360, 330 to 350, 330 to 340, 335 to 340, 335 to 380, 340 to 380, 350 to 380, 360 to 380, or 370 to 380 amino acid residues. In some preferred embodiments, the TH comprises 330 to 340 amino acid residues. In some more preferred embodiments, the TH comprises 335 to 340 amino acid residues, for example, 335, 336, 337, 338, 339, or 340 amino acid residues.

[0011] In some embodiments, the TH comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 11. In some embodiments, the GCH1 comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 9. In some embodiments, the AADC comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 7.

[0012] In some embodiments, the linker linking the TH, the GCH1, and/or the AADC includes a fusion linker, a 2A peptide linker, and/or an internal ribosome entry site (IRES).

[0013] In some embodiments, the fusion linker is a peptide linker. In some embodiments, the peptide linker is selected from (GGS)n (SEQ ID NO: 49), (GGGS)n (SEQ ID NO: 50), or (GGGGS)n (SEQ ID NO: 51), wherein n is an integer from 1 to 5. In some preferred embodiments, the fusion linker is (GGGGS)3.

[0014] In some embodiments, the 2A peptide linker comprises a 2A peptide selected from foot-and-mouth disease virus 2A peptide (F2A), porcine teschovirus 2A peptide (P2A), Thosea asign virus 2A peptide (T2A), and/or equine rhinitis virus 2A peptide (E2A). In some embodiments, the N-terminus and/or C-terminus, preferably the N-terminus, of the 2A peptide linker further comprises a GSG amino acid sequence. In some preferred embodiments, the 2A peptide linker comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 13 or SEQ ID NO: 15.

[0015] In some embodiments, the nucleic acid sequences encoding the TH, the GCH1, and the AADC in the AAV vector of the present invention are arranged from upstream to downstream in an order selected from any one of:

(1) TH, GCH1, AADC;

(2) AADC, GCH1, TH;

(3) AADC, TH, GCH1;

(4) GCH1, TH, AADC;

(5) GCH1, AADC, TH; or

(6) TH, AADC, GCH1.

the AADC comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 7.

[0087] In some embodiments, the linker linking the TH, the GCH1, and/or the AADC includes a fusion linker, a 2A peptide linker, and/or an internal ribosome entry site (IRES).

[0088] In some embodiments, the fusion linker is a peptide linker. In some embodiments, the peptide linker is selected from (GGS)n, (GGGS)n or (GGGGS)n, wherein n is an integer from 1 to 5. In some preferred embodiments, the fusion linker is (G4S)3 (SEQ ID NO: 17, GGGGSGGGGSGGGGS).

[0089] In some embodiments, the 2A peptide linker comprises a 2A peptide selected from foot-and-mouth disease virus 2A peptide (F2A), porcine teschovirus 2A peptide (P2A), Thosea asign virus 2A peptide (T2A), and/or equine rhinitis virus 2A peptide (E2A).

[0090] 2A Peptide is a short peptide (about 18-25 amino acids, e.g., ATNFSLLKQAGDVEENPGP (SEQ ID NO: 52)) derived from a virus, which can translate a transcript product to produce multiple polypeptide chains. The 2A peptide functions by allowing the ribosome to skip the synthesis of glycine and proline peptide bonds at the C-terminus of the 2A element, ultimately leading to the separation of the terminal of the 2A sequence and the downstream polypeptide chain. After translation of the two proteins linked by the nucleotide sequence encoding the 2A peptide, some additional 2A residues will be added to the C-terminus of the upstream protein, and an additional proline will be added to the N-terminus of the downstream protein.

[0091] In some embodiments, the N-terminus and/or C-terminus, preferably the N-terminus, of the 2A peptide linker further comprises a GSG amino acid sequence. In some preferred embodiments, the 2A peptide linker comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 13 or SEQ ID NO: 15.

[0092] In some embodiments, the nucleic acid sequences encoding the TH, the GCH1, and the AADC in the AAV vector of the present invention are arranged from upstream to downstream in an order selected from any one of:

(1) TH, GCH1, AADC;

(2) AADC, GCH1, TH;

(3) AADC, TH, GCH1;

(4) GCH1, TH, AADC;

CLAIMS

1. An adeno-associated virus (AAV) vector comprising nucleic acid sequences encoding tyrosine hydroxylase (TH), GTP-cyclohydrolase 1 (GCH1) and aromatic amino acid decarboxylase (AADC), wherein the nucleic acid sequences encoding the TH, the GCH1 and/or the AADC are in-frame linked by a nucleic acid sequence encoding a linker, wherein any one or more of the TH, the GCH1 and the AADC is a truncated form of wild type or a functional variant thereof and has the catalytic activity required in the dopamine synthesis pathway.

2. The AAV vector of claim 1, wherein the TH is a truncated form of wild-type TH or a functional variant thereof and has the activity of catalyzing the synthesis of levodopa from tyrosine; preferably wherein the TH comprises 330 to 390 amino acid residues.

3. The AAV vector of claim 1 or 2, wherein the TH comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 11; wherein the GCH1 comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 9; and/or wherein the AADC comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 7.

4. The AAV vector of any one of claims 1-3, wherein the linker comprises a fusion linker, a 2A peptide linker, and/or an internal ribosome entry site (IRES).

5. The AAV vector of claim 4, wherein the fusion linker is a peptide linker; preferably wherein the peptide linker is selected from (GGS)n, (GGGS)n or (GGGGS)n, wherein n is an integer from 1 to 5; and more preferably wherein the fusion linker is (G4S)3.

6. The AAV vector of claim 4, wherein the 2A peptide linker comprises a 2A peptide selected from foot-and-mouth disease virus 2A peptide (F2A), porcine teschovirus 2A peptide (P2A), Thosea asign virus 2A peptide (T2A), and equine rhinitis virus 2A peptide (E2A); preferably wherein the N-terminus and/or C-terminus, preferably the N-terminus, of the 2A peptide linker further comprises a GSG amino acid sequence; and preferably wherein the 2A peptide linker comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 13 or SEQ ID NO: 15.

7. The AAV vector of any one of claims 1-6, wherein the nucleic acid sequences encoding the TH, the GCH1, and the AADC are arranged from upstream to downstream in an order

selected from any one of:

(1) TH, GCH1, AADC;

(2) AADC, GCH1, TH;

(3) AADC, TH, GCH1;

(4) GCH1, TH, AADC;

(5) GCH1, AADC, TH; or

(6) TH, AADC, GCH1; preferably wherein the nucleic acid sequences encoding the TH, the GCH1, and the AADC are linked from upstream to downstream in a manner selected from any one of:

1) TH-F2A-GCH1-P2A-AADC;

2) GCH1-P2A-TH-P2A-AADC;

3) GCH1-P2A-AADC-P2A-TH;

4) AADC-E2A-GCH1-(G4S)3-TH;

5) GCH1-F2A-TH-F2A-AADC;

6) AADC-(G4S)3-TH-T2A-GCH1;

7) AADC-(G4S)4-TH-T2A-GCH1;

8) AADC-P2A-GCH1-P2A-TH;

9) AADC-T2A-GCH1-(G4S)3-TH;

10) GCH1-F2A-TH-P2A-AADC;

11) GCH1-F2A-AADC-F2A-TH;

12) AADC-(G4S)3-GCH1-F2A-TH;

13) GCH1-E2A-TH-T2A-AADC;

14) GCH1-F2A-AADC-P2A-TH;

15) AADC-(G4S)3-GCH1-P2A-TH;

16) AADC-(G4S)5-GCH1-P2A-TH;

17) TH-(G4S)3-GCH1-P2A-AADC;

18) GCH1-T2A-AADC-E2A-TH;

19) AADC-(G4S)3-GCH1-T2A-TH;

20) GCH1-E2A-TH-P2A-AADC;

21) AADC-(G4S)3-GCH1-E2A-TH;

22) GCH1-E2A-AADC-P2A-TH;

23) AADC-(G4S)3-GCH1-(G4S)3-TH;

24) GCH1-T2A-TH-E2A-AADC;

25) GCH1-(G4S)3-TH-T2A-AADC;

26) GCH1-(G4S)2-TH-T2A-AADC;

27) GCH1-P2A-AADC-T2A-TH;

28) TH-P2A-GCH1-P2A-AADC;

29) TH-(G4S)3-GCH1-T2A-AADC;

30) GCH1-(G4S)3-TH-E2A-AADC;

31) GCH1-T2A-TH-(G4S)3-AADC;

32) TH-P2A-GCH1-F2A-AADC;

33) GCH1-F2A-TH-(G4S)3-AADC;

34) TH-F2A-GCH1-F2A-AADC;

35) TH-(G4S)3-GCH1-E2A-AADC;

36) TH-G4S-GCH1-E2A-AADC;

37) GCH1-P2A-TH-(G4S)3-AADC;

38) AADC-F2A-GCH1-P2A-TH;

39) TH-T2A-GCH1-(G4S)3-AADC;

40) GCH1-E2A-TH-(G4S)3-AADC;

41) TH-E2A-GCH1-(G4S)3-AADC;

42) TH-T2A-GCH1-E2A-AADC;

43) AADC-F2A-GCH1-F2A-TH;

44) TH-F2A-GCH1-(G4S)3-AADC;

45) GCH1-(G4S)3-TH-(G4S)3-AADC;

46) TH-(G4S)3-GCH1-F2A-AADC;

47) TH-E2A-GCH1-T2A-AADC;

48) AADC-P2A-TH-(G4S)3-GCH1;

49) TH-(G4S)3-GCH1-(G4S)3-AADC;

50) AADC-F2A-TH-(G4S)3-GCH1;

51) TH-F2A-AADC-F2A-GCH1;

52) TH-(G4S)3-AADC-T2A-GCH1;

53) TH-(G4S)3-AADC-E2A-GCH1;

54) AADC-P2A-GCH1-(G4S)3-TH;

55) TH-F2A-AADC-(G4S)3-GCH1;

56) AADC-F2A-GCH1-(G4S)3-TH;

57) TH-P2A-AADC-F2A-GCH1;

58) TH-P2A-AADC-(G4S)3-GCH1;

59) GCH1-(G4S)3-TH-P2A-AADC;

60) TH-T2A-AADC-(G4S)3-GCH1;

61) TH-T2A-AADC-E2A-GCH1;

62) GCH1-(G4S)3-TH-F2A-AADC;

63) TH-E2A-AADC-(G4S)3-GCH1;

64) AADC-E2A-GCH1-T2A-TH;

65) GCH1-P2A-TH-F2A-AADC;

66) TH-(G4S)3-AADC-(G4S)3-GCH1;

67) TH-E2A-AADC-T2A-GCH1;

68) GCH1-P2A-AADC-F2A-TH;

69) AADC-T2A-TH-(G4S)3-GCH1;

70) AADC-T2A-GCH1-E2A-TH;

71) TH-F2A-AADC-P2A-GCH1;

72) AADC-E2A-TH-(G4S)3-GCH1;

73) AADC-P2A-GCH1-F2A-TH;

74) TH-(G4S)3-AADC-F2A-GCH1;

75) AADC-P2A-TH-F2A-GCH1;

76) AADC-(G4S)3-TH-P2A-GCH1;

77) AADC-T2A-TH-E2A-GCH1;

78) AADC-(G4S)3-TH-E2A-GCH1;

79) AADC-E2A-TH-T2A-GCH1; or

80) AADC-(G4S)3-TH-F2A-GCH1.

8. The AAV vector of claim 7, wherein the AAV vector comprises the nucleic acid sequence set forth in any one of SEQ ID NOs: 19-21.

9. The AAV vector of any one of claims 1-8, further comprising a promoter operably linked to the nucleic acid sequences encoding the TH, the GCH1, and the AADC; preferably, wherein

the promoter is selected from a CBH promoter, a chimeric Synapsin I promoter, a CMV promoter, a CAG promoter, a CAGG promoter and a CASI promoter.

10. The AAV vector of claim 9, wherein the promoter comprises a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 1-6; and preferably wherein the promoter comprises the nucleic acid sequence set forth in any one of SEQ ID NOs: 1-3; and more preferably wherein the promoter comprises the nucleic acid sequence set forth in SEQ ID NO: 1.

11. The AAV vector of any one of claims 1-10, wherein the AAV vector further comprises one or more elements selected from a KOZAK sequence, a polyadenylation signal of an SV40 virus (SV40 poly A), and an inverted terminal repeat (ITR).

12. The AAV vector of claim 11, wherein the AAV vector comprises the following elements: a CBH promoter, a Kozak sequence, a polyadenylation signal of an SV40 virus (SV40 poly A), and an inverted terminal repeat (ITR); and the nucleic acid sequence set forth in SEQ ID NO: 21.

13. An AAV virus particle comprising the AAV vector of any one of claims 1-12, and a capsid protein; preferably wherein the capsid protein is derived from an AAV selected from the following serotypes: AAV5, AAV9, AAVPHP.eB, AAVPHP.S or AAVPHP.B; preferably wherein the capsid protein comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 33-37; and preferably wherein the capsid protein comprises an amino acid sequence selected from SEQ ID NO: 34 or 37; and more preferably wherein the capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 34.

14. A composition comprising the AAV vector of any one of claims 1-12 and a packaging plasmid encoding a capsid protein, and optionally a helper plasmid and optionally further comprises a pharmaceutically acceptable carrier, diluent, or excipient.

15. The composition of claim 14, wherein the packaging plasmid further comprises a nucleic acid sequence encoding a Rep protein, preferably wherein the Rep protein is derived from an AAV of AAV2 or AAVPHP.B serotype, and more preferably wherein the Rep protein comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 45 or SEQ ID NO: 46.

16. A composition comprising the AAV virus particle of claim 13; optionally, the

composition further comprises a pharmaceutically acceptable carrier, diluent, or excipient; preferably wherein the composition comprises a phosphate buffer, sodium chloride, and a surfactant, and preferably wherein the surfactant is poloxamer 188.

17. A cell comprising the AAV vector of any one of claims 1-12, the AAV virus particle of claim 13, or the composition of any one of claims 14-16.

18. The cell of claim 17, wherein the cell is a nerve cell, as distinguished by function, the nerve cell is selected from: an inhibitory neuron (preferably, said cell is a medium spiny neuron, an interneuron), an excitatory neuron (preferably, said cell is a dopaminergic neuron), a microglia, an astrocyte, an oligodendrocyte, or a Schwann cell; or as distinguished by brain region, the cell is a midbrain striatal neuron or a glial cell.

19. The AAV vector of any one of claims 1-12, the AAV virus particle of claim 13, the composition of any one of claims 14-16, or the cell of claim 17 or 18, for use in therapy.

20. Use of the AAV vector of any one of claims 1-12, the AAV virus particle of claim 13, the composition of any one of claims 14-16, or the cell of claim 17 or 18 in the manufacture of a medicament for preventing and/or treating a disease caused by dopamine deficiency in a subject; preferably wherein the disease is Parkinson's disease.