

(19) **DANMARK**

(10) **DK/EP 3833746 T5**



Patent- og
Varemærkestyrelsen

(12) Rettet oversættelse af
europæisk patentskrift

-
- (51) Int.Cl.: **C 12 N 9/00 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2024-08-19**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2023-03-29**
- (86) Europæisk ansøgning nr.: **19746522.2**
- (86) Europæisk indleveringsdag: **2019-08-06**
- (87) Den europæiske ansøgnings publiceringsdag: **2021-06-16**
- (86) International ansøgning nr.: **EP2019071158**
- (87) Internationalt publikationsnr.: **WO2020030661**
- (30) Prioritet: **2018-08-08 EP 18306088**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **Genethon, 1 bis rue de, l'Internationale, 91000 Evry, Frankrig**
Institut National de la Santé et de la Recherche Médicale, 101, rue de Tolbiac, 75013 Paris, Frankrig
Sorbonne Université, 21 Rue de l'Ecole de Médecine, 75006 Paris, Frankrig
Universite d'Evry Val d'Essonne, 47 Boulevard François Mitterand, 91000 Evry, Frankrig
Association Institut de Myologie, 47, boulevard de l'Hôpital, 75013 Paris, Frankrig
- (72) Opfinder: **RONZITTI, Giuseppe, 4 Rue du Canada, 77300 Fontainebleau, Frankrig**
VIDAL, Patrice, 12 rue des Docks, 91130 Ris-Orangis, Frankrig
MINGOZZI, Federico, 604 Saint Andrews Road, Philadelphia, PA 19118, USA
- (74) Fuldmægtig i Danmark: **Plougmann Vingtoft A/S, Strandvejen 70, 2900 Hellerup, Danmark**
- (54) Benævnelse: **MINI-GDE TIL BEHANDLINGEN AF GLYKOGENLAGRINGSSYGDOM III**
- (56) Fremdragne publikationer:
WO-A1-2014/130722
PATRICE VIDAL ET AL: "Rescue of GSDIII Phenotype with Gene Transfer Requires Liver- and Muscle-Targeted GDE Expression", MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, vol. 26, no. 3, 27 December 2017 (2017-12-27), pages 890-901, XP055485928, US ISSN: 1525-0016, DOI: 10.1016/j.ymthe.2017.12.019
VIDAL P ET AL: "Adeno associated vector-based gene therapy strategy for type 3 glycogen storage disease", NEUROMUSCULAR DISORDERS, vol. 27, 1 May 2017 (2017-05-01), XP085172746, ISSN: 0960-8966, DOI: 10.1016/J.NMD.2017.06.544
BAODONG SUN ET AL: "Preclinical Development of New Therapy for Glycogen Storage Diseases", CURRENT GENE THERAPY, vol. 15, no. 4, 22 July 2015 (2015-07-22), pages 338-347, XP055397008, NL ISSN: 1566-5232, DOI: 10.2174/1566523215666150630132253
KYLE CHAMBERLAIN ET AL: "Expressing Transgenes That Exceed the Packaging Capacity of Adeno-Associated Virus Capsids", HUMAN GENE THERAPY METHODS, vol. 27, no. 1, 1 February 2016 (2016-02-01),

Fortsættes ...

DESCRIPTION

[0001] The present invention relates to the treatment of glycogen storage disease III (GSDIII).

BACKGROUND OF THE INVENTION

[0002] Mutations in the AGL gene cause genetic deficiency of glycogen debranching enzyme (GDE), or "amylo-alpha-1,6-glucosidase, 4-alpha-glucanotransferase", an enzyme involved in glycogen degradation. GDE has two independent catalytic activities which occur at different sites on the protein: a 4-alpha-glucotransferase activity and an amylo-1,6-glucosidase activity. Genetic deficiency of GDE causes an incomplete glycogenolysis in glycogen storage disease III (GSD III), resulting in accumulation of abnormal glycogen with short outer chain in various organs, mostly liver and muscle. The disease is characterized by hepatomegaly, hypoglycemia, short stature, variable myopathy and cardiomyopathy. Most patients have diseases involving both liver and muscle (type IIIa), while some patients (~15 percent) have only liver involvement (type IIIb). Liver symptoms normally occur in childhood. Liver cirrhosis and hepatocellular carcinoma have been reported in some cases (Chen et al., 2009, Scriver's Online Metabolic & Molecular Bases of inherited Disease, New York: McGraw-Hill; Kishnani et al., 2010, Genet Med 12, 446-463). Muscle weakness could be present during childhood. It becomes more prevalent in adults with onset in the third or fourth decade. There is significant morbidity from progressive muscle weakness and patients in later stages can become wheel chair bound. Patients can also develop cardiomyopathy. There is significant clinical variability in the severity of the symptoms that these patients develop. The progressive myopathy and/or cardiomyopathy and/or peripheral neuropathy are major causes of morbidity in adults (Kishnani et al., 2010, Genet Med 12, 446-463; Cornelio et al., 1984, Arch Neurol 41, 1027-1032; Coleman et al., 1992, Ann Intern Med 116, 896-900). Reports of possible neurological manifestations associated with the disease derive from clinicians working with GSDIII patients, who reported attention fluctuations, deficiencies in executive functions and impaired emotional skills (Michon et al., 2015, J Inherit Metab Dis, 38(3): 573-580). Accordingly, in the GDE-/- mouse model of the disease, an extensive accumulation of glycogen throughout the nervous system was documented (Pagliarani et al., 2014, Biochim Biophys Acta, 1842(11): 2318-2328; Liu et al., 2014, Mol Genet Metab, 111(4): 467-476) although a careful characterization of the phenotype associated with the accumulation of glycogen is still missing. Current treatment is symptomatic, and there is no effective therapy for the disease. Hypoglycemia can be controlled by frequent meals high in carbohydrates with cornstarch supplements or nocturnal gastric drip feedings. Patients with myopathy have been treated with a diet high in protein during the daytime plus overnight enteral infusion. In some patients transient improvement in symptoms has been documented, but there are no systemic studies or long-term data demonstrating that the high protein diet prevents or treats the progressive myopathy (Kishnani et al., 2010, Genet Med 12, 446-463). These approaches do little to alter the long term course and morbidity of these diseases. Vidal P et al., "Rescue of GSDIII phenotype with gene transfer requires liver- and muscle-targeted GDE expression", Molecular Therapy, vol. 26, no.3,

doi:10.1016/j.ymthe.2017.12.019, December 2017, describes gene therapy in a mouse model of glycogen storage disease III (Fabry Cori disease). Gene therapy with GDE expressed in liver in a dual vector system had direct impact on glucose metabolism. Proof of concept that GSDIII can be corrected with AAV vectors, but does not disclose improvements of vector therapy using shortened GDE. Baodong S et al., "Preclinical development of new therapy for glycogen storage diseases", *Curr Gen Therapy*, vol.15, no. 4, pp. 338-347, 15 July 2015, is a review on treatment options of glycogen storage diseases, among them GSDIII. Discussed is the possibility of gene therapy, it mentions the option of dual vector AAV-based therapy, but there is no hint to instead shorten GDE and use only one vector. It rather suggests as an alternative of dual vector therapy the administration of small molecules that penetrate into liver and muscle and inhibit their glycogen synthesis.

[0003] Therefore, there is still a need for a long-term treatment of GSD III. Gene therapy aiming to stably replace the GDE protein in the affected tissues appears as a potential therapeutic approach. However, the large size of the GDE transgene constitutes a major impediment since it cannot fit the size limit of most gene therapy vectors. Indeed, the human AGL gene is 85 kb in length and composed of 35 exons, encoding a 7.4-kb mRNA that includes a 4596-bp coding region and a 2371-bp 3' untranslated sequence to express a 175 kDa GDE protein (Bao Y et al., 1996, *Genomics*, 38(2):155-65). This constitutes a real issue since the minimum size of a GDE expression cassette (including for example at least a promoter, the GDE coding sequence, a polyA signal and the two ITRs for an AAV vector) would be larger than 5 kb, the genome size limit that can be packaged into an AAV gene therapy vector for in vivo gene delivery. The inventors have previously proposed the use of dual AAV vectors to overcome this size limitation. (Vidal P et al., "Adeno-associated vector-based gene therapy strategy for type glycogen storage disease", *Neuromuscular disorders*, vol. 27, doi:10.1016/J.NMD.2017.06.544, 01 May 2017).

[0004] Following this approach, two vectors, each containing a portion of the large transgene coding sequence, are used to transduce the same cell. Although the use of dual AAV vectors is promising, it would be preferable to provide a gene therapy strategy implementing only one viral vector for both economic and practical reasons.

[0005] There is therefore a need for novel strategies to improve gene therapy in the treatment of GSD III.

SUMMARY OF THE INVENTION

[0006] A first aspect of the invention relates to a functional truncated human GDE polypeptide, which is deleted of at least about 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or at least about 525 amino acids with respect to a reference full-length human GDE sequence. In a particular embodiment, the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41.

[0007] In particular embodiments:

1. (i) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1, and said truncated human GDE polypeptide comprises at least the amino acid residues at positions 429-666, 770-892, 1088-1194, and 1235-1532 with respect to SEQ ID NO:1 ;
2. (ii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40, and said truncated human GDE polypeptide comprises at least the amino acid residues at positions 412-649, 753-875, 1071-1177, 1218-1515 with respect to SEQ ID NO:40; or
3. (iii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41, and said truncated human GDE polypeptide comprises at least the amino acid residues at positions 413-650, 754-876, 1072-1178, 1219-1516 with respect to SEQ ID NO:41 .

[0008] In other embodiments:

1. (i) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1, and the deleted amino acids are at least one amino acid at positions 1-428, 668-769, 895-1087 and/or 1195-1232 with respect to SEQ ID NO:1 ;
2. (ii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40, and the deleted amino acids are at least one amino acid at positions 1-411, 651-752, 878-1070 and/or 1178-1215 with respect to SEQ ID NO:40; or
3. (iii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41, and the deleted amino acids are at least one amino acid at positions 1-412, 652-753, 879-1071 and/or 1179-1216 with respect to SEQ ID NO:41.

[0009] In yet other embodiments:

1. (i) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1, and said truncated human GDE polypeptide is deleted of :
 - at least one amino acid selected from amino acids at positions 1 to 428 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 428 with respect to SEQ ID NO:1 ; and/or
 - at least one amino acid selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1 ; and / or
 - at least one amino acid selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125,

- 150, 175, or at least 200 consecutive amino acids selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1 ; or
2. (ii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40, and said truncated human GDE polypeptide is deleted of :
- at least one amino acid selected from amino acids at positions 1 to 411 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 411 with respect to SEQ ID NO:40 ; and / or
 - at least one amino acid selected from amino acids at positions 651-752 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 651-752 with respect to SEQ ID NO:40 ; and / or
 - at least one amino acid selected from amino acids at positions 878-1070 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 200 consecutive amino acids selected from amino acids at positions 878-1070 with respect to SEQ ID NO:40 ; and / or
 - at least one amino acid selected from amino acids at positions 1178-1215 with respect to SEQ ID NO:40, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1178-1215 with respect to SEQ ID NO:40 ; or
3. (iii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41, and said truncated human GDE polypeptide is deleted of :
- at least one amino acid selected from amino acids at positions 1 to 412 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 412 with respect to SEQ ID NO:41 ; and / or
 - at least one amino acid selected from amino acids at positions 652-753 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 652-753 with respect to SEQ ID NO:41 ; and / or
 - at least one amino acid selected from amino acids at positions 879-1071 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 200 consecutive amino acids selected from amino acids at positions 879-1071 with respect to SEQ ID NO:41 ; and / or
 - at least one amino acid selected from amino acids at positions 1179-1216 with respect to SEQ ID NO:41, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1179-1216 with respect to SEQ ID NO:41.

[0010] In further embodiments:

1. (i) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:1, wherein the deletion is selected from the group consisting of :
 - deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1195 to 1232 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 223 to 320 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 360 to 428 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 15 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 30 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 81 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 103 with respect to SEQ ID NO:1 ; and
 - deletion of amino acids from position 1 to 129 with respect to SEQ ID NO:1 ; or
2. (ii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:40, wherein the deletion is selected from the group consisting of:
 - deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 344 to 411 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 651 to 752 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 878 to 1070 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1178 to 1215 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 206 to 303 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 343 to 411 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 652 to 703 with respect to SEQ ID NO:40 ;
 - 40deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 408 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 213 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 13 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 64 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 86 with respect to SEQ ID NO:40 ; and
 - deletion of amino acids from position 1 to 112 with respect to SEQ ID NO:40 ; or
3. (iii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:41, wherein the deletion is selected from

the group consisting of:

- deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 345 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 652 to 753 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 879 to 1071 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1179 to 1216 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 207 to 304 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 344 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 653 to 704 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 409 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 214 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 14 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 65 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 87 with respect to SEQ ID NO:41 ; and
- deletion of amino acids from position 1 to 113 with respect to SEQ ID NO:41.

[0011] In further particular embodiments:

1. (i) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:1, wherein the deletion is selected from the group consisting of :
 - deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1195 to 1232 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 223 to 320 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 360 to 428 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1 ;
 - deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1 ; and
 - deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1 ; or
2. (ii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:40, wherein the deletion is selected from the group consisting of:
 - deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 344 to 411 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 651 to 752 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 878 to 1070 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1178 to 1215 with respect to SEQ ID NO:40 ;

- deletion of amino acids from position 206 to 303 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 343 to 411 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 652 to 703 with respect to SEQ ID NO:40 ;
 - 40deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40 ;
 - deletion of amino acids from position 1 to 408 with respect to SEQ ID NO:40 ; and
 - deletion of amino acids from position 1 to 213 with respect to SEQ ID NO:40 ; or
3. (iii) the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41 and the functional truncated human GDE polypeptide comprises at least one deletion with respect to SEQ ID NO:41, wherein the deletion is selected from the group consisting of:
- deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 345 to 412 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 652 to 753 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 879 to 1071 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 1179 to 1216 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 207 to 304 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 344 to 412 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 653 to 704 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41 ;
 - deletion of amino acids from position 1 to 409 with respect to SEQ ID NO:41 ; and
 - deletion of amino acids from position 1 to 214 with respect to SEQ ID NO:41.

[0012] In another particular embodiment, the functional truncated human GDE polypeptide of the invention comprises a deletion or a combination of deletions as shown in table 2 below. In a particular embodiment, the functional truncated human GDE polypeptide comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 below, and
2. (ii) a deletion or a combination of deletions, as shown in table 3 below.

[0013] Such functional truncated human GDE polypeptides include, without limitation, those having a sequence selected from SEQ ID NO:2-10 and SEQ ID NO:48-52, in particular from SEQ ID NO:2-6. In a particular embodiment, the functional truncated human GDE polypeptide of the invention has a sequence comprising or consisting of the sequence selected from SEQ ID NO:2, 5 or 6. In a further particular embodiment, the functional truncated human GDE polypeptide of the invention has a sequence comprising or consisting of the sequence shown in SEQ ID NO:5.

[0014] In another aspect, the invention relates to a nucleic acid molecule encoding the functional truncated human GDE polypeptide disclosed herein.

[0015] In a further aspect, the invention relates to a nucleic acid construct, comprising,

preferably in this order:

- a promoter;
- optionally, an intron;
- the nucleic acid molecule disclosed herein, encoding the functional truncated human polypeptide of the invention; and
- a polyadenylation signal.

[0016] In yet another aspect, the invention relates to a vector comprising :

- the nucleic acid molecule of the invention; or
- the nucleic acid construct of the invention.

[0017] In certain embodiments, the vector may be a viral vector.

[0018] According to a further aspect, the invention relates to a viral vector comprising a nucleic acid construct encoding a functional non-human GDE polypeptide, wherein the functional non-human GDE polypeptide comprises less than about 1500 amino acids. The functional non-human GDE polypeptide may be selected, without limitation, in the group consisting of : horse GDE polypeptide of SEQ ID NO:11, gorilla GDE polypeptide of SEQ ID NO:12 , orangutan GDE polypeptide of SEQ ID NO:13, Pteropus alecto GDE polypeptide of SEQ ID NO:14, sooty mangabey GDE polypeptide of SEQ ID NO:15, platypus GDE polypeptide of SEQ ID NO:16, and duck GDE polypeptide of SEQ ID NO:17. In a particular embodiment, the functional non-human GDE polypeptide is the gorilla GDE polypeptide of SEQ ID NO:12.

[0019] In particular embodiments of the vectors of the invention, said vectors may be AAV vectors or retroviral vectors, such as a lentiviral vectors. In a particular embodiment, the vector is an AAV vector, such as a single-stranded or double-stranded self-complementary AAV vector, preferably an AAV vector with an AAV-derived capsid, such as an AAV1, AAV2, variant AAV2, AAV3, variant AAV3, AAV3B, variant AAV3B, AAV4, AAV5, AAV6, variant AAV6, AAV7, AAV8, AAV9, AAV9P1, AAV10 such as AAVcy10 and AAVrh10, AAVrh74, AAVdj, AAV-Anc80, AAV-LK03, AAV2i8, and porcine AAV, such as AAVpo4 and AAVpo6 capsid or with a chimeric capsid. In a particular embodiment, the AAV vector has an AAV9, AAV9P1 or AAV6 capsid.

[0020] In a further aspect, the invention relates to an isolated cell transformed with the nucleic acid molecule, the nucleic acid construct or the vector of the invention. The cell may be, for example, a liver cell, a muscle cell, a cardiac cell or a CNS cell.

[0021] Yet in another aspect, the invention relates to a pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the functional truncated human GDE polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the

invention.

[0022] The invention also relates, in a particular aspect, to the functional truncated human GDE polypeptide, the functional non-human GDE polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the invention, for use as a medicament.

[0023] In a further aspect, the invention relates to the functional truncated human GDE polypeptide, the functional non-human GDE polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the invention, for use in a method for treating GSDIII (Cori disease).

[0024] The present invention also relates to the gorilla GDE polypeptide of SEQ ID NO:12, for use in a method for treating GSDIII (Cori disease).

LEGENDS TO THE FIGURES

[0025]

Figure 1. Reduced size of non-human mammalian GDE sequences. The size, expressed as amino acids number, of different mammalian GDE proteins is reported (paGDE: Pteropus Alecto GDE sequence, oGDE: orangutan GDE sequence, gGDE: gorilla GDE sequence, hoGDE: horse GDE sequence, hGDE: human isoform 1 GDE sequence).

Figure 2. Schematic representation of the different truncations on the human GDE sequence. Three different human GDE truncations, $\Delta 1$, $\Delta 2-3$ and $\Delta 4$ are reported.

Figure 3. gGDE protein expression. GDE knock-out (KO) mice were injected with 1×10^{12} vg/mouse of a single AAV9 vector expressing gorilla GDE (gGDE). Three months after vector injection, animals were sacrificed and GDE was detected by western-blot in the heart. Wild-type (WT) GDE-KO animals were used as positive and negative controls, respectively.

Figure 4. AAV9-gGDE vector efficiently clears glycogen from quadriceps. GDE knock-out (KO) mice were injected with 1×10^{12} vg/mouse of a single AAV9 vector expressing gorilla GDE (AAV9-gGDE) or with 2×10^{12} vg/mouse of a dual AAV9 vectors expressing human GDE (AAV9-GDEov). Three months after vector injection, animals were sacrificed and glycogen accumulation was measured in the quadriceps. In parallel, glycogen was measured in age-matched wild-type (WT) and GDE knock-out (KO) animal. Statistical analysis was performed by ANOVA (** = $p < 0.001$, *** = $p < 0.0001$, ns = not significant).

Figure 5. AAV9-gGDE vector rescues muscle function in GSDIII mice. GDE knock-out (KO) mice were injected with 1×10^{12} vg/mouse of a single AAV9 vector expressing gorilla GDE (AAV9-gGDE) or with 2×10^{12} vg/mouse of a dual AAV9 vectors expressing human GDE (AAV9-GDEov). Three months after vector injection, muscle function was scored by wire-hang test. In

the graph are showed wire-hang performances measured in age-matched wild-type (WT) and GDE knock-out (KO) animal. Statistical analysis was performed by ANOVA (**** = $p < 0.0001$, ns = not significant).

Figure 6. Measurement of truncated GDEs activity in vitro. Huh-7 cells were transfected with plasmids expressing full-size human GDE (hGDE), one truncated human GDE (GDE $\Delta 4$) or gorilla GDE (gGDE) under the control of a CMV promoter. In parallel cells were transfected with a GFP-expressing plasmid as control. 48 hours after transfection, cytosolic extracts were prepared and GDE activity was measured. In the histogram are shown the levels of GDE activity expressed as glucose released from the digestion of limited dextrin. Statistical analysis was performed by ANOVA (* = $p < 0.05$ vs CMV-hGDE).

Figure 7. Truncated GDEs are produced in vivo. Tibialis anterior (TA) muscles of GDE knock-out mice were injected with 1×10^{11} vg/mouse of an AAV9 vector expressing a truncated human GDE (AAV9- $\Delta 1$ -GDE) or gorilla GDE (AAV9-gGDE) or with 2×10^{11} vg/mouse of a dual AAV9 vectors expressing human, full-size GDE (AAV9-GDEov). 15 days after the injection, TA were obtained and processed to analyze the expression of GDE by Western blot. Actin was used as loading control.

Figure 8. Truncated GDEs are active in vivo. Tibialis anterior (TA) muscles of GDE knock-out mice were injected with 1×10^{11} vg/mouse of an AAV9 vector expressing truncated human GDEs (AAV9- $\Delta 1$ -GDE, AAV9- $\Delta 4$ -GDE, AAV9- $\Delta 2/3$ -GDE) derived from either the wild type (wt) or a codon optimized (co) human GDE coding sequence. In parallel mice were injected with 1×10^{11} vg/mouse of an AAV9 vector expressing the codon optimized gorilla GDE (AAV9-gGDEco) or with PBS as control. 15 days after the injection, TA were obtained and processed to analyze the GDE activity.

Figure 9. Truncated GDEs are produced in vitro. HEK293T cells were transfected with plasmids expressing full-size GDE (GDEfs) or 5 truncated human GDEs ($\Delta 9 + \Delta 2/3$; $\Delta 10 + \Delta 2/3$; $\Delta 11 + \Delta 2/3$; $\Delta 12 + \Delta 2/3$; $\Delta 13 + \Delta 2/3$). GFP transfected cells were used as control. 3 days after transfection, cells were harvested and processed to analyze the expression of GDE by Western blot.

Figure 10. Truncated GDEs are produced in vivo. Tibialis anterior (TA) muscles of GDE knock-out mice were injected with 2×10^{11} vg/mouse of an AAV9 vector expressing full-size GDE (GDEfs) or 7 truncated human GDEs ($\Delta 2/3$; $\Delta 9 + \Delta 2/3$; $\Delta 10 + \Delta 2/3$; $\Delta 13 + \Delta 2/3$; and $\Delta 1$). 15 days after the injection, TA were obtained and processed to analyze the expression of GDE by Western blot.

DETAILED DESCRIPTION OF THE INVENTION

[0026] As used herein with respect to any disclosed values or ranges, the term "about"

indicates that the stated numerical value allows for slight imprecision, e.g., reasonably close to the value or nearly, such as plus or minus 10 %, in particular such as plus or minus 5%, of the stated values or ranges.

[0027] Despite the lack of knowledge regarding the three-dimensional structure of the GDE protein, the present inventors have identified GDE polypeptides whose coding sequences are small enough to be packaged into a gene therapy vector, while preserving the GDE functionality, otherwise referred to as "mini-GDE polypeptides".

[0028] By "gene therapy vector" is meant any vector suitable for gene therapy. In particular, the gene therapy vector may be a plasmid or a recombinant virus such as a viral vector derived from a retrovirus or a lentivirus. Preferably, the viral vector is an AAV vector, such as an AAV vector suitable for transducing liver tissues or muscle cells. Extensive experience in clinical trials and in preclinical model of muscle diseases indicates adeno-associated virus (AAV) as the vector of choice for in vivo gene therapy for GSDIII. These vectors efficiently transduce liver and muscle, their production is scalable and compared to other gene therapy vectors they have a relatively low immunogenicity profile. However, one of the biggest limitations in the use of AAV for gene replacement is their limited encapsidation size limit (about 5 kb). Indeed, during recombinant AAV production, genomes larger than 5 kb are encapsidated with low efficacy and the resulting AAV may contain fragmented genomes reducing the efficacy of gene transfer.

[0029] As will be explained in details below, in the context of the present invention, the expression "mini-GDE polypeptide" encompasses either (i) functional truncated human GDE polypeptides or (ii) functional non-human GDE polypeptides.

[0030] A first aspect of the present invention is thus a functional mini-GDE polypeptide whose coding sequence is small enough to be efficiently packaged into a single AAV vector.

[0031] By "functional" GDE polypeptide is meant a polypeptide that retains, at least in part, at least one of the enzymatic activities of the GDE protein, preferably all of the enzymatic activities of the GDE protein. As a consequence, the functional GDE polypeptide implemented in the present invention is able to rescue glycogen accumulation and muscle strength *in vivo*. As defined above, GDE enzymatic activities are a 4-alpha-glucotransferase activity and an amylo-1,6-glucosidase activity, involved in glycogen degradation. The transferase activity of GDE relocates three glucose units of glycogen from one chain to another. This leaves one glucose unit at the branch point, which is subsequently released as glucose by the glucosidase activity. In a particular embodiment, the functional mini-GDE polypeptide of the invention has the same functionality as a full-length GDE polypeptide, in particular as a full-length human GDE polypeptide. For example, a functional mini-GDE polypeptide of the invention may have an activity of at least 50 %, 60 %, 70 %, 80 %, 90 %, 95 %, or at least 99 % in relation to one, preferably both, enzymatic activities described above, or at least 100 %, as compared to a full-length human GDE protein, in particular the full-length human GDE protein of SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41. The activity of the mini-GDE protein of the invention may

even be of more than 100 %, such as of more than 110 %, 120 %, 130 %, 140 %, 150%, 200%, 500%, 700%, or even more than 1000 % of the activity of a full-length human GDE protein, in particular the full-length human GDE protein of SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41.

[0032] A skilled person is readily able to determine whether a polypeptide is a functional GDE polypeptide. Suitable methods would be apparent to those skilled in the art. For example, one suitable in vitro method involves inserting a nucleic acid encoding a polypeptide into a vector, such as a plasmid or viral vector, transfecting or transducing host cells, such as 293T or HeLa cells, or other cells such as Huh7, with the vector, and assaying for GDE activity. Suitable methods are described in more details in the experimental part below. For example, GDE activity may be determined by measuring the glucose produced after incubating homogenized mouse tissues with limit dextrin. Other methods include testing the GDE activity by determining GDE expression in tissues of a GDE KO animal, such as by western-blot, by following the glucose produced from glycogen phosphorylase-digested glycogen, by evaluating muscle strength of treated GDE-KO animals by wire-hang after administration of the vectors, such as after one, two or three months after administration, or by evaluating the rescue of glycogen accumulation in muscle and/or cardiac tissue.

[0033] In a first variant of the first aspect of the invention, the mini-GDE polypeptide is a functional truncated human GDE polypeptide, which is truncated with respect to a reference full-length human GDE sequence.

[0034] The term "truncated human GDE polypeptide" encompasses any human GDE polypeptide that is rendered shorter by amino acid deletion, with respect to a reference full-length human GDE sequence from which the truncated human GDE is derived. In particular, the functional truncated human GDE polypeptide is deleted of at least 1 amino acid with respect to a reference full-length human GDE sequence. Preferably, the functional truncated human GDE polypeptide is deleted of at least about 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or at least about 525 amino acids with respect to a reference full-length human GDE sequence. In a preferred embodiment, the functional truncated human GDE polypeptide is deleted of at least about 50, 100 or 150 amino acids with respect to a reference full-length human GDE sequence.

[0035] In a particular embodiment, the functional truncated human GDE polypeptide that is truncated with respect to a reference full-length human GDE sequence may comprise one or more additional amino acid modifications with respect to said reference full-length human GDE sequence. In particular, in addition to the deletion(s) that are further described below, the functional truncated human GDE polypeptide may comprise one or more amino acid modifications such as amino acid insertion, deletion and/or substitution as compared to the reference full-length human GDE sequence. For example, the functional truncated human GDE polypeptide may comprise from 1 to 10 (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) additional amino acid modifications, in particular from 1 to 5 (e.g. 1, 2, 3, 4 or 5) additional amino acid modifications, as long as the functionality of the truncated human GDE polypeptide is

preserved. In a particular embodiment, when the functional truncated human GDE polypeptide comprises a N-terminal deletion, a methionine can be added at the N-terminal end.

[0036] In the context of the present invention, "a reference full-length human GDE sequence" encompasses all native isoforms of human GDE. Bao and colleagues (Genomics, 1997, 38, 155-165) identified the presence of six transcript variants encoding for three GDE protein isoforms. Transcript variants 1-4 encode for the same protein, namely GDE isoform 1. Transcript variants 5 and 6 encode for GDE isoforms 2 and 3 respectively.

[0037] The term "reference full-length human GDE polypeptide" thus encompasses all native isoforms of human GDE including the precursor form, as well as modified or mutated by insertion(s), deletion (s) and/or substitution(s) GDE proteins or fragments thereof that are functional derivatives of GDE. In particular, the reference full-length human GDE sequence is selected from the group consisting of SEQ ID NO:1 (corresponding to GDE isoform 1), SEQ ID NO:40 (corresponding to GDE isoform 2) and SEQ ID NO:41 (corresponding to GDE isoform 3).

[0038] In a particular embodiment, the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:1, which corresponds to the GDE isoform 1.

[0039] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 429-666, 770-892, 1088-1194, 1235-1532 with respect to SEQ ID NO:1.

[0040] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 429-667, 770-894, 1088-1194, 1233-1532 with respect to SEQ ID NO:1.

[0041] In another particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of at least one amino acid with respect to SEQ ID NO:1, wherein the deleted amino acid(s) is at least one amino acid at positions 1-428, 668-769, 895-1087 and/or 1195-1232 with respect to SEQ ID NO:1. In a further particular embodiment, the functional truncated human GDE polypeptide is deleted of at least about 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or at least about 525 amino acids, wherein the deleted amino acid(s) are selected from any amino acids at positions 1-428, 668-769, 895-1087, and/or 1195-1232 with respect to SEQ ID NO:1. In this embodiment, the deleted amino acids may be consecutive amino acids or non-consecutive amino acids, as long as they are selected from any amino acids at positions 1-428, 668-769, 895-1087 and/or 1195-1232 with respect to SEQ ID NO:1

[0042] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 428 with respect to

SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 428 with respect to SEQ ID NO:1 ; and/or

- at least one amino acid selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 190 consecutive amino acids selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1.

[0043] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of :

- at least one amino acid selected from amino acids at positions 1 to 428 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 110 or at least 120 consecutive amino acids selected from amino acids at positions 1 to 428 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 190 consecutive amino acids selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1.

[0044] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 156 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 156 with respect to SEQ ID NO:1 ; and / or

- at least one amino acid selected from amino acids at positions 361 to 428 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 361 to 428 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 223 to 320 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 223 to 320 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 360 to 428 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 360 to 428 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 669 to 720 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 669 to 720 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 280 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 280 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 425 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 425 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 230 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 230 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 15, with respect to SEQ ID NO:1, preferably at least 10 consecutive amino acids selected from amino acids at positions 1 to 15 with respect to SEQ ID NO:1 ; and / or

- at least one amino acid selected from amino acids at positions 1 to 30 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 25 consecutive amino acids selected from amino acids at positions 1 to 30 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 81 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 81 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 103 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 103 with respect to SEQ ID NO:1 ; and/or
- at least one amino acid selected from amino acids at positions 1 to 129 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 129 with respect to SEQ ID NO:1.

[0045] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of :

- at least one amino acid selected from amino acids at positions 1 to 156 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 156 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 361 to 428 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 361 to 428 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 668 to 769 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 895 to 1087 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids selected from amino acids at positions 1195 to 1232 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 223 to 320 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive

amino acids selected from amino acids at positions 223 to 320 with respect to SEQ ID NO:1 ; and / or

- at least one amino acid selected from amino acids at positions 360 to 428 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 360 to 428 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 669 to 720 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 669 to 720 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 280 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 280 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 425 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 425 with respect to SEQ ID NO:1 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 230 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 230 with respect to SEQ ID NO:1.

[0046] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:1, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1195 to 1232 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 223 to 320 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 360 to 428 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 15 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 30 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 81 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 103 with respect to SEQ ID NO:1 ; and
- deletion of amino acids from position 1 to 129 with respect to SEQ ID NO:1.

[0047] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:1, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1195 to 1232 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 223 to 320 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 360 to 428 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1 ;
- deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1 ; and
- deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1.

[0048] For the sake of clarity, in this embodiment, the deletion relates to the deletion of all consecutive amino acids in the mentioned range of positions. For example, a functional truncated human GDE polypeptide comprising the deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 156 with respect to SEQ ID NO:1.

[0049] Also for the sake of clarity, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1 ; and
- the deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 280, since the range 1-156 is included in the range 1-280.

[0050] In addition, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1 ; and
- the deletion of amino acids from position 223 to 320 with respect to SEQ ID NO:1

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 320, since the range 1-280 overlaps the range 223-320.

[0051] In another embodiment, the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:40, which corresponds to GDE isoform 2.

[0052] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 412-649, 753-875, 1071-1177, 1218-1515 with respect to SEQ ID NO:40.

[0053] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 412-650, 753-877, 1071-1177, 1216-1515 with respect to SEQ ID NO:40.

[0054] In another particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of at least one amino acid with respect to SEQ ID NO:40, wherein the deleted amino acid(s) is at least one amino acid at positions 1-411, 651-752, 878-1070 and/or 1178-1215 with respect to SEQ ID NO:40. In a further particular embodiment, the functional truncated human GDE polypeptide is deleted of at least about 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or at least about 525 amino acids, wherein the deleted amino acid(s) are selected from any amino acids at positions 1-411, 651-752, 878-1070, and/or 1178-1215 with respect to SEQ ID NO:40. In this embodiment, the deleted amino acids may be consecutive amino acids or non-consecutive amino acids, as long as they are selected from any amino acids at positions 1-411, 651-752, 878-1070 and/or 1178-1215 with respect to SEQ ID NO:40.

[0055] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 411 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 411 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 651-752 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 651-752 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 878-1070 with respect to SEQ ID NO:40, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 190 consecutive amino acids selected from amino acids at positions 878-1070 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1178-1215 with respect to SEQ ID NO:40, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1178-1215 with respect to SEQ ID NO:40.

[0056] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 139 with respect to

SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 139 with respect to SEQ ID NO:40 ; and / or

- at least one amino acid selected from amino acids at positions 344 to 411 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 344 to 411 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 651 to 752 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 651 to 752 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 878 to 1070 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 878 to 1070 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1178 to 1215 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids selected from amino acids at positions 1178 to 1215 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 206 to 303 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 206 to 303 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 343 to 411 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 343 to 411 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 652 to 703 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 652 to 703 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 263 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 263 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 408 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 408 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 213 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 213 with respect to SEQ ID NO:40 ; and / or

- at least one amino acid selected from amino acids at positions 1 to 13 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 25 consecutive amino acids selected from amino acids at positions 1 to 13 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 64 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 64 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 86 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 86 with respect to SEQ ID NO:40 ; and/or
- at least one amino acid selected from amino acids at positions 1 to 112 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 112 with respect to SEQ ID NO:40.

[0057] In another particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of :

- at least one amino acid selected from amino acids at positions 1 to 139 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 139 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 344 to 411 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 344 to 411 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 651 to 752 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 651 to 752 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 878 to 1070 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 878 to 1070 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1178 to 1215 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids selected from amino acids at positions 1178 to 1215 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 206 to 303 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive

amino acids selected from amino acids at positions 206 to 303 with respect to SEQ ID NO:40 ; and / or

- at least one amino acid selected from amino acids at positions 343 to 411 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 343 to 411 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 652 to 703 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 652 to 703 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 263 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 263 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 408 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 408 with respect to SEQ ID NO:40 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 213 with respect to SEQ ID NO:40, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 213 with respect to SEQ ID NO:40.

[0058] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:40, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 344 to 411 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 651 to 752 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 878 to 1070 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1178 to 1215 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 206 to 303 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 343 to 411 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 652 to 703 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 408 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 213 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 13 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 64 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 86 with respect to SEQ ID NO:40 ; and
- deletion of amino acids from position 1 to 112 with respect to SEQ ID NO:40.

[0059] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:40, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 344 to 411 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 651 to 752 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 878 to 1070 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1178 to 1215 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 206 to 303 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 343 to 411 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 652 to 703 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40 ;
- deletion of amino acids from position 1 to 408 with respect to SEQ ID NO:40 ; and
- deletion of amino acids from position 1 to 213 with respect to SEQ ID NO:40.

[0060] For the sake of clarity, in this embodiment, the deletion relates to the deletion of all consecutive amino acids in the mentioned range of positions. For example, a functional truncated human GDE polypeptide comprising the deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 139 with respect to SEQ ID NO:40.

[0061] Also for the sake of clarity, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 139 with respect to SEQ ID NO:40 ; and
- the deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 263, since the range 1-139 is included in the range 1-263.

[0062] In addition, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 263 with respect to SEQ ID NO:40 ; and
- the deletion of amino acids from position 206 to 303 with respect to SEQ ID NO:40

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 303, since the range 1-263 overlaps the range 206-303.

[0063] In another embodiment, the reference full-length human GDE sequence has an amino acid sequence as shown in SEQ ID NO:41, which corresponds to GDE isoform 3.

[0064] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 413-650, 754-876, 1072-1178, 1219-1516 with respect to SEQ ID NO:41.

[0065] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least the amino acid residues at positions 413-651, 754-878, 1072-1178, 1217-1516 with respect to SEQ ID NO:41.

[0066] In another particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of at least one amino acid with respect to SEQ ID NO:41, wherein the deleted amino acid(s) is at least one amino acid at positions 1-412, 652-753, 879-1071 and/or 1179-1216 with respect to SEQ ID NO:41. In a further particular embodiment, the functional truncated human GDE polypeptide is deleted of at least about 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or at least about 525 amino acids, wherein the deleted amino acid(s) are selected from any amino acids at positions 1-412, 652-753, 879-1071, and/or 1179-1216 with respect to SEQ ID NO:41. In this embodiment, the deleted amino acids may be consecutive amino acids or non-consecutive amino acids, as long as they are selected from any amino acids at positions 1-412, 652-753, 879-1071 and/or 1179-1216 with respect to SEQ ID NO:41.

[0067] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 412 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350 or at least 400 consecutive amino acids selected from amino acids at positions 1 to 412 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 652-753 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 80 or at least 100 consecutive amino acids selected from amino acids at positions 652-753 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 879-1071 with respect to SEQ ID NO:41, preferably at least 10, 15, 20, 30, 40, 50, 80, 100, 125, 150, 175, or at least 190 consecutive amino acids selected from amino acids at positions 879-1071 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1179-1216 with respect to SEQ ID NO:41, preferably at least 10, 15, 20 or at least 30 consecutive amino acids selected from amino acids at positions 1179-1216 with respect to SEQ ID NO:41.

[0068] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 140 with respect to

SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 140 with respect to SEQ ID NO:41 ; and / or

- at least one amino acid selected from amino acids at positions 345 to 412 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 345 to 412 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 652 to 753 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 652 to 753 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 879 to 1071 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 879 to 1071 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1179 to 1216 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids selected from amino acids at positions 1179 to 1216 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 207 to 304 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 207 to 304 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 344 to 412 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 344 to 412 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 653 to 704 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 653 to 704 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 264 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 264 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 409 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 409 with respect to SEQ ID NO:41 ;
- at least one amino acid selected from amino acids at positions 1 to 214 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 214 with respect to SEQ ID NO:41; and / or

- at least one amino acid selected from amino acids at positions 1 to 14 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 25 consecutive amino acids selected from amino acids at positions 1 to 14 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 65 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 65 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 87 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 1 to 87 with respect to SEQ ID NO:41 ; and/or
- at least one amino acid selected from amino acids at positions 1 to 113 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 113 with respect to SEQ ID NO:41.

[0069] In a particular embodiment, the functional truncated human GDE polypeptide of the invention is deleted of:

- at least one amino acid selected from amino acids at positions 1 to 140 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 140 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 345 to 412 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 345 to 412 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 652 to 753 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 652 to 753 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 879 to 1071 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids, at least 100 consecutive amino acids, at least 150 consecutive amino acids, at least 175 consecutive amino acids or at least 190 consecutive amino acids selected from amino acids at positions 879 to 1071 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1179 to 1216 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids selected from amino acids at positions 1179 to 1216 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 207 to 304 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive

amino acids selected from amino acids at positions 207 to 304 with respect to SEQ ID NO:41 ; and / or

- at least one amino acid selected from amino acids at positions 344 to 412 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 344 to 412 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 653 to 704 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids or at least 50 consecutive amino acids selected from amino acids at positions 653 to 704 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 264 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 264 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 409 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 409 with respect to SEQ ID NO:41 ; and / or
- at least one amino acid selected from amino acids at positions 1 to 214 with respect to SEQ ID NO:41, preferably at least 15 consecutive amino acids, at least 50 consecutive amino acids or at least 100 consecutive amino acids selected from amino acids at positions 1 to 214 with respect to SEQ ID NO:41.

[0070] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:41, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 345 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 652 to 753 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 879 to 1071 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1179 to 1216 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 207 to 304 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 344 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 653 to 704 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 409 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 214 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 14 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 65 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 87 with respect to SEQ ID NO:41 ; and
- deletion of amino acids from position 1 to 113 with respect to SEQ ID NO:41.

[0071] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises at least one deletion with respect to SEQ ID NO:41, wherein the deletion is selected from the group consisting of:

- deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 345 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 652 to 753 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 879 to 1071 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1179 to 1216 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 207 to 304 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 344 to 412 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 653 to 704 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41 ;
- deletion of amino acids from position 1 to 409 with respect to SEQ ID NO:41 ; and
- deletion of amino acids from position 1 to 214 with respect to SEQ ID NO:41.

[0072] For the sake of clarity, in this embodiment, the deletion relates to the deletion of all consecutive amino acids in the mentioned range of positions. For example, a functional truncated human GDE polypeptide comprising the deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 140 with respect to SEQ ID NO:41.

[0073] Also for the sake of clarity, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 140 with respect to SEQ ID NO:41 ; and
- the deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 264, since the range 1-140 is included in the range 1-264.

[0074] In addition, a functional truncated human GDE polypeptide comprising for example :

- the deletion of amino acids from position 1 to 264 with respect to SEQ ID NO:41 ; and
- the deletion of amino acids from position 207 to 304 with respect to SEQ ID NO:41

corresponds to a GDE polypeptide which is deleted of all consecutive amino acids from position 1 to 304, since the range 1-264 overlaps the range 207-304.

[0075] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises a deletion or a combination of deletions with respect to SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41 wherein the deletion(s) is(are) selected from any deletion referred

to as $\Delta 1$, $\Delta 2$, $\Delta 3$, $\Delta 4$, $\Delta 5$, $\Delta 6$, $\Delta 7$ and $\Delta 8$ in table 1 :

Table 1 :

Deletion	Position of the deleted amino acids with respect to SEQ ID NO:1	Position of the deleted amino acids with respect to SEQ ID NO:40	Position of the deleted amino acids with respect to SEQ ID NO:41
$\Delta 1$	1-156	1-139	1-140
$\Delta 2$	361-428	344-411	345-412
$\Delta 3$	668-769	651-752	652-753
$\Delta 4$	895-1087	878-1070	879-1071
$\Delta 5$	223-320	206-303	207-304
$\Delta 6$	360-428	343-411	344-412
$\Delta 7$	669-720	652-703	653-704
$\Delta 8$	1-280	1-263	1-264

[0076] In a particular embodiment, the functional truncated human GDE polypeptide of the invention may comprise a combination of 2, 3, 4, 5, 6, 7 or 8 deletions, wherein the deletion(s) is(are) selected from any deletion referred to as $\Delta 1$, $\Delta 2$, $\Delta 3$, $\Delta 4$, $\Delta 5$, $\Delta 6$, $\Delta 7$ and $\Delta 8$ in table 1.

[0077] In a particular embodiment, the functional truncated human GDE polypeptide of the invention may comprise a deletion or a combination of deletions, as shown in table 2, wherein the deletion(s) is(are) as referred in table 1.

Table 2 :

$\Delta 1$	$\Delta 2 + \Delta 8$	$\Delta 1 + \Delta 2 + \Delta 7$
$\Delta 2$	$\Delta 3 + \Delta 4$	$\Delta 1 + \Delta 2 + \Delta 8$
$\Delta 3$	$\Delta 3 + \Delta 5$	$\Delta 1 + \Delta 3 + \Delta 4$
$\Delta 4$	$\Delta 3 + \Delta 6$	$\Delta 1 + \Delta 3 + \Delta 5$
$\Delta 5$	$\Delta 3 + \Delta 7$	$\Delta 1 + \Delta 3 + \Delta 6$
$\Delta 6$	$\Delta 3 + \Delta 8$	$\Delta 1 + \Delta 3 + \Delta 7$
$\Delta 7$	$\Delta 4 + \Delta 5$	$\Delta 1 + \Delta 3 + \Delta 8$
$\Delta 8$	$\Delta 4 + \Delta 6$	$\Delta 1 + \Delta 4 + \Delta 5$
$\Delta 1 + \Delta 2$	$\Delta 4 + \Delta 7$	$\Delta 1 + \Delta 4 + \Delta 6$
$\Delta 1 + \Delta 3$	$\Delta 4 + \Delta 8$	$\Delta 1 + \Delta 4 + \Delta 7$
$\Delta 1 + \Delta 4$	$\Delta 5 + \Delta 6$	$\Delta 1 + \Delta 4 + \Delta 8$
$\Delta 1 + \Delta 5$	$\Delta 5 + \Delta 7$	$\Delta 1 + \Delta 5 + \Delta 6$
$\Delta 1 + \Delta 6$	$\Delta 5 + \Delta 8$	$\Delta 1 + \Delta 5 + \Delta 7$
$\Delta 1 + \Delta 7$	$\Delta 6 + \Delta 7$	$\Delta 1 + \Delta 5 + \Delta 8$
$\Delta 1 + \Delta 8$	$\Delta 6 + \Delta 8$	$\Delta 1 + \Delta 6 + \Delta 7$

[0078] For the sake of clarity, Table 2 should be understood as follows. Taking as an example the functional truncated human GDE polypeptide comprising the following combination of deletions : " $\Delta 1 + \Delta 2 + \Delta 3$ ", the functional truncated human GDE polypeptide comprises deletions $\Delta 1$, $\Delta 2$ and $\Delta 3$ with respect to SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41, as referred in Table 1. In other words, in this example, when the reference full-length GDE sequence is SEQ ID NO:1, the functional truncated human GDE polypeptide " $\Delta 1 + \Delta 2 + \Delta 3$ " corresponds to a functional truncated human GDE polypeptide derived from SEQ ID NO:1, which is deleted of all consecutive amino acids from position 1 to 156, from position 361-428 and from position 668-769 with respect to SEQ ID NO:1. Accordingly, when the reference full-length GDE sequence is SEQ ID NO:40, the functional truncated human GDE polypeptide " $\Delta 1 + \Delta 2 + \Delta 3$ " corresponds to a functional truncated human GDE polypeptide derived from SEQ ID NO:40, which is deleted of all consecutive amino acids from position 1 to 139, from position 344-411 and from position 651-752 with respect to SEQ ID NO:40.

[0079] In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises the $\Delta 2$ and $\Delta 3$ deletions referred to in table 1. In a particular embodiment, the functional truncated human GDE polypeptide of the invention comprises the $\Delta 2$ and $\Delta 3$ deletions referred to in table 1 and is derived from SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41, in particular from SEQ ID NO:1.

[0080] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) a N-terminal deletion of at least one amino acid and of at most 132 amino acids selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1.

[0081] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) a N-terminal deletion of at least one amino acid selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 30 consecutive amino acids, at least 50 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 81 consecutive amino acids, at least 100 consecutive amino acids, at least 103 consecutive amino acids, at least 125 consecutive amino acids, or at least 132 consecutive amino acids selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1.

[0082] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a N-terminal deletion of at least one amino acid and of at most 132 amino acids selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1.

[0083] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a N-terminal deletion of at least one amino acid selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1, preferably at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 30 consecutive amino acids, at least 50 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 81 consecutive amino acids, at least 100 consecutive amino acids, at least 103 consecutive amino acids, at least 125 consecutive amino acids, or at least 132 consecutive amino acids selected from amino acids at positions 1 to 132 with respect to SEQ ID NO:1.

[0084] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40, and
2. (ii) a N-terminal deletion of at least one amino acid and of at most 115 amino acids selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:40.

[0085] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40, and
2. (ii) a N-terminal deletion of at least one amino acid selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:40, preferably at least 10 consecutive amino acids, at least 13 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 50 consecutive amino acids, at least 60 consecutive amino acids, at least 64 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 85 consecutive amino acids,

at least 86 consecutive amino acids, at least 100 consecutive amino acids, at least 110 consecutive amino acids, at least 112 consecutive amino acids, or at least 115 consecutive amino acids selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:40.

[0086] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) a N-terminal deletion of at least one amino acid and of at most 115 amino acids selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:40.

[0087] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) a N-terminal deletion of at least one amino acid selected from amino acids at positions 1 to Z_2 with respect to SEQ ID NO:40, preferably at least 10 consecutive amino acids, at least 13 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 50 consecutive amino acids, at least 60 consecutive amino acids, at least 64 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 85 consecutive amino acids, at least 86 consecutive amino acids, at least 100 consecutive amino acids, at least 110 consecutive amino acids, at least 112 consecutive amino acids, or at least 115 consecutive amino acids selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:40.

[0088] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:41, and
2. (ii) a N-terminal deletion of at least one amino acid and of at most 115 amino acids selected from amino acids at positions 1 to 115 with respect to SEQ ID NO:41.

[0089] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:41, and
2. (ii) a N-terminal deletion of at least one amino acid selected from amino acids at

positions 1 to 116 with respect to SEQ ID NO:41, preferably at least 10 consecutive amino acids, at least 14 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 50 consecutive amino acids, at least 60 consecutive amino acids, at least 65 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 85 consecutive amino acids, at least 87 consecutive amino acids, at least 100 consecutive amino acids, at least 110 consecutive amino acids, at least 113 consecutive amino acids, or at least 116 consecutive amino acids selected from amino acids at positions 1 to 116 with respect to SEQ ID NO:41.

[0090] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:41, and
2. (ii) aN-terminal deletion of at least one amino acid selected from amino acids at positions 1 to 116 with respect to SEQ ID NO:41, preferably at least 10 consecutive amino acids, at least 14 consecutive amino acids, at least 15 consecutive amino acids, at least 25 consecutive amino acids, at least 50 consecutive amino acids, at least 60 consecutive amino acids, at least 65 consecutive amino acids, at least 75 consecutive amino acids, at least 80 consecutive amino acids, at least 85 consecutive amino acids, at least 87 consecutive amino acids, at least 100 consecutive amino acids, at least 110 consecutive amino acids, at least 113 consecutive amino acids, or at least 116 consecutive amino acids selected from amino acids at positions 1 to 116 with respect to SEQ ID NO:41.

[0091] In a particular embodiment, the functional truncated human GDE polypeptide comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41, and
2. (ii) a deletion or a combination of deletions selected from any deletion referred to as $\Delta 9$, $\Delta 10$, $\Delta 11$, $\Delta 12$, and $\Delta 13$ in table 3, with respect to SEQ ID NO:1, SEQ ID NO:40 or SEQ ID NO:41.

Table 3 :

Deletion	Position of the deleted amino acids with respect to SEQ ID NO:1	Position of the deleted amino acids with respect to SEQ ID NO:40	Position of the deleted amino acids with respect to SEQ ID NO:41
$\Delta 9$	1-15	-	-
$\Delta 10$	1-30	1-13	1-14
$\Delta 11$	1-81	1-64	1-65

Deletion	Position of the deleted amino acids with respect to SEQ ID NO:1	Position of the deleted amino acids with respect to SEQ ID NO:40	Position of the deleted amino acids with respect to SEQ ID NO:41
$\Delta 12$	1-103	1-86	1-87
$\Delta 13$	1-129	1-112	1-113

[0092] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 9$ deletion, as referred in Table 3 with respect to SEQ ID NO:1 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 10$ deletion, as referred in Table 3 with respect to SEQ ID NO:1 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 11$ deletion, as referred in Table 3 with respect to SEQ ID NO:1 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 12$ deletion, as referred in Table 3 with respect to SEQ ID NO:1 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:1 and
2. (ii) the $\Delta 13$ deletion, as referred in Table 3 with respect to SEQ ID NO:1.

[0093] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 9$, $\Delta 10$, $\Delta 11$, $\Delta 12$ and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:1.

[0094] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 9$ deletion as referred in table 3 with respect to SEQ ID NO:1.

[0095] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 10$ deletion as referred in table 3 with respect to SEQ ID NO:1.

[0096] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 11$ deletion as referred in table 3 with respect to SEQ ID NO:1.

[0097] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 12$ deletion as referred in table 3 with respect to SEQ ID NO:1.

[0098] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) the $\Delta 13$ deletion as referred in table 3 with respect to SEQ ID NO:1.

[0099] In a further particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 9$, $\Delta 10$, and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:1.

[0100] In yet another particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:1 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 9$ and $\Delta 10$ deletions as referred in table 3 with respect to SEQ ID NO:1.

[0101] In another particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 10$ deletion, as referred in Table 3 with respect to SEQ ID NO:40 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 11$ deletion, as referred in Table 3 with respect to SEQ ID NO:40 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 12$ deletion, as referred in Table 3 with respect to SEQ ID NO:40 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:40 and
2. (ii) the $\Delta 13$ deletion, as referred in Table 3 with respect to SEQ ID NO:40.

[0102] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 10$, $\Delta 11$, $\Delta 12$ and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:40.

[0103] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 10$ deletion as referred in table 3 with respect to SEQ ID NO:40.

[0104] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 11$ deletion as referred in table 3 with respect to SEQ ID NO:40.

[0105] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 12$ deletion as referred in table 3 with respect to SEQ ID NO:40.

[0106] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) the $\Delta 13$ deletion as referred in table 3 with respect to SEQ ID NO:40.

[0107] In a further particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:40 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:40, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 10$, and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:40.

[0108] In another particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:41, and
2. (ii) the $\Delta 10$ deletion, as referred in Table 3 with respect to SEQ ID NO:41 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:41, and
2. (ii) the $\Delta 11$ deletion, as referred in Table 3 with respect to SEQ ID NO:41 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:41, and
2. (ii) the $\Delta 12$ deletion, as referred in Table 3 with respect to SEQ ID NO:41 ;

or

1. (i) a deletion or a combination of deletions, as shown in table 2 with respect to SEQ ID NO:41 and
2. (ii) the $\Delta 13$ deletion, as referred in Table 3 with respect to SEQ ID NO:41.

[0109] In a particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:41, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 9$, $\Delta 10$, $\Delta 11$, $\Delta 12$, and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:41.

[0110] In a further particular embodiment, the functional truncated human GDE polypeptide is derived from SEQ ID NO:41 and comprises :

1. (i) the $\Delta 2$ and $\Delta 3$ deletions as referred in table 1 with respect to SEQ ID NO:1, and
2. (ii) a deletion or a combination of deletions selected from the $\Delta 10$, and $\Delta 13$ deletions as referred in table 3 with respect to SEQ ID NO:41.

[0111] In another particular embodiment, the functional truncated human GDE polypeptide of the invention is selected from the group consisting of:

- SEQ ID NO:2 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1;
- SEQ ID NO:3 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1;
- SEQ ID NO:4 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;
- SEQ ID NO:5 : a functional truncated human GDE polypeptide comprising a first deletion

of amino acids from position 361 to 428 and a second deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;

- SEQ ID NO:6 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1;
- SEQ ID NO:7 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 223 to 320, a second deletion of amino acids from position 360 to 428, and a third deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1;
- SEQ ID NO:8: a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1;
- SEQ ID NO:9 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1;
- SEQ ID NO:10 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1;
- SEQ ID NO:48 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 1-15, a second deletion of amino acids from position 361 to 428 and a third deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;
- SEQ ID NO:49 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 1-30 a second deletion of amino acids from position 361 to 428 and a third deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;
- SEQ ID NO:50 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 1-81 a second deletion of amino acids from position 361 to 428 and a third deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;
- SEQ ID NO:51 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 1-103 a second deletion of amino acids from position 361 to 428 and a third deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1; and
- SEQ ID NO:52 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 1-129 a second deletion of amino acids from position 361 to 428 and a third deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1.

[0112] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention is selected from the group consisting of:

- SEQ ID NO:2 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 156 with respect to SEQ ID NO:1;
- SEQ ID NO:3 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 361 to 428 with respect to SEQ ID NO:1;
- SEQ ID NO:4 : a functional truncated human GDE polypeptide comprising a deletion of

- amino acids from position 668 to 769 with respect to SEQ ID NO:1;
- SEQ ID NO:5 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 361 to 428 and a second deletion of amino acids from position 668 to 769 with respect to SEQ ID NO:1;
 - SEQ ID NO:6 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 895 to 1087 with respect to SEQ ID NO:1;
 - SEQ ID NO:7 : a functional truncated human GDE polypeptide comprising a first deletion of amino acids from position 223 to 320, a second deletion of amino acids from position 360 to 428, and a third deletion of amino acids from position 669 to 720 with respect to SEQ ID NO:1;
 - SEQ ID NO:8: a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 280 with respect to SEQ ID NO:1;
 - SEQ ID NO:9 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1; and
 - SEQ ID NO:10 : a functional truncated human GDE polypeptide comprising a deletion of amino acids from position 1 to 230 with respect to SEQ ID NO:1.

[0113] When the deletion is a N-terminal deletion, a methionine may be added at the N-terminal end of the sequence. For example, SEQ ID NO:9 comprises a deletion of amino acids from position 1 to 425 with respect to SEQ ID NO:1 and an addition of a methionine at the N-terminal end of the sequence resulting from this deletion. The present application does disclose all the functional GDE truncated forms specifically disclosed therein, wherein said functional GDE truncated forms being with a methionine residue at its N-terminal end.

[0114] In a further particular embodiment, the functional truncated human GDE polypeptide of the invention comprises or consists of a sequence selected from SEQ ID NO:2 to 10 and SEQ ID NO:48 to 52, in particular a sequence selected from SEQ ID NO:2 to 10, in particular a sequence selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, more particularly SEQ ID NO:5. The functional truncated human GDE polypeptide may comprise one or more amino acid modifications such as amino acid insertion, deletion and/or substitution, as compared to SEQ ID NO:2 to 10 and SEQ ID NO:48 to 52, in particular a sequence selected from SEQ ID NO:2 to 10, in particular a sequence selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, more particularly SEQ ID NO:5. In particular, the functional truncated human GDE polypeptide may comprise 1, 2, 3, 4 or 5 amino acid modifications as compared to SEQ ID NO:2 to 10 and SEQ ID NO:48 to 52, in particular a sequence selected from SEQ ID NO:2 to 10, in particular a sequence selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, more particularly SEQ ID NO:5. In particular, the functional truncated human GDE polypeptide may have at least 80, 85, 90, 95, 96, 97, 98 or at least 99 percent sequence identity to SEQ ID NO:2 to 10 and SEQ ID NO:48 to 52, in particular a sequence selected from SEQ ID NO:2 to 10, in particular a sequence selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, more particularly SEQ ID NO:5.

[0115] In a second variant of the first aspect of the invention, the mini-GDE is a functional non-human GDE polypeptide.

[0116] The functional non-human GDE polypeptide of the invention can be any GDE polypeptide whose coding sequence is small enough to be packaged into a gene therapy vector, in particular into an AAV vector. Indeed, the present inventors have shown that non-human GDE polypeptides whose coding sequence is shorter than native human GDE coding sequence can be used for treating GSD III, using gene therapy vectors. In a particular embodiment, the functional non-human GDE polypeptide comprises less than about 1500, 1480, 1460, 1440, 1420, 1400, 1380, 1360, 1340, 1320, 1300, 1280, 1260, 1240, 1220, 1200, 1180, 1160, 1140, 1120, 1100, 1080, 1060, 1040, 1020, or less than about 1000 amino acids. In a particular embodiment, the functional non-human GDE polypeptide comprises between about 1000 and 1500 amino acids, between about 1000 and 1300 amino acids, between about 1300 and 1500 amino acids or between about 1300 and 1400 amino acids.

[0117] According to the invention, the functional non-human GDE polypeptide of the invention retains the biological function of human GDE polypeptide, as defined above. In particular, the non-human GDE polypeptide is able to rescue glycogen accumulation and muscle strength *in vivo*.

[0118] The amino acid sequence of the functional non-human GDE polypeptide or its coding sequence can derive from any non-human eukaryotic source, such as from yeast or non-human animals including non-human mammal or avian species. In a particular embodiment, the functional non-human GDE polypeptide is a non-human mammalian GDE polypeptide.

[0119] In addition, the non-human GDE polypeptide may be a functional variant of a wild type non-human GDE polypeptide, comprising one or more amino acid modifications such as amino acid insertion, deletion and/or substitution as compared to a reference native GDE polypeptide. For example, the non-human GDE polypeptide may be a functional derivative of a non-human GDE polypeptide, in particular of a non-human animal GDE polypeptide, such as the polypeptides of SEQ ID NO:11 to SEQ ID NO:17, having at least 80, 85, 90, 95, 96, 97, 98 or at least 99 percent sequence identity to these animal GDE polypeptides.

[0120] In a particular embodiment, the functional non-human GDE polypeptide or its coding sequence derives from horse, gorilla, orangutan, Pteropus alecto, sooty mangabey, platypus, duck or Tasmania devil.

[0121] In a particular embodiment, the non-human GDE polypeptide is selected in the group consisting of : horse GDE polypeptide of SEQ ID NO:11, gorilla GDE polypeptide of SEQ ID NO:12 , orangutan GDE polypeptide of SEQ ID NO:13, Pteropus alecto GDE polypeptide of SEQ ID NO:14, sooty mangabey GDE polypeptide of SEQ ID NO:15, platypus GDE polypeptide of SEQ ID NO:16, and duck GDE polypeptide of SEQ ID NO:17.

[0122] In a further particular embodiment, the non-human GDE polypeptide is a gorilla GDE

polypeptide, in particular the gorilla GDE polypeptide of SEQ ID NO:12.

[0123] In another aspect, the invention relates to a nucleic acid molecule encoding the mini-GDE polypeptide of the invention.

[0124] The term "nucleic acid molecule" (or nucleic acid sequence) refers to a DNA or RNA molecule in single or double stranded form, particularly a DNA encoding a functional truncated human GDE polypeptide or a functional non-human GDE polypeptide according to the invention.

[0125] According to the present invention, the nucleic acid molecule encoding the mini-GDE polypeptide is small enough to be packaged into a gene therapy vector, wherein the gene therapy vector is as defined above. In a preferred embodiment, the nucleic acid molecule encoding the mini-GDE polypeptide is small enough to be packaged into an AAV vector. Preferably, the size of the nucleic acid molecule encoding the mini-GDE polypeptide is less than about 5, 4.7, 4.5, 4.2, 4.1, 4, 3.7, 3.5, 3.2, 3, 2.7, 2.5, 2.2, 2, or 1.5 kb. Preferably, the nucleic acid molecule encoding the mini-GDE polypeptide is less than about 4.1 kb.

[0126] The sequence of the nucleic acid molecule of the invention, encoding a mini-GDE polypeptide may be optimized for expression of the GDE polypeptide *in vivo*. Sequence optimization may include a number of changes in a nucleic acid sequence, including codon optimization, increase of GC content, decrease of the number of CpG islands, decrease of the number of alternative open reading frames (ARFs) and decrease of the number of splice donor and splice acceptor sites. Because of the degeneracy of the genetic code, different nucleic acid molecules may encode the same protein. It is also well known that the genetic codes of different organisms are often biased towards using one of the several codons that encode the same amino acid over the others. Through codon optimization, changes are introduced in a nucleotide sequence that take advantage of the codon bias existing in a given cellular context so that the resulting codon optimized nucleotide sequence is more likely to be expressed in such given cellular context at a relatively high level compared to the non-codon optimized sequence. In a preferred embodiment of the invention, such sequence optimized nucleotide sequence encoding a mini-GDE polypeptide is codon-optimized to improve its expression in human cells compared to non-codon optimized nucleotide sequences coding for the same mini-GDE polypeptide, for example by taking advantage of the human specific codon usage bias. The nucleic acid sequence encoding the full-length human GDE isoform 1 is as shown in SEQ ID NO:37. Examples of corresponding codon optimized sequence is as shown in SEQ ID NO:38 or SEQ ID NO:39.

[0127] In a particular embodiment, the nucleic acid molecule of the invention comprises or consists of:

- the sequence shown in SEQ ID NO:18, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:2 ;
- the sequence shown in SEQ ID NO:20, encoding the functional truncated human GDE

- polypeptide having the amino acid sequence shown in SEQ ID NO:3 ;
- the sequence shown in SEQ ID NO:21, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:4 ;
 - the sequence shown in SEQ ID NO:22, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:5 ;
 - the sequence shown in SEQ ID NO:24, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:6 ;
 - the sequence shown in SEQ ID NO:26, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:7 ;
 - the sequence shown in SEQ ID NO:27, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:8 ;
 - the sequence shown in SEQ ID NO:28, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:9 ;
 - the sequence shown in SEQ ID NO:29, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:10 ;
 - the sequence shown in SEQ ID NO:53 or SEQ ID NO:59, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:48 ;
 - the sequence shown in SEQ ID NO:54 or SEQ ID NO:60, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:49 ;
 - the sequence shown in SEQ ID NO:55 or SEQ ID NO:61, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:50 ;
 - the sequence shown in SEQ ID NO:56 or SEQ ID NO:62, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:51 ; or
 - the sequence shown in SEQ ID NO:57 or SEQ ID NO:63, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:52.

[0128] In a further particular embodiment, the nucleic acid molecule of the invention comprises or consists of:

- the sequence shown in SEQ ID NO:18, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:2 ;
- the sequence shown in SEQ ID NO:20, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:3 ;
- the sequence shown in SEQ ID NO:21, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:4 ;
- the sequence shown in SEQ ID NO:22, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:5 ;
- the sequence shown in SEQ ID NO:24, encoding the functional truncated human GDE

- polypeptide having the amino acid sequence shown in SEQ ID NO: 7 ;
- the sequence shown in SEQ ID NO:26, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO: 7 ;
- the sequence shown in SEQ ID NO:27, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO: 7 ;
- the sequence shown in SEQ ID NO:28, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:9 ; or
- the sequence shown in SEQ ID NO:29, encoding the functional truncated human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:10.

[0129] As already mentioned, the above sequences may be codon-optimized. Sequences shown in SEQ ID NO:19, SEQ ID NO:23 and SEQ ID NO:25 are examples of codon-optimized sequences corresponding to SEQ ID NO:18, SEQ ID NO:22 and SEQ ID NO:24, respectively.

[0130] In another particular embodiment, the nucleic acid molecule of the invention comprises or consists of:

- the sequence shown in SEQ ID NO:30, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:11 ;
- the sequence shown in SEQ ID NO:31, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:12 ;
- the sequence shown in SEQ ID NO:32, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:13 ;
- the sequence shown in SEQ ID NO:33, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:14 ;
- the sequence shown in SEQ ID NO:34, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:15 ;
- the sequence shown in SEQ ID NO:35, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:16 ; or
- the sequence shown in SEQ ID NO:36, encoding the non-human GDE polypeptide having the amino acid sequence shown in SEQ ID NO:17.

[0131] The nucleic acid molecule encoding the mini-GDE polypeptide as defined above may have at least 90 or at least 95 percent identity to any of the nucleotide sequences of SEQ ID NO:18 to 26. In a particular embodiment, the nucleic acid molecule encoding the mini-GDE polypeptide as defined above may have at least 90 or at least 95 percent identity to any of the nucleotide sequences of SEQ ID NO:18 to 36 and SEQ ID NO:53 to 57. In a particular embodiment, the nucleic acid molecule of the invention has at least 95 percent identity, for example at least 96, 97, 98, 99 or 100 percent identity to any of the nucleotide sequences of SEQ ID NO:18 to 36. In a further particular embodiment, the nucleic acid molecule of the invention has at least 95 percent identity, for example at least 96, 97, 98, 99 or 100 percent

identity to any of the nucleotide sequences of SEQ ID NO:18 to 36 and SEQ ID NO:53 to 57.

[0132] The term "identical" and declinations thereof refers to the sequence identity between two nucleic acid molecules or between two polypeptide molecules. When a position in both of the two compared sequences is occupied by the same base or the same amino acid, then the molecules are identical at that position. The percent of identity between two sequences is a function of the number of matching positions shared by the two sequences divided by the number of positions compared X 100. For example, if 6 of 10 of the positions in two sequences are matched then the two sequences are 60% identical. Generally, a comparison is made when two sequences are aligned to give maximum identity. Various bioinformatics tools known to the one skilled in the art might be used to align nucleic acid sequences such as BLAST or FASTA.

[0133] The invention also relates to a nucleic acid construct comprising a nucleic acid molecule of the invention. The nucleic acid construct may correspond to an expression cassette comprising the nucleic acid sequence of the invention, operably linked to one or more expression control sequences and/or other sequences improving the expression. As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter, or another transcription regulatory sequence, is operably linked to a coding sequence if it affects the transcription of the coding sequence. Such expression control sequences are known in the art, such as promoters, enhancers (such as cis-regulatory modules (CRMs)), introns, polyA signals, etc.

[0134] In a particular embodiment, the expression cassette may include a promoter. The promoter may be an ubiquitous or tissue-specific promoter, in particular a promoter able to promote expression in cells or tissues in which expression of GDE is desirable such as in cells or tissues in which GDE expression is desirable in GDE-deficient patients.

[0135] In a particular embodiment, the promoter is a muscle-specific promoter. Non-limiting examples of muscle-specific promoters include the muscle creatine kinase (MCK) promoter. Non-limiting examples of suitable muscle creatine kinase promoters are human muscle creatine kinase promoters and truncated murine muscle creatine kinase [(tMCK) promoters] (Wang B et al, Construction and analysis of compact muscle-selective promoters for AAV vectors. Gene Ther. 2008 Nov;15(22):1489-99) (representative GenBank Accession No. AF188002). Human muscle creatine kinase has the Gene ID No. 1158 (representative GenBank Accession No. NC_000019.9, accessed on December 26, 2012). Other examples of muscle-specific promoters include a synthetic promoter C5.12 (spC5.12, alternatively referred to herein as "C5.12"), such as the spC5.12 or the spC5.12 promoter (disclosed in Wang et al., Gene Therapy volume 15, pages 1489-1499 (2008)), the MHCK7 promoter (Salva et al. Mol Ther. 2007 Feb;15(2):320-9), myosin light chain (MLC) promoters, for example MLC2 (Gene ID No. 4633; representative GenBank Accession No. NG_007554.1, accessed on December 26, 2012); myosin heavy chain (MHC) promoters, for example alpha-MHC (Gene ID No. 4624; representative GenBank Accession No. NG_023444.1, accessed on December 26, 2012);

desmin promoters (Gene ID No. 1674; representative GenBank Accession No. NG_008043.1, accessed on December 26, 2012); cardiac troponin C promoters (Gene ID No. 7134; representative GenBank Accession No. NG_008963.1, accessed on December 26, 2012); troponin I promoters (Gene ID Nos. 7135, 7136, and 7137; representative GenBank Accession Nos. NG_016649.1, NG_011621.1, and NG_007866.2, accessed on December 26, 2012); myoD gene family promoters (Weintraub et al., Science, 251, 761 (1991); Gene ID No. 4654; representative GenBank Accession No. NM_002478, accessed on December 26, 2012); alpha actin promoters (Gene ID Nos. 58, 59, and 70; representative GenBank Accession Nos. NG_006672.1, NG_011541.1, and NG_007553.1, accessed on December 26, 2012); beta actin promoters (Gene ID No. 60; representative GenBank Accession No. NG_007992.1, accessed on December 26, 2012); gamma actin promoters (Gene ID No. 71 and 72; representative GenBank Accession No. NG_011433.1 and NM_001199893, accessed on December 26, 2012); muscle-specific promoters residing within intron 1 of the ocular form of Pitx3 (Gene ID No. 5309) (Coulon et al; the muscle-selective promoter corresponds to residues 11219-11527 of representative GenBank Accession No. NG_008147, accessed on December 26, 2012); and the promoters described in US Patent Publication US 2003/0157064, and CK6 promoters (Wang et al 2008 doi: 10.1038/gt.2008.104). In another particular embodiment, the muscle-specific promoter is the E-Syn promoter described in Wang et al., Gene Therapy volume 15, pages 1489-1499 (2008), comprising the combination of a MCK-derived enhancer and of the spC5.12 promoter. In a particular embodiment of the invention, the muscle-specific promoter is selected in the group consisting of a spC5.12 promoter, the MHCK7 promoter, the E-syn promoter, a muscle creatine kinase myosin light chain (MLC) promoter, a myosin heavy chain (MHC) promoter, a cardiac troponin C promoter, a troponin I promoter, a myoD gene family promoter, an alpha actin promoter, an beta actin promoter, an gamma actin promoter, a muscle-specific promoter residing within intron 1 of the ocular form of Pitx3, a CK6 promoter, a CK8 promoter and an Acta1 promoter. In a particular embodiment, the muscle-specific promoter is selected in the group consisting of the spC5.12, desmin and MCK promoters. In a further embodiment, the muscle-specific promoter is selected in the group consisting of the spC5.12 and MCK promoters. In a particular embodiment, the muscle-specific promoter is the spC5.12 promoter.

[0136] In a particular embodiment, the promoter is a liver-specific promoter. Non-limiting examples of liver-specific promoters include the alpha-1 antitrypsin promoter (hAAT), the transthyretin promoter, the albumin promoter, the thyroxine-binding globulin (TBG) promoter, the LSP promoter (comprising a thyroid hormone-binding globulin promoter sequence, two copies of an alpha1-microglobulin/bikunin enhancer sequence, and a leader sequence - III, C. R., et al. (1997). Optimization of the human factor VIII complementary DNA expression plasmid for gene therapy of hemophilia A. Blood Coag. Fibrinol. 8: S23-S30.), etc. Other useful liver-specific promoters are known in the art, for example those listed in the Liver Specific Gene Promoter Database compiled the Cold Spring Harbor Laboratory (<http://rulai.cshl.edu/LSPD/>). A preferred liver-specific promoter in the context of the invention is the hAAT promoter.

[0137] In another particular embodiment, the promoter is a neuron-specific promoter. Non-limiting examples of neuron-specific promoters include, but are not limited to the following:

synapsin-1 (Syn) promoter, neuron-specific enolase (NSE) promoter (Andersen et al., *Cell. Mol. Neurobiol.*, 13:503-15 (1993)), neurofilament light-chain gene promoter (Piccioli et al., *Proc. Natl. Acad. Sci. USA*, 88:5611-5 (1991)), and the neuron-specific vgf gene promoter (Piccioli et al. *Neuron*, 15:373- 84 (1995)), among others which will be apparent to the skilled artisan. In a particular embodiment, the neuron-specific promoter is the Syn promoter. Other neuron-specific promoters include, without limitation: synapsin-2 promoter, tyrosine hydroxylase promoter, dopamine β -hydroxylase promoter, hypoxanthine phosphoribosyltransferase promoter, low affinity NGF receptor promoter, and choline acetyl transferase promoter (Bejanin et al., 1992; Carroll et al., 1995; Chin and Greengard, 1994; Foss-Petter et al., 1990; Harrington et al., 1987; Mercer et al., 1991; Patei et al., 1986). Representative promoters specific for the motor neurons include, without limitation, the promoter of the Calcitonin Gene-Related Peptide (CGRP), a known motor neuron-derived factor. Other promoters functional in motor neurons include the promoters of Choline Acetyl Transferase (ChAT), Neuron Specific Enolase (NSE), Synapsin and Hb9. Other neuron-specific promoters useful in the present invention include, without limitation: GFAP (for astrocytes), Calbindin 2 (for interneurons), Mnx1 (motoneurons), Nestin (neurons), Parvalbumin, Somatostatin and Plp1 (oligodendrocytes and Schwann cells).

[0138] In another particular embodiment, the promoter is a ubiquitous promoter. Representative ubiquitous promoters include the cytomegalovirus enhancer/chicken beta actin (CAG) promoter, the cytomegalovirus enhancer/promoter (CMV) (optionally with the CMV enhancer) [see, e.g., Boshart et al, *Cell*, 41:521-530 (1985)], the PGK promoter, the SV40 early promoter, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the dihydrofolate reductase promoter, the β -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 alpha promoter.

[0139] In addition, the promoter may also be an endogenous promoter such as the albumin promoter or the GDE promoter.

[0140] In a particular embodiment, the promoter is associated to an enhancer sequence, such as a cis-regulatory module (CRMs) or an artificial enhancer sequence. CRMs useful in the practice of the present invention include those described in Rincon et al., *Mol Ther.* 2015 Jan;23(1):43-52, Chuah et al., *Mol Ther.* 2014 Sep;22(9):1605-13 or Nair et al., *Blood.* 2014 May 15;123(20):3195-9. Other regulatory elements that are, in particular, able to enhance muscle-specific expression of genes, in particular expression in cardiac muscle and/or skeletal muscle, are those disclosed in WO2015110449. Particular examples of nucleic acid regulatory elements that comprise an artificial sequence include the regulatory elements that are obtained by rearranging the transcription factor binding sites (TFBS) that are present in the sequences disclosed in WO2015110449. Said rearrangement may encompass changing the order of the TFBSs and/or changing the position of one or more TFBSs relative to the other TFBSs and/or changing the copy number of one or more of the TFBSs. For example, a nucleic acid regulatory element for enhancing muscle-specific gene expression, in particular cardiac and skeletal muscle-specific gene expression, may comprise binding sites for E2A, HNH 1 , NF1 , C/EBP, LRF, MyoD, and SREBP; or for E2A, NF1 , p53, C/EBP, LRF, and SREBP; or for E2A,

HNH 1 , HNF3a, HNF3b, NF1 , C/EBP, LRF, MyoD, and SREBP; or E2A, HNF3a, NF1 , C/EBP, LRF, MyoD, and SREBP; or for E2A, HNF3a, NF1 , CEBP, LRF, MyoD, and SREBP; or for HNF4, NF1 , RSRFC4, C/EBP, LRF, and MyoD, or NF1 , PPAR, p53, C/EBP, LRF, and MyoD. For example, a nucleic acid regulatory element for enhancing muscle-specific gene expression, in particular skeletal muscle-specific gene expression, may also comprise binding sites for E2A, NF1 , SRFC, p53, C/EBP, LRF, and MyoD; or for E2A, NF1 , C/EBP, LRF, MyoD, and SREBP; or for E2A, HNF3a, C/EBP, LRF, MyoD, SEREBP, and Tal1_b; or for E2A, SRF, p53, C/EBP, LRF, MyoD, and SREBP; or for HNF4, NF1 , RSRFC4, C/EBP, LRF, and SREBP; or for E2A, HNF3a, HNF3b, NF1 , SRF, C/EBP, LRF, MyoD, and SREBP; or for E2A, CEBP, and MyoD. In further examples, these nucleic acid regulatory elements comprise at least two, such as 2, 3, 4, or more copies of one or more of the TFBSs recited before. Other regulatory elements that are, in particular, able to enhance liver-specific expression of genes, are those disclosed in WO2009130208.

[0141] In another particular embodiment, the nucleic acid construct comprises an intron, in particular an intron placed between the promoter and the GDE coding sequence. An intron may be introduced to increase mRNA stability and the production of the protein. In a further embodiment, the intron is a human beta globin b2 (or HBB2) intron, a coagulation factor IX (FIX) intron, a SV40 intron, a hCMV intron A (hCMVI), a TPL intron (TPLI), a CHEF1 gene intron1 (CHEFI), a MVM intron (Wu et al, 2008), a FIX truncated intron 1 (Wu et al., 2008, Mol Ther, 16(2):280-289 ; Kurachi et al., 1995, J Biol Chem., 270(10):5276-5281), a β -globin/immunoglobulin heavy chain hybrid intron (5'-donor site from a human β -globin intron and 3'-acceptor site from an immunoglobulin heavy chain variable region intron, Wu et al., 2008, Mol Ther, 16(2):280-289 ; Kurachi et al., 1995, J Biol Chem., 270(10):5276-5281), a hybrid intron consisting of an adenovirus splice donor and an immunoglobulin G splice (Wong et al., 1985, Chromosoma, 92(2):124-135 ; Yew et al., 1997, Hum Gene Ther, 8(5):575-584 ; Choi T. et al., 1991, Mol Cell Biol, 11(6):3070-3074 ; Huang et al., 1990, Mol Cell Biol., 10(4):1805-1810), a hybrid 19S/16S SV40 intron (5'-donor site from 19S intron and 3'-acceptor site from 16S intron, Yew et al., 1997, Hum Gene Ther, 8(5):575-584) or a chicken beta-globin intron. In another further embodiment, the intron is a modified intron (in particular a modified HBB2 or FIX intron) designed to decrease the number of, or even totally remove, alternative open reading frames (ARFs) found in said intron. Preferably, ARFs are removed whose length spans over 50 bp and have a stop codon in frame with a start codon. ARFs may be removed by modifying the sequence of the intron. For example, modification may be carried out by way of nucleotide substitution, insertion or deletion, preferably by nucleotide substitution. As an illustration, one or more nucleotides, in particular one nucleotide, in an ATG or GTG start codon present in the sequence of the intron of interest may be replaced resulting in a non-start codon. For example, an ATG or a GTG may be replaced by a CTG, which is not a start codon, within the sequence of the intron of interest.

[0142] The classical HBB2 intron is shown in SEQ ID NO:42. For example, this HBB2 intron may be modified by eliminating start codons (ATG and GTG codons) within said intron. In a particular embodiment, the modified HBB2 intron has the sequence shown in SEQ ID NO:43. The classical FIX intron is derived from the first intron of human FIX and is shown in SEQ ID

NO:44. FIX intron may be modified by eliminating start codons (ATG and GTG codons) within said intron. In a particular embodiment, the modified FIX intron has the sequence shown in SEQ ID NO:45. The classical chicken-beta globin intron used in nucleic acid constructs is shown in SEQ ID NO:46. Chicken-beta globin intron may be modified by eliminating start codons (ATG and GTG codons) within said intron. In a particular embodiment, the modified chicken-beta globin intron has the sequence shown in SEQ ID NO:47.

[0143] The inventors have previously shown in WO2015/162302 that such a modified intron, in particular a modified HBB2 or FIX intron, has advantageous properties and can significantly improve the expression of a transgene.

[0144] In a particular embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, a promoter optionally preceded by an enhancer, the coding sequence of the invention (i.e. the nucleic acid molecule encoding a mini-GDE polypeptide), and a polyadenylation signal such as the bovine growth hormone polyadenylation signal (bGH polyA), the SV40 polyadenylation signal, or another naturally occurring or artificial polyadenylation signal. In particular, the polyadenylation signal is the bGH polyA. In a preferred embodiment, a very short polyA signal is preferred. For example, a very short polyA signal comprising less than 20 nucleotides is preferred. In a particular embodiment, the polyadenylation signal is the human soluble neuropilin-1 (sNRP) polyadenylation signal (sNRP polyA; SEQ ID NO:58).

[0145] In a particular embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, a promoter optionally preceded by an enhancer, an intron, the coding sequence of the invention, and a polyadenylation signal. In another embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, a promoter, the coding sequence of the invention, and a polyadenylation signal. In another embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, an enhancer, a promoter, the coding sequence of the invention, and a polyadenylation signal. In another embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, a SpC5-12 promoter, the coding sequence of the invention, and a polyadenylation signal (such as a bGH polyA or a sNRP polyA, in particular a bGH polyA). In another embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, an enhancer, a SpC5-12 promoter, the coding sequence of the invention, and a polyadenylation signal (such as a bGH polyA or a sNRP polyA, in particular a bGH polyA). In a further particular embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, an enhancer, a promoter, an intron, the coding sequence of the invention, and a polyadenylation signal. In a further particular embodiment of the invention the expression cassette comprising, in the 5' to 3' orientation a promoter, an optional intron, the coding sequence of the invention and a polyA signal. In a further particular embodiment, the expression cassette comprises, in the 5' to 3' orientation: a SpC5-12 promoter; a SV40 intron; a sequence coding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:48, SEQ ID NO:49, SEQ ID

NO:50, SEQ ID NO:51 or SEQ ID NO:52, in particular SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, in particular SEQ ID NO:5; and a bGH polyA. In a further particular embodiment, the nucleic acid construct of the invention is an expression cassette comprising, in the 5' to 3' orientation, a promoter, the coding sequence of the invention, and a polyadenylation signal. In a further particular embodiment of the invention the expression cassette comprising, in the 5' to 3' orientation an enhancer, a promoter, the coding sequence of the invention and a polyA signal. In a further particular embodiment, the expression cassette comprises, in the 5' to 3' orientation: a SpC5-12 promoter; a sequence coding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51 or SEQ ID NO:52, in particular SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, in particular SEQ ID NO:5; and a bGH polyA or sNRP polyA, in particular a bGH polyA. In another embodiment, the expression cassette comprises, in the 5' to 3' orientation: a CMV promoter; a SV40 intron; a sequence coding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51 or SEQ ID NO:52, in particular SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, in particular SEQ ID NO:5; and a bGH polyA. In another embodiment, the expression cassette comprises, in the 5' to 3' orientation: a CMV promoter; a sequence coding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51 or SEQ ID NO:52, in particular SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, in particular SEQ ID NO:5; and a bGH polyA or sNRP polyA, in particular a bGH polyA. In a further particular embodiment, the expression cassette comprises, in the 5' to 3' orientation: a SpC5-12 promoter; a SV40 intron; a sequence coding the amino acid sequence of SEQ ID NO:12; and a bGH polyA. In a further particular embodiment, the expression cassette comprises, in the 5' to 3' orientation: a SpC5-12 promoter; a sequence coding the amino acid sequence of SEQ ID NO:12; and a bGH polyA or sNRP polyA, in particular a bGH polyA. In another embodiment, the expression cassette comprises, in the 5' to 3' orientation: a CMV promoter; a SV40 intron; a sequence coding the amino acid sequence of SEQ ID NO:12; and a bGH polyA. In another embodiment, the expression cassette comprises, in the 5' to 3' orientation: a CMV promoter; a sequence coding the amino acid sequence of SEQ ID NO:12; and a bGH polyA or sNRP polyA, in particular a bGH polyA.

[0146] In designing the nucleic acid construct of the invention, one skilled in the art will take care of respecting the size limit of the vector used for delivering said construct to a cell or organ. In particular, one skilled in the art knows that a major limitation of AAV vector is its cargo capacity which may vary from one AAV serotype to another but is thought to be limited to around the size of parental viral genome. For example, 5 kb, is the maximum size usually thought to be packaged into an AAV8 capsid (Wu Z. et al., Mol Ther., 2010, 18(1): 80-86; Lai Y. et al., Mol Ther., 2010, 18(1): 75-79; Wang Y. et al., Hum Gene Ther Methods, 2012, 23(4): 225-33). In addition, during recombinant AAV production, genomes larger than 5 kb are encapsidated with low efficacy and the resulting AAV may contain fragmented genomes reducing the efficacy of gene transfer. Accordingly, those skilled in the art will take care in practicing the present invention to select the components of the nucleic acid construct of the

invention so that the resulting nucleic acid sequence, including sequences coding AAV 5'- and 3'-ITRs to preferably not exceed 110 % of the cargo capacity of the AAV vector implemented, in particular to preferably not exceed 5 kb. AAV vectors having larger cargo capacity can also be used in the context on the present invention. For example AAV particles lacking Vp2 subunit are shown to successfully package larger genomes (i.e. 6 kb) while preserving integrity of encapsidated genomes (Grieger et al., 2005, J Virol., 79(15):9933-9944).

[0147] The present invention also relates to a vector comprising a nucleic acid molecule or construct as disclosed herein. In a particular embodiment, the vector comprises a nucleic acid molecule or construct encoding a functional truncated human GDE polypeptide as defined above. In another particular embodiment, the vector comprises a nucleic acid molecule or construct encoding a functional non-human GDE polypeptide as defined above.

[0148] In particular, the vector of the invention is a vector suitable for protein expression, preferably for use in gene therapy. In one embodiment, the vector is a plasmid vector. In another embodiment, the vector is a nanoparticle containing a nucleic acid molecule of the invention, in particular a messenger RNA encoding the mini-GDE polypeptide of the invention. In another embodiment, the vector is a system based on transposons, allowing integration of the nucleic acid molecule or construct of the invention in the genome of the target cell, such as the hyperactive Sleeping Beauty (SB100X) transposon system (Mates et al. 2009). In another embodiment, the vector is a viral vector suitable for gene therapy, targeting any cell of interest such as liver tissue or cells, muscle cell, CNS cells (such as brain cells), or hematopoietic stem cells such as cells of the erythroid lineage (such as erythrocytes). In this case, the nucleic acid construct of the invention also contains sequences suitable for producing an efficient viral vector, as is well known in the art.

[0149] Viral vectors are preferred for delivering the nucleic acid molecule or construct of the invention, such as a retroviral vector, for example a lentiviral vector, or a non-pathogenic parvovirus, more preferably an AAV vector. The human parvovirus Adeno-Associated Virus (AAV) is a dependovirus that is naturally defective for replication which is able to integrate into the genome of the infected cell to establish a latent infection. The last property appears to be unique among mammalian viruses because the integration occurs at a specific site in the human genome, called AAVS1, located on chromosome 19 (19q13.3-qter).

[0150] Therefore, AAV vectors have arisen considerable interest as potential vectors for human gene therapy. Among the favorable properties of the virus are its lack of association with any human disease, its ability to infect both dividing and non-dividing cells, and the wide range of cell lines derived from different tissues that can be infected.

[0151] Among the serotypes of AAVs isolated from human or non-human primates (NHP) and well characterized, human serotype 2 is the first AAV that was developed as a gene transfer vector. Other currently used AAV serotypes include AAV-1, AAV-2 variants (such as the quadruple-mutant capsid optimized AAV-2 comprising an engineered capsid with Y44+500+730F+T491V changes, disclosed in Ling et al., 2016 Jul 18, Hum Gene Ther

Methods.), -3 and AAV-3 variants (such as the AAV3-ST variant comprising an engineered AAV3 capsid with two amino acid changes, S663V+T492V, disclosed in Vercauteren et al., 2016, Mol. Ther. Vol. 24(6), p. 1042), -3B and AAV-3B variants, -4, -5, -6 and AAV-6 variants (such as the AAV6 variant comprising the triply mutated AAV6 capsid Y731F/Y705F/T492V form disclosed in Rosario et al., 2016, Mol Ther Methods Clin Dev. 3, p.16026), -7, -8, -9, -2G9, -10 such as cy10 and -rh10, -rh74, -dj, Anc80, LK03, AAV2i8, porcine AAV serotypes such as AAVpo4 and AAVpo6, and tyrosine, lysine and serine capsid mutants of the AAV serotypes, etc. In addition, other non-natural engineered variants and chimeric AAV can also be useful.

[0152] AAV viruses may be engineered using conventional molecular biology techniques, making it possible to optimize these particles for cell specific delivery of nucleic acid sequences, for minimizing immunogenicity, for tuning stability and particle lifetime, for efficient degradation, for accurate delivery to the nucleus.

[0153] Desirable AAV fragments for assembly into vectors include the cap proteins, including the vp1, vp2, vp3 and hypervariable regions, the rep proteins, including rep 78, rep 68, rep 52, and rep 40, and the sequences encoding these proteins. These fragments may be readily utilized in a variety of vector systems and host cells.

[0154] AAV-based recombinant vectors lacking the Rep protein integrate with low efficacy into the host's genome and are mainly present as stable circular episomes that can persist for years in the target cells. Alternatively to using AAV natural serotypes, artificial AAV serotypes may be used in the context of the present invention, including, without limitation, AAV with a non-naturally occurring capsid protein. Such an artificial capsid may be generated by any suitable technique, using a selected AAV sequence (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from a different selected AAV serotype, non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or from a non-viral source. An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a "humanized" AAV capsid.

[0155] In the context of the present invention, the AAV vector comprises an AAV capsid able to transduce the target cells of interest, i.e. cells of the tolerogenic tissue (for example hepatocytes) and cells of the tissue(s) of therapeutic interest such as muscle cells, CNS cells or cardiac cells.

[0156] According to a particular embodiment, the AAV vector is of the AAV-1, -2, AAV-2 variants (such as the quadruple-mutant capsid optimized AAV-2 comprising an engineered capsid with Y44+500+730F+T491V changes, disclosed in Ling et al., 2016 Jul 18, Hum Gene Ther Methods. [Epub ahead of print]), -3 and AAV-3 variants (such as the AAV3-ST variant comprising an engineered AAV3 capsid with two amino acid changes, S663V+T492V, disclosed in Vercauteren et al., 2016, Mol. Ther. Vol. 24(6), p. 1042), -3B and AAV-3B variants, -4, -5, -6 and AAV-6 variants (such as the AAV6 variant comprising the triply mutated AAV6 capsid Y731F/Y705F/T492V form disclosed in Rosario et al., 2016, Mol Ther Methods Clin Dev. 3,

p.16026), -7, -8, -9, -9P1, -2G9, -10 such as -cy10 and -rh10, -rh39, -rh43, -rh74, -dj, Anc80, LK03, AAV.PHP, AAV2i8, porcine AAV such as AAVpo4 and AAVpo6, and tyrosine, lysine and serine capsid mutants of AAV serotypes. In a particular embodiment, the AAV vector is of the AAV6, AAV8, AAV9, AAV9P1, AAVrh74 or AAV2i8 serotype (i.e. the AAV vector has a capsid of the AAV6, AAV8, AAV9, AAV9P1, AAVrh74 or AAV2i8 serotype). In a further particular embodiment, the AAV vector is a pseudotyped vector, i.e. its genome and capsid are derived from AAVs of different serotypes. For example, the pseudotyped AAV vector may be a vector whose genome is derived from one of the above mentioned AAV serotypes, and whose capsid is derived from another serotype. For example, the genome of the pseudotyped vector may have a capsid derived from the AAV6, AAV8, AAV9, AAV9P1, AAVrh74 or AAV2i8 serotype, and its genome may be derived from a different serotype. In a particular embodiment, the AAV vector has a capsid of the AAV6, AAV8, AAV9 or AAVrh74 serotype, in particular of the AAV6, AAV8, AAV9, or AAV9P1 serotype, more particularly of the AAV6, AAV9 or AAV9P1 serotype.

[0157] In a specific embodiment, wherein the vector is for use in delivering the therapeutic transgene to muscle cells, the AAV vector may be selected, among others, in the group consisting of AAV8, AAV9 and AAVrh74.

[0158] In another specific embodiment, wherein the vector is for use in delivering the transgene to liver cells, the AAV vector may be selected, among others, in the group consisting of AAV1, AAV5, AAV8, AAV9, AAVrh10, AAVrh39, AAVrh43, AAVrh74, AAV-LK03, AAV2G9, AAV.PHP, AAV-Anc80 and AAV3B.

[0159] In a further specific embodiment, wherein the vector is for use in delivering the transgene to the CNS, the AAV vector may be selected, among others, in the group consisting of AAV9, AAV9P1, AAV10 and AAV2G9.

[0160] In another embodiment, the capsid is a modified capsid. In the context of the present invention, a "modified capsid" may be a chimeric capsid or capsid comprising one or more variant VP capsid proteins derived from one or more wild-type AAV VP capsid proteins.

[0161] In a particular embodiment, the AAV vector is a chimeric vector, i.e. its capsid comprises VP capsid proteins derived from at least two different AAV serotypes, or comprises at least one chimeric VP protein combining VP protein regions or domains derived from at least two AAV serotypes. Examples of such chimeric AAV vectors useful to transduce liver cells are described in Shen et al., *Molecular Therapy*, 2007 and in Tenney et al., *Virology*, 2014. For example, a chimeric AAV vector can derive from the combination of an AAV8 capsid sequence with a sequence of an AAV serotype different from the AAV8 serotype, such as any of those specifically mentioned above. In another embodiment, the capsid of the AAV vector comprises one or more variant VP capsid proteins such as those described in WO2015013313, in particular the RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4 and RHM15-6 capsid variants, which present a high liver tropism.

[0162] In another embodiment, the modified capsid can be derived also from capsid

modifications inserted by error prone PCR and/or peptide insertion (e.g. as described in Bartel et al., 2011). In a particular embodiment, the capsid is modified includes the P1 modification, as described in as disclosed in PCT/EP2019/058560. In addition, capsid variants may include single amino acid changes such as tyrosine mutants (e.g. as described in Zhong et al., 2008)

[0163] In addition, the genome of the AAV vector may either be a single stranded or self-complementary double-stranded genome (McCarty et al., Gene Therapy, 2003). Self-complementary double-stranded AAV vectors are generated by deleting the terminal resolution site from one of the AAV terminal repeats. These modified vectors, whose replicating genome is half the length of the wild type AAV genome have the tendency to package DNA dimers. In a preferred embodiment, the AAV vector implemented in the practice of the present invention has a single stranded genome, and further preferably comprises an AAV8, AAV9, AAVrh74 or AAV2i8 capsid, in particular an AAV8, AAV9 or AAVrh74 capsid, such as an AAV8 or AAV9 capsid, more particularly an AAV9 capsid.

[0164] The AAV vector used for packaging the GDE sequence of the invention can also be modified in order to increase its cargo capacity. For example, AAV vectors lacking Vp2 subunit are shown to successfully package larger genomes (i.e. 6 kb) while preserving integrity of encapsidated genomes (Grieger et al., 2005).

[0165] As is known in the art, additional suitable sequences may be introduced in the nucleic acid construct of the invention for obtaining a functional viral vector. Suitable sequences include AAV ITRs.

[0166] In a particular embodiment, the AAV vector comprises a muscle-specific promoter as described above, in particular a muscle-specific promoter that presents some leakage of expression into liver cells.

[0167] In another particular embodiment of the invention, the AAV vector comprises a liver-specific promoter as described above. The protolerogenic and metabolic properties of the liver are advantageously implemented thanks to this embodiment to develop highly efficient and optimized vectors to express GDE in hepatocytes and to induce immune tolerance to the protein.

[0168] The invention also relates to a cell, in particular an isolated cell, for example a liver cell, a cardiac cell, a CNS cell or a muscle cell, that is transformed or transduced with the nucleic acid molecule, the construct or the vector of the invention. In a particular embodiment, the cell is an isolated human cell. In a further particular embodiment, the cell is not a human embryonic stem cell. The cell of the invention expresses a mini-GDE polypeptide. Cells of the invention may be delivered to the subject in need thereof, such as GDE-deficient patient, by any appropriate administration route such as via injection in the liver, in the CNS, in the heart, in the muscle(s) or in the bloodstream of said subject. In a particular embodiment, the invention involves transducing liver or muscle cells, in particular liver or muscle cells of the subject to be treated, and administering said transduced liver and/or muscle cells into which the nucleic acid

has been introduced to the subject. In a particular embodiment, the liver cells are liver cells from the patient to be treated, or are liver stem cells that are further transformed, and differentiated *in vitro* into liver cells, for subsequent administration to the patient. In another embodiment, the cell is a muscle cell from the patient to be treated, or is a muscle stem cell that is further transformed, and optionally differentiated *in vitro* into muscle cells, for subsequent administration to the patient.

[0169] The present invention also provides pharmaceutical compositions comprising the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, or the cell of the invention. Such compositions may comprise a therapeutically effective amount of the therapeutic (the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention), and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. or European Pharmacopeia or other generally recognized pharmacopeia for use in animals, and humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

[0170] The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. In a particular embodiment, the nucleic acid, vector or cell of the invention is formulated in a composition comprising phosphate-buffered saline and supplemented with 0.25% human serum albumin. In another particular embodiment, the nucleic acid, vector or cell of the invention is formulated in a composition comprising ringer lactate and a non-ionic surfactant, such as pluronic F68 at a final concentration of 0.01-0.0001%, such as at a concentration of 0.001%, by weight of the total composition. The formulation may further comprise serum albumin, in particular human serum albumin, such as human serum albumin at 0.25%. Other appropriate formulations for either storage or administration are known in the art, in particular from WO 2005/118792 or Allay et al., 2011.

[0171] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to, ease pain at the, site of the injection.

[0172] In an embodiment, the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention can be delivered in a vesicle, in particular a liposome. In yet another embodiment, the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention can be delivered in a controlled release system.

[0173] In a particular embodiment, the nucleic acid molecule is delivered as a mRNA, corresponding to the transcript encoding the mini-GDE polypeptide of the invention. In particular, the mRNA of the invention may be delivered using liposomes such as lipid nanoparticle (LNP).

[0174] Methods of administration of the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. In a particular embodiment, the administration is via the intravenous or intramuscular route. The nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention, whether vectorized or not, may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0175] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment, e.g. the liver or the muscle. This may be achieved, for example, by means of an implant, said implant being of a porous, nonporous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

[0176] In a particular embodiment, the mini-GDE polypeptide of the invention is used in enzyme replacement therapy (ERT), in particular for treating GSDIII. The term "enzyme replacement therapy" or "ERT" generally refers to the introduction of a purified enzyme into an individual having a deficiency in such enzyme. The administered polypeptide of the invention can be obtained from natural sources, by recombinant expression, produced in vitro, or purified from isolated tissue or fluid. In particular, when used in ERT, the polypeptide of the invention may be administered parenterally, such as via intraperitoneal, intramuscular, intravascular (i.e. intravenous or intraarterial) administration. In particular the polypeptide is administered by intravenous injection. Said administration may be repeated frequently, such as every day, every week, every two weeks or every month, in particular every week or every two

weeks.

[0177] The amount of the therapeutic (i.e. the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention) of the invention which will be effective in the treatment of GSDIII can be determined by standard clinical techniques. In addition, in vivo and/or in vitro assays may optionally be employed to help predict optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. The dosage of the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide or the cell of the invention administered to the subject in need thereof will vary based on several factors including, without limitation, the route of administration, the specific disease treated, the subject's age or the level of expression necessary to achieve the therapeutic effect. One skilled in the art can readily determine, based on its knowledge in this field, the dosage range required based on these factors and others. In case of a treatment comprising administering a viral vector, such as an AAV vector, to the subject, typical doses of the vector are of at least 1×10^8 vector genomes per kilogram body weight (vg/kg), such as at least 1×10^9 vg/kg, at least 1×10^{10} vg/kg, at least 1×10^{11} vg/kg, at least 1×10^{12} vg/kg at least 1×10^{13} vg/kg, or at least 1×10^{14} vg/kg.

[0178] Disclosed but not part of the invention is a method for treating GSDIII, which comprises a step of delivering a therapeutic effective amount of the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, the pharmaceutical composition or the cell of the invention to a subject in need thereof.

[0179] Cirrhosis and hepatocellular carcinoma can also develop in patients with GSD III. Thus, the invention also relates to a method for treating cirrhosis and hepatocellular carcinoma in a GSDIII patient which comprises a step of delivering a therapeutic effective amount of the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, the pharmaceutical composition or the cell of the invention to a subject in need thereof.

[0180] The invention also relates to a method for treating GSD III, said method inducing no immune response to the transgene (i.e. to the mini-GDE polypeptide encoded by the nucleic acid molecule), or inducing a reduced immune response to the transgene, comprising a step of delivering a therapeutic effective amount of the nucleic acid, the vector, the mini-GDE polypeptide, the pharmaceutical composition or the cell of invention to a subject in need thereof. The invention also relates to a method for treating GSD III, said method comprising repeated administration of a therapeutic effective amount of the nucleic acid, the vector, the mini-GDE polypeptide, the pharmaceutical composition or the cell of the invention to a subject in need thereof. In this aspect, the nucleic acid molecule, the nucleic acid construct or the vector of the invention comprises a promoter which is functional in liver cells, thereby allowing immune tolerance to the expressed mini-GDE polypeptide produced therefrom. As well, in this aspect, the pharmaceutical composition used in this aspect comprises a nucleic acid molecule,

a nucleic acid construct or a vector comprising a promoter which is functional in liver cells. In case of delivery of cells, in particular of liver, cardiac, CNS or muscle cells, said cells may be cells previously collected from the subject in need of the treatment and that were engineered by introducing therein the nucleic acid molecule, the nucleic acid construct or the vector of the invention to thereby make them able to produce the mini-GDE polypeptide. According to an embodiment, in the aspect comprising a repeated administration, said administration may be repeated at least once or more, and may even be considered to be done according to a periodic schedule, such as once per week, per month or per year. The periodic schedule may also comprise an administration once every 2, 3, 4, 5, 6, 7, 8, 9 or 10 year, or more than 10 years. In another particular embodiment, administration of each administration of a viral vector of the invention is done using a different virus for each successive administration, thereby avoiding a reduction of efficacy because of a possible immune response against a previously administered viral vector. For example, a first administration of an AAV vector comprising an AAV8 capsid may be done, followed by the administration of a vector comprising an AAV9 capsid.

[0181] According to the present invention, a treatment may include curative, alleviation or prophylactic effects. Accordingly, therapeutic and prophylactic treatment includes amelioration of the symptoms of GSD III or preventing or otherwise reducing the risk of developing a particular glycogen storage disease. The term "prophylactic" may be considered as reducing the severity or the onset of a particular condition. "Prophylactic" also includes preventing reoccurrence of a particular condition in a patient previously diagnosed with the condition. "Therapeutic" may also reduce the severity of an existing condition. The term "treatment" is used herein to refer to any regimen that can benefit an animal, in particular a mammal, more particularly a human subject.

[0182] The invention also relates to an *ex vivo* gene therapy method for the treatment of GSD III, comprising introducing the nucleic acid molecule, the nucleic acid construct or the vector of the invention into an isolated cell of a patient in need thereof, for example an isolated hematopoietic stem cell, and introducing said cell into said patient in need thereof.

[0183] The invention also relates to the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, the cell or the pharmaceutical composition of the invention for use as a medicament.

[0184] The invention also relates to the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, the cell or the pharmaceutical composition of the invention, for use in a method for treating a disease caused by a mutation in the GDE gene, in particular in a method for treating GSDIII (Cori disease).

[0185] The invention further relates to the use of the nucleic acid molecule, the nucleic acid construct, the vector, the mini-GDE polypeptide, the cell or the pharmaceutical composition of the invention, in the manufacture of a medicament useful for treating GSD III (Cori disease).

EXAMPLES

[0186] The invention is further described in detail by reference to the following experimental examples and the attached figures. These examples are provided for purposes of illustration only, and are not intended to be limiting.

MATERIAL AND METHODS

Western blot analysis

[0187] Mouse tissues were homogenized in DNase/RNase free water and protein concentration determined using a BCA Protein Assay. SDS-PAGE electrophoresis was performed in a 4-15% gradient polyacrylamide gel. After transfer, the membrane was blocked and incubated with an anti-GDE antibody and an anti-actin antibody. The membrane was washed, incubated with the appropriate secondary antibody, and visualized by Odyssey imaging system.

Enzyme activity measurements

[0188] Tissues homogenized as described above were incubated 3-16 hours at 37°C with limit dextrin dissolved in phosphate buffer pH 6.9. The reaction was stopped by incubating 10 min at 95°C and then centrifuged 10 min at 11000 × g. Supernatants were used to measure the glucose produced using a commercial glucose assay kit. The reaction was stopped with concentrated H₂SO₄ and the resulting absorbance was measured on an EnSpire alpha plate reader (Perkin-Elmer, Waltham, MA) at 540 nm.

Measurement of glycogen content

[0189] Glycogen content was measured indirectly in tissue homogenates as the glucose released after total digestion with *Aspergillus Niger* amyloglucosidase (Sigma Aldrich, Saint Louis, MO). Samples were incubated for 5 min at 95°C and then cooled at 4°C; 25 µl of amyloglucosidase diluted 1:50 in 0.1M potassium acetate pH5.5 were then added to each sample. A control reaction without amyloglucosidase was prepared for each sample. Both sample and control reactions were incubated at 37°C for 90 minutes. The reaction was stopped by incubating samples for 5 min at 95°C. The glucose released was determined with a commercial glucose assay kit (Sigma Aldrich, Saint Louis, MO) and the resulting absorbance was acquired on an EnSpire alpha plate reader (Perkin-Elmer, Waltham, MA) at a wavelength

of 540 nm.

Muscle function tests

[0190] To measure the mean hanging time, a three-minute lasting hanging test on a 4-mm wire was performed. At the beginning of the test, a "falling" score of 10 is attributed to each animal. A mouse is handled by the tail and brought near the wire. The operator suspends the animal by the fore limbs only. As soon as the animal is properly suspended, a 180-sec timer is started. If the animal falls, the timer is stopped, the falling score is diminished by 1 and the elapsed time is noted. The animal is then suspended by the fore limbs and the timer started again. The test is stopped either when the timer or the falling score reach 0. Results are expressed as number of falls per minute.

RESULTS

[0191] AAV are the vector of choice for in vivo gene therapy. One of the biggest limitations in the use of AAV for gene replacement is their encapsidation size that is limited to 5 Kb. Indeed, during recombinant AAV production, genomes larger than 5 Kb are encapsidated with low efficacy and the resulting AAV may contain fragmented genomes reducing the efficacy of gene transfer. Different approaches have been developed to overcome this limitation. In particular the use of dual AAV vectors has been reported. Following this approach, two vectors, each containing a portion of the large transgene coding sequence, are used to transduce the same cell. The recombination of the two vectors may occur through i) an overlapping sequence derived from the transgene, ii) internal terminal repeats (ITR) combined with a splicing donor and acceptor or iii) an heterologous highly recombinogenic sequence coupled with a splicing donor and acceptor. However, although dual AAV vectors demonstrated efficacy in different animal models they have some drawback. Here we report the first data on the use of a gorilla GDE that fits in a single AAV and rescue glycogen accumulation and muscle function in GSDIII mice with an efficacy similar to that of dual AAV vectors at a lower dose.

[0192] Figure 1 represents 4 different mammalian non-human GDE proteins smaller than human GDE (hGDE), this being not exhaustive.

[0193] In figure 2 are represented the truncated human GDE (hGDE) $\Delta 1$, $\Delta 2-3$, and $\Delta 4$ sequences.

[0194] First, we evaluated the effects induced in GSD III mice by a short non-human mammalian GDE. A transgene expression cassette composed of a muscle specific promoter (SpC5-12), SV40 intron, the coding sequence for gorilla GDE (gGDE) and the bGH polyA (AAV9-gGDE, total size: 5.1 Kb) was then used to produce an AAV9 vector by triple transfection and cesium chloride gradient purification.

[0195] The AAV9-gGDE vector was then injected in 3 month-old GSDIII mice at the dose of 1×10^{12} vg/mouse in parallel with a dual AAV vector expressing GDE under the translational control of CMV promoter at the dose of 2×10^{12} vg/mouse. Three months after vector injection, mice were sacrificed and tissues were analyzed to evaluate the biochemical correction of GSDIII. Western blot performed on the heart of mice treated as described above with an antibody specific for GDE indicates that the injection of AAV9-gGDE induces the expression of a protein smaller than GDE (estimated size ~130 KDa) and recognized by a specific anti-GDE antibody (Figure 3). We then evaluated glycogen accumulation in the quadriceps of GDE-KO animals injected with AAV9-gGDE in comparison with a dual AAV9 vector expressing GDE under the transcriptional control of CMV (Dual-GDE). In the graph of figure 4 are reported the levels of glycogen measured in AAV-treated animals and in untreated wild-type (WT) and KO animals. The treatment with a single vector expressing gGDE cleared glycogen accumulation to levels comparable to those observed with dual AAV vectors. Additionally, the measurement of muscle function by wire-hang indicates that both approaches are equally efficient in the rescue of the muscle strength (Figure 5). Taken together, these data indicate that AAV-mediated gGDE expression in the muscle rescues glycogen accumulation and muscle strength of GSDIII mice.

[0196] We then evaluated the activity of truncated forms of the human GDE sequence both in vitro and in vivo. We first transfected liver hepatoma cells (Huh-7) with plasmids encoding one truncated form derived from the human GDE under the transcriptional control of a CMV promoter. The expression cassette also contained a SV40 intron and a bGH polyadenylation signal. Two days after transfection, the activity was measured in cytosolic extracts obtained from those cells. The activity test, based on the release of glucose from limited dextrin allowed for the detection of a basal activity in the cell line, due to the endogenous expression of GDE. Full-size human GDE overexpression resulted in an increased GDE activity. Similar results were obtained with the $\Delta 4$ truncated human GDE but not with the gorilla GDE (Figure 6). We then obtained results in vivo by intramuscular injection of AAV vectors expressing GDEs. GDE-KO animals were injected intramuscularly with AAV9 vectors expressing a human truncated GDE ($\Delta 1$), the gorilla GDE or a dual vector expressing human full size GDE. 15 days after the injection, the tibialis anterior was dissected and analyzed for GDE expression and activity (Figure 7). Western blot analysis with an anti-GDE antibody clearly demonstrated the presence of a band with a molecular weight lower than the full-size GDE (Figure 7).

[0197] AAV vectors expressing three different truncated hGDE either wild-type (wt) or codon optimized (co) under the transcriptional control of SpC5.12 promoter were derived. These vectors were injected directly in the right tibialis anterior (TA) of female GDE-KO mice at the dose of 1×10^{11} vg/mouse. Fifteen days after the injection, GDE activity was measured in extracts obtained from the TA of the injected mice. GDE activities measured in the left TA that did not receive any injection were used as control (Agl^{-/-}, CTRL). Protein extracts obtained from the TA of mice treated with single vectors AAV expressing the different truncated forms of GDE showed higher levels of GDE activity compared to the control (Figure 8).

[0198] Figure 9 demonstrates the successful expression of additional human truncated GDEs : $\Delta 9 + \Delta 2/3$; $\Delta 10 + \Delta 2/3$; $\Delta 11 + \Delta 2/3$; $\Delta 12 + \Delta 2/3$; $\Delta 13 + \Delta 2/3$, by transfection in HEK293 cells.

[0199] Figure 10 further demonstrates the successful expression of additional human truncated GDEs : $\Delta 2/3$; $\Delta 9 + \Delta 2/3$; $\Delta 10 + \Delta 2/3$; $\Delta 13 + \Delta 2/3$, in the tibialis anterior of GDE-KO mice, 15 days after the injection with AAV9 vectors expressing said human truncated GDE.

[0200] Data shown clearly demonstrates that shorter forms of GDE, either truncated forms of human GDE or non-human GDE, can be expressed both in vitro and in vivo in an active form and that can be used to degrade glycogen accumulated in GSDIII mice.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [US20030157064A \[0135\]](#)
- [WO2015110449A \[0140\] \[0140\]](#)
- [WO2009130208A \[0140\]](#)
- [WO2015162302A \[0143\]](#)
- [WO2015013313A \[0161\]](#)
- [EP2019058560W \[0162\]](#)
- [WO2005118792A \[0170\]](#)

Non-patent literature cited in the description

- **CHEN et al.** Scriver's Online Metabolic & Molecular Bases of inherited Disease McGraw-Hill 2009 0000 [\[0002\]](#)
- **KISHNANI et al.** Genet Med, 2010, vol. 12, 446-463 [\[0002\]](#) [\[0002\]](#) [\[0002\]](#)

- CORNELIO et al. Arch Neurol, 1984, vol. 41, 1027-1032 [0002]
- COLEMAN et al. Ann Intern Med, 1992, vol. 116, 896-900 [0002]
- MICHON et al. J Inherit Metab Dis, 2015, vol. 38, 3573-580 [0002]
- PAGLIARANI et al. Biochim Biophys Acta, 2014, vol. 1842, 112318-2328 [0002]
- LIU et al. Mol Genet Metab, 2014, vol. 111, 4467-476 [0002]
- VIDAL P et al. Rescue of GSDIII phenotype with gene transfer requires liver- and muscle-targeted GDE expression Molecular Therapy, 2017, vol. 26, 3 [0002]
- BAODONG S et al. Preclinical development of new therapy for glycogen storage diseases Curr Gen Therapy, 2015, vol. 15, 4338-347 [0002]
- BAO Y et al. Genomics, 1996, vol. 38, 2155-65 [0003]
- VIDAL P et al. Adeno-associated vector-based gene therapy strategy for type glycogen storage disease Neuromuscular disorders, 2017, vol. 27, [0003]
- BAO Genomics, 1997, vol. 38, 155-165 [0036]
- WANG B et al. Construction and analysis of compact muscle-selective promoters for AAV vectors Gene Ther., 2008, vol. 15, 221489-99 [0135]
- WANG et al. Gene Therapy, 2008, vol. 15, 1489-1499 [0135] [0135]
- SALVA et al. Mol Ther., 2007, vol. 15, 2320-9 [0135]
- WEINTRAUB et al. Science, 1991, vol. 251, 761- [0135]
- ILL, C. R. et al. Optimization of the human factor VIII complementary DNA expression plasmid for gene therapy of hemophilia A Blood Coag. Fibrinol., 1997, vol. 8, S23-S30 [0136]
- ANDERSEN et al. Cell. Mol. Neurobiol., 1993, vol. 13, 503-15 [0137]
- PICCIOLI et al. Proc. Natl. Acad. Sci. USA, 1991, vol. 88, 5611-5 [0137]
- PICCIOLI et al. Neuron, 1995, vol. 15, 373-84 [0137]
- BOSHART et al. Cell, 1985, vol. 41, 521-530 [0138]
- RINCON et al. Mol Ther., 2015, vol. 23, 143-52 [0140]
- CHUAH et al. Mol Ther., 2014, vol. 22, 91605-13 [0140]
- NAIR et al. Blood., 2014, vol. 123, 203195-9 [0140]
- WU et al. Mol Ther, 2008, vol. 16, 2280-289 [0141] [0141]
- KURACHI et al. J Biol Chem., 1995, vol. 270, 105276-5281 [0141] [0141]
- WONG et al. Chromosoma, 1985, vol. 92, 2124-135 [0141]
- YEW et al. Hum Gene Ther, 1997, vol. 8, 5575-584 [0141] [0141]
- CHOI T. et al. Mol Cell Biol, 1991, vol. 11, 63070-3074 [0141]
- HUANG et al. Mol Cell Biol., 1990, vol. 10, 41805-1810 [0141]
- WU Z. et al. Mol Ther., 2010, vol. 18, 180-86 [0146]
- LAI Y. et al. Mol Ther., 2010, vol. 18, 175-79 [0146]
- WANG Y. et al. Hum Gene Ther Methods, 2012, vol. 23, 4225-33 [0146]
- GRIEGER et al. J Virol., 2005, vol. 79, 159933-9944 [0146]
- LING et al. Hum Gene Ther Methods, 2016, [0151] [0156]
- VERCAUTEREN et al. Mol. Ther., 2016, vol. 24, 61042- [0151] [0156]
- ROSARIO et al. Mol Ther Methods Clin Dev., 2016, vol. 3, 16026- [0151] [0156]
- SHEN et al. Molecular Therapy, 2007, [0161]
- TENNEY et al. Virology, 2014, [0161]
- MCCARTY et al. Gene Therapy, 2003, [0163]

Patentkrav

- 1.** Funktionelt trunkeret humant GDE-polypeptid, som har en deletion på mindst omtrent 10, 20, 30, 40, 50, 60, 75, 90, 100, 125, 150, 175, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 eller mindst omtrent 525
- 5 aminosyrer i forhold til en human GDE-referencesekvens med fuld længde, især en human GDE-referencesekvens med fuld længde, som har en aminosyresekvens som vist i SEQ ID NO:1, SEQ ID NO:40 eller SEQ ID NO:41.
- 2.** Det funktionelle trunkerede humane GDE-polypeptid ifølge krav 1, hvor:
- 10 (i) den humane GDE-referencesekvens med fuld længde har en aminosyresekvens som vist i SEQ ID NO:1, og nævnte trunkerede humane GDE-polypeptid omfatter mindst aminosyrerester ved positionerne 429-666, 770-892, 1088-1194 og 1235-1532 i forhold til SEQ ID NO:1;
- (ii) den humane GDE-referencesekvens med fuld længde har en
- 15 aminosyresekvens som vist i SEQ ID NO:40, og nævnte trunkerede humane GDE-polypeptid omfatter mindst aminosyreresterne ved positionerne 412-649, 753-875, 1071-1177, 1218-1515 i forhold til SEQ ID NO:40; eller
- (iii) den humane GDE-referencesekvens med fuld længde har en
- 20 aminosyresekvens som vist i SEQ ID NO:41, og nævnte trunkerede humane GDE-polypeptid omfatter mindst aminosyreresterne ved positionerne 413-650, 754-876, 1072-1178, 1219-1516 i forhold til SEQ ID NO:41.
- 25 **3.** Det funktionelle trunkerede humane GDE-polypeptid ifølge krav 1 eller 2, omfattende en deletion eller en kombination af deletioner, som vist i tabel 2.
- 4.** Det funktionelle trunkerede humane GDE-polypeptid ifølge et hvilket som helst af kravene 1 til 3, som har en sekvens valgt fra SEQ ID NO:2-6 og SEQ ID NO:48-
- 30 52.
- 5.** Det funktionelle trunkerede humane GDE-polypeptid ifølge et hvilket som helst af kravene 1 til 4, som har en aminosyresekvens bestående af SEQ ID NO:5.

6. Nukleinsyremolekyle som koder for det funktionelle trunkerede humane GDE-polypeptid ifølge et hvilket som helst af kravene 1 til 5.

7. Nukleinsyrekonstrukt, omfattende, fortrinsvis i denne rækkefølge:

- 5 - en promotor;
 - eventuelt, et intron;
 - nukleinsyremolekylet ifølge krav 6; og
 - et polyadenyleringssignal.

10 **8.** Vektor, især en virusvektor, omfattende:

- nukleinsyremolekylet ifølge krav 6; eller
 - nukleinsyrekonstruktet ifølge krav 7.

9. Virusvektor omfattende et nukleinsyrekonstrukt, som koder for et funktionelt ikke-humant GDE-polypeptid, hvor det funktionelle ikke-humane GDE-polypeptid omfatter mindre end omtrent 1500 aminosyrer.

10. Virusvektoren ifølge krav 9, hvor det funktionelle ikke-humane GDE-polypeptid er valgt fra gruppen bestående af: heste-GDE-polypeptid af SEQ ID NO:11, gorilla-GDE-polypeptid af SEQ ID NO:12, orangutang-GDE-polypeptid af SEQ ID NO:13, sort flyvende ræv-GDE-polypeptid af SEQ ID NO:14, sodet mangabey-GDE-polypeptid af SEQ ID NO:15, næbdyr-GDE-polypeptid af SEQ ID NO:16 og ande-GDE-polypeptid af SEQ ID NO:17, idet det funktionelle ikke-humane GDE-polypeptid fortrinsvis er gorilla-GDE-polypeptidet af SEQ ID NO:12.

25

11. Vektoren ifølge et hvilket som helst af kravene 8 til 10, hvilken er en AAV-vektor, og hvor nukleinsyremolekylet, som koder for GDE-polypeptidet, er lille nok til at kunne pakkes i AAV-vektoren.

30 **12.** Isoleret celle transformeret med nukleinsyremolekylet ifølge krav 6, nukleinsyrekonstruktet ifølge krav 7 eller vektoren ifølge et hvilket som helst af kravene 8 til 11, hvor cellen især er en levercelle, en muskelcelle, en hjertecelle eller CNS-celle.

13. Det funktionelle trunkerede humane GDE-polypeptid ifølge et hvilket som helst af kravene 1 til 5, det funktionelle ikke-humane GDE-polypeptid som defineret i krav 9, nukleinsyremolekylet ifølge krav 6, nukleinsyrekonstruktet ifølge krav 7, vektoren ifølge et hvilket som helst af kravene 8 til 11, eller cellen 5 ifølge krav 12, til anvendelse som er medikament.

14. Det funktionelle trunkerede humane GDE-polypeptid ifølge et hvilket som helst af kravene 1 til 5, det funktionelle ikke-humane GDE-polypeptid som defineret i krav 9, nukleinsyremolekylet ifølge krav 6, nukleinsyrekonstruktet af 10 krav 7, vektoren ifølge et hvilket som helst af kravene 8 til 11, eller cellen ifølge krav 12, til anvendelse i en fremgangsmåde til behandling af GSDIII (Cori-sygdom).

15. Gorilla-GDE-polypeptid af SEQ ID NO:12, til anvendelse i en fremgangsmåde 15 til behandling af GSDIII (Cori-sygdom).

DRAWINGS

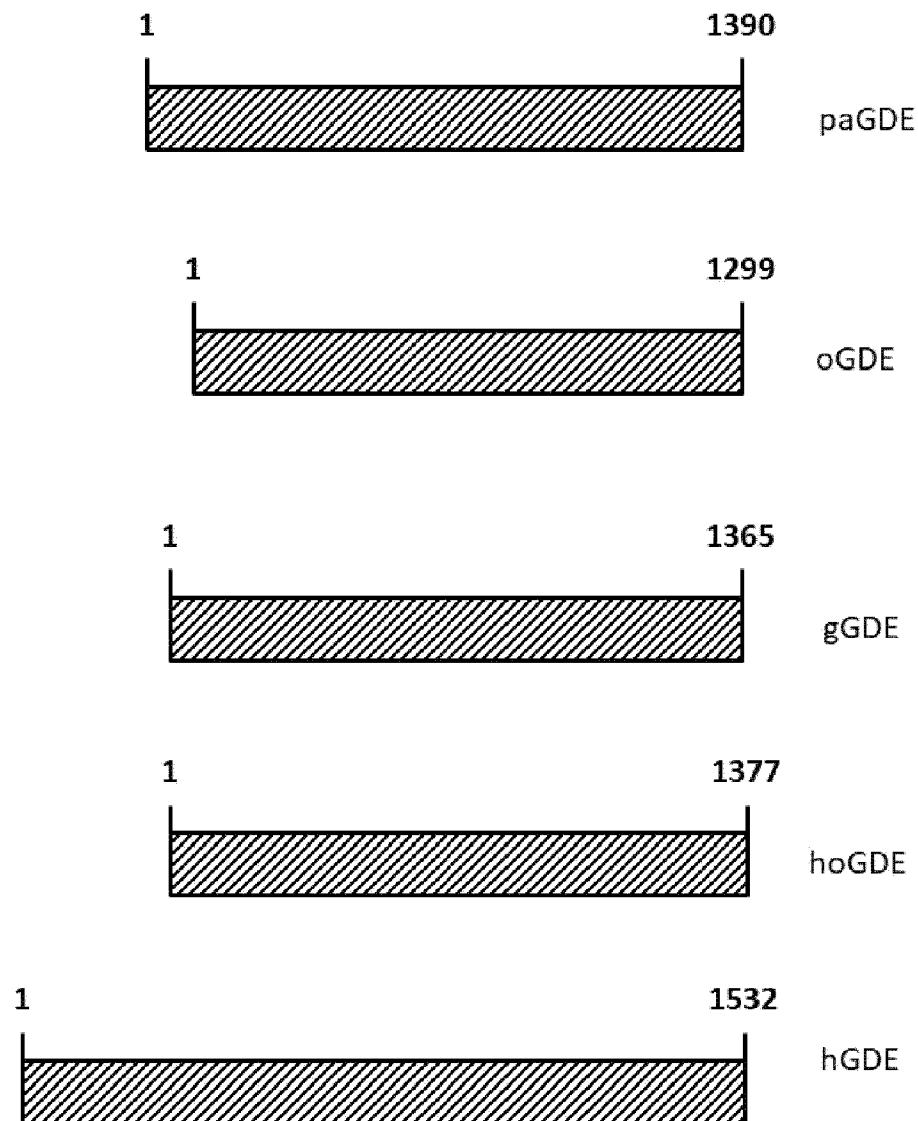


FIGURE 1

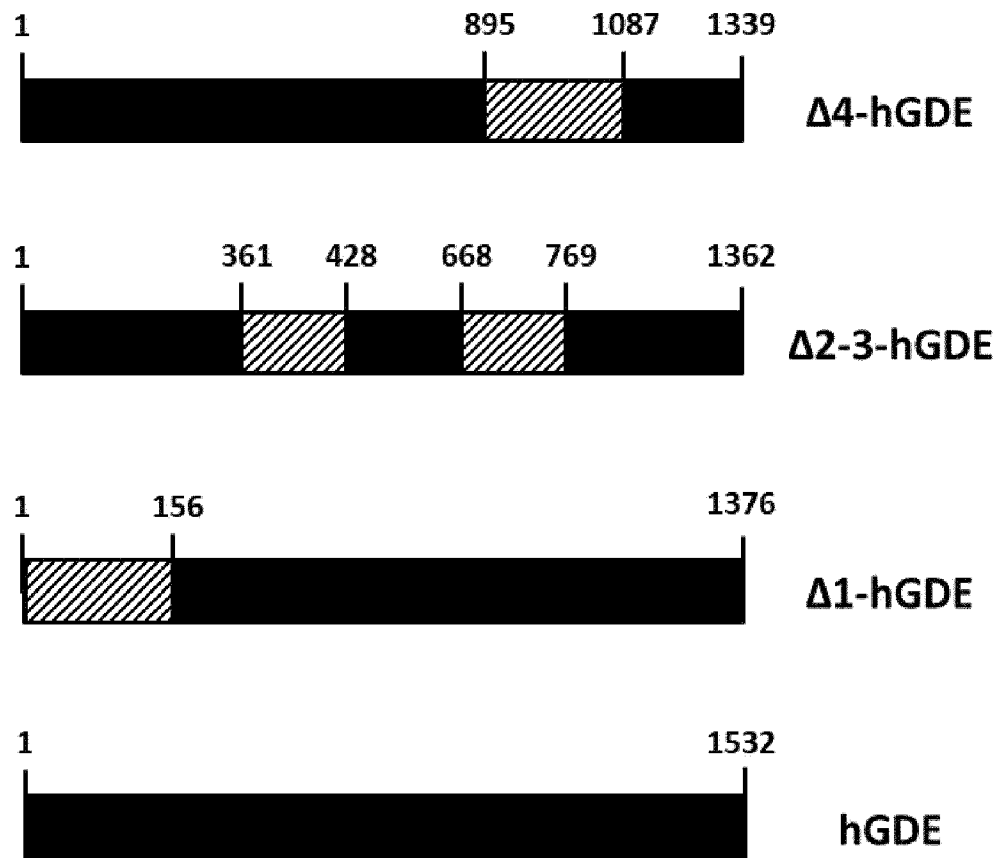


FIGURE 2

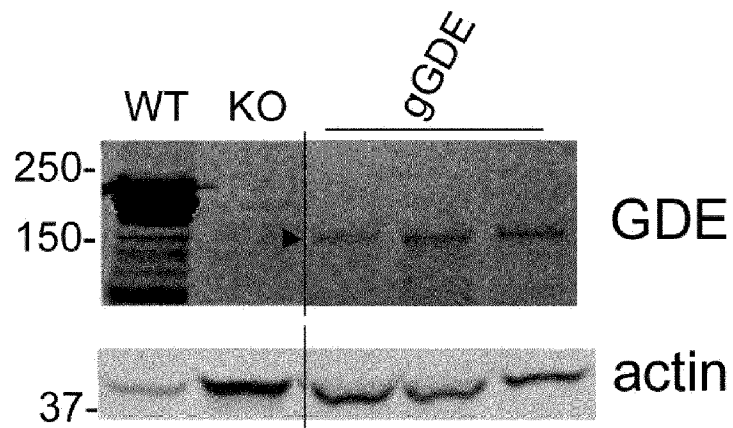


FIGURE 3

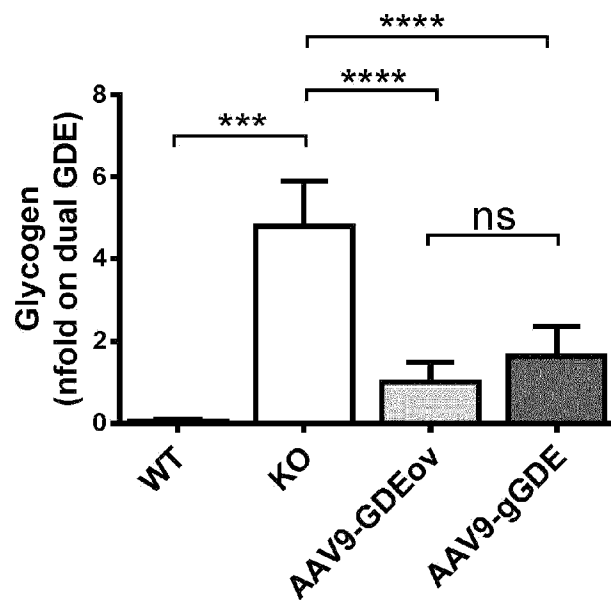


FIGURE 4

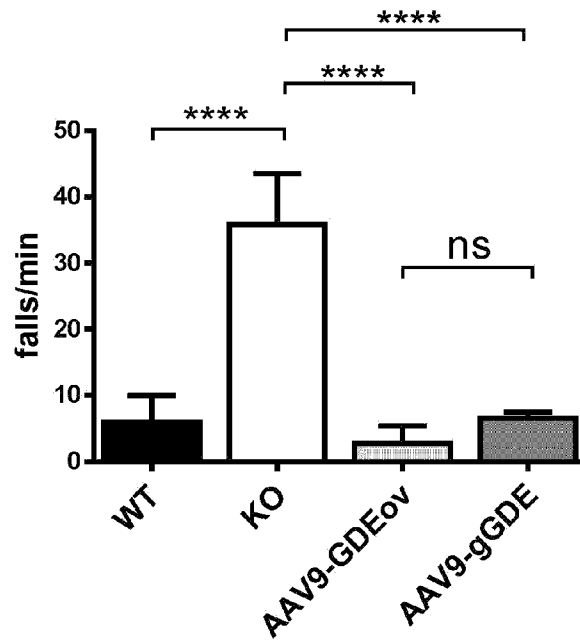


FIGURE 5

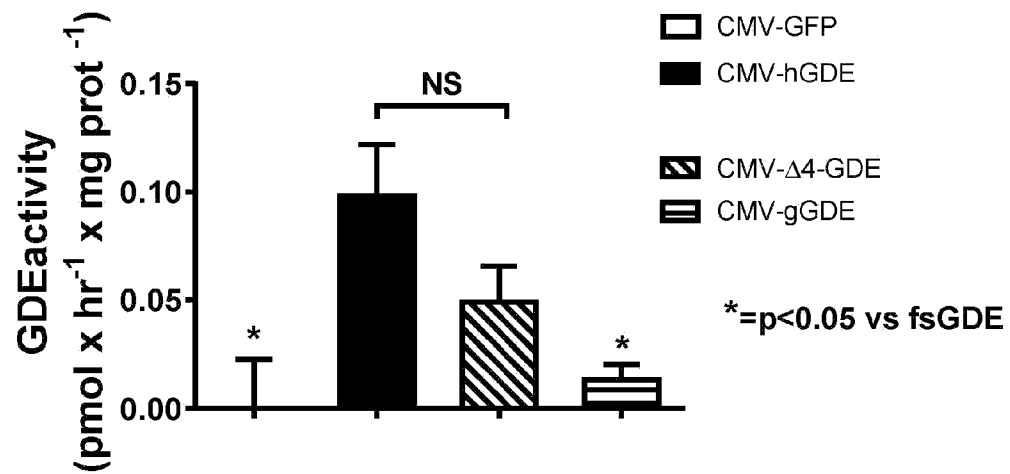


FIGURE 6

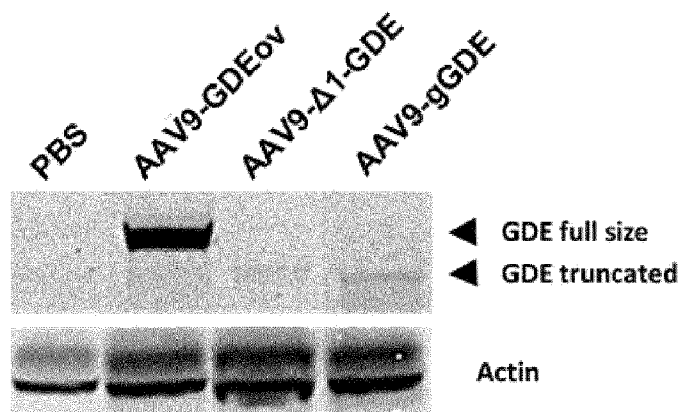


FIGURE 7

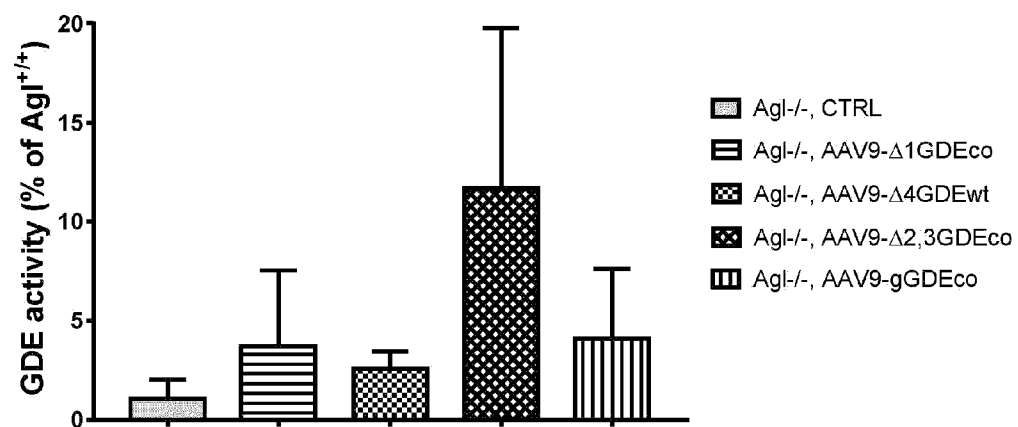


FIGURE 8

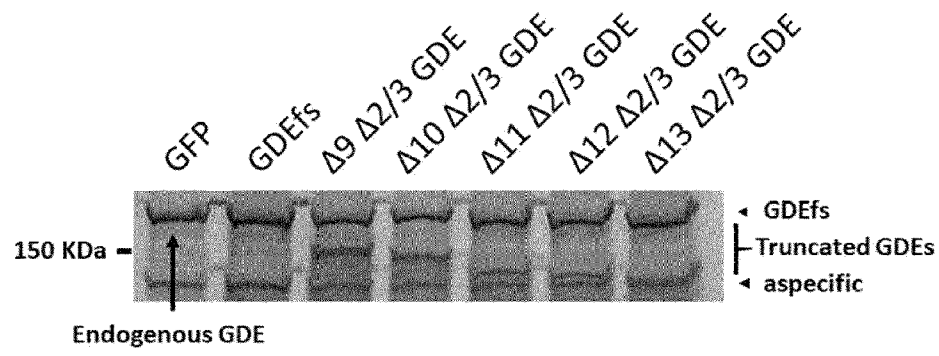


FIGURE 9



FIGURE 10

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

