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 having at least 80% sequence identity to SEQ ID NO: 7.

2. The rAAV vector of claim 1, wherein the codon optimized PAH sequence comprises a variant having at least 85% sequence identity to SEQ ID NO: 7.

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

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

5. The rAAV vector according to claim 1, wherein the codon optimized PAH sequence comprises SEQ ID NO: 7.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

24. The rAAV vector according to claim 22, 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 AAV 5' ITR and/or AAV3' ITR is from AAV2.

27. The rAAV vector according to any one of claims 1-26, 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. The rAAV vector according to claim 1, 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23; or (b) a variant thereof 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

29. The rAAV vector according to claim 1, 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

30. The rAAV vector according to claim 1, wherein the vector genome sequence comprises a variant 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

31. The rAAV vector according to claim 1, wherein the vector genome sequence comprises a variant 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

32. The rAAV vector according to claim 1, wherein the vector genome sequence comprises a variant 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

33. The rAAV vector according to claim 1, wherein the vector genome sequence comprises a variant 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; SEQ ID NO: 21; SEQ ID NO: 22; or SEQ ID NO: 23.

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

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

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

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

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

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

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

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

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

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

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

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

48. The use of any one of claims 45-47, 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.