of rAAV9-WT as compared to rAAV9-null (null vector) and untreated control. **FIG. 3E** shows expression of alpha galactosidase in colon, post administration of rAAV9 as compared to rAAV9-null and untreated control. **FIG. 3F** shows the levels of GB3 in serum as compared to ERT and untreated controls. **FIG. 3G** shows the levels of GB3 in liver as compared to ERT and untreated controls. **FIG. 3H** shows the levels of GB3 in kidney as compared to ERT and untreated controls. **FIG. 3I** shows the levels of GB3 in heart as compared to ERT and untreated controls. **FIG. 3J** shows the levels of lysoGB3 in serum as

CLAIMS

1. A recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

   a. a 5’ inverted terminal repeat (ITR);

   b. a ubiquitous promoter;

   c. a nucleotide sequence encoding α-GAL enzyme;

   d. a poly A; and

   e. a 3’ ITR.

2. A recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

   a. a 5’ inverted terminal repeat (ITR);

   b. a ubiquitous promoter;

   c. a nucleotide sequence encoding α-GAL enzyme;

   d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE);

   e. a poly A; and

   f. a 3’ ITR.

3. The recombinant rAAV vector of claim 1 or 2, wherein the AAV capsid is a wide-tropism AAV capsid selected from an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, AAV8 capsid, or AAV9 capsid.

4. The recombinant rAAV vector of claim 3, wherein the wide-tropism AAV capsid is AAV9.

5. The rAAV vector of claim 1, wherein the ubiquitous promoter is selected from chicken β actin (CBA) promoter, EF-1α promoter, PGK promoter, UBC promoter, LSE beta-glucuronidase (GUSB) promoter, or ubiquitous chromatin opening element (UCOE) promoter.

6. The rAAV vector of claim 1, wherein the ubiquitous promoter comprises a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron.

7. The rAAV vector of claim 1, wherein the ubiquitous promoter comprises a shortened EF-1α promoter and one or more introns.

8. The rAAV vector of claim 7, wherein the one or more introns are from chicken β-actin and/or rabbit β-globin genes.

9. The rAAV vector of claim 4, wherein the AAV9 capsid is naturally occurring or modified.

10. The rAAV vector of claim 2, wherein the WPRE sequence is modified.

11. The rAAV vector of claim 10, wherein the WPRE sequence is WPRE mut6delATG.

12. The rAAV vector of claim 1 or 2, wherein the poly A is bovine growth hormone (BGH) poly A.

13. The rAAV vector of any one of the proceeding claims, wherein the nucleotide sequence encoding α-GAL enzyme is codon optimized.

14. The rAAV vector of claim 13, wherein the nucleotide sequence encoding α-GAL enzyme is codon optimized for human cells.

15. The rAAV vector of any one of claims 1-13, wherein the α-GAL enzyme has an unmodified sequence.

16. A pharmaceutical composition comprising a recombinant adeno-associated viral vector (rAAV) of any one of the preceding claims for use in therapy.

17. A pharmaceutical composition comprising the rAAV vector of any one of claims 1-15.

18. A cell comprising the rAAV vector of any one of claims 1-15.

19. Use of a recombinant adeno-associated viral vector (rAAV) packaged in a capsid with broad tissue tropism in the manufacture of a pharmaceutical composition for treating Fabry disease, the vector comprising:

a. a 5’ inverted terminal repeat (ITR);

b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a poly A; and

e. a 3’ ITR,

wherein the use comprises that the pharmaceutical composition is to be administered to a subject in need thereof.

20. Use of a recombinant adeno-associated viral vector (rAAV) packaged in a capsid with broad tissue tropism in the manufacture of a pharmaceutical composition for treating Fabry disease, the vector comprising:

a. a 5’ inverted terminal repeat (ITR);

b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE);

e. a poly A; and

f. a 3’ ITR,

wherein the use comprises that the pharmaceutical composition is to be administered to a subject in need thereof.

21. The use according to claim 19 or 20, wherein the AAV capsid is a wide-tropism AAV capsid selected from an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, AAV8 capsid, or AAV9 capsid.

22. The use according to claim 21, wherein the wide-tropism AAV capsid is AAV9.

23. The use according to claim 19 or 20, wherein the nucleotide sequence encoding α-GAL enzyme is codon optimized.

24. The use according to claim 19 or 20, wherein the nucleotide sequence encoding α-GAL enzyme is engineered.

25. The use according to claim 24, wherein the nucleotide sequence encoding α-GAL enzyme is engineered and codon optimized.

26. The use according to any one of claims 19-22, wherein the α-GAL enzyme has an unmodified sequence.

27. The use according to claim 20, wherein the WPRE is WPRE mut6delATG.

28. The use according to claim 20, wherein the poly A is a bovine growth hormone (BGH) poly A.

29. The use according to any one of claims 19-28, wherein the rAAV vector is to be administered by intravenous, subcutaneous, or transdermal administration.

30. The use according to claim 29, wherein the transdermal administration is by gene gun.

31. The use according to any one of claims 19-30, wherein the rAAV vector is episomal following administration.

32. The use according to claim 19 or 20, wherein the rAAV vector achieves a therapeutic effect at a lower dose than a rAAV vector comprising an AAV1 capsid, AAV2 capsid, AAV3 capsid, AAV4 capsid, AAV5 capsid, AAV6 capsid, AAV7 capsid, or AAV8 capsid which is specifically targeted to the liver using a liver-specific promoter.

33. The use according to any one of claims 19-32, wherein following administration of the rAAV vector, the subject has detectable α-GAL in the serum for at least 5 weeks, 10 weeks, 15 weeks, 26 weeks, 1 year, 5 years, 10 years or 15 years.

34. The use according to claim 33, wherein the subject has detectable α-GAL in the serum for greater than 15 weeks.

35. The use according to any one of claims 19-34, wherein administration results in α-GAL enzyme expression in one or more of liver, kidney, heart, and gastrointestinal tract, of the subject.

36. The use according to any one of claims 19-34, wherein administration of the rAAV vector results in reduced levels of globotriaosylceramide (gb3) in one or more of liver, heart, kidney and GI tract of the subject.

37. Use of a rAAV vector packaged in an AAV9 capsid in the manufacture of a pharmaceutical composition for expressing α-GAL enzyme in a cell, said vector comprising:

a. a 5’ inverted terminal repeat (ITR);

b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a bovine growth hormone (BGH) poly A; and

e. a 3’ ITR.

38. Use of a rAAV vector packaged in an AAV9 capsid in the manufacture of a pharmaceutical composition for expressing α-GAL enzyme in a cell, said vector comprising:

a. a 5’ inverted terminal repeat (ITR);

b. a ubiquitous promoter comprising a cyto-megalo-virus (CMV) enhancer, chicken beta actin promoter, and a rabbit beta globin intron;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) having mut6delATG mutation;

e. a bovine growth hormone (BGH) poly A; and

f. a 3’ ITR.

39. A recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

a. a 5’ inverted terminal repeat (ITR);

b. a liver specific promoter;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a poly A; and

e. a 3’ ITR.

40. A recombinant adeno-associated virus (rAAV) vector packaged in an AAV capsid having broad tissue tropism, said vector comprising

a. a 5’ inverted terminal repeat (ITR);

b. a liver-specific promoter;

c. a nucleotide sequence encoding α-GAL enzyme;

d. a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE);

e. a poly A; and

f. a 3’ ITR.

41. The vector of claim 39 or 40, wherein the nucleotide sequence encoding α-GAL enzyme is codon optimized.

42. The vector of claim 39 or 40, wherein the nucleotide sequence encoding α-GAL enzyme is engineered.

43. The vector of claim 39 or 40, wherein the nucleotide sequence encoding α-GAL enzyme is both codon optimized and engineered.