CLAIMS

1. A recombinant adenovirus associated (rAAV) vector comprising in its genome:

(a) 5' and 3' AAV inverted terminal repeats (ITR) sequences, and

(b) located between the 5' and 3' ITRs, a heterologous nucleic acid sequence encoding all or a portion of an endogenous GAA signal peptide, a heterologous signal peptide and an alpha-glucosidase (GAA) polypeptide,

in which the GAA polypeptide comprises amino acid residues 28-952 of SEQ ID NO: 1, 57-952 of SEQ ID NO: 1, or comprises a N-terminal GAA polypeptide fragment comprising amino acids 28, 28-29, 28-30, 28-31, 28-32, or 28-33 of SEQ ID NO: 1 and a deletion of any number of amino acids from the next about 5 amino acids to about 40 amino acids after the N terminal GAA polypeptide fragment of SEQ ID NO: 1, in which the heterologous signal peptide can be inserted immediately after the N-terminal GAA polypeptide fragment and before the remaining amino acids of the GAA polypeptide, in which the heterologous signal peptide is optionally fused at position 57 of the remaining amino acids of the GAA polypeptide, and where the GAA polypeptide can extend to amino acid 952 of SEQ ID NO: 1, or a functional fragment thereof, and

in which the nucleic acid sequence encoding the GAA polypeptide can be codon optimized, and in which the heterologous nucleic acid is operatively linked to a liver-specific promoter.

2. A recombinant AAV vector according to Claim 1, in which the codon optimized nucleic acid sequence encoding the GAA polypeptide is selected from the group consisting of SEQ ID NO: 1-18, or functional fragments thereof, or

in which the nucleic acid encoding SEQ ID NO: 3 is wildtype, or

further comprising at least one of a UTR or a reverse RNA polII terminator sequence, or

which comprises the nucleic acid sequence of SEQ ID NO: 23, or a functional variant thereof, or

in which the heterologous nucleic acid sequence encodes a GAA protein comprising a signal peptide fused to the GAA polypeptide, in which the signal peptide is an endogenous GAA signal peptide, or a heterologous signal peptide, or a combination thereof.

3. A recombinant AAV vector according to Claim 1 or 2, in which the AAV genome comprises, in the 5’ to 3’ direction:

(a) a 5’ ITR,

(b) a liver-specific promoter sequence,

(c) an 5’ UTR sequence,

(d) a nucleic acid encoding a portion or all of the endogenous GAA signal peptide,

(e) a nucleic acid encoding a heterologous signal peptide or the N-terminal GAA polypeptide fragment,

(f) a nucleic acid encoding an alpha-glucosidase (GAA) polypeptide, in which the GAA polypeptide is functionally active,

(g) a poly A sequence, and

(h) a reverse RNA pol II terminator sequence.

4. A recombinant AAV vector according to Claim 1, 2 or 3, in which the UTR is 5’ or 3’, and/or in which nucleic acid encoding the signal peptide encodes a signal sequence is selected from any of:

an endogenous GAA signal peptide, a fibronectin signal peptide (FN1), a IL-2 wt signal peptide, modified IL-2 signal peptide, IL2(1-3) signal peptide, IgG signal peptide, a AAT signal peptide, a A2M signal peptide, or a PZP signal peptide, or an active fragment thereof having signal peptide activity, and/or

in which the nucleic acid sequence encodes a GAA polypeptide having the amino acid sequence of SEQ ID NO: 1, or a polypeptide having at least 80% sequence identity to SEQ ID NO: 1 where amino acid residue 199 is a R (199R), amino acid residue 223 is a H (223H) and amino acid residue 780 is a I (780I), and/or

in which the nucleic acid sequence encoding the GAA polypeptide is SEQ ID NO: 3, or a nucleic acid sequence having at least 80%, or at least 85%, or at least 90% sequence identity to SEQ ID NO: 3 that encodes a GAA polypeptide having at least 80% sequence identity to SEQ ID NO: 1 where amino acid residue 199 is a R (199R), amino acid residue 223 is a H (223H) and amino acid residue 780 is a I (780I), or

in which the 5’ UTR sequence comprises SEQ ID NO: 41, or a nucleic acid having at least 80% sequence identity to SEQ ID NO: 41, and/or

in which the 5’ UTR sequence comprises SEQ ID NO: 40, or a nucleic acid having at least 80% sequence identity to SEQ ID NO: 40, and/or

further comprising an intron sequence located 5’ of the nucleic acid sequence encoding the signal peptide, and 3’ of the promoter, optionally

in which the intron sequence is selected from the group consisting of: MVM sequence, a HBB2 sequence, an CMVIE intron sequence, or a UBC intron sequence or a SV40 sequence, and/or

in which the GAA polypeptide is a N-terminal truncated GAA polypeptide selected from any disclosed in Table 1, and/or

further comprising at least one polyA sequence located 3’ of the nucleic acid encoding the GAA gene and 5’ of the 3’ ITR sequence, and/or

in which the heterologous nucleic acid sequence further comprises a 3’ UTR sequence, in which the 3’ UTR sequence is located 3’ of the nucleic acid encoding the GAA polypeptide and 5’ of the 3’ ITR sequence, or is located between the nucleic acid encoding the GAA polypeptide and the poly A sequence, or RNA pol II terminator sequence, and/or

in which the heterologous nucleic acid sequence further comprises a 3’ intron sequence, in which the 3’ intron sequence is located 3’ of the nucleic acid encoding the GAA polypeptide and 5’ of the 3’ ITR sequence, or is located between the nucleic acid encoding the GAA polypeptide and the poly A sequence or RNA polII terminator sequence, and/or

in which the ITR comprises an insertion, deletion or substitution, optionally

in which one or more CpG islands in the ITR are removed.

5. A recombinant AAV vector according to any preceding claim, in which

(a) the nucleic acid encoding the signal peptide is selected from any of the group consisting of:

AAT signal peptide (e.g., SEQ ID NO: 67), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 67;

a fibronectin signal peptide (FN1) (e.g., SEQ ID NO: 73-75), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 73-75;

an endogenous GAA signal peptide (SEQ ID NO: 51), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 51;

an hIGF2 signal peptide (e.g., SEQ ID NO: 72), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 72;

a IgG1 (201) signal peptide (SEQ ID NO: 54), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 54;

wtIL2 leader peptide (SEQ ID NO: 55), or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 55;

mutant IL2 leader peptide (SEQ ID NO: 56) or an active fragment thereof having secretory signal activity, e.g., a nucleic acid encoding an amino acid sequence that has at least about 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 56; and

(b) the nucleic acid encoding the GAA polypeptide is selected from any of the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO:

12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 or a nucleic acid sequence having at least 60%, or 70%, or 80%, 85% or 90% or 95%, or 98%, or 99% sequence identity to SEQ ID NOS: 1-18, optionally

in which the nucleic acid encoding the GAA polypeptide is selected from SEQ ID NO: 3, or a nucleic acid sequence having at least 60%, or 70%, or 80%, 85% or 90% or 95%, or 98%, or 99% sequence identity to SEQ ID NO: 3 which encodes a GAA polypeptide at least 85% sequence identity to SEQ ID NO: 1 where amino acid residue 199 is a R (199R), amino acid residue 223 is a H (223H) and amino acid residue 780 is a I (780I), and/or

in which the nucleic acid encoding the GAA polypeptide encodes a GAA polypeptide beginning at any of amino acid residues 35, 40, 50, 57, 60, 68, 69, 70, 72, 74, 779, 790, 791, 792, 793, or 796 of SEQ ID NO: 1 or a sequence 80% identical to SEQ ID NO: 1 where amino acid residue 199 is a R (199R), amino acid residue 223 is a H (223H) and amino acid residue 780 is a I (780I), optionally

in which the GAA polypeptide has an endogenous GAA signal peptide attached, or a heterologous signal peptide attached to the N-terminal of the GAA polypeptide, in which the endogenous signal peptide has the amino acid sequence of SEQ ID NO: 59 or a sequence at least 80% sequence identity to SEQ ID NO: 59, and the heterologous signal peptide is selected from the group consisting of: SEQ ID NO: 60 (201 IgG signal peptide), or an IL2 wild type signal peptide (SEQ ID NO: 61), modified IL2 signal peptide (SEQ ID NO: 62), A2M signal peptide (SEQ ID NO: 63), or PZP signal peptide (SEQ ID NO: 64), or artificial signal peptide (SEQ ID NO: 65), or cathpetsin L signal peptide (SEQ ID NO: 66) or signal peptides at least 90% sequence identity to SEQ ID NOS: 60-66, and/or

in which the liver specific promoter is selected from any of: SEQ ID NOS: 86, 88, 91-96, 146-150 or 439-441, or a liver specific promoter having at least 80% sequence identity to SEQ ID NOs: 86, 88, 91-96, 146-150 or 439-441, or

in which the liver specific promoter is selected from any of: SEQ ID NOS: 98 or 99, or a liver specific promoter having at least 80% sequence identity to SEQ ID NOs: 98 or 99, or

in which the liver specific promoter is SEQ ID NOS: 97, or a liver specific promoter having at least 80% sequence identity to SEQ ID NO: 97.

6. A recombinant AAV vector according to any preceding claim, in which the recombinant vector is manufactured from the plasmid of SEQ ID NO: 27, and/or

in which the nucleic acid comprises SEQ ID NO: 25, or a functional fragment thereof, and/or

in which the recombinant AAV vector is a chimeric AAV vector, haploid AAV vector, a hybrid AAV vector or polyploid AAV vector, and/or

in which the recombinant AAV vector is a rational haploid vector, a mosaic AAV vector, a chemically modified AAV vector, or a AAV vector from any AAV serotypes, and/or

in which the recombinant AAV vector is selected from the group consisting of: a AAVXL32 vector, a AAVXL32.1 vector, a AAV8 vector, or a haploid AAV8 vector comprising at least one AAV8 capsid protein, and/or

in which the serotype is AAV3b, optionally

in which the AAV3b serotype comprises one or mutations in a capsid protein selected from any of: 265D, 549A, Q263Y, optionally

in which the AAV3b serotype is selected from any of: AAV3b265D, AAV3b265D549A, AAV3b549A or AAV3bQ263Y, or AAV3bSASTG.

7. A recombinant AAV vector according to any preceding claim, in which the poly A sequence is a full length HGF poly A sequence, optionally

in which poly A sequence is selected from SEQ ID NO: 42, 43 or 44, or a nucleic acid sequence at least 80% sequence identity to SEQ ID NOS: 42-44, and/or

in which the reverse RNA pol II terminator sequence is SEQ ID NO: 45, or a nucleic acid sequence at least 80% sequence identity to SEQ ID NOS: 45.

8. A pharmaceutical composition comprising the recombinant AAV vector according to any preceding claim in a pharmaceutically acceptable carrier.

9. A recombinant AAV vector, or the rAAV genome or a nucleic acid sequence according to any preceding claim for use in a method to treat a subject with Pompe Disease, or a

glycogen storage disease type II (GSD II, Acid Maltase Deficiency) or having a deficiency in alpha-glucosidase (GAA) polypeptide, in which any of the recombinant AAV vector, or the rAAV genome or the nucleic acid sequence are to be administered to the subject, optionally

in which the AAV vector manufactured from the plasmid of SEQ ID NO: 27.

10. A recombinant AAV vector, or a rAAV genome or a nucleic acid sequence for use according to Claim 9, where the recombinant AAV vector comprises the nucleic acid sequence of SEQ ID NO: 3, or a functional fragment thereof, and/or

where the recombinant AAV vector comprises the nucleic acid sequence of SEQ ID NO: 23, or a functional variant thereof, and/or

in which GAA polypeptide is secreted from the subject's liver and there is uptake of the secreted GAA by skeletal muscle tissue, cardiac muscle tissue, diaphragm muscle tissue or a combination thereof, in which uptake of the secreted GAA results in a reduction in lysosomal glycogen stores in the tissue(s), optionally

in which the administering to the subject is selected from any of: intramuscular, subcutaneous, intraspinal, intracisternal, intrathecal, intravenous administration, and/or

where the recombinant AAV vector is a chimeric AAV vector, haploid AAV vector, a hybrid AAV vector or polyploid AAV vector, and/or

where the recombinant AAV vector is a rational haploid vector, a mosaic AAV vector, a chemically modified AAV vector, or a AAV vector from any AAV serotypes, and/or

where the recombinant AAV vector is a AAVXL32 vector or a AAVXL32.1 vector or a AAV8 vector, or a haploid AAV8 vector comprising at least one AAV8 capsid protein, and/or

where the recombinant AAV vector is a AAV8 vector, and/or

where the recombinant AAV vector is to be administered at a dosage range of between 1.0E11vg/kg and 5.0E13 vg/kg, and/or

further comprising receiving GAA protein enzyme replacement therapy, and withdrawing GAA protein enzyme replacement therapy (ERT) on the same day, a day after or, any time between day 1 and 26 weeks after administration of the recombinant AAV vector.

11. A nucleic acid construct comprising SEQ ID NO: 3, or a nucleic acid sequence at least 80% sequence identity to SEQ ID NOS: 3, optionally

in which the expression of the nucleic acid sequence of SEQ ID NO: 3 or a nucleic acid having 80% sequence identity thereto encodes a GAA polypeptide having at least 80% sequence identity to SEQ ID NO: 1 and in which there is R at position 199, a H at position 223 and I at position 780.

12. A nucleic acid construct comprising SEQ ID NO: 23, or a nucleic acid sequence at least 80% sequence identity to SEQ ID NO: 23, optionally

comprising SEQ ID NO: 3 or SEQ ID NO: 25, or a nucleic acid sequence at least 80% sequence identity to SEQ ID NOS: 3 or 25, optionally

in which the expression of the nucleic acid sequence of SEQ ID NO: 3 or a nucleic acid having 80% sequence identity thereto encodes a GAA polypeptide having at least 80% sequence identity to SEQ ID NO: 1 and in which there is R at position 199, a H at position 223 and I at position 780.

13. A recombinant AAV comprising a nucleic acid construct according to Claim 11 or 12.

14. A recombinant AAV vector according to Claim 1, in which the AAV lacks at least 1 amino acids of the GAA N terminus.

15. A recombinant AAV vector according to Claim 1, in which the AAV lacks at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or mor amino acids of the GAA N terminus, or

in which the heterologous signal peptide is inserted immediately after the endogenous GAA signal peptide or a potion thereof.