[0069] Figure 30 shows that ENU2 mice treated with AAV5 increases the rate of gain in body weight.

[0070] Figure 31 shows that the coat color of ENU2 mice returns to wild type color when treated with AAV5-PAH.

[0071] Figure 32A-E shows that high dose AAV-PAH normalizes brain amino acids Phe (A) and Tyr (B), and neurotransmitter levels (C-D) of ENU2 mice to wildtype levels. Chemical pathway is also described (E).

[0072] Figure 33 shows that ENU2 mice treated with ApoE-HCR-hAAT.GI.muPAH.bGH vector reduced phenylalanine levels for a duration of 60 weeks.

[0073] Figure 34 shows minimal change in Phe levels caused by increased PAH protein in wild-type mice treated with ApoE-HCR-hAAT.GI.hPAH.bGH vector or ApoE-HCR-hAAT.GI.muPAH.bGH vector.

[0074] Figure 35 is a western blot which shows increased levels of the PAH protein in the treated mice of Figure 34 despite minimal Phe level change.

[0075] Figure 36A-B depicts quantification of staining (A) and staining (B) that shows no significant TUNEL staining in the livers of animals treated with AAV5-hPAH at 2E13 or 2E14 vg/kg 12 weeks post-dose.

[0076] Figure 37 is a schematic representation of the ApoE-HCR-hAAT.hPAH-tI2.bGH vector.

[0077] Figure 38 is a graph that shows plasma Phe levels were reduced in mice receiving the ApoE-HCR-hAAT.hPAH-tI2.bGH vector in a dose-dependent fashion.

[0078] Figure 39A-D are graphs that show high dose ApoE-HCR-hAAT.hPAH-tI2.bGH vector corrects (A-C) amino acid levels and (D) neurotransmitter levels in the brain.

[0079] Figure 40 is a schematic representation of ApoE-HCR-hAAT.cG-AIATI.hPAH.bGH vector.

[0080] Figure 41 shows the sequence alignment of codop-hPAH DNA sequences. hPAH (SEQ ID NO: 1), NW2-Cop (SEQ ID NO: 9), NW-RCG (SEQ ID NO: 10), D20-Op (SEQ ID NO: 11), D20-Cop1 (SEQ ID NO: 12), JCAT (SEQ ID NO: 13), Operon-1 (SEQ ID NO: 7), and Operon-2 (SEQ ID NO: 8).

[0081] Figure 42A-C analyzes the codop-hPAH sequences with charts that compare percent sequence identities (A), cladogram relationships (B), and GC content (C).

[0082] Figure 43A-B shows AAV5-PAH vector designs and DNA structure.

[0083] Figure 44A-B is a comparison of codon optimized hPAH versions by HEPG2 liver cell transduction or transfection.

CLAIMS

1. A recombinant adeno-associated virus (rAAV) vector genome comprising:

(a) a promoter; and

(b) a codon optimized sequence encoding a human phenylalanine hydroxylase (hPAH) wherein the codon optimized sequence comprises SEQ ID NO: 7 or a ~~variant thereof~~ sequence having at least ~~80%~~ 95% sequence identity to SEQ ID NO: 7; and

(c) an AAV inverted terminal repeat (ITR) sequence.

2. ~~The~~ A rAAV vector genome comprising: ~~of claim 1,~~

(a) a promoter; and

(b) a codon optimized sequence encoding a hPAH wherein the codon optimized PAH sequence comprises SEQ ID NO: 7 or a ~~variant~~ sequence having at least ~~85%~~ 95% sequence identity to SEQ ID NO: 7; and

(c) an AAV 5' ITR sequence and an AAV 3' ITR sequence.

3. The rAAV vector genome of claim 1 or claim 2, wherein the codon optimized hPAH sequence comprises a ~~variant~~ sequence having at least ~~90%~~ 98% sequence identity to SEQ ID NO: 7.

4. The rAAV vector genome of claim 1 or claim 2, wherein the codon optimized hPAH sequence comprises ~~a variant having at least 95% sequence identity to~~ SEQ ID NO: ~~7~~ 8.

5. The rAAV vector genome of claim 1 or claim 2, wherein the codon optimized hPAH sequence comprises SEQ ID NO: 13

~~5.~~ 6. The rAAV vector genome according to claim 1 or claim 2, wherein the codon optimized hPAH sequence comprises SEQ ID NO: 7.

7. The rAAV vector genome according to claim 1 or claim 2, wherein the codon optimized hPAH sequence consists of SEQ ID NO: 7.

8. The rAAV vector genome according to claim 2, wherein the AAV 5' ITR and/or AAV3' ITR is from AAV2.

~~6.~~ 9. The rAAV vector genome according to any one of claims 1-~~5~~ 8, wherein the promoter is a liver-specific promoter.

~~7.~~ 10. The rAAV vector genome according to any one of claims 1-~~6~~ 9, wherein the promoter comprises a synthetic promoter, constitutive promoter, regulatable promoter, or a promoter responsive to physiologic cues.

~~8.~~ 11. The rAAV vector genome according to claim ~~6~~ 9, wherein the promoter is a thyroxine binding globulin (TBG) promoter, a transthyretin (TTR) promoter, a human albumin (humAlb) promoter; a Liver Specific promoter (LSP), or a hepatitis B virus core promoter.

~~9.~~ 12. The rAAV vector genome according to any one of claims 1-~~6~~ 9, wherein the promoter is synthetic promoter sequence comprising portions of an hAAT promoter, a hepatic control region (HCR) enhancer, and an ApoE enhancer.

~~10.~~ 13. The rAAV vector genome according to any one of claims 1-~~6~~ 9, wherein the promoter comprises SEQ ID NO: 6 or a ~~variant thereof~~ sequence having at least 80% sequence identity to SEQ ID NO: 6.

~~11~~ 14. The rAAV vector genome according to claim ~~10~~ 13, wherein the promoter comprises a ~~variant thereof~~ sequence having at least 85% sequence identity to SEQ ID NO: 6.

~~12.~~ 15. The rAAV vector genome according to claim ~~10~~ 13, wherein the promoter comprises of a ~~variant~~ sequence having at least 90% sequence identity to SEQ ID NO: 6.

~~13.~~ 16. The rAAV vector genome according to claim ~~10~~ 13, wherein the promoter comprises of a ~~variant~~ sequence having at least 95% sequence identity to SEQ ID NO: 6.

~~14.~~ 17. The rAAV vector genome according to claim ~~10~~ 13, wherein the promoter comprises SEQ ID NO: 6.

~~15.~~ 18. The rAAV vector genome according to any one of claims 1-~~14~~ 17, wherein the vector genome further comprises an intron.

~~16.~~ 19. The rAAV vector genome according to claim ~~15~~ 18, wherein the intron is a composite globin/AIAT intron sequence.

~~17.~~ 20. The rAAV vector genome according to claim ~~15~~ 18, wherein the intron sequence comprises SEQ ID NO: 14 or a ~~variant thereof~~ sequence having at least 80% sequence identity to SEQ ID NO: 14.

~~18.~~ 21. The rAAV vector genome according to claim ~~17~~ 20, wherein the intron sequence comprises a ~~variant thereof~~ sequence having at least 85% sequence identity to SEQ ID NO: 14.

~~19.~~ 22. The rAAV vector genome according to claim ~~17~~ 20, wherein the intron sequence comprises ~~SEQ ID NO: 14 or~~ a ~~variant thereof~~ sequence having at least 90% sequence identity to SEQ ID NO: 14.

~~20.~~ 23. The rAAV vector genome according to claim ~~17~~ 20, wherein the intron sequence comprises ~~SEQ ID NO: 14 or~~ a ~~variant thereof~~ sequence having at least 95% sequence identity to SEQ ID NO: 14.

~~21.~~ 24. The rAAV vector genome according to claim ~~17~~ 20, wherein the intron sequence comprises SEQ ID NO: 14.

~~22.~~ 25. The rAAV vector genome according to any one of claims 1-~~21~~ 24, wherein the vector genome further comprises a polyadenylation signal sequence.

~~23.~~ 26. The rAAV vector genome according to claim ~~22~~ 25, wherein the polyadenylation signal sequence is a bovine growth hormone (bGH) poly(A), SV40 late poly(A), rabbit beta-globin (rBG) poly(A), or thymidine kinase (TK) poly(A) sequence~~s, or any variants thereof~~.

~~24.~~ 27. The rAAV vector genome according to claim ~~22~~ 25, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal (bGH).

~~25. The rAAV vector according to any one of claims 1-24, wherein the vector genome further comprises an AAV 5' inverted terminal repeat (ITR) sequence and/or an AAV 3' ITR.~~

~~26. The rAAV vector according to claim 25, wherein the AVV 5' ITR and/or AAV3' ITR is from AAV2.~~

~~27.~~ 28. The rAAV vector genome according to any one of claims 1-~~26~~ 27, wherein the vector genome is ~~about~~ 2 kb to ~~about~~ 3 kb, ~~about~~ 3 kb to ~~about~~ 4 kb, or ~~about~~ 4 kb to ~~about~~ 5 kb in size.

~~28.~~ 29. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome sequence comprises (a) ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23;~~ or (b) a ~~variant thereof~~ sequence having at least 80% sequence identity to ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

~~29.~~ 30. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome sequence comprises ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

~~30.~~ 31. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome sequence comprises a ~~variant~~ sequence having at least 80% sequence identity to ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

~~31.~~ 32. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome ~~sequence~~ comprises a ~~variant~~ sequence having at least 85% sequence identity to ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

~~32.~~ 33. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome ~~sequence~~ comprises a ~~variant~~ sequence having at least 90% sequence identity to ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

~~33.~~ 34. The rAAV vector genome according to claim ~~1~~ 2, wherein the vector genome ~~sequence~~ comprises a ~~variant~~ sequence having at least 95% sequence identity to ~~SEQ ID NO: 15;~~ SEQ ID NO: 16; ~~SEQ ID NO: 17;~~ SEQ ID NO: 18; ~~SEQ ID NO: 19;~~ SEQ ID NO: 20; or SEQ ID NO: 21; ~~SEQ ID NO: 22; or SEQ ID NO: 23~~.

35. The rAAV vector genome according to claim 2, wherein the vector genome comprises SEQ ID NO: 16.

36. The rAAV vector genome according to claim 2, wherein the vector genome sequence comprises SEQ ID NO: 18.

~~34.~~ 37. A recombinant adeno-associated virus (rAAV) particle comprising an AAV capsid and the vector genome of any one of claims 1-~~33~~ 36.

~~35.~~ 38. The rAAV particle according to claim ~~34~~ 37, wherein the AAV capsid is an AAV5 capsid.

~~36.~~ 39. An isolated nucleic acid molecule comprising a nucleotide sequence having at least ~~80%~~ 95% homology to the nucleotide sequence of SEQ ID NO: 7 and which encodes functional PAH.

40. An isolated nucleic acid molecule comprising a nucleotide sequence having at least 98% homology to the nucleotide sequence of SEQ ID NO: 7 and which encodes functional hPAH.

41. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 7 and which encodes functional hPAH.

~~37. A codon optimized PAH nucleic acid comprising a reduced CpG di-nucleotide content.~~

~~38. The nucleic acid according to claim 37, comprising a GC content of less than 65%, less than 60%, or less than 58%.~~

~~39.~~ 42. A method of producing an rAAV particle of claim ~~34 or 35~~ 37 or 38, comprising the steps of (a) culturing a cell transfected with a rAAV vector genome of any one of claims 1-~~33~~ 36, wherein the cell expresses AAV capsid and rep proteins; and (b) recovering rAAV particles from the supernatant of the cell.

~~40.~~ 43. The method of claim ~~39~~ 42, wherein said cell is an insect cell.

~~41.~~ 44. The method of claim ~~40~~ 43, wherein said insect cell is an SF9 cell or subclone thereof.

~~42.~~ 45. The method of any one of claims ~~39-41~~ 42-44, wherein the transfection of said cell with the rAAV vector genome was accomplished by infection of said cell by a baculovirus comprising said rAAV vector genome.

~~43.~~ 46. The method of any one of claims ~~39-42~~ 42-45, wherein the ~~the AAV vector genome was a~~ the cell has been co-transfected with a second vector comprising AAV rep and cap genes.

~~44.~~ 47. A pharmaceutical composition comprising the rAAV vector genome of any one of claims 1-~~33~~ 36, or the rAAV particle of claim ~~34 or 35~~ 37 or 38, and a pharmaceutically acceptable carrier.

~~45.~~ 48. Use of the rAAV particle of claim ~~34 or 35~~ 37 or 38, ~~in the manufacture of a medicament~~ for the expression of PAH protein in a subject.

~~46.~~ 49. Use of the rAAV vector genome of any one of claims 1-~~33~~ 36, the rAAV particle of claim ~~34 or 35~~ 37 or 38, or the pharmaceutical composition of claim ~~44~~ 47, for the manufacture of a medicament for the treatment of ~~treating~~ PKU in a subject.

~~47.~~ 50. The use of claim ~~46~~ 49, wherein the rAAV particle is in an aqueous suspension ~~delivered~~ at a concentration of ~~about~~ 1 x 10^{12} to ~~about~~ 1 x 10^{15} vg/kg ~~in an aqueous suspension~~.

~~48.~~ 51. The use of any one of claims ~~45-47~~ 48-50, wherein the subject is a human.

~~49. A method of measuring a neurotransmitter or neurotransmitter metabolite level in a biological sample from a subject comprising:~~

~~obtaining the biological sample from the subject;~~

~~precipitating the sample with cold acetonitrile containing a heavy-labeled internal standard;~~

~~pelleting the sample by centrifugation;~~

~~transferring the supernatant to a fresh container;~~

adding sodium carbonate and benzoyl chloride or ethyl chloroformate and pyridine to the supernatant;

adding formic acid to the supernatant after it has reacted with the sodium carbonate and benzoyl chloride or ethyl chloroformate and pyridine; and

analyzing the resultant reactant supernatant by LC/MS, thereby detecting and measuring the level of the neurotransmitter or neurotransmitter metabolite.

50. Use of an effective amount of a PKU therapeutic for the manufacture of a medicament for the treatment of phenylketonuria (PKU) in a subject, wherein the neurotransmitter or neurotransmitter metabolite level of the subject has been determined by the method of claim 49, and wherein an effective amount of the PKU therapeutic is one which alters the levels of one or more neurotransmitters or neurotransmitter metabolites in a biological sample of the subject.

51. Use of an effective amount of a PKU therapeutic for the manufacture of a medicament for ameliorating a neurocognitive symptom of phenylketonuria (PKU) in a subject, wherein the neurotransmitter or neurotransmitter metabolite level of the subject has been determined by the method of claim 49, and wherein an effective amount of the PKU therapeutic is one which alters the levels of one or more neurotransmitters or neurotransmitter metabolites in a biological sample of the subject.

52. The use of claim 51, wherein the neurocognitive symptom is selected from the group consisting of decreased IQ, attention deficits, and deficits in executive functions including strategic planning, inhibitory control, working memory, and cognitive flexibility.

53. The use of any one of claims 50-53, wherein the one or more neurotransmitters or neurotransmitter metabolites are selected from the group consisting of phenethylamine, phenylethanolamine, tyramine, dopamine, norepinephrine, epinephrine, tryptamine,

~~hydroxytryptamine, phenylacetic acid, phenylacetylglutamine, mandelic acid, hydroxyphenylacetic acid, DOPAC, homovanillic acid, DOMA, MOPEG, vanillylmandelic acid, indoleactic acid, and 5-hydroxyindoleacetic acid.~~

~~54. The use of any one of claims 50-53, wherein the biological sample of the subject is selected from the group consisting of blood, serum, plasma, cerebrospinal fluid (CSF), and urine.~~

~~55. The use of any one of claims 50-54, wherein the PKU therapeutic is selected from the group consisting of sapropterin, pegvaliase-pqpz, and a PKU gene therapy.~~

~~56. The use of claim 55, wherein the PKU gene therapy comprises an adeno associated viral (AAV) vector that expresses a transgene comprising phenylalanine hydroxylase (PAH).~~

~~57. The use of claim 56, wherein the effective amount of PKU gene therapy is greater than 1E12 vg/kg body weight of the subject.~~

~~58. The use of any one of claims 50-57, wherein the levels of one or more neurotransmitters or neurotransmitter metabolites are measured at least one month after, at least three months after, or at least six months after administering the PKU therapeutic.~~

~~59. The use of any one of claims 50-58, wherein the altered levels of the one or more neurotransmitters is within 5%, 10%, or 20% of a reference level of the one or more neurotransmitters, wherein the reference level is obtained as the average of levels of one or more neurotransmitters obtained from at least ten subjects that are neurotypical.~~

~~60. The use of any one of claims 50-59, wherein the subject is a human.~~