compared to polynucleotides comprising a wild type GCase encoding polynucleotides. Such a gene therapy approach would avoid the need for frequent and lifelong intravenous injections of GCase.

Summary

The present application demonstrates that specific modifications to a GBA nucleotide sequence encoding for GCase can help to improve the expression level and the activity of the expressed GCase polypeptide *in vitro* and/or *in vivo*. For example, the present application demonstrates that using a codon-optimised GBA nucleotide sequence can improve the expression and/or activity of the encoded GCase protein. Such modified (*i.e.* non wild-type) and/or codon-optimised GBA nucleotide sequences may be further modified to provide further improvements in the expression and/or activity of the encoded GCase protein. Further modifications may include providing further modifications in the GBA nucleotide sequence such as the removal of CpG motifs, and/or the use of particular gene regulatory elements comprising specific promoter and/or enhancer sequences. It is believed that such improvements to a GBA nucleotide sequence can improve the efficacy of such a nucleotide in the treatment of GD.

These modifications provide a GBA nucleotide sequence which is expressed highly, for example in the liver, and which encodes a GCase polypeptide or fragment thereof. As demonstrated in the Examples, the polynucleotides of the disclosure express GCase activity at higher levels than wild type GBA.

In one aspect, the present disclosure refers to a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein and wherein the GBA nucleotide sequence comprises a sequence that is: (i) 100% identical to SEQ ID NO: 1 or SEQ ID NO: 5;
(ii) at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identical to SEQ ID NO: 1 or SEQ ID NO: 5, wherein the GCase encoded by the GBA nucleotide sequence has GCase activity and expresses in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide; and/or
(iii) a variant of SEQ ID NO: 1 or SEQ ID NO: 5 encoding a GCase protein having GCase activity, wherein the variant is identical to SEQ ID NO: 1 or SEQ ID NO: 5 respectively except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25, further wherein the GCase encoded by the GBA nucleotide expresses in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide.

In another aspect, the present disclosure refers to a viral particle comprising a recombinant genome comprising the polynucleotide as disclosed herein.

In another aspect, the present disclosure refers to a composition comprising the polynucleotide or viral particle as disclosed herein and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to the polynucleotide, viral particle or composition of any one of the preceding claims for use in therapy.

In another example, the present disclosure refers to the use of the polynucleotide, viral particle, or composition as disclosed herein in the manufacture of a medicament for treating a disease associated with GCase deficiency.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle, or composition as disclosed herein in the manufacture of a medicament for treating Parkinson's disease.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Gaucher disease. .

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject, wherein achieving a stable GCase activity leads to the treatment of Gaucher disease in the subject.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for expressing the GBA nucleotide sequence and providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration, wherein achieving a stable GCase activity leads to the treatment of Gaucher disease in the subject.

In yet another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for reducing hexosylceramide and/or hexosylsphingosine levels in a subject suffering from Parkinson's disease or Gaucher disease.

Accordingly, in a first example of the disclosure, there is provided a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein or fragment thereof and wherein at least a portion of the GBA nucleotide sequence is not wild type.

In a second example of the disclosure, there is provided a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a GCase protein or a fragment thereof and comprises a sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 1000, at least 1200, at least 1300, less than 1494, less than 1611, between 1000 and 1494, between 1000 and 1611, between 1300 and 1494, between 1300 and 1611, or around 1494 nucleotides of SEQ ID NO: 1-8.

In a third example of the disclosure, there is provided a viral particle comprising a recombinant genome comprising the polynucleotide of the disclosure.

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

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

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

In a seventh example of the disclosure, there is provided the use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for achieving a stable GCase activity in a subject.

In an eighth example of the disclosure, there is provided the use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for providing greater GCase bioavailability in a subject compared to the bioavailability from

Claims

1. A polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein and wherein the GBA nucleotide sequence comprises a sequence that is:

(i) 100% identical to SEQ ID NO: 1 or SEQ ID NO: 5;

(ii) at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identical to SEQ ID NO: 1 or SEQ ID NO: 5, wherein the GCase encoded by the GBA nucleotide sequence has GCase activity and expresses in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide; and/or

(iii) a variant of SEQ ID NO: 1 or SEQ ID NO: 5 encoding a GCase protein having GCase activity, wherein the variant is identical to SEQ ID NO: 1 or SEQ ID NO: 5 respectively except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25, further wherein the GCase encoded by the GBA nucleotide expresses in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide.

2. The polynucleotide of claim 1, wherein the variant is a variant of SEQ ID NO: 1 and the variant of SEQ ID NO: 1:

(i) is identical to SEQ ID NO: 1 except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;

(ii) has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 20, or up to 30 nucleotide substitutions relative to the sequence of SEQ ID NO: 1;

(iii) has 1, up to 2, up to 3, up to 4, up to 5, or up to 6 nucleotide substitutions relative to the sequence of SEQ ID NO: 1;

(iv) has up to 4 nucleotide substitutions relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 3 amino acid substitutions relative to the wild type amino acid GCase sequence of SEQ ID NO: 25;

(v) has up to 3 nucleotide substitutions relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or

(vi) has 1 nucleotide substitution relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

3. The polynucleotide of claim 1, wherein the variant is a variant of SEQ ID NO: 5 and the variant of SEQ ID NO: 5:

(i) is identical to SEQ ID NO: 5 except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;

(ii) has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 20, or up to 30 nucleotide substitutions relative to the sequence of SEQ ID NO: 5;

(iii) has 1, up to 2, up to 3, up to 4, up to 5, or up to 6 nucleotide substitutions relative to the sequence of SEQ ID NO: 5;

(iv) has up to 4 nucleotide substitutions relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 3 amino acid substitutions relative to the wild type amino acid GCase sequence of SEQ ID NO: 25;

(v) has up to 3 nucleotide substitutions relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or

(vi) has 1 nucleotide substitution relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

4. The polynucleotide of any one of the preceding claims, wherein the variant has:
(i) up to 3 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;
(ii) up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or
(iii) up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

5. The polynucleotide of any one of the preceding claims, wherein the GCase encoded by the GBA nucleotide sequence expresses in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide and wherein:
(a) the GBA nucleotide sequence is codon-optimised for expression in human liver cells; and/or
(b) the GBA nucleotide sequence comprises a reduced number of CpGs compared to a wild type GBA nucleotide sequence; optionally wherein the GBA nucleotide sequence comprises less than 40, less than 20, less than 18, less than 10, or less than 5 CpGs, more optionally wherein the GBA nucleotide sequence comprises less than 5, less than 4, less than 3, or less than 2 CpGs per 100 nucleotides, more optionally wherein the GBA nucleotide sequence is CpG-free, preferably wherein the wild type GBA nucleotide sequence is SEQ ID NO: 9.

6. The polynucleotide of any one of the preceding claims, wherein the polynucleotide further comprises a transcription regulatory element.

7. The polynucleotide of claim 6, wherein the transcription regulatory element comprises a liver-specific promoter and/or an enhancer.

8. The polynucleotide of claim 6 or 7, wherein the transcription regulatory element comprises an A1AT promoter.

9. The polynucleotide of claim 8, wherein:

(a) the A1AT promoter is at least 100, at least 120, at least 150, at least 180, less than 255, between 100 and 255, between 150 and 225, between 150 and 300, or between 180 and 255 nucleotides in length;

(b) the A1AT promoter is at least 200, at least 250, at least 300, less than 500, between 200 and 500, between 250 and 500, between 350 and 450, or 418 nucleotides in length; and/or

(c) the polynucleotide comprises a promoter that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 15 or SEQ ID NO: 12.

10. The polynucleotide of any one of claims 6 to 9, wherein the enhancer is an HCR enhancer.

11. The polynucleotide of claim 10, wherein:

(a) the HCR enhancer is at least 80, at least 90, at least 100, less than 192, between 80 and 192, between 90 and 192, between 100 and 250, or between 117 and 192 nucleotides in length;

(b) the HCR enhancer is at least 150, at least 190, at least 230, less than 400, between 150 and 400, between 190 and 370, between 230 and 340, between 250 and 340, or 321 nucleotides in length; or

(c) the polynucleotide comprises an enhancer that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 16 or SEQ ID NO: 11.

12. The polynucleotide of claim 6 or 7, wherein the transcription regulatory element is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 14 or 10.

13. The polynucleotide of any one of the preceding claims, wherein the GCase encoded by the GBA nucleotide sequence expresses in human liver cells at least 1.1x, at least 1.2x, at least 1.3x, at least 1.4x, or at least 1.5x higher compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide.

14. The polynucleotide of claim 13, wherein the reference polynucleotide comprises a wild type GBA nucleotide sequence of SEQ ID NO: 9, optionally wherein the reference polynucleotide comprises a promoter of SEQ ID NO: 13.

15. A viral particle comprising a recombinant genome comprising the polynucleotide of any one of the preceding claims.

16. The viral particle of claim 15, which is an AAV, adenoviral, or lentiviral viral particle, optionally which is an AAV viral particle.

17. The viral particle of claim 15 or 16, wherein the viral particle comprises a liver-tropic or CNS-tropic capsid.

18. The viral particle of claim 17, wherein the liver-tropic capsid comprises a sequence at least 98%, at least 99%, or at least 99.5% identical to a sequence 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: 19, 20 or 24, optionally wherein the liver-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 19 or 20.

19. The viral particle of claim 17, wherein the CNS-tropic capsid comprises a sequence at least 98%, at least 99%, or at least 99.5% identical to a sequence 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: 21, optionally wherein the CNS-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 21.

20. The viral particle of any one of claims 15 to 19, wherein the recombinant genome further comprises:

a) AAV2 ITRs;

b) a poly A sequence; and/or

c) an intron;

optionally wherein the recombinant genome is single-stranded.

21. The viral particle of any one of claims 15 to 20, wherein on transduction into Huh-7 cells, the viral particle expresses GCase such that the GCase activity in the transduced cell is greater than the activity of GCase in a cell transduced with an otherwise identical viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9, optionally wherein on transduction into Huh-7 cells, the viral particle expresses GCase such that the GCase activity in the transduced cell is at least 2x, at least 3x, at least 4x, at least 5x, at least 10x, or at least 20x greater than the activity of GCase in a cell transduced with an otherwise identical viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9, more optionally wherein the activity is measured using a fluorometric substrate which is specific for GCase.

22. A composition comprising the polynucleotide or viral particle of any one of the preceding claims and a pharmaceutically acceptable excipient.

23. The polynucleotide, viral particle or composition of any one of the preceding claims for use in therapy.

24. The polynucleotide, viral particle or composition for use of claim 23, wherein the polynucleotide, composition or viral particle of any one of claims 1 to 22 is to be administered to a patient.

25. Use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Parkinson's disease.

26. Use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Gaucher disease.

27. The use of claim 26, wherein:
(i) the Gaucher disease is Gaucher disease type I, II or III; and/or
(ii) 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.

28. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject, wherein achieving a stable GCase activity leads to the treatment of Gaucher disease in the subject.

29. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for expressing the GBA nucleotide sequence and providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration, wherein providing greater GCase bioavailability leads to the treatment of Gaucher disease in the subject.

30. The use of claim 28 or 29, wherein:
(i) the GCase activity is stable at a level of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 µmol/h/ml in the subject;
(ii) the GCase activity is stable at a level of at least 3 µmol/h/ml in the subject;
(iii) the GCase activity is stable at a level of at least 5 µmol/h/ml in the subject;
(iv) the GCase activity is stable at a level of at least 9 µmol/h/ml in the subject;

(v) an effective dose of the polynucleotide, viral particle or composition is to be administered to the subject;

(vi) the stable GCase activity is a GCase activity of at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% relative to the GCase activity of a healthy subject;

(vii) the stable GCase activity is a GCase activity of between 10% and 100%, between 20% and 90%, between 30% and 70%, between 40% and 70%, or between 50% and 70% relative to the GCase activity of a healthy subject;

(viii) the stable GCase activity is stable for at least 5 weeks from administration;

(ix) the stable GCase activity is stable for at least 10 weeks from administration;

(x) the stable GCase activity is stable for at least 15 weeks from administration;

(xi) the stable GCase activity is stable for at least 20 weeks from administration;

(xii) the stable GCase activity is stable for at least 25 weeks from administration;

(xiii) the stable GCase activity is stable for at least 30 weeks from administration;

(xiv) the stable GCase activity is stable for at least 35 weeks from administration;

(xv) the stable GCase activity is stable for at least 40 weeks after administration;

(xvi) the GCase activity in the liver, spleen, and/or bone marrow of the subject at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, or at least 35 weeks after administration is greater when compared to the activity measured in a subject administered an effective dose of a GCase enzyme replacement therapy, when measured in the same assay at the same time point after administration; and/or

(xvii) the GCase bioavailability in the liver, spleen, and/or bone marrow of the subject over a period of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, or at least 35 weeks after administration is greater when compared to the bioavailability measured in a subject administered an effective dose of a GCase enzyme replacement therapy, when measured in the same assay at the same time point after administration.

31. The use of any of claims 28 to 30, wherein the Gaucher disease is Gaucher disease type I, II or III.

32. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for reducing hexosylceramide and/or hexosylsphingosine levels in a subject suffering from Parkinson's disease or Gaucher disease.

33. The use of claim 32, wherein reducing hexosylceramide and/or hexosylsphingosine levels leads to the treatment of Parkinson's disease or Gaucher disease.

34. The use of claim 32 or 33, wherein:

(i) the hexosylceramide and/or hexosylsphingosine levels are reduced by 2 times or more, 3 times or more, 4 times or more, 5 times or more, 6 times or more, 2 to 3 times, 2 to 4 times, 2 to 5 times, 2 to 6 times, or 3 to 5 times when compared to the hexosylceramide and/or hexosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of any one of claims 1 to 22;

(ii) the reduction in hexosylceramide and/or hexosylsphingosine levels is greater than the reduction achieved in a subject administered an effective dose of a GCase enzyme replacement therapy, optionally when the hexosylceramide and/or hexosylsphingosine levels are measured at least 6 weeks, at least 8 weeks, at least 10 weeks or at least 12 weeks after administration;

(iii) the hexosylceramide and/or hexosylsphingosine levels are measured in the macrophages of the subject;

(iv) the hexosylceramide and/or hexosylsphingosine levels are measured in the spleen of the subject;

(v) the hexosylceramide and/or hexosylsphingosine levels are measured in the liver of the subject;

(vi) the hexosylceramide and/or hexosylsphingosine levels are measured in the serum of the subject;

(vii) the hexosylceramide and/or hexosylsphingosine levels are measured by mass spectrometry; and/or

(viii) the Gaucher disease is Gaucher disease type I, II or III.

35. The polynucleotide, viral particle, or composition for use, or use of any one of claims 23 to 34, 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.

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