CLAIMS

1. A polynucleotide comprising a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence encoding α-galactosidase A (GLA), wherein said nucleic acid sequence is at least 85% identical to the sequence of SEQ ID NO: 15, and wherein said GLA has a sequence at least 95% identical to the sequence of SEQ ID NO: 100;

b) a nucleic acid sequence encoding α-galactosidase A (GLA), wherein said nucleic acid sequence comprises an intron in the coding sequence for said GLA, and wherein said GLA has a sequence at least 95% identical to the sequence of SEQ ID NO: 100;

c) a nucleic acid sequence encoding a precursor α-galactosidase A (GLA) comprising an amino-terminal signal peptide, wherein said signal peptide has a sequence at least 80% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63; and wherein said GLA has a sequence at least 95% identical to the sequence of SEQ ID NO: 100; and

d) a nucleic acid sequence encoding an α-galactosidase A (GLA) having an amino acid sequence differing from SEQ ID NO: 100 by 1 to 7 amino acids, wherein at least one of said 1 to 7 amino acids is a substitution selected from the group consisting of Gln57Lys, Gln111Glu, Lys213Glu, Lys237Gln, Phe248Thr, Gly334Glu, and Gly346Asn.

2. The polynucleotide of claim 1, wherein said polynucleotide comprises a nucleic acid sequence having at least 95% identity to the sequence of SEQ ID NO: 15.

3. The polynucleotide of claim 2, wherein said nucleic acid sequence encodes GLA comprising the sequence of SEQ ID NO: 100.

4. The polynucleotide of any one of claims 1-3, wherein said nucleic acid sequence contains fewer than 14 CpG dinucleotides.

5. The polynucleotide of claim 1, wherein said nucleic acid sequence has a sequence of any one of SEQ ID NOs: 15-19, or bases 1-1194 of any one of SEQ ID NOs: 15-19.

6. The polynucleotide of claim 1, wherein said nucleic acid sequence has a sequence at least 95% identical to the sequence of SEQ ID NO: 15, and wherein said GLA has a sequence at

least 98% identical to the sequence of SEQ ID NO: 100.

7. The polynucleotide of claim 1, wherein said nucleic acid sequence encodes a GLA protein having an amino acid sequence of SEQ ID NO: 100 having one or more amino acid substitutions selected from the group consisting of Gln57Lys, Gln111Glu, Lys213Glu, Lys237Gln, Phe248Thr, Gly334Glu, and Gly346Asn.

8. The polynucleotide of claim 7, wherein said GLA comprises the amino acid sequence of SEQ ID NO: 48.

9. The polynucleotide of claim 8, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO: 47 or bases 1-1194 of SEQ ID NO: 47.

10. The polynucleotide of any one of claims 1-9, wherein said polynucleotide further comprises a second nucleic acid sequence, wherein said second sequence encodes a signal peptide sequence positioned at the 5’ end of the first nucleic acid sequence.

11. The polynucleotide of claim 10, wherein said signal peptide sequence is selected from the group consisting of human chymotrypsinogen B2 signal peptide (SEQ ID NO: 41), AHSG signal peptide (SEQ ID NO: 57), CD300 signal peptide (SEQ ID NO: 58), GLA signal peptide (SEQ ID NO: 59), LAMP1 signal peptide (SEQ ID NO: 60), Notch 2 signal peptide (SEQ ID NO: 61), ORM1 signal peptide (SEQ ID NO: 62), TF signal peptide (SEQ ID NO: 63), and wild-type GLA signal peptide (SEQ ID NO: 59), or a variant thereof having a sequence at least 90% identical to the sequence of any of SEQ ID NOs: 41, and 57-63.

12. The polynucleotide of claim 11, wherein said signal peptide is human chymotrypsinogen B2 signal peptide (SEQ ID NO: 41).

13. The polynucleotide of claim 12, wherein said nucleic acid encoding said signal peptide has a sequence of any one of SEQ ID NOs: 1-5.

14. The polynucleotide of any one of claims 1-13, wherein said polynucleotide further comprises an AAV inverted repeat (ITR) flanking the 5’ terminus of said polynucleotide and/or an AAV ITR flanking the 3’ terminus of said polynucleotide.

15. An expression cassette comprising the polynucleotide of any one of claims 1-14, wherein said GLA encoding sequence is operably linked to an expression control element, said expression control element comprises a promoter positioned 5’ of the nucleic acid encoding GLA, and a poly-adenylation sequence positioned 3’ of the nucleic acid encoding GLA.

16. The expression cassette of claim 15, wherein the expression control element and/or poly-adenylation sequence is CpG-reduced compared to the wild-type expression control element or polyadenylation sequence.

17. The expression cassette of any one of claims 15-16, wherein the expression control element comprises an ApoE/hAAT enhancer/promoter sequence of SEQ ID NO: 38 and the poly-adenylation sequence comprises a bovine growth hormone (bGH) polyadenylation sequence of SEQ ID NO: 20.

18. The expression cassette of claim 17, wherein said expression cassette further comprising an intron positioned between the 3’ end of the promoter and the 5’ end of the polynucleotide, wherein the intron comprises an hBB2m1 intron (SEQ ID NO: 39).

19. The expression cassette of any one of claims 15-18, wherein said expression cassette further comprises an ITR flanking the 5’ of said expression cassette and/or an ITR flanking the 3’ terminus of said expression cassette.

20. An AAV plasmid genome comprising the polynucleotide of claim 14 or the expression cassette of claim 19, and an origin of replication.

21. An adeno-associated virus (AAV) vector comprising the polynucleotide of claim 14 or the expression cassette of claim 19 and a capsid.

22. The AAV vector of claim 21, wherein said AAV vector comprises said expression cassette, and said expression cassette further comprises said ITR flanking the 5’ of said expression cassette and said ITR flanking the 3’ terminus of said expression cassette.

23. The AAV vector of claims 21 or 22, wherein said 5’ ITR and said 3’ ITR are selected

from the group consisting of a AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 and AAV3B ITRs.

24. The AAV vector of claims 22 or 23, wherein at least one or more of said 5' ITR and 3' ITR is modified to have reduced CpGs.

25. The AAV vector of claim 24, wherein said vector comprises a sequence having a sequence identity of at least 95% with a sequence of any one of SEQ ID NOs: 21-34, 53-56 and 91-99.

26. The AAV vector of claim 25, wherein said vector consists of a sequence having a sequence identity of at least 95% with a sequence of any one of SEQ ID NOs: 91-95.

27. The AAV vector of any one of claims 21-26, wherein said capsid comprises a AAV VP1, VP2 or VP3 capsid having 90% or more sequence identity to VP1, VP2 or VP3 capsid of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 (SEQ ID NO: 35), AAV3B, LK03 (SEQ ID NO: 42), AAV-2i8; or SEQ ID NO: 110, SEQ ID NO: 36 or SEQ ID NO: 37.

28. The AAV vector of claim 27, wherein said capsid is a AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 (SEQ ID NO: 35), AAV3B, LK03 (SEQ ID NO: 42) or AAV-2i8 capsid; or a capsid comprising SEQ ID NO: 110, SEQ ID NO: 36, and/or SEQ ID NO: 37.

29. A pharmaceutical composition comprising a plurality of AAV vectors of any one of claims 21-28 in a biologically compatible carrier or excipient.

30. The pharmaceutical composition of claim 29, wherein said plurality of AAV vectors is an effective amount to increase GLA activity in a human subject.

31. Use of the polynucleotide or expression cassette of any one of claims 1-19 or the AAV vector of any one of claims 20-28, or the pharmaceutical composition of claims 29 or 30 for the manufacture of a medicament for treating a subject in need of α-galactosidase A (GLA), wherein the GLA is expressed in the subject.

32. The use of claim 31, wherein the subject has Fabry disease.

33. The use of claims 31 or 32, wherein said use reduces, decreases or inhibits one or more symptoms of Fabry disease.

34. The use of any one of claims 31-33, wherein said AAV is to be administered to the subject in a range from about $1x10^{8}$ to about $1x10^{14}$ vector genomes per kilogram (vg/kg) of the weight of the subject.

35. The use of any one of claims 31-34, wherein said subject is a human.

36. A method of producing the AAV vector of any one of claims 21-28, comprising (a) introducing an AAV vector genome plasmid of claim 20 into a packaging helper cell; and (b) culturing the helper cell under conditions to produce the AAV vector.

37. A polypeptide selected from the group consisting of:

a) a precursor α-galactosidase A comprising a signal peptide joined to the amino terminus of α-galactosidase A (GLA), wherein said signal peptide has a sequence at least 80% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63; and said GLA has a sequence at least 95% identical to SEQ ID NO: 100; and

b) an α-galactosidase A (GLA) having an amino acid sequence differing from SEQ ID NO: 100 by 1 to 7 amino acids, wherein at least one of said 1 to 7 amino acids is a substitution selected from the group consisting of Gln57Lys, Gln111Glu, Lys213Glu, Lys237Gln, Phe248Thr, Gly334Glu, and Gly346Asn.

38. The polynucleotide or expression cassette of any one of claims 1-19 or the AAV vector of any one of claims 20-28, or the pharmaceutical composition of claims 29 or 30 for use in therapy.