

Modified GAA proteins have been proposed in the past to improve lysosomal storage disease treatment. In particular, application WO2004064750 and Sun et al. 2006, disclose a chimeric GAA polypeptide comprising a signal peptide operably linked to GAA as a way to enhance targeting of the protein to the secretory pathway.

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However, therapies available to the patient are not entirely satisfactory and improved GAA polypeptides and GAA production is still a need in the art. In particular, a need still exists of a long term efficacy of the treatment with GAA, of high level GAA production, of improved immunological tolerance to the produced GAA polypeptide, and of improved uptake of GAA by the cells and tissues in need thereof. In addition, in WO2004064750 and Sun et al., 2006, tissue distribution of the chimeric GAA polypeptide disclosed therein is not entirely satisfactory. Therefore, a need still exists for a GAA polypeptide that would be fully therapeutic, by allowing a correction of glycogen accumulation in most if not all tissues of interest.

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## 15 SUMMARY OF THE INVENTION

The present invention relates to GAA variants that are expressed and secreted at higher levels compared to the wild type GAA protein and that elicit improved correction of the pathological accumulation of glycogen body-wide and results in the induction of immunological tolerance to GAA.

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In one aspect, there is provided a nucleic acid molecule encoding a functional chimeric GAA protein, comprising a signal peptide moiety and a functional GAA moiety, in particular a GAA moiety corresponding to a truncated form of a parent GAA, wherein the signal peptide moiety has an amino acid sequence selected in the group consisting of SEQ ID NO:2 to 4, preferably SEQ ID NO:2.

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In another aspect, there is provided a nucleic acid construct, comprising the nucleic acid molecule as described herein, which is an expression cassette comprising said nucleic acid molecule operably linked to a promoter, such as a liver-specific promoter preferably selected in the group consisting of the alpha-1 antitrypsin promoter (hAAT), the transthyretin promoter, the albumin promoter and the thyroxine-binding globulin (TBG) promoter, wherein said nucleic acid construct optionally further comprises an intron, in particular an intron selected in the group consisting of a human beta globin b2 (or HBB2) intron, a FIX intron, a chicken beta-globin intron and a SV40 intron, wherein said intron is optionally a modified intron such as a modified HBB2 intron of SEQ ID NO:7, a modified FIX intron of SEQ ID NO:9, or a modified chicken beta-globin intron of SEQ ID NO:11.

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In another aspect, there is provided a vector comprising the nucleic acid molecule or the nucleic acid construct as described herein, which is a viral vector, preferably a retroviral vector, such as a lentiviral vector, or an AAV vector, such as a single-stranded or double-stranded self-complementary AAV vector, preferably an AAV vector with an AAV-derived capsid, such as an AAV1, AAV2, variant AAV2, AAV3, variant AAV3, AAV3B, variant AAV3B, AAV4, AAV5, AAV6, variant AAV6, AAV7, AAV8, AAV9, AAV10 such as AAVcy10 and AAVrh10, AAVrh74, AAVdj, AAV-Anc80, AAV-LK03, AAV2i8, and porcine AAV, such as AAVpo4 and AAVpo6 capsid or with a chimeric capsid, wherein the AAV vector has more particularly an AAV8, AAV9, AAVrh74 or AAV2i8 capsid, in particular an AAV8, AAV9 or AAVrh74 capsid, more particularly an AAV8 capsid.

In another aspect, there is provided a cell transformed with the nucleic acid molecule, the nucleic acid construct or the vector as described herein, wherein the cell is in particular a liver cell or a muscle cell.

In another aspect, there is provided a chimeric GAA polypeptide, comprising a signal peptide moiety and a functional GAA moiety, wherein the signal peptide moiety is selected in the group consisting of SEQ ID NO:2 to 4, wherein in particular the GAA moiety is a truncated form of a parent GAA polypeptide, such as a GAA moiety having 1 to 75 consecutive amino acids deleted at its N-terminal end as compared to a parent GAA polypeptide, in particular 6, 7, 8, 9, 10, 20, 27, 28, 29, 30, 31, 41, 42, 43, 44, 45 or 45, more particularly 6, 7, 8, 9, 10, 20, 41, 42, 43 or 44 consecutive amino acids deleted at its N-terminal end as compared to a parent GAA polypeptide, wherein the GAA moiety is in particular a truncated form of the human GAA protein of SEQ ID NO:5 or SEQ ID NO:36, in particular of SEQ ID NO:5.

In another aspect, there is provided a pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the nucleic acid sequence, the nucleic acid construct, the vector, the cell, or the chimeric polypeptide as described herein.

In another aspect, there is provided the nucleic acid sequence, the nucleic acid construct, the vector, the cell, or the chimeric polypeptide as described herein, for use as a medicament.

In another aspect, there is provided use of the nucleic acid sequence, the nucleic acid construct, the vector, the cell, or the chimeric polypeptide as described herein, in the manufacture of a medicament for treating a glycogen storage disease, such as GSDI (von Gierke's disease), GSDII (Pompe disease), GSDIII (Cori disease), GSDIV, GSDV, GSDVI, GSDVII, GSDVIII and lethal congenital glycogen storage disease of the heart, more particularly GSDI, GSDII or GSDIII, even more particularly GSDII and GSDIII, and most particularly GSDII.

According to one embodiment, the invention provides a nucleic acid molecule encoding a functional chimeric GAA polypeptide, comprising a signal peptide moiety and a functional GAA moiety. In the encoded chimeric GAA polypeptide, the endogenous (or natural) signal peptide of a GAA polypeptide is replaced with the signal peptide of another protein. The nucleic acid molecule therefore encodes a  
5 chimeric GAA polypeptide comprising a signal peptide from another protein than a GAA, operably linked to a GAA polypeptide. The encoded chimeric polypeptide is a functional GAA protein wherein the amino acid sequence corresponding to the natural signal peptide of GAA (such as that corresponding to nucleotides 1 to 81 of SEQ ID NO: 1 which is a wild-type nucleic acid encoding human GAA) is replaced by the amino acid sequence of a different protein. In a preferred  
10 embodiment, the encoded signal peptide has an amino acid sequence selected in the group consisting of SEQ ID NO:2 to 4. In a particular embodiment, the GAA moiety is a N-terminally truncated form of a parent GAA polypeptide.

In a particular embodiment, the GAA moiety has 1 to 75 consecutive amino acids deleted at its N-  
15 terminal end as compared to a parent GAA polypeptide, wherein the parent polypeptide corresponds to a precursor form of a GAA polypeptide devoid of its signal peptide. In a particular embodiment, said truncated GAA polypeptide has at least 2, in particular at least 2, in particular at least 3, in particular at least 4, in particular at least 5, in particular at least 6, in particular at least 7, in particular at least 8

In yet another embodiment, the invention relates to a nucleic acid construct, comprising the nucleic acid molecule of the invention operably linked to one or more regulatory sequences such as a promoter, an intron, a polyadenylation signal and/or an enhancer (for example a cis-regulatory module, or CRM). In a particular embodiment, the promoter is a liver-specific promoter preferably  
 5 selected in the group consisting of the alpha-1 antitrypsin promoter (hAAT), the transthyretin promoter, the albumin promoter and the thyroxine-binding globulin (TBG) promoter. In another particular embodiment, the promoter is a muscle-specific promoter, such as the Spc5-12, MCK and desmin promoters. In another embodiment, the promoter is an ubiquitous promoter such as the CMV, CAG and PGK promoters. The nucleic acid construct may further optionally comprises an intron, in  
 10 particular an intron selected in the group consisting of a human beta globin b2 (or HBB2) intron, a FIX intron, a chicken beta-globin intron and a SV40 intron, wherein said intron is optionally a modified intron such as a modified HBB2 intron of SEQ ID NO:7, a modified FIX intron of SEQ ID NO:9, or a modified chicken beta-globin intron of SEQ ID NO:11.

In another particular embodiment, the nucleic acid construct comprises, preferably in this order,: an enhancer; an intron; a promoter, in particular a liver-specific promoter; the nucleic acid sequence encoding the chimeric GAA polypeptide; and a polyadenylation signal, the construct comprising preferably, in this order: an ApoE control region; a HBB2 intron, in particular a modified HBB2 intron; a hAAT promoter; the nucleic acid sequence encoding the chimeric GAA polypeptide; and  
 20 bovine growth hormone polyadenylation signal. In specific embodiment, the nucleic acid construct comprises a nucleotide sequence selected in the group consisting of the combinations of sequences shown in table 2, table 2' or table 2", in particular in table 2' or 2", more particularly the nucleotide sequence of SEQ ID NO:17 (corresponding to the fusion of SEQ ID NO:26 and SEQ ID NO:32), 18 (corresponding to the fusion of SEQ ID NO:27 and SEQ ID NO:32) or 19 (corresponding to the fusion  
 25 of SEQ ID NO:28 and SEQ ID NO:32).

According to another embodiment, the invention relates to a vector comprising the nucleic acid molecule or the nucleic acid construct according to the invention. In a particular embodiment, the vector is a viral vector, preferably a retroviral vector, such as a lentiviral vector, or an AAV vector.

30 According to another embodiment, the viral vector is a single-stranded or double-stranded self-complementary AAV vector, preferably an AAV vector with an AAV-derived capsid, such as an AAV1, AAV2, variant AAV2, AAV3, variant AAV3, AAV3B, variant AAV3B, AAV4, AAV5, AAV6, variant AAV6, AAV7, AAV8, AAV9, AAV10 such as AAVcy10 and AAVrh10, AAVrh74,  
 35 AAVdj, AAV-Anc80, AAV-LK03, AAV2i8, and porcine AAV, such as AAVpo4 and AAVpo6 capsid or with a chimeric capsid.

According to a further particular embodiment, the AAV vector has an AAV8, AAV9, AAVrh74 or AAV2i8 capsid, in particular an AAV8, AAV9 or AAVrh74 capsid, more particularly an AAV8 capsid.

- 5 In another embodiment, the invention relates to a cell transformed with the nucleic acid molecule, the nucleic acid construct or the vector of the invention. In a particular embodiment, the cell is a liver cell or a muscle cell.

According to another embodiment, the invention relates to a chimeric GAA polypeptide, comprising a  
 10 signal peptide moiety and a functional GAA moiety. The signal peptide moiety is selected in the group consisting of SEQ ID NO:2 to 4, preferably SEQ ID NO:2. Furthermore, the GAA moiety may be a truncated form of a parent GAA polypeptide, such as a GAA moiety having 1 to 75 consecutive amino acids truncated at its N-terminal end as compared to a parent GAA polypeptide, in particular 6, 7, 8, 9,  
 15 10, 20, 41, 42, 43 or 44 consecutive amino acids truncated at its N-terminal end as compared to a parent GAA polypeptide, such as 8 or 42 consecutive amino acids truncated at its N-terminal end as compared to a parent GAA polypeptide, wherein the GAA moiety is in particular a truncated form of the human GAA protein of SEQ ID NO:5 or SEQ ID NO:36, in particular of SEQ ID NO:5. In a particular embodiment, the GAA moiety has 8 consecutive amino acids truncated at its N-terminal end as compared to a parent GAA polypeptide (more particularly the parent GAA polypeptide of SEQ ID  
 20 NO:5 or SEQ ID NO:36, in particular of SEQ ID NO:5). In a particular embodiment of the invention, the chimeric GAA polypeptide of the invention is selected in the group consisting of the combinations of amino acid sequences shown in table 1, table 1' or table 1'', in particular in table 1' or table 1''. Further particular embodiments of the chimeric GAA polypeptide comprising a truncated for of a parent GAA polypeptide are disclosed in the following detailed description.

- 25 In another embodiment, the invention relates to a pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the nucleic acid sequence, the nucleic acid construct, the vector, the cell or the chimeric polypeptide disclosed herein.

- 30 Another embodiment of the invention relates to the nucleic acid sequence, the nucleic acid construct, the vector, the cell, or the chimeric polypeptide of the invention, for use as a medicament.

In yet another embodiment, the invention relates to the nucleic acid sequence, the nucleic acid construct, the vector, the cell, or the chimeric polypeptide of the invention, for use in a method for  
 35 treating a glycogen storage disease. In a particular embodiment, the glycogen storage disease is GSDI, GSDII, GSDIII, GSDIV, GSDV, GSDVI, GSDVII, GSDVIII or lethal congenital glycogen storage disease of the heart. In a more particular embodiment, the glycogen storage disease is selected in the group consisting of

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|-------------------------------------------|-------------------------------------------------------------------------------------|
| SEQ ID NO:2<br>SEQ ID NO:3<br>SEQ ID NO:4 | truncated hGAA deleted for 43 consecutive N-terminal amino acids; e.g. SEQ ID NO:42 |
| SEQ ID NO:2<br>SEQ ID NO:3<br>SEQ ID NO:4 | truncated hGAA deleted for 47 consecutive N-terminal amino acids; e.g. SEQ ID NO:43 |

14. A pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the nucleic acid sequence of any one of claims 1 to 6, the nucleic acid construct of any one of claims 7 to 8, the vector of claim 9, the cell according to claim 10, or the chimeric polypeptide according to any one of claims 11 to 13.

15. The nucleic acid sequence of any one of claims 1 to 6, the nucleic acid construct of any one of claims 7 to 8, the vector of claim 9, the cell according to claim 10, or the chimeric polypeptide according to any one of claims 11 to 13, for use as a medicament.

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16. Use of the nucleic acid sequence of any one of claims 1 to 6, the nucleic acid construct of any one of claims 7 to 8, the vector of claim 9, the cell according to claim 10, or the chimeric polypeptide according to any one of claims 11 to 13, in the manufacture of a medicament for treating a glycogen storage disease, such as GSDI (von Gierke's disease), GSDII (Pompe disease), GSDIII (Cori disease), GSDIV, GSDV, GSDVI, GSDVII, GSDVIII and lethal congenital glycogen storage disease of the heart, more particularly GSDI, GSDII or GSDIII, even more particularly GSDII and GSDIII, and most particularly GSDII.

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