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.

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.

SUMMARY

The present disclosure 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.

In one aspect, the present disclosure refers to a truncated GAA polypeptide, comprising a deletion of at least one amino acid from the N-terminal end of a parent GAA polypeptide, wherein the parent polypeptide corresponds to a precursor form of a GAA polypeptide devoid of its signal peptide,
wherein said truncated GAA polypeptide has 1 to 75 consecutive amino acids deleted at its N-terminal end as compared to the parent GAA polypeptide, and
wherein said truncated GAA polypeptide further comprises a signal peptide fused to its N-terminal end.

In one aspect, the present disclosure refers to a nucleic acid molecule encoding a truncated GAA polypeptide of the invention, said nucleic acid molecule being in particular a nucleotide sequence optimized to improve the expression of and/or improve immune tolerance to the truncated GAA polypeptide *in vivo*, such as the nucleic acid sequence shown in SEQ ID NO:12, SEQ ID NO:13 SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50 or SEQ ID NO:51.

In one aspect, the present disclosure refers to a nucleic acid construct, comprising the nucleic acid molecule of the invention, comprising said nucleic acid molecule operably linked to a promoter, in particular a promoter that is 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 further optionally 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:17, a modified FIX intron of SEQ ID NO:19, or a modified chicken beta-globin intron of SEQ ID NO:21.

In one aspect, the present disclosure refers to a vector comprising the nucleic acid molecule or the nucleic acid construct according to the invention, which is a viral vector, preferably a retroviral vector, such as a lentiviral vector, or an AAV vector.

In one aspect, the present disclosure refers to a cell transformed with the nucleic acid molecule of the invention, the nucleic acid construct of the invention or the vector of the invention, wherein the cell is in particular a liver cell or a muscle cell.

In one aspect, the present disclosure refers to a pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the truncated GAA polypeptide of the invention, the nucleic acid molecule of the invention, the nucleic acid construct of the invention, the vector of the invention, or the cell according to the invention.

According to one example, the invention relates to a truncated GAA polypeptide, comprising a deletion of at least one amino acid from the N-terminal end of 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 example, 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 consecutive amino acids deleted at its N-terminal end as compared to the parent GAA polypeptide. In another example, said truncated GAA polypeptide has at most 75, in particular at most 70, in particular at most 60, in particular at most 55, in particular at most 50, in particular at most 47, in particular at most 46, in particular at most 45, in particular at most 44, in particular at most 43 consecutive amino acids deleted at its N-terminal end as compared to the parent GAA polypeptide. In a further particular example, said truncated GAA polypeptide has at most 47, in particular at most 46, in particular at most 45, in particular at most 44, in particular at most 43 consecutive amino acids deleted at its N-terminal end as compared to the parent GAA polypeptide. In another particular example, said truncated GAA polypeptide has 1 to 75, in particular 1 to 47, in particular 1 to 46, in particular 1 to 45, in particular 1 to 44, in particular 1 to 43 consecutive amino acids deleted at its N-terminal end as compared to the parent

GAA polypeptide. In another example, said truncated GAA polypeptide has 2 to 43, in particular 3 to 43, in particular 4 to 43, in

particular 5 to 43, in particular 6 to 43, in particular 7 to 43, in particular 8 to 43 consecutive amino acids deleted at its N-terminal end as compared to the parent GAA polypeptide. In a more particular example, said truncated GAA polypeptide has 6, 7, 8, 9, 10, 27, 28, 29, 30, 31, 40, 41, 42, 43, 44, 45, 46 or 47 consecutive amino acids deleted at its N-terminal end as compared to a parent GAA polypeptide, in particular 7, 8, 9, 28, 29, 30, 41, 42, 43 or 44, more particularly 8, 29, 42 or 43 consecutive amino acids truncated at its N-terminal end as compared to a parent GAA polypeptide. In a further particular example, the parent polypeptide is a human GAA (hGAA), in particular a hGAA having the amino acid sequence shown in SEQ ID NO:1 or SEQ ID NO:33, in particular SEQ ID NO:1.

In a particular example, the truncated GAA polypeptide of the invention has the sequence shown in SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:34 and SEQ ID NO:35.

Moreover, the truncated GAA polypeptide of the invention may further comprise a signal peptide fused to its N-terminal end, in particular a signal peptide selected in the group consisting of SEQ ID NO:3 to 7, in particular the signal peptide of SEQ ID NO:3.

In another example, the invention relates to a nucleic acid molecule encoding a truncated GAA polypeptide as described above, optionally fused to a signal peptide via its N-terminal end. In some examples, the nucleic acid molecule has a nucleotide sequence optimized to improve the expression of and/or improve immune tolerance to the truncated GAA polypeptide *in vivo*, in particular in a human subject.

In yet another example, 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 example, the promoter is 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. In another particular example, the promoter is a muscle-specific promoter, such as the Spc5-12, MCK and desmin promoters. In another example, 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 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:17, a modified FIX intron of SEQ ID NO:19, or a modified chicken beta-globin intron of SEQ ID NO:21. In a particular example of the nucleic acid construct of the invention, said construct comprises, preferably in this order,: an enhancer; an intron; a promoter, in particular a

liver-specific promoter; the nucleic acid sequence encoding the GAA protein; 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 truncated GAA polypeptide; and a bovine growth hormone polyadenylation signal. In specific examples, said nucleic acid construct more particularly comprises the nucleotide sequence of any one of SEQ ID NO:22 to 26.

In another example, the invention relates to a vector comprising the nucleic acid molecule or the nucleic acid construct herein disclosed. The vector of the invention may be in particular a viral vector, preferably a retroviral vector, such as a lentiviral vector, or an AAV vector. Preferably, the 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, AAVdj, AAV-Anc80, AAV-LK03, AAV2i8, a porcine AAV capsid, such as AAVpo4 and AAVpo6 capsid, or with a chimeric capsid. In a specific example, the vector is an AAV vector with an AAV8, AAV9, AAVrh74 or AAV2i8 capsid, in particular an AAV8, AAV9 or AAVrh74 capsid, more particularly an AAV8 capsid.

In yet another example, the invention provides a cell transformed with the nucleic acid molecule, the nucleic acid construct or the vector of the invention. More particularly, the cell is a liver cell or a muscle cell.

In a particular example, the invention provides a pharmaceutical composition, comprising, in a pharmaceutically acceptable carrier, the truncated GAA polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the invention.

The invention further relates to the truncated GAA polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the invention, for use as a medicament.

The invention further provides the truncated GAA polypeptide, the nucleic acid molecule, the nucleic acid construct, the vector, or the cell of the invention, for use in a method for treating a glycogen storage disease. In a particular example, 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 example, the glycogen storage disease is selected in the group consisting of GSDI, GSDII and GSDIII, more particularly in the group consisting of GSDII and GSDIII. In an even more particular example, the glycogen storage disease is GSDII.