emerged as the standard of care for type 1 GD. In addition to its high cost (~$200,000 or ~£150,000/patient/year), enzyme replacement therapy treatment in GD generally requires one or more injections every other week for life. This leads to a high proportion of GD patients displaying high levels of treatment burden.

Accordingly, there is a need to provide improved and effective therapy for the treatment of GD, *i.e.* one that increases the availability of functioning GCase to affected target organs, which would avoid the need for frequent and lifelong intravenous injections of GCase.

Summary

The present disclosure relates to a modified β-Glucocerebrosidase (GCase) polypeptide which comprises one or more amino acid substitution mutations.

In one aspect, the present disclosure refers to a modified β-Glucocerebrosidase (GCase) polypeptide which comprises at least one mutation, wherein the at least one mutation comprises: (i) a mutation at a position corresponding to position 351 of SEQ ID NO: 1 that is a substitution with cysteine; and (ii) a mutation at a position corresponding to position 380 of SEQ ID NO: 1 that is a substitution with cysteine, wherein the modified GCase polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 2, and wherein the modified GCase polypeptide has increased stability and has higher effective activity relative to a reference GCase polypeptide, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3, 4, or 5.

Accordingly, in an example of the disclosure, there is provided a modified β-Glucocerebrosidase (GCase) polypeptide which comprises at least one mutation, wherein the at least one mutation:

(i) provides higher effective activity; and/or
(ii) provides increased stability; and/or
(iii) provides structural stabilisation at physiological pH; and/or
(iv) provides a longer half-life; and/or
(v) provides increased thermostability.

In an example of the disclosure, there is provided a modified β-Glucocerebrosidase (GCase) polypeptide comprising at least one mutation at a position corresponding to a position selected from the group consisting of 351, 380, 272, 262, 313, 404, 407, 482, 484, 490, 494, 503, and 534 of SEQ ID NO: 1.

In one aspect, there is provided a polynucleotide comprising a modified glucocerebrosidase (GBA) nucleotide sequence, wherein the modified GBA nucleotide sequence encodes the modified GCase polypeptide as described herein.

In one aspect, there is provided a viral particle comprising a recombinant genome comprising the polynucleotide as described herein.

In an example of the disclosure, there is provided a composition comprising the modified GCase polypeptide, polynucleotide, or viral particle of the disclosure and a pharmaceutically acceptable excipient.

In an example of the disclosure, there is provided the modified GCase polypeptide, polynucleotide, viral particle, or composition of the disclosure for use in a method of treatment.

In an example of the disclosure, there is provided a method of treatment comprising administering an effective amount of the modified GCase polypeptide, polynucleotide, viral particle, or composition of the disclosure to a patient.

In an example of the disclosure, there is provided a use of the modified GCase polypeptide, polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for use in a method of treatment.

In an example of the disclosure, there is provided the modified GCase polypeptide, polynucleotide, viral particle, or composition of the disclosure for use in treating a disease, wherein the modified GCase polypeptide or the encoded modified GCase polypeptide has increased stability at pH 7.4 compared to a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided a method of treating a disease by administering the modified GCase polypeptide, polynucleotide, viral particle or composition of the disclosure, wherein the modified GCase polypeptide or encoded modified GCase polypeptide has increased stability at pH 7.4 compared to a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided a use of the modified GCase polypeptide, polynucleotide, viral particle or composition of the disclosure in treating a disease, wherein the modified GCase polypeptide or encoded modified GCase polypeptide has increased stability at pH 7.4 compared to a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided the modified GCase polypeptide, polynucleotide, viral particle, or composition of the disclosure for use in treating a disease, wherein treating the disease comprises administering a lower dose of the modified GCase polypeptide, polynucleotide, viral particle or composition compared to the dose required to be administered of an equivalent polypeptide, polynucleotide, viral particle or composition comprising or encoding a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided a method of treating a disease by administering the GCase polypeptide, polynucleotide, viral particle or composition of the disclosure, wherein treating the disease comprises administering a lower dose of the modified GCase polypeptide, polynucleotide, viral particle or composition compared to the dose required to be administered of an equivalent polypeptide, polynucleotide, viral particle or composition comprising or encoding a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided a use of the modified GCase polypeptide, polynucleotide, viral particle or composition of the disclosure in treating a disease, wherein treating the disease comprises administering a lower dose of the modified GCase polypeptide, polynucleotide, viral particle or composition compared to the dose required to be administered of an equivalent polypeptide, polynucleotide, viral particle or composition comprising or encoding a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.

In an example of the disclosure, there is provided a modified GCase polypeptide which comprises at least one mutation, wherein the modified GCase polypeptide:

(i) has higher effective activity; and/or

(ii) has increased stability; and/or

(iii) has structural stabilisation at physiological pH; and/or

(iv) has a longer half-life; and/or

(v) provides increased thermostability.

In an example of the disclosure, there is provided a modified GCase polypeptide which comprises at least one mutation, wherein the at least one mutation reduces the number of Human leukocyte antigen (HLA)-I and/or HLA-II binders.

In an example of the disclosure, there is provided a modified GCase polypeptide which comprises at least one mutation, wherein the modified GCase polypeptide has a reduced number of HLA-I and/or HLA-II binders.

In an example, there is provided a use of the modified GCase polypeptide, the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for:

(i) reducing the level of hexosylsphingosine and/or hexosylceramide;

(ii) reducing the number of storage cells; and/or

(iii) reducing the level of activated macrophages;

in a subject suffering from a disease or condition associated with GCase deficiency.

In an example, there is provided a method of

(i) reducing the level of hexosylsphingosine and/or hexosylceramide;

(ii) reducing the number of storage cells; and/or

(iii) reducing the level of activated macrophages;

in a subject suffering from a disease or condition associated with GCase deficiency by administering to the subject the modified GCase polypeptide, the polynucleotide, viral particle, or composition of the disclosure.

In an example, there is provided the modified GCase polypeptide, the polynucleotide, viral particle, or composition of the disclosure, for use in a method of

(iv) reducing the level of hexosylsphingosine and/or hexosylceramide;

(v) reducing the number of storage cells; and/or

(vi) reducing the level of activated macrophages;

in a subject suffering from a disease or condition associated with GCase deficiency, optionally wherein

(i) reducing the level of hexosylsphingosine and/or hexosylceramide;

(ii) reducing the number of storage cells; and/or

(iii) reducing the level of activated macrophages;

leads to the treatment of the disease or condition associated with GCase deficiency.

GCase is a lysosomal protein which normally functions and is stable at an acidic pH. It is usually unstable at physiological pH (*e.g.* pH 7.4) with a half-life of only a few minutes. For example, see Example 2 and Figure 1 showing that the activity of VPRIV (velaglucerase alfa, a commercially available ERT which is a recombinant GCase having an identical amino acid sequence as a naturally-occurring human GCase polypeptide) dramatically reduces following incubation at pH 7.4. SEQ ID NO: 3 comprises the sequence of velaglucerase alfa. Existing enzyme replacement treatment (ERT) is the standard treatment approach for those with GD. In ERT, the GCase enzymes are usually provided intravenously, which means that such treatments also encounter issues with effective delivery of the GCase enzymes to the target organs due to the distance that they need to travel and their instability in blood. The present inventors have developed modified GCase polypeptides with increased stability. The present inventors have identified amino acid substitutions in the GCase polypeptide that are able to increase the stability of the GCase polypeptide.

Without wishing to be bound by theory, it is believed that the instant amino acid mutations may serve to enhance the stability at physiological pH (*e.g.* pH 7.4), thereby extending the

time for which the GCase is stable, and thus provide higher effective activity. The modified GCase polypeptide according to the disclosure may have a higher residual activity than a wild-type GCase polypeptide under various pHs (for example see Examples 3 and 4). The modified GCase polypeptide according to the disclosure may have a longer half-life under various pHs than a wild-type GCase polypeptide (for example see Example 5). The modified GCase polypeptide according to the disclosure may show a higher GCase effective activity than a wild-type GCase polypeptide (for example see Examples 8 and 9). The modified GCase polypeptide of the disclosure may have improved thermostability (for example see Example 12). The modified GCase polypeptide according to the disclosure may be provided as a protein for enzyme replacement therapy. Also, the modified GCase polypeptide of the disclosure may be provided by expression in the liver (such as in the form of gene therapy) and allowing the protein to be transported through blood to reach the target organs. Provision of the modified GCase polypeptide according to the disclosure (such as in the form of gene therapy) may result in a higher uptake in relevant tissues than provision of a wild-type GCase polypeptide (for example see Example 10). The present inventors have also found that the modified GCase polypeptide according to the disclosure may have a reduced potential immunogenicity risk compared to a wild type GCase polypeptide. The modified GCase polypeptide according to the disclosure may have a lower predicted number of “strong” binders to HLA-I and a lower predicted number of strong binders to HLA-II (for example see Example 7).

By extending the time for which the GCase is stable, and thus providing a higher effective activity, the modified GCase polypeptide of the disclosure may achieve a greater therapeutic effect when administered at a dose which is the same or similar to the dose of a GCase of lower stability (*e.g.* wild-type GCase). Furthermore, a lower dose of the modified GCase polypeptide of the disclosure may be able to achieve the same therapeutic effect as a GCase of lower stability (*e.g.* wild-type GCase) when the modified GCase polypeptide of the disclosure is administered at a lower dose than the GCase of lower stability. Thus, the costs associated with therapy may be reduced and the safety profile may be improved.

Description of the figures

CLAIMS

1. A modified β-Glucocerebrosidase (GCase) polypeptide which comprises at least one mutation, wherein the at least one mutation comprises:

(i) a mutation at a position corresponding to position 351 of SEQ ID NO: 1 that is a substitution with cysteine; and

(ii) a mutation at a position corresponding to position 380 of SEQ ID NO: 1 that is a substitution with cysteine,

wherein the modified GCase polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 2, and

wherein the modified GCase polypeptide has increased stability and has higher effective activity relative to a reference GCase polypeptide, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3, 4, or 5.

2. The modified GCase polypeptide of claim 1, wherein the effective activity of the modified GCase polypeptide is at least 1.2 fold, at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 3.5 fold, at least 4 fold, at least 4.5 fold, at least 5 fold, at least 5.5. fold, at least 6 fold, at least 6.5 fold, at least 7 fold, at least 7.5 fold, at least 8 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 35 fold, at least 40 fold, at least 45 fold, or at least 50 fold higher than the effective activity of the reference GCase polypeptide.

3. The modified GCase polypeptide of claim 1 or 2, wherein:

(i) the modified GCase polypeptide is more structurally stable at physiological pH relative to a reference GCase polypeptide, wherein the reference GCase is the polypeptide of SEQ ID NO: 3, 4, or 5, optionally wherein the pH is pH 7.4; or

(ii) the increased stability is increased stability at pH 7.4; or

(iii) the modified GCase polypeptide retains at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% activity compared to the initial activity when measured after at least 10 minutes, at least 30 minutes, at least 60 minutes, at least 120 minutes, at least 1 day, at least 2 days, at least 3

days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days of incubation at pH 7.4 and 37 degrees Celsius; or

(iv) the modified GCase polypeptide retains activity which is at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.8 fold, at least 2 fold, at least 2.5 fold, at least 2.8 fold, or at least 3 fold higher than the activity of a reference GCase polypeptide when measured after 120 minutes of incubation at pH 7.4 and 37 degrees Celsius, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3, 4, or 5.

4. The modified GCase polypeptide of any one of the preceding claims, wherein:
   (i) the modified GCase polypeptide retains at least 70%, at least 75%, at least 80%, or at least 85% activity compared to the initial activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius; or
   (ii) the modified GCase polypeptide retains at least 40%, at least 45%, at least 50%, at least 55%, or at least 60% activity compared to the initial activity when measured after 72 hours of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in PBS; or
   (iii) the modified GCase polypeptide retains at least 15%, or at least 20% activity compared to the initial activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in serum or plasma; or
   (iv) the modified GCase polypeptide retains at least 40% activity compared to the initial activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in serum or plasma; or
   (v) the modified GCase polypeptide retains at least 60% activity compared to the initial activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius; or
   (vi) the modified GCase polypeptide retains at least 80% activity compared to the initial activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius; or
   (vii) the activity, effective activity, and/or stability is determined using a fluorometric assay.

5. The modified GCase polypeptide of any one of the preceding claims having a longer half-life relative to a reference GCase polypeptide, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3, 4, or 5, optionally wherein the modified GCase polypeptide has a half-life of at least 1.2 fold, at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, or at least 10 fold longer than the half-life of the reference GCase polypeptide.

6. The modified GCase polypeptide of claim 5, wherein:
   a. the longer half-life is longer half-life at pH 7.4 or pH 5.6; or
   b. the modified GCase polypeptide has a half-life at pH 5.6 of at least 20 fold longer than the half-life of the reference GCase polypeptide; or
   c. the longer half-life is longer half-life in serum or plasma; or
   d. the half-life is determined using a fluorometric assay.

7. The modified GCase polypeptide of any one of the preceding claims, wherein:
   a. the at least one mutation comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 272 of SEQ ID NO: 1 is a substitution with glutamine, further optionally E272Q; or
   b. the at least one mutation comprises a mutation at a position corresponding to position 262 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 262 of SEQ ID NO: 1 is a substitution with asparagine or tyrosine; or
   c. the at least one mutation comprises a mutation at a position corresponding to position 262 of SEQ ID NO: 1, wherein the mutation at a position corresponding to position 262 of SEQ ID NO: 1 is a substitution with asparagine, optionally H262N; or
   d. the at least one mutation comprises a mutation at a position corresponding to position 313 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 313 of SEQ ID NO: 1 is a substitution with asparagine, further optionally H313N; or
   e. the at least one mutation comprises a mutation at a position corresponding to position 404 of SEQ ID NO: 1, optionally wherein the mutation at a position

corresponding to position 404 of SEQ ID NO: 1 is a substitution with lysine, further optionally H404K; or

f. the at least one mutation comprises a mutation at a position corresponding to position 490 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 490 of SEQ ID NO: 1 is a substitution with lysine, further optionally H490K; or

g. the at least one mutation comprises a mutation at a position corresponding to position 534 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 534 of SEQ ID NO: 1 is a substitution with asparagine, further optionally R534N.

8. The modified GCase polypeptide of any one of the preceding claims, wherein:

(a) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 482 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 503 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 482 of SEQ ID NO: 1 is a substitution with cysteine, optionally an aspartic acid to cysteine mutation; and

(ii) the mutation at a position corresponding to position 503 of SEQ ID NO: 1 is a substitution with cysteine, optionally a serine to cysteine mutation; or

(b) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 494 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 534 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 494 of SEQ ID NO: 1 is a substitution with cysteine, optionally a serine to cysteine mutation; and

(ii) the mutation at a position corresponding to position 534 of SEQ ID NO: 1 is a substitution with cysteine, optionally an arginine to cysteine mutation; or

(c)

(i) the mutation at a position corresponding to position 351 of SEQ ID NO: 1 is a tryptophan to cysteine mutation; and

(ii) the mutation at a position corresponding to position 380 of SEQ ID NO: 1 is an alanine to cysteine mutation; or

(d) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 407 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 484 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 407 of SEQ ID NO: 1 is a substitution with cysteine, optionally an isoleucine to cysteine mutation; and

(ii) the mutation at a position corresponding to position 484 of SEQ ID NO: 1 is a substitution with cysteine, optionally an aspartic acid to cysteine mutation; or

(e) the at least one mutation comprises a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3; or

(f) the at least one mutation comprises:

(i) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(ii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1;

and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, wherein the reference GCase polypeptide the polypeptide of SEQ ID NO: 3; or

(g) the at least one mutation comprises:

(i) a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, optionally E272Q; and

(ii) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(iii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1; or

(h) the at least one mutation comprises:

(i) a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, optionally E272Q; and

(ii) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(iii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1;

and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, wherein the reference GCase polypeptide is the polypeptide of SEQ ID NO: 3; or

(i) the modified GCase polypeptide has higher effective activity and/or increased stability compared to a reference GCase polypeptide, wherein the reference GCase polypeptide is selected from any one of SEQ ID NOs: 1 to 5.

9. The modified GCase polypeptide of any one of the preceding claims, wherein:

(i) the modified GCase polypeptide comprises an amino acid sequence at least 95%, at least 98%, or at least 99% identical to SEQ ID NO: 1 or SEQ ID NO: 2; or

(ii) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to a fragment of between 400 and 536 amino acids of SEQ ID NO: 1; or

(iii) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to a fragment of between 400 and 497 amino acids of SEQ ID NO: 2; or

(iv) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to SEQ ID NO: 1; or

(v) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to SEQ ID NO: 2; or

(vi) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises the at least one mutation defined in any one of the preceding claims; or

(vii) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1; or

(viii) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a substitution with cysteine at a position corresponding to position 351 of SEQ ID NO: 1 and at a position corresponding to position 380 of SEQ ID NO: 1; or

(ix) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a substitution with cysteine at a position corresponding to position 351 of SEQ ID NO: 1 and at a position corresponding to position 380 of SEQ ID NO: 1, and the modified GCase polypeptide comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1.

10. A polynucleotide comprising a modified glucocerebrosidase (GBA) nucleotide sequence, wherein the modified GBA nucleotide sequence encodes the modified GCase polypeptide of any one of the preceding claims.

11. The polynucleotide of claim 10, wherein:

(i) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 750, at least 850, at least 950, at least 1000, at least 1200, at least 1400, or at least 1494 nucleotides of any one of SEQ ID NOs: 14 to 29; or

(ii) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 18; or

(iii) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 22 or SEQ ID NO: 26.

12. The polynucleotide of claim 10 or 11, wherein the modified GBA nucleotide sequence comprises a sequence that is at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 18.

13. A viral particle comprising a recombinant genome comprising the polynucleotide of any one of claims 10 to 12.

14. The viral particle of claim 13:

(i) which is an AAV, adenoviral, or lentiviral viral particle, optionally wherein the viral particle is an AAV viral particle; or

(ii) wherein the viral particle comprises a liver-tropic or CNS-tropic capsid, optionally wherein the liver-tropic capsid comprises a sequence at least 98%, at least 99%, or at least 99.5% identical to a fragment of at least 600, at least 650, at least 700, between 600 and 736, between 650 and 736, or between 700 and 736 amino acids of SEQ ID NO: 36, 37 or 38; or

(iii) wherein the viral particle further comprises:

a) AAV2 ITRs;
b) a polyA sequence; and/or
c) an intron; or

(iv) wherein the recombinant genome is single-stranded; or

(v) wherein the effective activity of the modified GCase polypeptide is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the effective activity of the GCase polypeptide encoded by the sequence of SEQ ID NO: 14 or 18.

15. The viral particle of claim 13 or 14, wherein:

(i) the viral particle is an AAV viral particle, and the viral particle comprises a CNS-tropic capsid;

(ii) the polynucleotide further comprises a transcription regulatory element;

(iii) the modified GBA nucleotide sequence is codon-optimised and/or the modified GBA nucleotide sequence comprises a sequence that is at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 18; and

(iv) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a substitution with cysteine at a position corresponding to position 351 of SEQ ID NO: 1 and at a position corresponding to position 380 of SEQ ID NO: 1.

16. The viral particle of claim 13 or 14, wherein:

(i) the viral particle is an AAV viral particle, and the viral particle comprises a liver-tropic capsid, optionally wherein the liver-tropic capsid comprises a sequence 100% identical to SEQ ID NO: 37;

(ii) the polynucleotide further comprises a transcription regulatory element, wherein (a) the transcription regulatory element comprises a liver-specific promoter and/or (b) the transcription regulatory element comprises a sequence that is 100% identical to SEQ ID NO: 35; and

(iii) the modified GBA nucleotide sequence is codon-optimised and/or the modified GBA nucleotide sequence comprises a sequence that 100% identical to a nucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 18.

17. A composition comprising the modified GCase polypeptide, polynucleotide, or viral particle of any one of the preceding claims and a pharmaceutically acceptable excipient.

18. The modified GCase polypeptide, polynucleotide, viral particle, or composition of any one of the preceding claims for use in therapy.

19. Use of the modified GCase polypeptide, polynucleotide, viral particle or composition of any one of claims 1 to 17 in the manufacture of a medicament for:

(i) treating a synucleinopathy; or

(ii) treating Parkinson's disease; or

(iii) treating Gaucher disease, optionally wherein the Gaucher disease is Gaucher disease type I, II or III; or

(iv) treating Gaucher disease and wherein the patient has antibodies or inhibitors to a recombinant GCase with which the patient has previously been treated as part of an enzyme replacement therapy, optionally wherein the Gaucher disease is Gaucher disease type I, II or III.