

Vectors encoding a glucose-6-phosphatase (G6Pase-a) for gene therapy

Field of the invention

The invention relates to an adeno-associated virus (AAV) comprising a nucleic acid construct
5 for the expression of a glucose-6-phosphatase-a (G6Pase-a) in a cell, useful in the treatment
of glycogen storage disease Ia (GSD-Ia), wherein the nucleic acid sequence encoding the
G6Pase-a is operably linked to a human alpha-1 antitrypsin (hAAT) promoter.

Background of the invention

10 Glycogen storage disease type Ia (GSD-Ia or von Gierke disease) is caused by a deficiency in
glucose-6-phosphatase-a (G6Pase-a), an enzyme that is expressed primarily in the liver,
kidney, and intestine. G6Pase-a, encoded by the *G6PC* gene, is a hydrophobic protein
anchored in the endoplasmic reticulum (ER) by nine transmembrane helices. This enzyme
catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate in
15 the terminal step of glycogenolysis and gluconeogenesis. Patients affected by GSD-Ia are
unable to maintain glucose homeostasis and present with fasting hypoglycemia, growth
retardation, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic
acidemia.

Most of the time, hypoglycemia can be managed using dietary therapies that enable patients
20 to attain near normal growth and pubertal development. However, the long-term clinical
complications, and their underlying pathological processes, remain uncorrected. One of the
most significant chronic risks is hepatocellular adenoma (HCA), that develops in 70-80% of
GSD-I patients over 25 year-old. HCAs in GSD-Ia patients are multiple and non-
encapsulated, with complications including local compression and intratumoral hemorrhage.
25 In 10% of GSD-Ia patients, HCAs undergo malignant transformation to hepatocellular
carcinoma (HCC).

Thus, a need exists for an improved therapy vector for the treatment of GSD-Ia and its
associated complications.

Gene therapy studies using recombinant adeno-associated virus (AAV) carrying G6Pase-a
30 have previously been performed in animal models of GSD-Ia. In particular, the prior art

discloses the use of nucleic acid constructs comprising a nucleic acid sequence encoding the G6Pase-a which is operably linked to a *G6PC* promoter/enhancer (GPE).

Complications of treatment related to immune responses against the vector represent serious obstacles for the use of AAV vectors in gene therapy. The administration of lower doses of AAV vectors is, in general, associated to a lower activation of the immune response and a better stability of transgene expression [15]. In order to decrease the appearance of side effects associated with the use of AAV vectors, it is therefore preferable to administer the lowest amount of AAV vector that is sufficient to obtain a therapeutically effective effect in a subject.

- Thus, there is a need for improved nucleic acid constructs that increases G6Pase-a expression and activity in gene therapy, allowing to decrease the amount of AAV vector that is necessary to achieve a therapeutic effect in a subject.

Summary of the invention

- The inventors have surprisingly shown that, in particular in an AAV gene therapy setting, a nucleic acid encoding G6Pase-a under the control of a hAAT promoter leads to increased G6Pase-a expression and G6Pase-a activity that permits phenotypical rescue in GSDIa mice compared to hGPE promoter. This was particularly unexpected in view of the prior art that used different forms of G6Pase native promoter (GPE) derived from different species to treat GSDIa.

- In addition, the inventors have also shown that the same nucleic acid encoding G6Pase-a under the control of a hAAT promoter decreases the risk of tumor formation, such as HCA and HCC, when compared to hGPE promoter in gene therapy. This was also particularly unexpected in view of the prior art that predicts that promoters with higher activity are more likely to increase the risk of HCA and HCC after gene therapy [1].

- Thus, in a first aspect, the invention relates to an adeno-associated virus (AAV) vector comprising a nucleic acid construct for the expression of a glucose-6-phosphatase-a (G6Pase-a) in a cell, the construct comprising a nucleic acid sequence encoding the G6Pase-a, wherein the nucleic acid sequence encoding the G6Pase-a is operably linked to a human alpha-1 antitrypsin (hAAT) promoter.

In a second aspect, the invention relates to a cell transformed with the vector of the invention.

In a third aspect, the invention relates to a composition comprising the vector of the invention, or the cell of the invention.

- 5 In a fourth aspect, the invention relates to the vector, the cell or the composition of the invention, for use as a medicament, in particular for use in the treatment of glycogen storage disease Ia (GSD-Ia).

Legends to the figures

- 10 **Figure 1** shows the correction of the liver phenotype in GSD-Ia mice and WT mice after 15 days of treatment with AAV vectors or PBS. **A.** is the scheme of the protocol. **B.** shows the glycemia measured after 6h of fasting at the end of the protocol. **C.** shows G6Pase activity measured in liver tissues. **D.** shows glycogen content of liver tissues. **E.** shows hepatomegaly reported as the percentage of liver/body weight. **F.** shows the vector genome copy number per diploid genome measured in liver of AAV-treated mice. Statistical analyses were performed by ANOVA in A-E (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice) and by t-test in F (ns, not significant).

- 20 **Figure 2** shows the long-term correction of the liver phenotype in GSD-Ia mice. **A.** represents the scheme of the protocol. **B.** shows the glycemia measured after 6h of fasting at the end of the protocol, i.e. 7 months after vector injection. **C.** shows the G6Pase activity measured in liver tissues collected at sacrifice. **D.** shows the glycogen content measured on liver tissues. **E.** shows the hepatomegaly reported as the percentage of liver/body weight at sacrifice. **F.** shows the vector genome copy number per diploid genome measured in liver of AAV-treated mice. Statistical analyses were performed by ANOVA (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice; † P<0.05 as indicated) and by t-test in F (* P<0.05).

- 30 **Figure 3** shows that hGPE-directed gene therapy promotes hepatic tumor formation in L.G6pc^{-/-} mice fed a high fat high sucrose diet. **A.** represents the scheme of the protocol. **B.** shows the glycemia measured after 6h of fasting at the end of the protocol i.e. 8 months after vectors injection. **C.** shows the G6Pase-a activity assay performed on liver tissues collected at sacrifice. **D.** shows the vector genome copy number per diploid genome measured in liver. **E.** shows the number of tumors larger than 2 mm observed in PBS or AAV

vector -treated L.G6pc^{-/-} mice 8 months after the start of the HF/HS regimen. Statistical analyses were performed by ANOVA in B and C (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice), by t-test in D (ns, not significant) and by non-parametric ANOVA in E (* P<0.05 vs. PBS-injected L.G6pc^{+/+} mice).

5 **Figure 4** shows the higher G6Pase-a activity achieved by the AAV9 vector bearing the hAAT promoter compared to the AAV9 vector expressing wild-type G6pc in a larger cohort of L.G6pc^{-/-} animals injected at 2.5×10^{11} vg/mouse. The activity assay was performed on liver tissues collected at sacrifice. Statistical analyses were performed by ANOVA (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice, X P<0.05 as indicated).

Figure 5 shows the G6Pase-a activity obtained with different AAV vectors encoding the human G6pc gene injected in L.G6pc^{-/-} mice. PBS-injected L.G6pc^{+/+} and L.G6pc^{-/-} mice were used as controls. The activity assay was performed on liver tissues collected 15 days after vector injection. Statistical analyses were performed by ANOVA (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice, + P<0.05 as indicated).

Figure 6 shows the comparison of the efficacy of three different codon-optimized sequences. **A.** shows the G6Pase-a activity assay, expressed as percent of L.G6pc^{+/+} G6Pase-a activity, of the wild-type (G6pc wt), codon-optimized 1 and 2 (G6pc co1 and G6pc co2) G6pc sequences expressed with AAV8 in L.G6pc^{-/-} mice injected at a dose of 1×10^{12} vg/kg and followed-up for 15 days. **B.** shows the G6Pase-a activity assay, expressed as percent of L.G6pc^{+/+} G6Pase-a activity, of the wild-type (G6pc wt) and codon-optimized 3 (G6pc co3) G6pc sequences in L.G6pc^{-/-} mice injected with AAV9 at a dose of 1×10^{11} vg/mouse and followed-up for 15 days. Statistical analyses were performed by ANOVA (# P<0.05 vs. PBS-injected L.G6pc^{+/+} mice; * P<0.05 vs. PBS-injected L.G6pc^{-/-} mice ; + P<0.05 as indicated).

Detailed description of the invention

Definitions

The terms "glucose-6-phosphatase alpha" or "G6Pase-a" relates to an enzyme encoded by the *G6pc* gene. This enzyme catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate in the terminal step of glycogenolysis and gluconeogenesis. According to the invention, G6Pase-a may be a wild-type G6Pase-a or a modified G6Pase-a, in particular a modified G6Pase-a having increased phosphohydrolase activity. Modified G6Pase-a are disclosed in WO2016106303 and [16]. For example, G6Pase-a may be SEQ ID NO: 1, SEQ ID NO: 12, or any the modified G6Pase-a having an amino acid sequence selected from SEQ ID NO: 29 to SEQ ID NO: 44.

According to the present invention, the "identity" is calculated by comparing two aligned sequences in a comparison window. The alignment of the sequences makes it possible to determine the number of positions (nucleotides or amino acids) in common for the two sequences in the comparison window. The number of positions in common is therefore divided by the total number of positions in the comparison window and multiplied by 100 to obtain the percentage of identity. The determination of the percentage of sequence identity can be carried out manually or by means of well-known computer programs. In a particular embodiment of the invention, the identity or the homology corresponds to at least one substitution, for example 1, 2, 3, 4, 5 substitutions, of an amino acid residue, without appreciable loss of interactive binding capacity. Preferably, the at least one substitution is a conservative amino acid substitution. By "conservative amino acid substitution", it is meant that an amino acid can be replaced with another amino acid having a similar side chain. Families of amino acid having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, cysteine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

According to the invention, the term "nucleic acid sequence" refers to a DNA or RNA molecule in single or double stranded form, particularly a DNA.

According to the invention, the term "nucleic acid sequence encoding a G6Pase-a" refers to a nucleic acid sequence encoding a G6Pase-a, either a wild-type G6Pase-a or a modified G6Pase-a. Modified nucleic acid sequence encoding a modified G6Pase-a are disclosed in reference [16] and WO2016106303. For example, a nucleic acid sequence encoding a wild-type G6Pase-a have the nucleotide sequence SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 45,
5 or SEQ ID NO: 46. For example, a nucleic acid sequence encoding a modified G6Pase-a have the nucleotide sequence SEQ ID NO: 4 or SEQ ID NO: 5. For example, a nucleic acid sequence encoding a G6Pase-a may therefore be SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 45, SEQ ID NO: 46, or any of the nucleic acid sequences
10 encoding a modified G6Pase-a selected from SEQ ID NO: 13 to SEQ ID NO: 28.

The term "nucleic acid construct" refers to an artificially constructed segment of nucleic acid that is to be transplanted into a target cell for expressing a transgene, e.g. for the expression of a G6Pase-a in a cell. The nucleic acid construct may comprise one or more expression control nucleic acid sequences and/or other nucleic acid sequences improving the
15 expression of G6Pase-a and/or nucleic acid sequences enhancing the secretion of G6Pase-a and/or nucleic acid sequences enhancing the tissue uptake of G6Pase-a, said nucleic acid sequences may be operably linked to the sequence encoding the transgene (e.g. G6Pase-a). As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a
20 functional relationship with another nucleic acid sequence. For instance, a promoter, or another transcription regulatory nucleic acid sequence, is operably linked to a nucleic acid sequence encoding a transgene (e.g. G6Pase-a) if it affects the transcription of the nucleic acid sequence. Such expression control nucleic acid sequences are known in the art, such as promoters, enhancers (such as cis-regulatory modules (CRMs)), introns, polyA signals, etc.

25 The term "alpha-1 antitrypsin" or "AAT" relates to a protein belonging to the serpin superfamily. It is encoded in humans by the SERPINA1 gene. The term "hAAT" relates to the human AAT.

The term "promoter" refers to a region of DNA that directs/initiates transcription of a nucleic acid sequence (e.g. a gene). A promoter includes necessary nucleic acid sequences near the
30 start site of transcription. Typically, promoters are located near the genes they transcribe.

The term "hAAT promoter" relates to the promoter of hAAT, either a wild-type hAAT promoter or a modified hAAT promoter.

The term "vector" according to the invention means a vector suitable for a transgene expression in gene therapy, e.g. for G6Pase-a expression in gene therapy.

In the context of the present invention, the term "gene therapy" refers to treatment of a subject which involves delivery of a gene / nucleic acid into an individual's cells for the purpose of treating a disease.

The term "subject", "patient" or "individual", as used herein, refers to a human or non-human mammal (such as a rodent (mouse, rat), a feline, a canine, or a primate) affected or likely to be affected with a glycogen storage disease Ia (GSD-Ia). Preferably, the subject is a human, man or woman.

The term "glycogen storage disease" or "GSD" refers to a metabolic disorder caused by enzyme deficiencies affecting glycogen synthesis, glycogen breakdown or glycolysis, typically within muscles and/or liver cells. GSD is classified in different types, from GSD type 0 to GSD type XV. GSD-I consists of two autosomal recessive disorders, GSD-Ia and GSD-Ib. GSD-Ia results from a deficiency in glucose-6-phosphatase-a. Deficiencies in the glucose-6-phosphate transporter (G6PT) are responsible for GSD-Ib. According to the invention, the GSD is GSD-Ia (von Gierke's disease; OMIM # 232240).

The term "treating" or "treatment" means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. In particular, the treatment of the disorder may consist in treating dysfunctions in GSD, preferably improving the glycaemia control in a subject.

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.

A "pharmaceutical composition" means a composition comprising pharmaceutically acceptable carrier. For example, a carrier can be 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. When the pharmaceutical composition is adapted for oral administration, the tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or another suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. The composition according to the invention is preferably a pharmaceutical composition.

Nucleic acid construct

The description relates to a nucleic acid construct for the expression of a glucose-6-phosphatase (G6Pase-a) in a cell, the construct comprising a nucleic acid sequence encoding the G6Pase-a, wherein the nucleic acid sequence encoding the G6Pase-a is operably linked to a human alpha-1 antitrypsin (hAAT) promoter.

According to the description, the nucleic acid sequence may encode a wild-type G6Pase-a, e.g. the G6Pase-a having the amino acid sequence SEQ ID NO: 1, or a modified G6Pase-a, preferably a modified G6Pase-a having increased phosphohydrolase activity, e.g. a G6Pase-a comprising or having an amino acid sequence at least 90% identical to SEQ ID NO: 1. The modified G6Pase-a can include one or more amino acid modification(s), such as substitution or deletion, so long as the protein retains enzymatic activity. In some embodiments, the modified G6Pase-a comprises a serine to cysteine substitution at amino acid 298 of human G6Pase-a (the amino acid sequence of wild type human G6Pase-a is set forth herein as SEQ ID NO: 1). The modified G6Pase-a can include modifications at other residues so long as the protein retains enzymatic activity. For example, the modified G6Pase-a can include

substitutions at other residues, such residues include positions 3, 54, 139, 196, 199, 242, 247, 292, 301, 318, 324, 332, 347, 349, 350 and/or 353 of the human G6Pase-a (set forth as SEQ ID NO: 1). Modified G6Pase-a are disclosed in WO2016/106303 and [16]. For example, G6Pase-a may be SEQ ID NO: 1, SEQ ID NO: 12, or any modified G6Pase-a having
5 an amino acid sequence selected from SEQ ID NO: 29 to SEQ ID NO: 44.

The G6Pase-a may comprise or may have an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1.

The nucleic acid sequence encoding the G6Pase-a may therefore encode either a wild-type
10 G6Pase-a (SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 45, or SEQ ID NO: 46, preferably SEQ ID NO: 45) or a modified G6Pase-a. Nucleic acid sequence encoding a modified G6Pase-a may be any of the nucleic acid sequences selected from SEQ ID NO: 4, SEQ ID NO: 5, any of SEQ ID NO: 13 to SEQ ID NO: 28, SEQ ID NO: 52, or SEQ ID NO: 54.

The nucleic acid sequence encoding the G6Pase-a may comprise a nucleotide sequence
15 having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 2.

For example, a nucleic acid sequence encoding a G6Pase-a may therefore be SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any of nucleic acid sequences n°6-7 of WO2016106303.

The nucleic acid sequence encoding the G6Pase-a may comprise a nucleotide sequence
20 having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 45.

The nucleic acid sequence encoding the G6Pase-a may comprise a nucleotide sequence
25 having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 46.

The nucleic acid sequence encoding a G6Pase-a is operably linked to a human alpha-1 antitrypsin (hAAT) promoter.

The hAAT promoter may be a wild-type hAAT promoter, e.g. the hAAT promoter having the
30 nucleic acid sequence SEQ ID NO: 8, or a modified hAAT promoter, e.g. a hAAT promoter comprising or having a nucleic acid sequence at least 90% identical to SEQ ID NO: 8. The

modified hAAT promoter can include one or more nucleic acid modification(s), such as substitution or deletion, so long as the promoter still directs/initiates transcription of the G6Pase-a.

The hAAT promoter may comprise or may have a nucleotide sequence having at least 90%,
5 at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 8.

The hAAT promoter is preferably preceded by an enhancer, such as ApoE enhancer (e.g. SEQ ID NO: 9). For example, the nucleic acid construct comprises SEQ ID NO: 7.

The nucleic acid construct may comprise an intron, in particular an intron placed between
10 the hAAT promoter and the nucleic acid sequence encoding a G6Pase-a. An intron may be introduced to increase mRNA stability and the production of G6Pase-a. Advantageously, the nucleic acid construct comprises an intron derived from the human β globin gene (e.g. HBB2) placed between the hAAT promoter and the nucleic acid sequence encoding a G6Pase-a, preferably the intron comprised in the nucleic acid construct has the sequence
15 shown in SEQ ID NO: 47. The intron having the sequence SEQ ID NO: 47 is disclosed in WO2015/162302. Alternatively, the intron is placed after the 3'-end of the nucleic acid sequence encoding a G6Pase-a.

The nucleic acid construct of the description may comprise, in the 5' to 3' orientation, the hAAT promoter optionally preceded by an enhancer, such as ApoE enhancer (e.g. SEQ ID
20 NO: 9), optionally an intron, such as an intron of the human β globin gene (e.g. SEQ ID NO: 47), the nucleic acid sequence encoding the G6Pase-a and a polyadenylation signal (such as the bovine growth hormone polyadenylation signal, the HBB2 polyadenylation signal, the SV40 polyadenylation signal, or another naturally occurring or artificial polyadenylation signal). Advantageously, the nucleic acid construct of the invention comprises, in the 5' to 3'
25 orientation, the hAAT promoter optionally preceded by an enhancer, such as ApoE enhancer (e.g. SEQ ID NO: 9), an intron (in particular an intron as defined above), a nucleic acid molecule encoding the G6Pase-a, and a polyadenylation signal.

The nucleic acid construct may comprise or may have a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at
30 least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 11, with SEQ ID NO: 48, with SEQ ID NO: 49, with SEQ ID NO: 50, with SEQ ID NO: 51, with SEQ ID NO: 53, or with SEQ ID NO: 55, or with SEQ ID NO: 56. In a preferred embodiment, the nucleic acid

construct may comprise or may have a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 48.

The term nucleic acid construct is to be transferred into a target cell for the expression of a G6Pase-a in said cell. Preferably, said target cell is a liver cell, a kidney cell or an intestinal cell.

Vector

The terms "nucleic acid construct of the description" or "nucleic acid construct according to the description" means the nucleic acid construct disclosed in the present description, particularly the nucleic acid construct disclosed above.

The description also relates to a vector comprising the nucleic acid construct of the description.

The vector may be a plasmid vector. The vector may also be a nanoparticle containing the nucleic acid construct of the invention. The vector may also be a system based on transposons, allowing integration of the nucleic acid construct of the invention in the genome of the target cell, such as the hyperactive Sleeping Beauty (SB100X) transposon system [2]. The vector may be a viral vector suitable for gene therapy. The vector may target any cell of interest such as liver cells, kidney cells or intestinal cells.

The vector may be a viral vector, such as lentiviral vector or adeno-associated virus (AAV) vector.

The invention relates to an AAV vector comprising the nucleic acid construct of the description. In a particularly preferred embodiment, the AAV vector implemented in the practice of the present invention is AAV8 or AAV9, preferably AAV8.

Thus, the nucleic acid construct of the description may also contain sequences suitable for producing an efficient viral vector, as it is well disclosed in the art.

The nucleic acid construct may be inserted in the vector, such as a lentiviral vector according to the description, or an AAV vector according to the invention, such as a single-stranded or double-stranded self-complementary AAV vector. In a much preferred embodiment of the present invention, the AAV vector is an AAV vector suitable for transducing liver cells, more particularly an AAV-1, AAV-2 and AAV-2 variants (such as the quadruple-mutant capsid

optimized AAV-2 comprising an engineered capsid with Y44+500+730F+T491V changes, disclosed in [3]), AAV-3 and AAV-3 variants (such as the AAV3-ST variant comprising an engineered AAV-3 capsid with two amino acid changes, S663V+T492V, disclosed in Vercauteren et al. [4], AAV-3B and AAV-3B variants, AAV-4, AAV-5, AAV-6 and AAV-6
5 variants (such as the AAV-6 variant comprising the triply mutated AAV-6 capsid Y731F/Y705F/T492V form disclosed in [5], AAV-7, AAV-8, AAV-9, AAV-10 such as AAV-cy10 and AAV-rh10, AAV-rh74, AAV-dj, Anc80, LK03, AAV-2i8, porcine AAV serotypes such as AAV-po4 and AAV-po6, etc.. As it is known in the art, depending on the specific viral vector considered for use, additional suitable sequences will be introduced in the nucleic acid
10 construct of the description for obtaining a functional viral vector. Suitable sequences include AAV ITRs for an AAV vector, or LTRs for lentiviral vectors. As such, the description also relates to a nucleic acid construct as described above, flanked by an ITR or an LTR on each side.

In addition, other non-natural engineered variants and chimeric AAV can also be useful. AAV
15 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. Desirable AAV fragments for assembly into vectors include the cap proteins, including the vp1, vp2, vp3 and hypervariable regions, the rep
20 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. 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
25 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
30 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.

In the context of the present invention, the AAV vector comprises an AAV capsid able to transduce the target cells of interest, in particular hepatocytes. 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.

According to a particular embodiment, the AAV capsid is selected from AAV-1, -2, AAV-2
5 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, -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, -3B and AAV-3B variants, -4, -5, -6 and AAV-6 variants (such as the AAV6 variant comprising the triply
10 mutated AAV6 capsid Y731F/Y705F/T492V form disclosed in Rosario et al., 2016), -7, -8, -9 and AAV-9 variants (such as AAVhu68), -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 addition, the AAV capsid is selected from other non-natural engineered variants (such as AAV-spark100), chimeric AAV
15 or AAV serotypes obtained by shuffling, rationale design, error prone PCR, and machine learning technologies. In a particular embodiment, the Cap gene encodes VP capsid proteins derived from at least two different AAV serotypes, or encodes at least one chimeric VP protein combining VP protein regions or domains derived from at least two AAV serotypes. For example a chimeric AAV capsid can derive from the combination of an AAV8 capsid
20 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. In a particular embodiment, the capsid of the AAV vector is
25 a hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins. For example, the AAV serotype may be a -rh74-9 serotype as disclosed in WO2019/193119 (such as the Hybrid Cap rh74-9 serotype described in examples of WO2019/193119; a rh74-9 serotype being also referred to herein as "-rh74-9", "AAVrh74-9" or "AAV-rh74-9") or a -9-rh74 serotype as disclosed in WO2019/193119 (such as the Hybrid Cap 9-rh74 serotype
30 described in the examples of WO2019/193119; a -9-rh74 serotype being also referred to herein as "-9-rh74", "AAV9-rh74", "AAV-9-rh74", or "rh74-AAV9"). For example, the capsid of the AAV vector is a peptide-modified hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins, as described in PCT/EP2019/076958, such as an AAV9-rh74 hybrid capsid or AAVrh74-9 hybrid capsid modified with the P1 peptide described

in the examples of PCT/EP2019/076958. For another example, the AAV serotype may be a hybrids AAV2/13 as disclosed in PCT/EP2020/061380.

For example, the genome of the pseudotyped vector may have a capsid derived from the AAV8, AAV9, AAVrh74 or AAV2i8 serotype, and its genome may be derived from and
5 different serotype. In a particular embodiment, the AAV vector has a capsid of the AAV8, AAV9 or AAVrh74 serotype, in particular of the AAV8 or AAV9 serotype, more particularly of the AAV8 serotype.

In a specific embodiment, the vector is the vector referred to "AAV8mut5". AAV8mut5 vector has the amino acid sequence SEQ ID NO: 57 and is encoded by the polynucleotide of SEQ ID
10 NO: 58.

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

In another specific embodiment, wherein the vector is for use in delivering the transgene to
15 liver cells, the AAV vector may be selected, among others, in the group consisting of AAV5, AAV8, AAV9, AAV-LK03, AAV-Anc80 and AAV3B.

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.

20 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 [6] and in [7]. For example a chimeric AAV vector can derive from the
25 combination of an AAV8 or AAV9 capsid sequence with a sequence of an AAV serotype different from the AAV8 or AAV9 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
30 present a high liver tropism.

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 [8], or in [9]. In addition, capsid variants may include single amino acid changes such as tyrosine mutants (e.g. as described in [10]). Another example is the fusion of Anthopleurin-B to the N-terminus of AAV VP2 capsid protein described in [11].

In addition, the genome of the AAV vector may either be a single stranded or self-complementary double-stranded genome [12]. Self-complementary double-stranded AAV vectors are generated by deleting the terminal resolution site (trs) 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, AAVmut5, AAVrh74 or AAV2i8 capsid, in particular an AAV8, AAV9 or AAVrh74 capsid, such as an AAV8 or AAV9 capsid, more particularly an AAV8 capsid.

15 Cell

The terms "vector of the description" or "vector according to the description" means the vector disclosed in the present description, particularly the vector disclosed above.

The description relates to a cell transformed with the nucleic acid molecule of the description or the vector according to the description.

20 The invention relates to a cell transformed with the AAV vector of the invention.

For example, the host cell can be a cell (or cell line) appropriate for production of the vector, e.g. for production of AAV. In some examples, the host cell is a mammalian cell, such as a HEK-293, BHK, Vero, RD, HT-1080, A549, Cos-7, ARPE-19, or MRC-5 cell.

The host cell can also be a cell which is the target for a gene therapy, such as a liver cell, a kidney cell or an intestinal cell.

In some embodiments, the cell is an isolated cell.

Composition

The terms "cell of the description" or "cell according to the description" means the cell disclosed in the present description, particularly the cell disclosed above.

The description relates to a composition comprising the nucleic acid construct of the description, the vector of the description, or the cell of the description. The composition of the description is preferably a pharmaceutical composition.

The invention relates to a composition comprising the vector of the invention or the cell of the invention. The composition of the invention is preferably a pharmaceutical composition.

The composition can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like.

Such composition will contain a therapeutically effective amount of the nucleic acid construct of the invention, of the vector of the invention, or of the cell of the invention, 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 construct, 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 construct, 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.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous or intrathecal 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.

Method of treatment

The terms "composition of the description" or "composition according to the description" means the composition disclosed in the present description, particularly the composition disclosed above.

The description relates to the nucleic acid construct of the description, the vector of the description, the cell of the description or the composition of the description, for use as a medicament.

5 The description also relates to the nucleic acid construct of the description, the vector of the description, the cell of the description or the composition of the description, for use in the treatment of glycogen storage disease Ia (GSD-Ia).

The invention relates to the vector of the invention, the cell of the invention or the composition of the invention, for use as a medicament.

10 The invention also relates to the vector of the invention, the cell of the invention or the composition of the invention, for use in the treatment of glycogen storage disease Ia (GSD-Ia).

The nucleic acid construct, the vector, the cell or the composition of the invention may be administered to a subject by any effective route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, intrathecal, and intravenous), oral, intraductal, sublingual, rectal, transdermal, intranasal, vaginal and inhalation routes.

20 The nucleic acid construct, the vector, the cell or the composition may be administered to a subject in a therapeutically effective amount, i.e. in an amount sufficient to achieve the desired effect in a subject, or in the cell, being treated. The effective amount of the nucleic acid construct, the vector, the cell or the composition will be dependent on several factors, including, but not limited to the subject or cells being treated, and the manner of administration.

25 The amount of the therapeutic (i.e. a nucleic acid construct, vector or cell) which will be effective in the treatment of a disease, such as GSD-Ia, 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 composition 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, the vector or the cell 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 require 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.

The therapeutic (i.e. a nucleic acid construct, vector or cell) of the invention can be administered in a single dose, or in multiple doses (such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses) as needed for the desired therapeutic results.

10 G6Pase-a codon optimized

The nucleic acid sequence encoding a G6Pase-a may be optimized for expression of the G6Pase-a *in vivo*. Sequence optimization may include a number of changes in a nucleic acid sequence, including codon optimization, increase of GC content, decrease of CG dimers, decrease of the number of CpG islands, decrease of the number of alternative open reading frames (ARFs) and/or 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. Of course, as is well known to those skilled in the art, sequence optimization is a balance between all these parameters, meaning that a sequence may be considered optimized if at least one of the above parameters is improved while one or more of the other parameters is not, as long as the optimized sequence leads to an improvement of the transgene, such as an improved expression and/or a decreased immune response to the transgene *in vivo*.

The nucleic acid sequence encoding a G6Pase-a may be codon-optimized to improve its expression in human cells compared to non-codon optimized nucleotide sequences coding for the same G6Pase-a. A wild type nucleic acid sequence encoding a wild type G6Pase-a is as shown in SEQ ID NO: 2. Examples of codon optimized sequences encoding the same G6Pase-a are as shown in SEQ ID NO: 3, SEQ ID NO: 45 or SEQ ID NO: 46. Other examples of sequence optimized nucleic acid encoding a modified G6Pase-a are SEQ ID NO: 5, SEQ ID

NO: 52 and SEQ ID NO: 54 encoding the modified G6Pase of SEQ ID NO: 12, all optimized with respect to one of the above parameters compared to the nucleic acid sequence of SEQ ID NO: 4.

5 The nucleic acid sequence of the invention encoding a G6Pase may be codon optimized and/or may have a decreased GC content and/or has a decreased number of CG dimers as compared to the wild type nucleotide sequence encoding the same G6Pase-a amino acid sequence such as the G6Pase-a of SEQ ID NO: 1. The nucleic acid sequence encoding a G6Pase may also be codon optimized and/or may have a decreased GC content and/or has a decreased number of CG dimers compared to the nucleotide sequence of SEQ ID NO: 2.
10 Alternatively, such nucleic acid sequence encoding a G6Pase may have the sequence of SEQ ID NO: 45 or SEQ ID NO: 46, preferably SEQ ID NO: 45.

The present description also discloses a nucleic acid sequence encoding a G6Pase-a comprising a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or
15 100% identity with SEQ ID NO: 45.

The present description also discloses a nucleic acid construct for the expression of a G6Pase-a in a cell, the construct comprising a nucleic acid sequence encoding the G6Pase-a, wherein the nucleic acid sequence encoding a G6Pase-a comprises a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,
20 at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 45. The nucleic acid sequence encoding the G6Pase-a is preferably operably linked to a promoter.

The present description also discloses a vector comprising the nucleic acid construct disclosed in this section (i.e. in section "G6Pase-a codon optimized"). The vector may be a
25 lentiviral vector or an adeno-associated virus (AAV) vector, such as AAV8, AAV9 or AAVmut5, preferably AAV8.

The present description also discloses a cell transformed with the nucleic acid molecule disclosed in this section or the vector disclosed in this section. The cell may be a liver cell, an intestinal cell or a kidney cell.

30 The present description also discloses a composition comprising the nucleic acid construct, the vector, or the cell disclosed in this section.

The present description also discloses the nucleic acid construct, the vector, the cell or the composition disclosed in this section, for use as a medicament.

The present description also discloses the nucleic acid construct, the vector, the cell or the composition disclosed in this section, for use in the treatment of glycogen storage disease Ia (GSD-Ia).

5

Examples

Materials and methods

In Examples 1, 2 and 3, we have prepared two nucleic acid constructs (hereafter "transgene expression cassettes") comprising the human G6pc gene wt (SEQ ID NO: 2) encoding G6Pase-a, under the control of different promoters:

- The alpha-1-anti-trypsin (hAAT) promoter. The nucleic acid sequence of the expression cassette is SEQ ID NO: 11.
- The endogenous promoter of the human G6pc gene (hGPE). The nucleic acid sequence of the expression cassette is SEQ ID NO: 10.

10 The transgene expression cassettes are represented in Figure 1A.

The two transgene expression cassettes were pseudotyped in AAV9 to obtain two different AAV vectors, namely AAV9-hAAT-hG6PC and AAV9-hGPE-hG6PC.

The two vectors or PBS (negative control) were independently tested in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS.

In Example 4, we have prepared a nucleic acid construct (hereafter "transgene expression cassettes") comprising the human G6pc gene wt (SEQ ID NO: 2) encoding G6Pase-a, under the control of the alpha-1-anti-trypsin (hAAT) promoter. The nucleic acid sequence of the expression cassette was SEQ ID NO: 56.

The expression cassette for human G6pc gene wt was pseudotyped in AAV9, AAV8 and AAVmut5.

The three vectors or PBS (negative control) were independently tested in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS.

In Example 5, we have prepared four nucleic acid constructs (hereafter "transgene expression cassettes") comprising the human G6pc gene wt (SEQ ID NO: 2) and two codon optimized human G6pc gene (co1, co2 and co3, respectively SEQ ID NO: 45, SEQ ID NO: 46

and SEQ ID NO: 3), each encoding G6Pase-a, under the control of the alpha-1-anti-trypsin (hAAT) promoter.

The nucleic acid sequence of the expression cassette for human G6pc gene wt was SEQ ID NO: 11 or SEQ ID NO: 56. The nucleic acid sequence of the expression cassettes for human
5 G6pc gene co1, human G6pc gene co2 and human G6pc gene co3 were respectively SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50.

The expression cassette for human G6pc gene wt was pseudotyped in AAV8 (SEQ ID NO: 56) and AAV9 (SEQ ID NO: 11).

The expression cassettes for human G6pc gene co1 and G6pc gene co2 were pseudotyped in
10 AAV8.

The expression cassette for human G6pc gene co3 was pseudotyped in AAV9.

The five vectors or PBS (negative control) were independently tested in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS.

15 In vivo studies

Mice were fed either a standard chow diet (A04 diet, Safe) or a high fat/high sucrose diet (made by INRAE, Jouy en Josas), known to accelerate hepatic tumor development in L.G6pc^{-/-} mice. Proneness to tumor formation was induced in mice by feeding them with a modified chow diet composed of 36% fat (INRA) [14].

20 AAV vectors were administered intravenously via the tail vein of L.G6pc^{-/-} mice. Wild type mice (C57Bl/6J mice, Charles Rivers) were injected with PBS via the tail vein. Glycemia was measured on peripheral blood after a 6-hour fasting using a glucometer (Roche Diagnostic). At sacrifice, livers were harvested and weighed to measure the liver/body weight ratio and snap-frozen for further evaluations.

25 Tumors were evaluated in GSDIa mice at sacrifice by visual inspection. Only tumors larger than 2 mm were considered in the count.

AAV production

HEK293T cells were grown in suspension in 250 mL of serum-free medium. The cells were transfected with 3 plasmids: i) a transgene plasmid, containing AAV2 ITRs flanking an

expression cassette ii) the helper plasmid pXX6, containing adenoviral sequences necessary for AAV production, and iii) a plasmid containing AAV Rep and Cap genes, defining the serotype of AAV. Two days after transfection, the cells were lysed to release the AAV particles.

- 5 The viral lysate was purified by affinity chromatography. Viral genomes were quantified by a TaqMan real-time PCR assay using primers and probes corresponding to the ITRs of the AAV vector genome [17].

G6Pase enzyme activity measurement

- 10 G6Pase enzyme activity was measured in homogenates from freeze-clamped livers as already reported in [13]. Briefly, tissues homogenates were incubated with glucose-6-phosphate (Sigma) for fifteen minutes at 37°C. The reaction was stopped by adding trichloroacetic acid and the released phosphate was measured by complexation with ammonium molybdate and citrate arsenite. The resulting absorbance was measured on at 700 nm.

15 Measurement of glycogen content

- Glycogen content was measured indirectly in tissue homogenates as the glucose released after total digestion with *Aspergillus Niger* amyloglucosidase (Sigma). Samples were incubated for 20 min at 95°C in the presence of 0.3 M NaOH and then cooled at 4°C. Samples were then added with amyloglucosidase and incubated at 37°C for 90 minutes. The
20 glucose released was determined with a commercial glucose assay kit.

Vector genome copy number (VGCN) quantification

- For vector genome copy number (VGCN) quantification in samples, DNA was extracted from samples using KingFisher (Thermo Fisher Scientific). Real-time PCR was performed on 1µL of DNA, using the protocol for AAV vectors titration described above. Exon Mex5 of titin gene
25 was used as genomic DNA loading control.

Example 1 : Correction of the liver phenotype in GSD-Ia mice 15 days after intravenous injection of AAV vectors expressing human G6Pase-a

- 30 The two AAV9 vectors were independently injected in L.G6pc^{-/-} mice fed a standard diet at the dose of 1x10¹¹ vg/mouse (Figure 1A).

Fifteen days after vectors injection, the concentration of glucose in the blood was measured after 6 hours of fasting. Glycaemia was completely normalized in mice injected with the two vectors (Figure 1B).

G6PC activity was then measured in liver. The AAV9-hAAT-hG6PC vector achieved
5 supraphysiological activity and showed the highest activity when compared to AAV9-hGPE-hG6PC vector (Figure 1C).

Glycogen concentration was also measured. Complete correction of glycogen accumulation and hepatomegaly was obtained with the AAV9-hAAT-hG6PC but not with the vector bearing the hGPE promoter (Figure 1D, E respectively), reflecting the higher G6Pase activity achieved
10 in mice treated with the AAV9 vector bearing hAAT promoter. The similar vector genome copy numbers per diploid genome measured in mice injected with the two AAV vectors (Figure 1F) indicates a similar transduction efficacy in liver.

Example 2: long-term correction of the liver phenotype in GSD-Ia mice after
15 **intravenous injection of AAV vectors expressing human G6Pase-a**

To assess the long-term efficacy of the two vectors, we performed a 7-months study. Vectors were injected in L.G6pc^{-/-} mice fed a standard diet at a dose of 2.5x10¹¹ vg/mouse (Figure 2A). Seven months after vectors injection, the concentration of glucose in the blood was measured after 6 hours of fasting. Glycemia was corrected in all the animals who
20 received the G6PC-expressing vectors (Figure 2B). Importantly, L.G6pc^{-/-} mice treated with AAV9-hAAT-hG6PC showed supraphysiological liver G6Pase-a activity that was significantly higher of the activity measured in AAV9-hGPE-hG6PC -treated L.G6pc^{-/-} mice (Figure 2C).

Glycogen accumulation and hepatomegaly were completely rescued in animals injected with the two vectors (Figure 2D, E). Higher vector genome copy numbers per diploid genome
25 were measured in mice injected with the AAV9-hGPE-hG6PC vector (Figure 2F) possibly reflecting a slightly lower liver transduction achieved with the AAV9-hAAT-hG6PC vector.

Taken together, these results indicate that G6Pase-a expression is increased with the hAAT promoter. Thus, hAAT promoter is suitable for AAV gene therapy for GSD-Ia with a potency superior to the hGPE promoter.

Example 3: hGPE-directed gene therapy promotes hepatic tumor formation in L.G6pc^{-/-} mice fed a high fat/high sucrose diet.

Adenoma formation is one of the hallmarks of GSD-Ia in humans, reported in most of the affected individuals in the second and third decades of life. In L.G6pc^{-/-} mice, almost all the mice fed a standard diet developed liver adenomas by 18 months of age [13]. This slow process can be accelerated by high fat/high sucrose (HF/HS) diet. Approximately 85 % of L.G6pc^{-/-} mice fed a HF/HS diet developed multiple hepatic tumors at nine months of age [14]. Thus, L.G6pc^{-/-} mice fed with HF/HS diet represent a robust model to evaluate the efficacy and the safety of gene replacement strategies in the prevention of tumors formation in GSDIa.

We therefore tested the AAV9-hAAT-hG6PC and the AAV9-hGPE-hG6PC vectors in L.G6pc^{-/-} mice fed a HF/HS diet (Figure 3A). Eight months after treatment, only the vector bearing the hAAT promoter completely rescued glycemia (Figure 3B). Under HF/HS diet, the levels of G6Pase activity in AAV-treated animals were significantly lower than those measured in L.G6pc^{+/+} animals. Although AAV9-hAAT-hG6PC –treated animals showed higher G6Pase activity levels, statistical significance was not reached when compared to L.G6pc^{-/-} mice treated with AAV9-hGPE-hG6PC vector (Figure 3C). Similar vector genome copies per diploid genome were measured in AAV-treated mice (Figure 3D).

Interestingly, necropsy of the mice revealed a higher number of tumors in the livers of mice treated with the hGPE-bearing AAV vectors compared to those that received the AAV9-hAAT-hG6PC (Figure 3E). These results suggest that the use of AAV9-hAAT-hG6PC may decrease the transformation rate of hepatocytes thus resulting in a decreased frequency of adenomas compared to AAV9-hGPE-hG6PC.

The data obtained for Figure 3A were completed to increase the number of mice per group (Figure 4 and Table 1). Figure 4 shows that, in a larger cohort, the hAAT driven vector achieved a significantly higher G6Pase activity. The data presented in Table 1 confirm that the use of AAV9-hAAT-hG6PC resulted in a decreased frequency of adenomas compared to AAV9-hGPE-hG6PC.

	L. G6pc ^{+/+} PBS	L. G6pc ^{-/-} PBS	hAAT	hGPE
Total number of mice	24	17	17	18
Number of mice that developed macroscopic tumors (%)	2 (8%)	5 (29%)	2 (12%)	5 (28%)

Table 1: frequency of adenomas**Example 4: Expression of G6Pase wt in murine liver with different AAV serotypes**

We produced three different AAV vectors, namely AAV9, AAV8, AAVmut5 expressing wild-type human G6pc gene wt (SEQ ID NO: 2) under the control of the hAAT promoter.

The three vectors or PBS (negative control) were independently tested in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice at the dose of 1x10¹² vg/kg. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS. Fifteen days after vector infusion, G6Pase activity was assessed for each vector in liver. The three AAV vector-injected groups showed a similar G6Pase activity with levels higher than those measured in wild-type animals (Figure 5) thus confirming the possibility to express the transgene in the mouse liver through different AAV vectors.

Example 5: Expression of G6Pase in murine liver with different codon-optimized G6pc sequences

We performed two independent experiments to evaluate the efficacy of codon optimization with CG dimers reduction in the expression of G6Pase in mouse liver.

The G6pc wt sequence (SEQ ID NO: 2) was codon optimized using different methods. The G6pc co1 has the highest similarity with the wt sequence, and the lower CG dimers content. The G6pc co2 has the highest GC content and an increased CG dimers content compared to the G6pc co1. The G6pc co3 was less similar to the wt sequence, and has the highest CG dimers content (Table 2).

G6pc sequence	% vs wt	% vs co1	% vs co2	SD (score>0.8) ^a	SA (score>0.8) ^a	GC content (%) ^b	CG dimers	CpG islands ^c
wt	-	-	-	0	4	52.16	24	0
co1	97.49	-	-	1	3	50.74	0	0
co2	81.88	83.41	-	1	3	59.22	11	0
co3	75.02	74.84	76.49	0	1	48.46	48	0

Table 2. Comparative analysis of the codon-optimized sequences. Sequence analysis has been performed on the wild-type hG6pc sequence (wt) and on the three codon optimized (co1, co2 and co3).

^aSplicing donor (SD) and splicing acceptor (SA) were predicted using an online tool (www.fruitfly.org) with a minimum score of 0.8.

^b GC content was calculated using the online molecular biology tool (www.genscript.com).

^c CpG islands smaller than 100 bp and with a GC content threshold of 60% were predicted with the online tool MethPrimerDB (www.urogene.org).

In the first experiment we produced three AAV8 vectors, expressing the following transgenes under the control of the hAAT promoter:

- hG6pc : human G6pc gene wt (SEQ ID NO: 2),
- hG6pc Co1 : human G6pc gene codon optimized (SEQ ID NO: 45), and
- hG6pc Co2 : human G6pc gene codon optimized (SEQ ID NO: 46).

We then tested the three AAV8 vectors in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice at the dose of 1x10¹² vg/kg. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS. Fifteen days after vector infusion, G6Pase activity was assessed in liver. The three AAV8 vector-injected groups showed supra-physiological G6Pase activity with levels higher than those measured in wild-type animals (Figure 6A). Importantly, the AAV8 vector expressing hG6pc Co1 showed significantly increased levels of G6Pase activity when compared to the same vector expressing hG6pc Co2 (Figure 6A).

For the second experiment we produced two AAV9 vectors, expressing the following transgenes under the control of the hAAT promoter:

- hG6pc : human G6pc gene wt (SEQ ID NO: 2),
- hG6pc Co3 : human G6pc gene codon optimized (SEQ ID NO: 3).

We then tested the two AAV9 vectors in a liver-specific G6pc knockout mouse model (L.G6pc^{-/-}, [13]), i.e. GSD-Ia mice at the dose of 1x10¹¹ vg/mouse. As a positive control, WT mice (L.G6pc^{+/+}) were infused with PBS. Fifteen days after vector infusion, G6Pase activity was assessed in liver. Importantly, only the group of animals injected with the AAV9 vector bearing the hG6pc wild-type sequence showed supra-physiological G6Pase activity with levels higher than those measured in wild-type animals (Figure 6B). Animals injected with the AAV9 vector expressing hG6pc Co3 showed a tendency to a decreased G6Pase activity when compared to animals injected with the AAV9 vector expressing the wild-type version of hG6pc (p=0.06, Figure 6B).

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Sequence listing

SEQ ID NO :	Description	Sequence
1	hG6Pase-a wt (amino acid)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILVSVIADLRNIFYVLFPIWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYSNTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKPTYRFRCLNVILWLGFVAVQLNVCLSRILAAHFPHQVAVGLSGIAVAETFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRWCEQPEWVHIDTTPFASLLKNLGLTFLGLGLALNSSMYRECKGKLSKWLPFRLSSIVASLVLLHVFDSLKPPSQVELVFYVLSFCKSAVVPLASVSVIPYCLAQVLGQPHKSL
2	hG6pc wt (nucleic acid) (Encoding SEQ ID NO : 1)	atggaggaaggaatgaatgttctccatgactttgggatccagtcacacattacctccaggtgaattaccaagactcccaggactggttcattctggtgtccgtgatcgacacctcaggaatgccttctacgtccttccccatctggttccatcttcaggaagctgtggcattaaactcctttggtagctgtgattggagactggctcaacctcgtcttaagtggattctctttggacagcgctccatctggtgggtttggatactgactactacagcaacacttccgtgccccctgataaagcaggttcctgtaacctgtgagactggaccaggagccccctctggccatgccatgggcacagcaggtgtatactacgtgatggtcacatctactcttccatcttccagggaagataaagccgacctacagatttcggtgcttgatgtcattttgtggttgggattctgggctgtgcagctgaatgtctgtctgtcacgaatctacctgtcgtcattttctcatcaagttgtgtcggagctcgtcaggcattgctgttcagaaactttcagccacatccacagcatctataatgccagcctcaagaaatattttctcattaccttctcgttcagcttcgccatcgattttatctgtcgtcaagggactgggtgtagacctcgtgactcggagaaagcccagaggtggtgcgagcagccagaatgggtccacattgacaccacacctttgccagcctcctcaagaacctgggcagcctctttggcctgggctggctcctcaactccagcatgtacagggagagctgcaaggggaaactcagcaagtggctccattccgctcagctctattgtagcctcctcgtcctcctgcacgtctttgactccttgaaccctcatcccaagtcgagctggtcttctacgtctgtcctctgcaagagtgcggtagtgccctggcatccgtcagtgatcctccctactgcctcgccaggctcctgggcagccgcacaagaagtcgttgtaa
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	(nucleic acid) (identical to SEQ ID NO: 2 except for a c >g mutation at position 893) (Encoding SEQ ID NO : 12)	gaattaccaagactcccaggactggttcattcttggtgtccgtgatcgagacctcaggaatg ccttctacgtcctcttccccatctggttccatcttcaggaagctgtgggcattaaactccttgg gtagctgtgattggagactggctcaacctcgtctttaagtggattctcttggacagcgcca tactggtgggttttgatactgactactacagcaacactccgtgcccctgataaagcagttc cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcaggt gtatactacgtgatggtcacatctactcttccatcttcagggaagataaagccgacctac agatttcggtgctgaatgtcattttgtggttgggattctgggtgtgcagctgaatgtctgtc tgtcacgaatctacctgtctgctcattttcctcatcaagttgtgtgagctctgtcaggcatt gctgttgagaaactttcagccacatccacagcatctataatgccagcctcaagaaatatttt ctcattaccttctctgttcagcttcgcatcgattttatctgtctcaagggactgggtgt agacctcctgtggactctggagaagcccagagggtgtgcgagcagccagaatgggtcc acattgacaccacaccttggcagcctcctaagaacctgggcagcgtcttggcctgggg ctggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctc ccattccgctcagctgtattgtagcctccctcgtcctcctgcagctcttggactcctgaaacc cccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtcggtagtgtccct ggcatccgtcagtgatcatcccctactgctcgtcccagggtcctgggcccagccgcacaagaag tcgttgtaa
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10	hGPE_HBB2_ hG6pc wt_bGH (expression cassette used in examples 1, 2 and 3)	tggaggaagcagaaggggctggcaggtggaagatgaggaccagctcatcgtctcat gactatgaggtgctctgatccagaggtgccccctgcctggtggccaccgcaggaaga ctccactgtccctggatgccagagtggatgtcaactccatcattatcaactcctatcc ataggggtattcttctgaggcgtctcagaaaacagggccctcccatatgctgaccacata atagaacccctcccaactcagagacctggctgctagctgcctggcatgaccagacagt ggcctttgtatgtttttagactcaccttgactcacctctgaccatagaaactcctatccag aggtcactgcaatagttaactcacaacagaggcttatctgggtagagggaggctccctacc tatggccagcagccctgacagtgcagatcacatataccccacgccccagcactgctgcc acgcatgggcttactttacccccccacagtcaccaacacattacctgctctccaaggta ggcgtggcaggagaagttgtctggaccagcagaacccatgcagtcaggacaactgga gtcagcatgggctgggtgcgagcccttggtgggtggggaggagactccaggctacacct cctggaggatgttttaatttccagcatggaatgctgtcaacttttgccacagattcattag ctctgagttctttttctgtccccagctacccttacatgtcaatatggacttaatgatggaa attcaggcaagttttaaacattttatccccctggctcttatctcaaaaaatgcatgaattg gaggcagtggtcatgacctgtaatccaatgctttgctaggttgaggcgggaggatcactt gaagccagggaatttgagaccagcctgggcccgcagatgagagccccgtttctacaaaaata aataaataaataaataaataatagtgatatgaagcatgattaaatagccctatttttaaatg catgagttcgttacctgattcattccctgggttctttcacagtcctccgtgacccaagtgttag gttttggtctctactattttaggctgatataatagatatacacacacacacacacacata acacacacacagtgatcttgagctttctttgtatatctacacacatatgtataagaagctc aagatatagaagcccttttcaaaaataactgaaagtttcaaactcttaagctccagttac cattttgctggtattcttatttgaaccatacattcatatattgtgcacagtaagactataca ttcattattttgcttaaacgtatgagttaaaacacttgccaggcatggtggttcacacctgta atccagagctttgggaagccaagactggcagtaactcttgagctcaggaattcaagacca gcctgggcaacatggaaaaacccatctctacaaaagatagaaaaattagccaggcatg gtggcgtgtgctgtggtcccagctactcaggaggctgagggtgggaggatcacattagcc caggaggttgaggctgcagtgcagccgtgattatgccactgcactccagcctgggagacag agtgcagccctgtttcaaaaaaagagagagaaaaattaaaaaagaaaacaacaccaa gggctgtaactttaaggtcattaaatgaattaatcactgcattcaaaaacgattactttctg ccctaagagacatgaggcaataaccaggaagggggtgatctccaaaccagaggcag accctagactctaatacagtttaaggaaagaccagcaagatgatagtcaccaatacaatag aagtactatattttattgtgttttctttgtttgtttgtttgtttgtttgttttagagactgg ggtcttgctgattgccaggctgtagtgcagcgggtgggacaatagctcactgcagactcc aactcctgggctcaagcaatcctcctgcctcagcctcctgaatagctgggactacaaggt acaccatcacacacacaaaacaatttttaaattttgtgtagaacgagggtcgtgtgtgt tgccaggctggtctcaactcctggttcaaggatcctcccacctcagcctcccaaattgc tgggattacaggtgtgagccaccacaaccagccagaacttactaattttaaaattaagaa cttaaaactgaaatgctagagcaccagaatttttcttgcctccaaataagtgagttgcag gcatagaaaaatcgacatctttgcaagaatcatcgtggatgtagactctgtcctgtgtcctg gcttggttcggggaccaggagggcagaccctgactgccaagaagcatgccaaagtta atcattggccctgctgagtacatggccgatcaggctgtttgtgtgctgctgttttctatttacg taaatcaccctgaaacatgtttgcatcaacctactggtgatgcactttgatcaatacttttag acaaacgtggtttttgacccaaagatcagggcgtgggttgactgaatactggatacaggg catataaaaacaggggcaaggcacagactcgggacccttgatgttttcttccccctcttttcta tgggtaagttcatgtcataggaaggggagaagtaacagggtacacatattgaccaaataca qqqtaattttgcatttgtaatttttaaaaaatgcttctcttttaataatactttttgtttatctattt

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11	hAAT_HBB2_ hG6pc wt (expression cassette used in examples 1, 2, 3 and 5)	<p>ggatcaaggctcagaggcacacaggagtttctgggctcacctgcccccttcaaccctc agttccatcctccagcagctgttctgtgtgctcctgaagtcacactgaacaaactcag cctactcatgtccctaaaatgggcaacattgcaagcagcaaacagcaaacacagccc tccctgctgctgaccttgagctgggagaggtcagagacctctctgggccatggcac ctcaacatccactcgaccccttgaatttcggtggagaggagcagaggtgtcctgtgcgt ggttaggtagtgtgagaggggtaccggggtctgtaccagtggaacagccactaag gattctgcagtgcagcagaggccagcctaagtgttactctccagagactgtctgactca cgccacccctccacttggacacaggacgctgtggttctgagccaggtacaatgactct ttcggttaagtgcagtgaagctgtactgcccaggcaagcgtccgggcagcgtaggc gggagactcagatccagcagtgacttagccctgttctcctccgataactggggtg acctgtgtaattaccagcagcctccccgttggccctctggtccactgttaatacg gacgaggacagggcctgtcctcagcttcaggcaccactgacctgggacagtga tagatcctgagaactcagggtgagtctatgggacctgtgttttcttccctcttctat ggttaagttcatgtcataggaaggggagaagtaacagggtacacatattgacaaatcag ggtaatgttcattgtaattttaaaaaatgcttctctttaaatacttttggttatcttattc taatactttccctaactcttcttccagggaatattgatacaatgtatcttgccctttgcacca ttctaaagaataacagtgataatttctgggtaaggcaatagcaatatttctgcatataaata tttctgcatataaattgtaactgatgtaagaggttcatattgctaatagcagctacaatccag ctaccattctgctttatttctggttgggataaggctggattattctgagtcgaagctaggccc tttctaatctgttcatacctcttatcttctccacagctcctgggcaacctgctgtctct gctggcccatcatttggcaaagcacgcgtgccaccatggagggaaggaatgaatgttctcc atgactttgggatccagtcacacattacctcagggtgaattaccaagactcccaggactg gttcatcttgggtccgtgatcgagacctcaggaatgccttctacgtcctcttccccatctgg ttccatctcaggaagctgtgggcattaaactccttgggtagctgtgattggagactggctc aacctcgtcttaagtgattcttcttggacagcgtccatactggtgggttttgatactgact</p>

		actacagcaacactccgtgccctgataaagcagttccctgtaacctgtgagactggacca gggagcccoctctggccatgccatgggacagcaggtgtatactacgtgatggtcacatcta ctctttccatctttcagggaaagataaaagccgacctacagatttcgggtgcttgatgtcattt gtgggtgggattctgggctgtgcagctgaatgtctgtctgtcacgaatctacctgtctcat tttctcatcaagttgttgctggagtcctgtcaggcattgtctgttcagaaaacttcagccaca tccacagcatctataatgccagcctcaagaaatattttctcattaccttctcctgttcagcttc gccatcggattttatctgctgctcaagggactgggtgtagacctcctgtggactctggagaa agcccagaggtgggtgcgagcagccagaatgggtccacattgacaccacaccctttgccag cctcctcaagaacctgggcacgctctttggcctggggctggctcactccagcatgtaca gggagagctgaaggggaaactcagcaagtggctccattccgcctcagctctattgtag cctccctcgtcctcctgcacgtctttgactcctgaaacccccatcccaagtcgagctggctt ctacgtctgtccttctgaagagtgcggtagtgtcccctggcatccgtcagtgcatccccta ctgcctcgcccaggtcctgggcccagccgcacaagaagtcggtgtaaagatctgaattcacc ccaccagtgcaggctgcctatcagaaagtggtgctggtgtggctaattgcctggcccaca agtatcactaagctcgtcttctgtctccaatttctattaaggttcctttgttcctaagtcca actactaaactggggatattatgaagggccttgagcatctggattctgctaataaaaaa cattattttcattgcaatgatgtatttaaattatttctgaatattttactaaaaaggaatgtgg gaggtcagtgcatthaaacataaagaatgaagagctagttcaaaccttgggaaaatac actatatcttaactccatgaagaaggtgaggctgcaaacagctaattgcacattggcaac agcccctgatgcctatgccttattcatccctcagaaaaggattcaagtagaggcttgatttg aggttaaagtttgctatgctgtattttacattactattgttttagctgcctcatgaatgtctt tcactaccatttgctatcctgcatctcagcctgactccactcagttctcttgcttagagat accacctttccctgaagtgtccttccatgttttacggcgagatggtttctcctcgctggcca ctcagccttagttgtctgtgttcttatagaggtctactgaagaaggaaaacagggggc atgggttgactgtcctgtgagccctcttccctgctccccactcaagtgaccgggaatccc tcgacatggcag
12	hG6PC mut (amino acid)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILVSVIADLRNAFYVLP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGIKIP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVVAGVLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWLEKAQRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRESCGKLSKWLP FRLSCIVASLVLLHVFDLSLKPPSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQVLGQPHKKSL
13	Nucleic acid coding modified G6Pase-a (R3K)	Atggagaagaatgaatgttctccatgactttgggatccagtcacacattacctccaggt gaattaccaagactcccaggactggttcatcttggtgctcgtgatcgagacctcaggaatg ccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttgg gtagctgtgattggagactggctcaacctcgtcttaagtggattctctttggacagcgtcca tactggtgggttttgatactgactactacagcaacactccgtgcccctgataaagcagttc cctgtaacctgtgagactggaccaggaggccctctggccatgccatgggcacagcaggt gtatactacgtgatggtcacatctactctttccatcttccagggaaagataaagccgacctac agatttcgggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgtc tgtcacgaatctacctgtctcattttctcatcaagttgtgtcggagtcctgtcaggcatt gctgttcagaaaacttcagccacatccacagcatctataatgcagcctcaagaaatatttt ctcattaccttctcctgttcagcttcgcatcgattttatctgtctgctcaagggactgggtgt agacctcctgtggactctggagaaagcccagaggtggtgcgagcagccagaatgggtcc acattgacaccacaccctttgccagcctcctcaagaacctgggcacgctctttggcctgggg ctggctcctaactccagatgtacagggaagagctgaaggggaaactcaggaagtggtctc ccattccgcctcagctctattgtagctccctcgtcctcctgcacgctcttggactcctgaaacc cccatcccaagtgcagctggtcttctacgtctgtcctctgcaagagtgcggtagtgtccct ggcatccgtcagtgcatcccctactgcctcgcccagggtcctgggcccagccgcacaagaag tcgttataa

14	Nucleic acid coding modified G6Pase-a (Q54R)	<p>Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcattcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggtccatcttcgtgaagctgtgggcattaaactcctttg ggtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagagccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct cagatttcggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctaccttgctgctcattttcctcatcaagttgttgctggagctctgtcaggcat tgctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttctctgttcagcttcgcatcgattttatctgtctgctcaagggactgggtgt agacctctgtggactctggagaagcccagagggtggtgcgagcagccagaatgggtcc acattgacaccacacctttgccagcctcctcaagaacctgggcacgctcttggcctgggg ctggctcctcaactccagcatgtacagggagagctgaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctctgctcctctgcagctcttgactccttgaacc ccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtgcggtagtgtccct ggcatccgtcagtgatcatcccctactgctcgtcccagggtctggggccagccgcacaagaag tcgttgtaa</p>
15	Nucleic acid coding modified G6Pase-a (Q139R)	<p>Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcattcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggtccatcttcaggaagctgtgggcattaaactcctttg ggtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagagccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcggggaaagataaagccgacct cagatttcggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctaccttgctgctcattttcctcatcaagttgttgctggagctctgtcaggcat tgctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttctctgttcagcttcgcatcgattttatctgtctgctcaagggactgggtgt agacctctgtggactctggagaagcccagagggtggtgcgagcagccagaatgggtcc acattgacaccacacctttgccagcctcctcaagaacctgggcacgctcttggcctgggg ctggctcctcaactccagcatgtacagggagagctgaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctctgctcctctgcagctcttgactccttgaacc ccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtgcggtagtgtccct ggcatccgtcagtgatcatcccctactgctcgtcccagggtctggggccagccgcacaagaag tcgttgtaa</p>
16	Nucleic acid coding modified G6Pase-a (I142K)	<p>Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcattcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggtccatcttcaggaagctgtgggcattaaactcctttg ggtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagagccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagaaaaagccgacct cagatttcggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctaccttgctgctcattttcctcatcaagttgttgctggagctctgtcaggcat tgctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttctctgttcagcttcgcatcgattttatctgtctgctcaagggactgggtgt agacctctgtggactctggagaagcccagagggtggtgcgagcagccagaatgggtcc acattgacaccacacctttgccagcctcctcaagaacctgggcacgctcttggcctgggg ctggctcctcaactccagcatgtacagggagagctgaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctctgctcctctgcagctcttgactccttgaacc ccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtgcggtagtgtccct</p>

		ggcatccgtcagtgatcatcccactactgcctcgcccagggtcctgggcccagccgcacaagaagtcgttgtaa
17	Nucleic acid coding modified G6Pase-a (S196R)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcatcttgggtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgtcttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct aagatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacttgcgtcatttctcatcaagttgttctgagctgtcagggat tgctgttcagaaaacttccgccacatccacagcatctataatgccagcctcaagaaatatt tctcattaccttcttctgttcagctcgccatcgattttatctgtctgaagggactgggtg agacctcctgtgactctggagaagcccagagggtgtgcgagcagccagaatgggtcc acattgacaccacaccttggcagcctcctcaagaacctgggcagcgtcttggcctggg ctggctcaactccagcatgtacaggagagctgaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctcgtcctcctgcagcgtcttgcacttgaacc cccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtgcggtagtgcctt ggcatccgtcagtgatcatcccactactgcctcgcccagggtcctgggcccagccgcacaagaagtcgttgtaa
18	Nucleic acid coding modified G6Pase-a (H199Q)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcatcttgggtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgtcttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct aagatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacttgcgtcatttctcatcaagttgttctgagctgtcagggat tgctgttcagaaaacttccgccacatccagagcatctataatgccagcctcaagaaatatt tctcattaccttcttctgttcagctcgccatcgattttatctgtctgaagggactgggtg agacctcctgtgactctggagaagcccagagggtgtgcgagcagccagaatgggtcc acattgacaccacaccttggcagcctcctcaagaacctgggcagcgtcttggcctggg ctggctcaactccagcatgtacaggagagctgaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctcgtcctcctgcagcgtcttgcacttgaacc cccatcccaagtcgagctggtcttctacgtcttgccttctgcaagagtgcggtagtgcctt ggcatccgtcagtgatcatcccactactgcctcgcccagggtcctgggcccagccgcacaagaagtcgttgtaa
19	Nucleic acid coding modified G6Pase-a (Q242R)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcatcttgggtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgtcttaagtggattctcttggacagcgtcc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct aagatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacttgcgtcatttctcatcaagttgttctgagctgtcagggat tgctgttcagaaaacttccgccacatccacagcatctataatgccagcctcaagaaatatt tctcattaccttcttctgttcagctcgccatcgattttatctgtctgaagggactgggtg agacctcctgtgactctggagaagcccagagggtgtgcgagcagccagaatgggtcc acattgacaccacaccttggcagcctcctcaagaacctgggcagcgtcttggcctggg ctggctcaactccagcatgtacaggagagctgaaggggaaactcagcaagtggctc

		ccattccgctcagctctattgtagcctcctcgctcctgacgctttgactccttgaacc cccatccaagtcgagctggtcttctacgtcttgccttctgcaagagtcggtagtgccct ggcatccgtcagtgatccctactgctcgcccaggctctgggcccagccgcacagaag tcgttgtaa
20	Nucleic acid coding modified G6Pase-a (Q247R)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcacacattacctccagg tgaattaccaagactcccaggactggtcatcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgctttaaagtggaattctcttggacagcgctc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct cagatttcggtgcttgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctcattttctcatcaagttgttctggtgagctctgtcaggcat tgctgttcagaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctattaccttcttctgtcagctcgccatcggaatttatctgctgctcaagggactgggtgt agacctcctgtggactctggagaagcccagagggtggtgcgagcggccagaatgggtcc acattgacaccacaccttggccagcctctcaagaacctgggcacgctcttggcctgggg ctggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctcgctcctgacgctttgactccttgaacc cccatccaagtcgagctggtcttctacgtcttgccttctgcaagagtcggtagtgccct ggcatccgtcagtgatccctactgctcgcccaggctctgggcccagccgcacagaag tcgttgtaa
21	Nucleic acid coding modified G6Pase-a (L292F)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcacacattacctccagg tgaattaccaagactcccaggactggtcatcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgctttaaagtggaattctcttggacagcgctc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct cagatttcggtgcttgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctcattttctcatcaagttgttctggtgagctctgtcaggcat tgctgttcagaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctattaccttcttctgtcagctcgccatcggaatttatctgctgctcaagggactgggtgt agacctcctgtggactctggagaagcccagagggtggtgcgagcggccagaatgggtcc acattgacaccacaccttggccagcctctcaagaacctgggcacgctcttggcctgggg ctggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctc ccattccgctcagctctattgtagcctcctcgctcctgacgctttgactccttgaacc cccatccaagtcgagctggtcttctacgtcttgccttctgcaagagtcggtagtgccct ggcatccgtcagtgatccctactgctcgcccaggctctgggcccagccgcacagaag tcgttgtaa
22	Nucleic acid coding modified G6Pase-a (S298C)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcacacattacctccagg tgaattaccaagactcccaggactggtcatcttggtgtccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgctttaaagtggaattctcttggacagcgctc atactggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaaagataaagccgacct cagatttcggtgcttgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctcattttctcatcaagttgttctggtgagctctgtcaggcat tgctgttcagaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctattaccttcttctgtcagctcgccatcggaatttatctgctgctcaagggactgggtgt agacctcctgtggactctggagaagcccagagggtggtgcgagcggccagaatgggtcc

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23	Nucleic acid coding modified G6Pase-a (A301V)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactgggtcatcttggtgccgtgatcgagacctcaggaat gccttctacgtcctctccccatctgggtccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgcc atactgggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggcacatctactcttccatcttcagggaagataaagccgacctac cagatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctcatttctcatcaagttgtgctggagtcctgtcaggcat tgctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttcttctgtcagcttcgcatcggtttatctgtctgaaggactgggtgt agacctcctgtggactctggagaagcccagagggtggcgagcagccagaatgggtcc acattgacaccacaccctttgccagcctcctcaagaacctgggcacgctctttggcctggg ctggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctc ccattccgcctcagctctattgtagtcctcctcgctcctgcacgctttgactccttgaacc cccattcccaagtcgagctggcttctacgtcttgccttctgcaagagtcggtagtgccct ggcatccgtcagtgatccctactgcctcgcccaggctctgggcccagccgcacaagaag tcgttgtaa
24	Nucleic acid coding modified G6Pase-a (V318T)	Atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactgggtcatcttggtgccgtgatcgagacctcaggaat gccttctacgtcctctccccatctgggtccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgcc atactgggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggcacatctactcttccatcttcagggaagataaagccgacctac cagatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctcatttctcatcaagttgtgctggagtcctgtcaggcat tgctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttcttctgtcagcttcgcatcggtttatctgtctgaaggactgggtgt agacctcctgtggactctggagaagcccagagggtggcgagcagccagaatgggtcc acattgacaccacaccctttgccagcctcctcaagaacctgggcacgctctttggcctggg ctggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctc ccattccgcctcagctctattgtagtcctcctcgctcctgcacgctttgactccttgaacc cccattccaaactgagctggcttctacgtcttgccttctgcaagagtcggtagtgccct ggcatccgtcagtgatccctactgcctcgcccaggctctgggcccagccgcacaagaag tcgttgtaa
25	Nucleic acid coding modified G6Pase-a (V324T)	atggaggaaggaatgaatgttctccatgactttgggatccagtcaacacattacctccagg gaattaccaagactcccaggactgggtcatcttggtgccgtgatcgagacctcaggaatg ccttctacgtcctctccccatctgggtccatcttcaggaagctgtgggcattaaactccttgg gtagctgtgattggagactggctcaacctcgctttaagtggattctcttggacagcgcca tactgggtgggttttgatactgactactacagcaacactccgtgccctgataaagcagttc cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg gtatactacgtgatggcacatctactcttccatcttcagggaagataaagccgacctac agatttcggtgctgaatgtcatttgggtgggattctgggctgtgcagctgaatgtctgtc tgtcacgaatctacctgtctgctcatttctcatcaagttgtgctggagtcctgtcaggcat gctgttcagaaaacttcagccacatccacagcatctataatgccagcctcaagaaatattt

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26	Nucleic acid coding modified G6Pase-a (V332A)	Atggaggaaggaatgaatgttctccatgacttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcattcttggtgccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgtctttaagtggattctcttggacagcgctc atactggtgggttttgatactgactactacagcaacactccgtgcccctgataaagcagtt cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaagataaagccgacct cagatttcggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctatttctcatcaagttgttctggaagtctgtcaggcat tgctgttcagaaaactttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttcttctgttcagcttcgccatcgattttatctgtctgctcaagggactgggtgt agacctcctgtgactctggagaaagcccagagggtggtgcgagcagccagaatgggtcc acattgacaccacaccctttgccagcctcctcaagaacctgggcacgctctttggcctgggg ctggctcctaactccagcatgtacaggagagctgcaaggggaaactcagcaagtgggtc ccattccgctcagctctattgtagcctcctcgtcctcctgcacgtctttgactccttgaacc cccattcccaagtcgagctgggtcttctacgtcttgccttctgcaagagtgcggtagtccct ggcatccgtcagtgatccccctactgcctcgcccaggctcctgggcccagccgcacaagaag tcgttgtaa
27	Nucleic acid coding modified G6Pase-a (Q347R)	Atggaggaaggaatgaatgttctccatgacttgggatccagtcaacacattacctccagg tgaattaccaagactcccaggactggttcattcttggtgccgtgatcgagacctcaggaat gccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttg ggtagctgtgattggagactggctcaacctcgtctttaagtggattctcttggacagcgctc atactggtgggttttgatactgactactacagcaacactccgtgcccctgataaagcagtt cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactacgtgatggtcacatctactcttccatcttcagggaagataaagccgacct cagatttcggtgctgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgt ctgtcacgaatctacctgtctgctatttctcatcaagttgttctggaagtctgtcaggcat tgctgttcagaaaactttcagccacatccacagcatctataatgccagcctcaagaaatattt tctcattaccttcttctgttcagcttcgccatcgattttatctgtctgctcaagggactgggtgt agacctcctgtgactctggagaaagcccagagggtggtgcgagcagccagaatgggtcc acattgacaccacaccctttgccagcctcctcaagaacctgggcacgctctttggcctgggg ctggctcctaactccagcatgtacaggagagctgcaaggggaaactcagcaagtgggtc ccattccgctcagctctattgtagcctcctcgtcctcctgcacgtctttgactccttgaacc cccattcccaagtcgagctgggtcttctacgtcttgccttctgcaagagtgcggtagtccct ggcatccgtcagtgatccccctactgcctcgcccgggtcctgggcccagccgcacaagaag tcgttgtaa
28	Nucleic acid coding modified G6Pase-a (L349F)	atggaggaaggaatgaatgttctccatgacttgggatccagtcaacacattacctccagg gaattaccaagactcccaggactggttcattcttggtgccgtgatcgagacctcaggaatg ccttctacgtcctctccccatctggttccatcttcaggaagctgtgggcattaaactccttgg gtagctgtgattggagactggctcaacctcgtctttaagtggattctcttggacagcgcca tactggtgggttttgatactgactactacagcaacactccgtgcccctgataaagcagttc cctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagggt gtatactacgtgatggtcacatctactcttccatcttcagggaagataaagccgacctac agatttcggtgcttgaatgtcattttgtggtgggattctgggctgtgcagctgaatgtctgtc

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29	Modified G6Pase-a (R3K)	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDLSKPPSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVGQPHKKSL</p>
30	Modified G6Pase-a (Q54R)	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP IWFHLREAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDLSKPPSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVGQPHKKSL</p>
31	Modified G6Pase-a (Q139R)	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFRGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDLSKPPSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVGQPHKKSL</p>
32	Modified G6Pase-a (I142K)	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKKKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDLSKPPSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVGQPHKKSL</p>
33	Modified G6Pase-a (S196R)	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFRHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDLSKPPSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVGQPHKKSL</p>
34	Modified	<p>MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVFLFP</p>

	G6Pase-a (H199Q)	IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIQSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
35	Modified G6Pase-a (Q242R)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKARRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
36	Modified G6Pase-a (Q247R)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRW CERPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
37	Modified G6Pase-a (L292F)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
38	Modified G6Pase-a (S298C)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSCIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
39	Modified G6Pase-a (A301V)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGTFLGLGLALNSSMYRECKGKLSKWLP FRLSSIVSLVLLHVFDSLKPSSQVELVFYVLSFCKSAVVPLASVSVIPYCL AQLGQPHKKSL
40	Modified G6Pase-a (V318T)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNAPYVFLFP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRIYLAHFPHQVAVGLSGIAVAE

		TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQTELVFYVLSFCKSAVPLASVSVIPYCL AQLGQPHKKSL
41	Modified G6Pase-a (V324T)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVLP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLGQPHKKSL
42	Modified G6Pase-a (V332A)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVLP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLGQPHKKSL
43	Modified G6Pase-a (Q347R)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVLP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVPLASVSVIPYCL ARVLGQPHKKSL
44	Modified G6Pase-a (L349F)	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILSVIADLRNIFYVLP IWFHLQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYS NTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYYVMVTSTLSIFQGKIKP TYRFRCLNVILWLGFWAVQLNVCLSRILAAHFPHQVAVGLSGIAVAE TFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRW CEQPEWVHIDTTPFASLLKNLGLTFLGLALNSSMYRESCKGKLSKWLP FRLSSIVASLVLLHVFDSLKPSSQVELVFYVLSFCKSAVPLASVSVIPYCL AQLVFGQPHKKSL
45	Codon optimised G6pc (co1) – used in example 5	atggaggaaggaatgaatgttctcatgacttgggatccagtcacacattacctccaggt gaattaccaagactcccaggactggtcatcttggtgtctgtgattgcagacctcaggaatg ccttctatgtcctctcccatctggttccatcttcaggaagctgtgggcattaaactccttgg gtagctgtgattggagactggctcaacctggtcttaagtggattctcttggacagaggcc atactggtgggttttgatactgactactacagcaacactctgtgcccctgataaagcagtt ccctgtaacctgtgagactggaccaggagcccctctggccatgccatgggcacagcagg tgtatactatgtgatggtcacatctactcttccatcttcagggaagataaagcccacctac agatttaggtgctgaatgtcattttgtggttgggattctgggctgtgcagctgaatgtctgtc tgtcaaggatctacctgtgctcattttctcatcaagttgttgcaggagctgtcaggcatt gctgttgagaaactttcagccacatccacagcatctataatgccagcctcaagaaatatttt ctcattaccttctcgttgcagcttgccattggatttatctgctgctcaagggaactgggtgta gacctctgtgactctggagaaagcccagagggtggtgtgagcagccagaatgggtcca cattgacaccacacctttgccagcctctcaagaacctgggaccctcttggcctggggc tggctctcaactccagcatgtacaggagagctgcaaggggaaactcagcaagtggctcc cattcaggctcagctctattgtagcctcctggtctctctgcatgtcttggactcctgaaacc

		ccatccaagtggagctggtcttctatgtcttgcctctgcaagagtgtgtagtggccctgg catctgtcagtgatcatccctactgcctggcccaggctctggccagccccacagaagtcc ttgtaa
46	Codon optimised G6pc (co2) – used in example 5	atggaggaggggatgaacgtgtgcacgacttgggatccagagcaccactacctgca ggtgaactaccaggacagccaggactggtttatcctggtgtctgtgattgtgacctgagg aacgccttctacgtgctgttccctatctggtccacctgcaggaggctgtggggatcaagct gctgtgggtgctgtgattggggactggctgaacctggtgtcaagtggatcctgtttggcc agaggccctactggtgggtgctggacacagactactacagcaacacctgtgcccctgat caagcagttccctgtgacctgtgagacaggccctggcagccctctggccacgctatggc acagctgggtgtactacgtgatggtaccagcacctgtctatctccagggaagatca agcccactacaggttcagggtgcctgaacgtgatcctgtggtggtcttgggctgtgca gctgaacgtgtgctgagcaggatctacctggctgccacttccccaccagggtggtggt gggtgtgtctgggattgctgtggtgagacctcagccacatccactctatcacaacgc cagcctgaagaagtacttctgatcaccttctcctgttcagcttctgtattgcttctacgtg gctgaaggcctgggggtgacgtgctgtggaccctggagaaggccagaggtggtgtg agcagcctgagtggtgcacattgacaccaccccttgcagcctgtgtaagaacctggg caccctgttggcctggcctggcctgaacagctctatgtacaggagagctgcaaggg caagctgagcaagtggctgcccctcaggctgagctctattgtgcccagcctggtgctgctg acgtgttgacagcctgaagccccagccaggtggagctggtgtctacgtgctgagcttc tgcaagtctgctgtggtgcccctggcctgtgtctgtgatccctactgcctggcccagggtg ctggccagccccacagaagagcctgtag
47	Codon optimised intron of the β-globin gene (HBB2 co) – used in examples 4 and 5	gtacacatattgacaaatcagggttaatttgcatttgtaattttaaaaaatgcttctctttaa atatactttttgtttatcttatttctaatacttccctaatacttcttcttcagggaataatgatac aatgtatcatgcctctttgcaccattctaagaataacagtgataatttctgggttaaggcaa tagcaatatttctgcatataaatatttctgcatataaatgttaactgatgaagaggttcatat tgctaatagcagctacaatccagctaccattctgcttttatttctggtgggataaggctgga ttattctgagccaagctaggccctttgctaactgttctacaccttcttctctccacagct cctgggaacctgctggtctctctgctgcccacatttggcaag
48	hAAT_HBB2C o_hG6pc wt co1 (expression cassette used in example 5)	aaggctcagaggcacacaggagtttctgggtcacctgccccctccaaccctcagttcc catcctccagcagctgtttgtgtgctgctgaagtccacactgaacaaactcagcctact catgtccctaaaatgggcaacattgcaagcagcaaacagcaaacacagccctccctg cctgtgaccttgagctggtggcagaggtcagagacctctctggcccatgccacctcaa catccactcgaccccttggaatttcggtggagaggagcagaggtgtcctgctggtgtta ggtagtgtgagaggggtacccgggactgtgctaccagtgaacagcagcctaaggattct gcagtgtgagcagagggccagctgaagtgttactctccagagactgtgactacgcca ccccctccacctggacacaggacgctgtggttctgagccaggatcaatgactccttctggt aagtgcagtgtgaagctgtacactgcccaggcaaacgctccgggcagcgtaggcgggcg actcagatccagccagtggaacttagcccctgttctcctccgataactgggtgacctg gttaatatccacagcagcctccccgttggccctctggatccactgctaaatacggacga ggacagggccctgtctcctcagcttcaggcaccaccactgacctgggacagtgaatagatc ctgagaactcagggtgagctatgggacccttgatgtttcttcccttcttctatggttaa gttcatgtcataggaaggggagaagtaacagggtacacatattgacaaatcagggtaat tttgatttgaattttaaaaaatgcttcttctttaaataacttttgtttatcttatttctaatact ttccctaactcttcttctcagggaataatgatacaatgatcatgccttctgaccattctaa agaataacagtgaataatttctgggttaaggcaatagcaatatttctgcatataaatatttctg catataaatgtaactgatgaagaggttcatattgtaatagcagctacaatccagctacc attctgttttatttctggttgggataaggctggattattctgagccaagctaggccctttg ctaactgttctacaccttcttctctccacagctcctgggaacctgctgtctctctgctg gcccacacttggcaagcagcgtgccaccatggaggaaggaatgaatgtctccatg acttgggatccagtcacacattacctcagggtgaattaccaagactcccaggactggttc atcttggtgtctgtgattgcagacctcaggaatgccttctatgtccttctcccatctggtcca

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50	<p>hAAT_HBB2C o_hG6pc wt co3 (expression cassette used in example 5)</p> <p>Comprising SEQ ID NO: 3</p>	<p>aaggctcagaggcacacaggagtttctgggctcacctgccccctccaaccctcagttcc catctccagcagctgtttgtgtgctgctgaagtccacactgaacaaactcagcctact catgtccctaaaatgggcaacattgcaagcagcaaacagcaaacacacagccctccctg cctgtgaccttgagctggggcagaggtcagagacctctctggcccatgccacctcaa catccactcgaccccttggaatttcggtggagaggagcagaggtgtcctggcgtggtta ggtagtgtgagaggggtacccggggtactgtctaccagtgaacagcagcctaaggattct gcagtggagcagaggggcagctgaagtgtgactctccagagactgtgactacgcca ccccctccaccttgacacaggacgctgtggttctgagccaggatcaatgactccttcggt aagtgagtggaagctgtacactgcccaggcaagcgtccgggagcgtagggcg actcagatcccagccagtgacttagcccctgttctcctccgataactgggtgacctg gttaatatccacagcagcctccccctgtgcccctctggatccactgctaaatcggacga ggacagggccctgtctcctcagcttcaggcaccaccactgacctgggacagtgaatagatc ctgagaactcagggtgagctatgggaccctgatgttttcttccccttctttctatggttaa gttcatgtcataggaaggggagaagtaacagggtagacatattgacaaatcagggtaat tttgatttgaattttaaaaaatgcttcttctttaataactttttgttattcttataact ttccctaactcttcttccagggaatattgatacaatgtatctgtccttctgaccattctaaa gaataacagtgataatttctgggttaaggcaatagcaatatttctgcatataaatatttctgc atataaattgtaactgatgaagaggttcatattgctaatagcagctacaatccagctacca ttctgcttttatttctggtgggataaggctggattattctgagtcgaagctagggcctttgt aatctgttcatacctcttatcttctccacagctcctgggcaacctgctggtctctgtg cccatcatttggcaagcagcgtgcccacatggaggagggaatgaatgtgtgcacga cttcgggattcagctaccactatctcaagtcaattatcaagacagtcagattggttcat cctgtctccgtatagctgacctcagggaatgttttatgtactgttccaatatggttcatct gcaagaagcagttggaattaaactgctgtgggtggccgtaacggagactggtgaaact ggtgttcaaatggatccttttgggtcagaggccatattggtgggtctggacaccgactatta tagcaacacatctgtacccctgataaagcaattccctgtaacgtgtgaaactgggctgggt</p>

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51	<p>hAAT_HBB2 co_hG6pc mut (expression cassette)</p> <p>Comprising SEQ ID NO: 5</p>	<p>aaggctcagaggcacacaggagtttctgggctcaccctgcccccttcaaccctcagttcc catctccagcagctgtttgtgtgctgctcgaagtccacactgaacaaactcagcctact catgtccataaatgggcaaacattgcaagcagcaaacacagcaaacacagccctcctg cctgctgaccttgagctggggcagaggtcagagacctctgtggcccatgccactccaa catccactcgacccttggaatttcggtggagaggagcagaggtgtcctggcgtgtgta ggtagtgtagaggggtaccggggtctgtaccagtgaacagccactaaggattct gcagtgaagcagagggcgactgaagtgtactctccagagactgtctgactacgcca ccccctccacttgacacaggacgctgtgtgttctgagccaggtacataactccttccgt aagtgcagtggaaagctgtacactgcccaggcaagcgtccggcgagcgtgagcggcg actcagatccagccagtgacttagcccctgttctcctccgataactggggtgacctg gttaattaccagcagcctccccgttgcctctggatccactgcttaaatcggacga ggacagggccctgtctcctcagctcaggcaccaccactgacctgggacagtgaatagatc ctgagaactcagggtgagctatggacccttgatgtttcttcccttctttctatggttaa gttcatgtcataggaaggggagaagtaacagggtagacataattgacaaatcagggtaat tttgatttgaattttaaaaaatgcttctctttaaataactttttgttatcttatttctaatact ttccctaattcttcttccagggcaataatgatacaatgtatcatgctcttgcaccattctaa agaataacagtataatttctgggttaaggcaatagcaatatttctgcatataaatatttctg catataaattgtaactgatgaagaggttcatattgtaatagcagctacaatccagctacc attctgctttatttctggttgggataaggctgattattctgagccaagctaggccctttg ctaattgttcatactcttctctcctccacagctcctgggcaacctgctggtctctgctg gcccacactttggcaagcagcgtgccaccatggaggaaggaatgaatgtctccatg acttgggatccagtcaacacattactccaggtgaattaccaagactcccaggactggttc atcttggtgctgctgagcagacctcagggaatgccttctacgtctcttccccactggttcc atcttcaggaagctgtgggcattaaactcttgggtagctgtgattggagactggctaac ctgctcttaagtggattcttggacagcgtccatactggtgggttggatactgactact acagcaacactccgtgcccctgataaagcagttccctgtaacctgtgagactggaccagg gagcccctctggccatgccatgggacagcaggtgtatactcgtgatggtcacatctactc ttccatcttccagggaagataaagccgacctacagatttcggtgctgaatgtcatttgtg</p>

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Claims

1. An adeno-associated virus (AAV) vector comprising a nucleic acid construct for the expression of a glucose-6-phosphatase-a (G6Pase-a) in a cell, the construct
5 comprising a nucleic acid sequence encoding the G6Pase-a, wherein the nucleic acid sequence encoding the G6Pase-a is operably linked to a human alpha-1 antitrypsin (hAAT) promoter.
2. The AAV vector according to claim 1, wherein the G6Pase-a has an amino acid sequence at least 90% identical to SEQ ID NO: 1.
- 10 3. The AAV vector according to any of claims 1 or 2, wherein the nucleic acid sequence encoding the G6Pase-a comprises a nucleotide sequence having at least 90% identity with SEQ ID NO: 2.
4. The AAV vector according to any of claims 1 to 3, wherein the nucleic acid sequence encoding the G6Pase-a is codon optimized, preferably the nucleic acid sequence
15 encoding the G6Pase-a is codon optimized by decreasing the content of GC and decreasing GC dimers in said nucleic acid sequence encoding the G6Pase-a.
5. The AAV vector according to any of claims 1 to 4, wherein the hAAT promoter comprises a nucleotide sequence having at least 90% identity with SEQ ID NO: 8.
6. AAV vector according to any of claims 1 to 4, wherein the hAAT promoter is preceded
20 by an enhancer, such as ApoE enhancer (e.g. SEQ ID NO: 9), preferably the nucleic acid construct comprises SEQ ID NO: 7.
7. The AAV vector according to any of claims 1 to 6, wherein the nucleic acid construct comprises a nucleotide sequence having at least 90% identity with with SEQ ID NO: 11, with SEQ ID NO: 48, with SEQ ID NO: 49, with SEQ ID NO: 50, with SEQ ID NO: 51, with SEQ ID NO: 53, or with SEQ ID NO: 55, or with SEQ ID NO: 56, preferably
25 having at least 90% identity with SEQ ID NO: 48.
8. The AAV vector according to any of claims 1 to 7, wherein the nucleic acid construct comprises, in the 5' to 3' orientation :

- (i) the hAAT promoter preceded by an enhancer, such as ApoE enhancer (e.g. SEQ ID NO: 9) ;
- (ii) optionally an intron, such as an intron of the human β globin gene (e.g. SEQ ID NO: 47) ;
- 5 (iii) the nucleic acid sequence encoding the G6Pase-a ; and
- (iv) a polyadenylation signal, such as the bovine growth hormone polyadenylation signal, the HBB2 polyadenylation signal, the SV40 polyadenylation signal, or another naturally occurring or artificial polyadenylation signal.
- 10 **9.** The AAV vector according to any of claims 1 to 8, wherein the cell is a liver cell, a kidney cell or an intestine cell.
- 10.** The vector of any of claims 1 to 9, which is an AAV serotype 8 (AAV8) vector, an AAV9 vector, AAVrh74 vector, an AAV2i8 vector or an AAVmut5 vector, preferably an AAV8 vector.
- 11.** A cell transformed with the vector of any one of claims 1 to 10.
- 15 **12.** The cell according to claim 11, which is a liver cell, an intestinal cell or a kidney cell.
- 13.** A composition comprising the vector of any one of claims 1 to 10, or the cell of any one of claims 11 to 12.
- 14.** The vector of any one of claims 1 to 10, the cell according to any one of claims 11 to 12 or the composition according to claim 13, for use as a medicament.
- 20 **15.** The vector of any one of claims 1 to 10, the cell according to any one of claims 11 to 12 or the composition according to claim 13, for use in the treatment of glycogen storage disease (GSD), such as GSD-Ia.