CLAIMS

1. An isolated nucleic acid encoding a β-glucocerebrosidase (GBA) protein comprising the nucleotide sequence of SEQ ID NO: 1773 or a nucleotide sequence that is at least 90% identical thereto.

2. The isolated nucleic acid of claim 1, comprising a nucleotide sequence that is at least 92% identical or at least 95% identical to the nucleotide sequence of SEQ ID NO: 1773.

3. The isolated nucleic acid of claim 1 or claim 2, further comprising a nucleotide sequence encoding a signal sequence, wherein the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 1850 or a nucleotide sequence that is at least 95% identical thereto.

4. The isolated nucleic acid of any one of claims 1-3, comprising the nucleotide sequence of SEQ ID NO: 1772 or a nucleotide sequence that is at least 90% identical thereto.

5. A recombinant viral genome comprising the isolated nucleic acid of any one of claims 1-4.

6. The recombinant viral genome of claim 5, further comprising:
(i) at least one inverted terminal repeat (ITR) sequence;
(ii) a promoter operably linked to the nucleotide sequence encoding the GBA protein;
(iii) an enhancer;
(iv) an intron;
(v) at least one microRNA (miRNA) binding site; and/or
(vi) a polyadenylation (polyA) sequence.

7. The recombinant viral genome of claim 6, wherein the promoter comprises a chicken beta actin (CB) promoter.

8. The recombinant viral genome of claim 7, wherein the CB promoter comprises the nucleotide sequence of SEQ ID NO: 1834 or a nucleotide sequence that is at least 95% identical thereto.

9. The recombinant viral genome of any one of claims 6-8, wherein the enhancer comprises a CMV immediate-early (CMVie) enhancer comprising the nucleotide sequence of SEQ ID NO: 1831 or a nucleotide sequence that is at least 95% identical thereto.

10. The recombinant viral genome of any one of claims 6-9, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 1842 or a nucleotide sequence that is at least 95% identical thereto.

11. The recombinant viral genome of any one of claims 6-10, wherein the polyA sequence comprises the nucleotide sequence of SEQ ID NO: 1846 or a nucleotide sequence that is at least 95% identical thereto.

12. The recombinant viral genome of any one of claims 6-11, wherein the at least one ITR sequence comprises:

(i) the nucleotide sequence of SEQ ID NO: 1829 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1829; and/or

(ii) the nucleotide sequence of SEQ ID NO: 1830 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1830.

13. The recombinant viral genome of any one of claims 6-12, wherein the at least one ITR sequence comprises a 5' ITR sequence and a 3' ITR sequence, wherein the 5' ITR sequence comprises the nucleotide sequence of SEQ ID NO: 1829 and the 3' ITR sequence comprises the nucleotide sequence of SEQ ID NO: 1830.

14. The recombinant viral genome of any one of claims 6-13, wherein the at least one miRNA binding site comprises at least one miR183 binding site comprising:

(i) the nucleotide sequence of SEQ ID NO: 1847;

(ii) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 1847; or

(iii) a nucleotide sequence having up to three modifications relative to SEQ ID NO: 1847.

15. The recombinant viral genome of any one of claims 6-14, wherein the at least one miRNA binding site comprises at least four miR183 binding sites, wherein each of the at least four miR183 binding sites is separated by a spacer.

16. The recombinant viral genome of claim 15, wherein the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications relative to SEQ ID NO: 1848.

17. The recombinant viral genome of any one of claims 6-13, wherein the at least one miRNA binding site comprises a miR183 binding site series comprising:

(i) the nucleotide sequence of SEQ ID NO: 1849;

(ii) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 1849; or

(iii) a nucleotide sequence having up to ten modifications relative to SEQ ID NO: 1849.

18. A recombinant viral genome comprising:

(i) a 5' inverted terminal repeat (ITR) sequence comprising the nucleotide sequence of SEQ ID NO: 1829 or a nucleotide sequence that is at least 95% identical thereto;

(ii) a CMV immediate-early (CMVie) enhancer comprising the nucleotide sequence SEQ ID NO: 1831 or a nucleotide sequence that is at least 95% identical thereto;

(iii) a chicken beta actin (CB) promoter comprising the nucleotide sequence of SEQ ID NO: 1834 or a nucleotide sequence that is at least 95% identical thereto;

(iv) an intron comprising the nucleotide sequence of SEQ ID NO: 1842 or a nucleotide sequence that is at least 95% identical thereto;

(v) a nucleotide sequence encoding a signal sequence, wherein the nucleotide sequence encoding the signal sequence comprises SEQ ID NO: 1850 or a nucleotide sequence that is at least 95% identical thereto;

(vi) a nucleotide sequence encoding a β-glucocerebrosidase (GBA) protein, wherein the nucleotide sequence encoding the GBA protein comprises the nucleotide sequence of SEQ ID NO: 1773 or a nucleotide sequence that is at least 90% identical thereto;

(vii) a polyadenylation (polyA) sequence comprising the nucleotide sequence of SEQ ID NO: 1846 or a nucleotide sequence that is at least 95% identical thereto; and

(viii) a 3' ITR sequence comprising the nucleotide sequence of SEQ ID NO: 1830 or a nucleotide sequence that is at least 95% identical thereto.

19. The recombinant viral genome of any one of claims 5-13 or claim 18, comprising the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 95% identical thereto.

20. A recombinant viral genome comprising:

(i) a 5' inverted terminal repeat (ITR) sequence comprising the nucleotide sequence of SEQ ID NO: 1829 or a nucleotide sequence that is at least 95% identical thereto;

(ii) a CMV immediate-early (CMVie) enhancer comprising the nucleotide sequence of SEQ ID NO: 1831 or a nucleotide sequence that is at least 95% identical thereto;

(iii) a chicken beta actin (CB) promoter comprising the nucleotide sequence of SEQ ID NO: 1834 or a nucleotide sequence that is at least 95% identical thereto;

(iv) an intron comprising the nucleotide sequence of SEQ ID NO: 1842 or a nucleotide sequence that is at least 95% identical thereto;

(v) a nucleotide sequence encoding a signal sequence, wherein the nucleotide sequence encoding the signal sequence comprises SEQ ID NO: 1850 or a nucleotide sequence that is at least 95% identical thereto;

(vi) a nucleotide sequence encoding a β-glucocerebrosidase (GBA) protein, wherein the nucleotide sequence encoding the GBA protein comprises the nucleotide sequence of SEQ ID NO: 1773 or a nucleotide sequence that is at least 90% identical thereto;

(vii) a miR183 binding site series comprising the nucleotide sequence of SEQ ID NO: 1849; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 1849; or a nucleotide sequence having up to ten modifications relative to SEQ ID NO: 1849;

(viii) a polyadenylation (polyA) sequence comprising the nucleotide sequence of SEQ ID NO: 1846 or a nucleotide sequence that is at least 95% identical thereto; and

(ix) a 3' ITR sequence comprising the nucleotide sequence of SEQ ID NO: 1830 or a nucleotide sequence that is at least 95% identical thereto.

21. The recombinant viral genome of any one of claims 5-19, comprising the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 95% identical thereto.

22. An AAV particle comprising an AAV capsid protein and the recombinant viral genome of any one of claims 5-21.

23. The AAV particle of claim 22, wherein the AAV capsid protein is an AAV9 capsid protein or a variant thereof; or wherein the AAV capsid protein is an AAV5 capsid protein or a variant thereof.

24. A vector comprising the recombinant viral genome of any one of claims 5-20, or the isolated nucleic acid of any one of claims 1-4.

25. A cell comprising the recombinant viral genome of any one of claims 5-21, the isolated nucleic acid of any one of claims 1-4, or the vector of claim 24.

26. A method of making an AAV particle, the method comprising:

(i) providing a host cell comprising the recombinant viral genome of any one of claims 5-20;

(ii) incubating the host cell under conditions suitable to encapsulate the recombinant viral genome in an AAV capsid protein;

thereby making the AAV particle.

27. A pharmaceutical composition comprising the AAV particle of claim 22 or claim 23 and a pharmaceutically acceptable excipient.

28. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 for use in delivering a nucleic acid sequence encoding a β-glucocerebrosidase (GBA) protein to a subject.

29. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 for use in increasing the level of a β-glucocerebrosidase (GBA) protein in the central nervous system of a subject.

30. Use of the pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 in the manufacture of a medicament for delivering a nucleic acid sequence encoding a β-glucocerebrosidase (GBA) protein to a subject.

31. Use of the pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 in the manufacture of a medicament for increasing the level of a β-glucocerebrosidase (GBA) protein in the central nervous system of a subject.

32. The use of claim 30 or claim 31, wherein the subject has a β-glucocerebrosidase (GBA)-related disorder.

33. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 for use in treating a subject having a β-glucocerebrosidase (GBA)-related disorder, wherein an effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

34. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 for use in treating a subject having Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy Bodies (DLB), Gaucher disease (GD), spinal muscular atrophy (SMA), multiple system atrophy (MSA), or multiple sclerosis (MS).

35. The pharmaceutical composition of claim 27 or the AAV particle of claim 23 or claim 23 for use in treating a subject having Parkinson's disease (PD), wherein an effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

36. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 for use in treating a subject having Gaucher's disease (GD), wherein an effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

37. The pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23, wherein the pharmaceutical composition or AAV particle is formulated for intravenous administration.

38. Use of the pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 in the manufacture of a medicament for treating a subject having a β-glucocerebrosidase (GBA)-related disorder, wherein an effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

39. The use of claim 32 or 38, wherein the GBA-related disorder is Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy Bodies (DLB), Gaucher disease (GD), spinal muscular atrophy (SMA), multiple system atrophy (MSA), or multiple sclerosis (MS).

40. Use of the pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 in the manufacture of a medicament for treating a subject having Parkinson's disease (PD), wherein an effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

41. The use of claim 39 or claim 40, wherein the PD is:
(i) associated with a mutation in a GBA gene;
(ii) early onset PD (e.g., before 50 years of age) or juvenile PD (e.g., before 20 years of age);
(iii) a tremor dominant, postural instability gait difficulty PD (PIGD); or
(iv) a sporadic PD (e.g., a PD not associated with a mutation).

42. Use of the pharmaceutical composition of claim 27 or the AAV particle of claim 22 or claim 23 in the manufacture of a medicament for treating a subject having Gaucher's disease (GD) wherein an

effective amount of the pharmaceutical composition or the AAV particle is to be administered to the subject.

43. The use of claim 39 or 42, wherein the GD is:

(i) neuronopathic GD (e.g., affects a cell or tissue of the CNS, e.g., a cell or tissue of the brain and/or spinal cord), non-neuronopathic GD (e.g., does not affect a cell or tissue of the CNS), or combination thereof; or

(ii) a Type I GD (GD1), a Type 2 GD (GD2), or a Type 3 GD (GD3), optionally wherein the GD1 is non-neuronopathic GD and the GD2 is a neuronopathic GD.

44. The use of any one of claims 30-32 and 38-43, wherein the medicament is formulated for intravenous administration.

45. The use of any one of claims 30-32 and 38-44, wherein the pharmaceutical composition or AAV particle is used in combination with an additional therapeutic agent and/or therapy suitable for treatment or prevention of the β-glucocerebrosidase (GBA)-related disorder, PD, or GD, optionally wherein the additional therapeutic agent comprises enzyme replacement therapy (ERT) (e.g., imiglucerase, velaglucerase alfa, or taliglucerase alfa); substrate reduction therapy (SRT) (e.g., eliglustat or miglustat), blood transfusion, levodopa, carbidopa, Safinamide, dopamine agonists (e.g., pramipexole, rotigotine, or ropinirole), anticholinergics (e.g., benztropine or trihexyphenidyl), cholinesterase inhibitors (e.g., rivastigmine, donepezil, or galantamine), an N-methyl-d-aspartate (NMDA) receptor antagonist (e.g., memantine), or a combination thereof.