GENE THERAPY

Field of the invention

The present invention relates to recombinant adeno-associated virus (rAAV) particles for use in a method of treating a disease. For example, the rAAV may be used to treat a disease associated with a loss of solute carrier family 6 member 1 (SLC6A1) function such as myoclonic atonic epilepsy (MAE), MAE- like and other epilepsy indications such as Lennox-Gastaut Syndrome as well as autism spectrum disorder and schizophrenia.

Background to the Invention

To date, thousands of genes have been associated with neurodevelopmental disorders and with the aid of clinical genetic testing, syndromes are increasingly defined by the mutated gene rather than their clinical characteristics. Disruption of the gene SLC6A1 has been identified as a prominent cause of a wide range of neurodevelopmental disorders, including autism spectrum disorder (ASD), intellectual disability (ID), and seizures of varying types and severity. SLC6A1 encodes GAT-1, a member of the gamma-amino butyric acid (GABA) transporter family expressed in the central nervous system (Brder S. and Gather U. 2012. Br J Pharmacol 167: 256-278). The SLC6A1 gene was first cloned in 1990 (Guastella J. et al. 1990. Science 249: 1303-1306) and belongs to a family of 20 paralogs. The proteins encoded by 13 of these genes exhibit above 80% sequence identity to one another and six of them are able to transport GABA with different degrees of substrate specificity.

GAT-1 is expressed broadly and exclusively in the mammalian central nervous system, predominantly in the frontal cortex in the adult human brain (Gamazon E.R. et al. 2018. Nat Genet 50: 956-967). Unlike other GABA transporters, GAT-1 is almost exclusively expressed in GABAergic axon terminals and astrocytes. In the developing brain, GABA exerts an excitatory action, but later becomes the main inhibitory neurotransmitter in the central nervous system. The onset of GABAergic inhibition is important to counterbalance neuronal excitation, and when significantly disrupted, it negatively impacts brain development leading to attention and cognitive deficits as well as seizures.

The GAT-1 protein is composed of 12 transmembrane domains that come together to form a single chain transporter. The primary function of GABA transporters is to lower the concentration of GABA in the extracellular space (Scimemi A. 2014. Front Cell Neurosci 8). This task is accomplished by coupling the translocation of GABA across the cell membrane with the dissipation of the electrochemical gradient for sodium and chloride (Figure 1). By moving these ions across the membrane in fixed ratio with GABA (1 GABA: 2 Na+: 1 Cl'), GAT-1 generates a stoichiometric current (Lester H.A. et al. 1994. Annual Review of Pharmacology and Toxicology 34: 219-249). At rest, in the pre-synaptic terminal of GABAergic neurons, the driving force for sodium and chloride forces these ions to move from the extracellular space towards the cell cytoplasm, thus carrying GABA in the same direction. The translocation of GABA across the membrane is relatively rapid, allowing GABA to be removed from the extracellular space within few milliseconds after its release (Isaacson et al. 1993. Neuron 10: 165-175). In addition to regulating the transport of GABA, GAT-1 also behaves as an ion channel, and generates two ionic currents that are not stoichiometrically coupled to the movement of GABA across the membrane. The first is a sodium inward current activated by GABA binding to GAT-1 (Risse et al. 1996. J Physiol 490: 691-702). The second is a leak current that can be detected even in the absence of GABA and is mediated, in vitro, by alkali ions like lithium and caesium (MacAulay et al. 2002. J Physiol (Lend) 544: 447-458). Last, in the absence of GABA, GAT-1 generates sodium-dependent capacitive currents (Mager et al. 1993. Neuron 10: 177-188). Through the coordinated activation of these currents, GAT-1 activation can generate a local shunt (i.e. a change in membrane resistance) or membrane depolarization.

As in the case of many other neurodevelopmental disorder-associated genes, patient variants within SLC6A1 are broadly distributed along its sequence (Johannesen et al. 2018. Epilepsia 59: 389-402). Two types of variants have been observed in patients: (i) protein truncating variants that stop the protein production for one of the two SLC6A1 gene alleles inherited and (ii) missense variants in critical regions of the protein such as GABA binding sites and transmembrane domains. Thus, the expected molecular pathological mechanism of SLC6A1 disorders is a loss of function or haploinsufficiency. The disease-model is supported by experiments in both wild type and GAT-1$^{-/-}$ mice, as well as studies on recombinant GAT-1 proteins from individuals with SLC6A1 mutations. However, the

mechanisms by which the haploinsufficiency lead to the clinical manifestations are not well understood. Recently, experimental evidence showed that SLC6A1 variants identified in epilepsy patients reduce GABA transport in vitro (Mattison et al., 2018; Cai et al., 2019. Epilepsia 59: e135-e141). Other evidence suggests that SLC6A1 mutations may also cause impaired protein trafficking (Cai et al. 2019 Experimental Neurology 320: 112973).

Gene therapy has strong potential for treating neurological and neurodevelopmental disorders, and a large number of preclinical and clinical studies are currently in place for addressing this need. Adeno-associated virus (AAV) has been the predominant choice for central or peripheral nervous system-focused clinical trials. Vectors based on AAV are particularly promising gene delivery vehicles in large part because they exhibit limited immunogenicity, have a low risk of insertional mutagenesis and can mediate long-term gene expression in both dividing and non-dividing cells (Ojala D.S. et al, 2015).

WO 2022/074105 describes intracerebroventricular (ICV) administration of an AAV-based gene therapy in neonatal mice. ICV administration in neonate mice offers an approach to achieve large brain distribution of a viral vector although the spread of the vector is more limited when administered ICV in older mice. To date, intraparenchymal administration has been a commonly employed route for AAV-based gene therapy delivery to the brain (Wood et al, 2022). This approach circumvents the biological transport barrier (the so called “blood-brain barrier”) and further reduces the risk of vector neutralisation by circulating antibodies. To date, intraparenchymal administration has been found to have significant drawbacks. Poor vector spread limits transgene expression to the vicinity of the administration site, a major shortcoming for diseases that affect multiple regions of the central nervous system.

WO 2022/074105 describes an AAV-based gene therapy for use in the treatment and/or prevention of disease associated with a loss of solute carrier family 6 member 1 (SLC6A1) function.

There remains a need for identifying the most effective route of administration for AAV-based gene therapies for neurological and neurodevelopmental disorders, particularly where a distribution across multiple key brain areas is beneficial for therapeutic efficacy. In particular, there remains a need to identify the most effective route of administration for the

treatment of disease associated with a loss of solute carrier family 6 member 1 (SLC6A1) function.

Summary of the invention

The present invention relates to novel routes of administration for an AAV-based gene therapy for the treatment of a disease, such as a genetic disorder associated with impaired GABA uptake or a disease characterised by SLC6A1 haploinsufficiency. The invention is based on the surprising finding that recombinant adeno-associated virus (rAAV) particles comprising AAV capsids were able, when administered to the thalamus or striatum, to spread to key brain areas relevant for the control of the disease symptoms and promote expression of the SLC6A1 transgene comprised in said particle throughout the brain (and in particular in the thalamus and frontal cortex) of a subject. The inventors have also unexpectedly found that the administration of rAAV particles comprising an SLC6A1 transgene to the thalamus or striatum results in a decrease in seizures in a mouse model of disease associated with a loss of solute carrier family 6 member 1 (SLC6A1) function.

In a first aspect, the present invention provides a method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn;

Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

In a second aspect, the present invention provides a recombinant adeno-associated virus (rAAV) particle for use in a method of treating a disease, the method comprising administering the rAAV particle to a subject, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

In a third aspect, the present invention provides a pharmaceutical composition comprising a recombinant adeno-associated virus (rAAV) particle and one or more carriers and/or excipients for use in a method of treating a disease, the method comprising administering the rAAV particle to a subject in need thereof, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the pharmaceutical composition to the thalamus of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

In a fourth aspect, the present invention provides a method of delivering a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle to the central nervous system of a subject, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus of the subject, and wherein the transgene encodes a polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile;

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

In a fifth aspect, the present invention provides a method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid comprising or consisting of the amino acid sequence of SEQ ID NO: 21; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) comprising or consisting of the nucleic acid sequence of SEQ ID NO: 9 and a 3' ITR comprising or consisting of the nucleic acid sequence of SEQ ID NO: 10;

ii) a gamma butyric acid (GABA) transporter protein 1 (GAT-1), wherein the GAT-1 comprises or consists of the amino acid sequence of SEQ ID NO: 1; and

iii) an endogenous hSCL6A1 promoter comprising or consisting of the nucleic acid sequence of SEQ ID NO: 20, wherein the endogenous hSCL6A1 promoter controls expression of the transgene;

wherein the method comprises administering the rAAV particle to the thalamus of the subject.

Description of the Figures

Figure 1: Cartoon illustrating the SLC6A1 encoded GAT-1 transporter and its function. GAT- 1 is a solute carrier protein which regulates the uptake of extracellular GABA. Stoichiometry of GAT-1: one molecule of inhibitory neurotransmitter GABA is co-transported together with two sodium cations and one chloride anion along the electrochemical gradient.

Figure 2: One representative picture is shown per condition for the AAV9-HA vector. Intrastriatal and intra-thalamic administration resulted in the highest brain coverage and highest HA signal intensity. ICV: intracerebroventricular, STR: striatal, Ect: Ectorhinal cortex, TH: thalamus, TH+CTX: thalamus and frontal cortex.

Figure 3: Comparison of the brain distribution of the HA-tag between AAV9 and AAVTT -HA administered through intrastriatal delivery.

Figure 4: HA-hGAT1 levels in the thalamus and cortex regions from all dose groups and compared side by side from the same dose group in AAV9 or AAVTT.

Figure 5: Viral genome copies (A) and human slc6a1 mRNA levels (B) in the cortex from all dose groups and compared side by side from the same dose group in AAV9 or AAVTT.

Figure 6: Levels of mGAT1 in the cortex and the thalamus in the vehicle vs AAV9-ENDO-hSLC6A1-HA treated groups.

Figure 7: (A) Average number of SWDs in SLC6A1+/S295L mice injected with vehicle-PBS (n=17), AAV9 ENDO-HA-HSLC6A1 (n=19), AAVTT ENDO-HA-HSLC6A1 (n=17). SWDs were analyzed 6 weeks after injection over a period of 5 hours between 1pm and 6pm for 7 consecutive days. The difference between groups was analyzed using a negative binomial regression model on the cumulative number of seizures across the 7-day recording period. (***$p<0.001$). (B) Time course of SWD reduction at 1, 3 and 6 weeks after AAV9-ENDO-hSLC6A1-HA injection.

Figure 8: Comparison of the brain distribution of the HA-tag between AAV9 and AAVTT-Endo-hSLC6A1-HA administered through intra-thalamic delivery. On left, HA-tag stained brain sections from 2 representative animals are shown per condition (AAV9 and AAVTT-Endo-hSLC6A1-HA). On the right, the graph illustrates the quantification of the HA immunoreactive signal, indicating that AAVTT led to a larger rostrocaudal brain coverage compared to AAV9.

Figure 9: Vector genome copies in brain tissue by region**.** Vector genomes (vg) were detected across all brain regions. The highest vector genome copies (vgc) were detected at the injection site, in the thalamus, (1E8vg/µg *g*DNA). The vgc across all other brain areas ranged from 1E5 to 5E6vg/µg *g*DNA with values in the prefrontal, temporal cortex, and other cortical areas of approx. 3E6vg/µg *g*DNA. In the figure, ug is equivalent to µg. 1001 and 1002 refer to two separate NHP subjects dosed with AAV vectors.

Figure 10: Vector genome copies in spinal cord and DRG regions. Vector genome copy numbers were detected in the tissues from spinal cord and DRG (A) and peripheral tissues (B)

after necropsy. In peripheral tissues only spleen showed a low detectable vgc count (1E4vg/µg *g*DNA), in all other tissues (heart, liver and testes) the counts were below the limit of quantification. Vgc were determined in blood and CSF at day 4 after dose and at day 28. At day 4 the CSF vgc count (1.45 & 2.27E7vg copies/ml CSF) were higher than blood (1.5 & 7.7E5vg copies/ml blood), and the vgc for both biofluids were below the limit of quantification at day 28, the terminal collection timepoint (C).

Figure 11: Immunohistochemistry analysis for mCherry protein following intra-thalamic administration of AAVTT. The mCherry reporter transgene expression determined by immunohistochemistry showed efficient transduction of cells and expression of the mCherry protein in the thalamic parenchyma, as well as across brain areas with thalamic projections such as the dorsal cerebral cortex, basal ganglia nuclei, caudate, deep cerebellar nuclei (DCN), and specific nuclei of the midbrain, pons, and medulla.

Figure 12: Cortical mCherry transgene expression - level 1, 2 and 3. AAV vector comprising an mCherry transgene was administered to the thalamus of non-human primates and expression measured by immunohistochemistry. In the cerebral cortex mCherry-expressing cells were predominantly localized in the dorsal areas across the anteroposterior extent of the cortex.

Figure 13: mCherry cortical layer expression. Following administration of AAV vector comprising an mCherry transgene to the thalamus of non-human primates mCherry signal was observed in deep cortical layers, V and VI layers, as anticipated from corticothalamic connections.

Figure 14: mCherry protein expression on thalamic projections following intra-thalamic administration of AAVTT. Immunohistochemical analysis is shown for the primary motor cortex, ventral anterior thalamic nucleus and reticular thalamus, caudate nucleus, and globus pallidus.

Figure 15: Quantification of % transduced cells following intra-thalamic injection (whole sections across the brain). Quantification of percentage of cells transduced (mCherry positive cells number over total cell number) along the rostro-caudal axis of the brain indicated that

the highest percentage of cell transduction was observed in the thalamus, close to the injection site, with values ranging from 7 to 13% of the cells transduced, as expected by the high vg copy number determined in the area.

Figure 16: Quantification of transduced cells (%) in the different brain regions (left) and in dorsal and ventral cortex (right). The highest percentage of transfected cells (40%) was seen in the thalamus, with between 0 and 5% transfected cells across many brain regions. A higher percentage of transfected cells was seen in the dorsal cortex compared to the ventral cortex.

Figure 17: Average number of SWDs in SLC6A1$^{+/S295L}$ mice injected with vehicle-PBS, AAVTT-HA-HSLC6A1 MAX dose, AAVTT-HA-HSLC6A1 MID dose, AAVTT-HA-HSLC6A1 LOW dose and AAVTT-HSLC6A1 MAX dose. SWDs were analyzed 6 weeks after injection over a period of 24 hours for 7 consecutive days. HA=hemagglutinin; mid=middle; SWD=spike-wave-discharge.

Figure 18: Analysis of SWD reductions in SLC6A1+/S295L mice at 6 weeks post treatment with AAVTT-HA-SLC6A1 and AAVTT-SLC6A1. CI=confidence interval; HA=hemagglutinin; mid=middle; SWD=spike-wave-discharge. Note: The SWD data were analyzed using a generalized linear model with a negative binomial distribution for the errors and a log link function. Low dose=5.28e9vg, mid dose =1.58e10vg, high dose=2.64e10vg.

Figure 19: Viral vector genome copy number and human slc6a1 mRNA levels in the isocortex from mice injected with vehicle-PBS, AAVTT ENDO-HA-HSLC6A1 MAX dose, AAVTT-ENDO-HA-HSCL6A1 MID dose, AAVTT ENDO-HA-HSLC6A1 LOW dose and AAVTT-ENDO-HSCL6A1 non-tagged MAX dose in the POM5 study cohorts A.

Figure 20: Levels of HA-hGAT1 protein in the isocortex and thalamus samples from animals injected with AAVTT-HA-hslc6a1 MAX dose, AAVTT-HA-hslc6a1 MID dose, AAVTT-HA-hslc6a1 LOW dose in the POM5 study cohorts A and B. HA-GAT1 ng/ 100ug total protein is being analysed using an analysis of variance (ANOVA) model suitable for a randomised block design, fitting Group & Batch as fixed effects.The analysis is performed on the log transformed data.

Detailed Description

General Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by a person skilled in the art to which this invention belongs.

In general, the term "*comprising*" is intended to mean including but not limited to. For example, the phrase "*a viral particle comprising a capsid*" should be interpreted to mean that the viral particle has a capsid, but the viral particle may comprise further elements. In some embodiments of the invention, the word "*comprising*" may be replaced with the phrase "*consisting of*". The term "*consisting of*" is intended to be limiting. For example, the phrase "*a viral particle consisting of a capsid and a viral genome*" should be interpreted to mean that the viral particle has a capsid and a viral genome and contains no further components.

In some embodiments of the invention, the word "*comprising*" may be replaced with the phrase "*consisting essentially of*". The term "*consisting essentially of*" means that specific further components can be present, namely those not materially affecting the essential characteristics of the subject matter.

The singular forms "*a*", "*an*", and "*the*" include plural referents unless the context clear dictates otherwise. Thus, for example, reference to "*an amino acid*" includes two or more instances or versions of such amino acids.

The term "*fragment*" as used herein refers to a contiguous portion of a reference sequence. For example, a fragment of a GAT-1 polypeptide may refer to at least 100, at least 200, at least 300, at least 400, or at least 500 contiguous amino acids of said GAT-1 polypeptide.

The term "*variant*" as used herein refers to a nucleic acid or amino acid sequence which is modified (e.g. has one or more substitutions, deletions or insertions) relative to a reference

sequence. For example, a GAT-1 variant may refer to an amino acid sequence having one or more mutations compared to the amino acid sequence of SEQ ID NO: 1. A variant of a GAT-1 polypeptide may comprise conservative mutations which do not significantly impact the activity of the polypeptide.

For the purpose of this invention, in order to determine the percent identity of two sequences (such as two polynucleotide or two polypeptide sequences), the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in a first sequence for optimal alignment with a second sequence). The nucleotide or amino acid residues at each position are then compared. When a position in the first sequence is occupied by the same amino acid as the corresponding position in the second sequence, then the amino acids are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical positions /total number of positions in the reference sequence x 100).

Typically, the sequence comparison is carried out over the length of the reference sequence. For example, if the user wished to determine whether a given ("test") sequence has at least 80% identity to SEQ ID NO: 1, SEQ ID NO: 1 would be the reference sequence. To assess whether a sequence has at least 80% identity to SEQ ID NO: 1 (an example of a reference sequence), the skilled person would carry out an alignment over the length of SEQ ID NO: 1, and identify how many positions in the test sequence were identical to those of SEQ ID NO: 1. If at least 80% of the positions are identical, the test sequence is at least 80% identical to SEQ ID NO: 1. If the sequence is shorter than SEQ ID NO: 1, the gaps or missing positions should be considered to be non-identical positions.

To assess whether a sequence is at least 80% identical to a fragment of 450 amino acids of SEQ ID NO:1, the skilled person would align SEQ ID NO: 1 to the test sequence, and determine which 450 amino acids of SEQ ID NO: 1 best align to the test sequence. The skilled person would then determine the number of positions in the test sequence that are identical to the 450 amino acids of SEQ ID NO: 1 which best align to the test sequence, and calculate the percentage identity as indicated above.

The skilled person is aware of different computer programs that are available to perform an alignment between two sequences. An alignment between two sequences can be accomplished using a mathematical algorithm. For example, an alignment may be performed using the Needleman and Wunsch algorithm (Needleman and Wunsch, 1970, J Mol Biol.;48(3):443- 53) which aligns the sequences optimally over the entire length). Sequences of substantially different lengths may alternatively be aligned using a local alignment algorithm (e.g.15 Smith and Waterman algorithm (Smith and Waterman, 1981, J Theor Biol. ;91 (2):379-80) or Altschul algorithm (Altschul SF et al., 1997, Nucleic Acids Res.;25(17):3389-402,; Altschul SF et al., 2005, Bioinformatics.;21(8):1451-6). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

A method of treating a disease in a subject

The present invention relates to a method of treating a disease in a subject. In some embodiments the disease is a genetic disorder associated with impaired GABA uptake. In some embodiments, the disease is a disease associated with a loss of solute carrier family 6 member 1 (SLC6A1) function. In some embodiments, the disease comprises a single-gene epilepsy accompanied by cognitive, motor behavioural comorbidities, early onset developmental and epileptic encephalopathy, epileptic encephalopathy, childhood onset Epilepsy Syndromes, myoclonic atonic epilepsy (MAE), an MEA-like indication or another epilepsy indication, optionally wherein the other epilepsy indication is Lennox Gastaut Syndrome, autism spectrum disorder and/or schizophrenia.

The terms "*treatment*", "*treating*" and the like refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptoms thereof from appearing or worsening and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease.

In some embodiments, the disease is characterised by SLC6A1 haploinsufficiency.

In some embodiments, the disease is associated with at least one mutation in a patient which leads to a pathological GAT-1 variant. In some embodiments, the pathological GAT-1 variant comprises one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from R44W, R44Q, R50L, D52E, D52V, F53S, S56F, G63S, N66D, G75R, G79R, G79V, F92S, G94E, G105S, Q106R, G112V, Y140C, C173Y, G232V, F270S, R277H, A288V, S295L, G297R, A305T, G307R, V323I, A334P, A367T, V342M, A357V, G362R, L366V, F385L, G393S, S456R, S459R, M487T, V511L, and G550R.

In some embodiments of the invention, treatment of a disease may comprise a reduction in seizures, for example a decrease in the severity or number of seizures. Desired therapeutic results include a significant reduction in frequency or duration of different seizure types, for example atonic seizures (drop attacks), myoclonic seizures, generalised seizures, partial seizures, febrile seizures or infantile spasms, a significant achievement of sustained seizure freedom, and a significant impact on the progression of neurodevelopmental symptoms such as developmental delay, intellectual disability, language impairment, cognitive impairment, involuntary movements, gait disturbance, or autistic features.

In some embodiments, the rAAV particle is able to reduce seizures after intra-thalamic or intra-striatal delivery, for example in mice. In some embodiments, the rAAV particle is able to reduce seizures by at least 10%, at least 20% or at least 40%. Whether or not an rAAV particle is able to reduce seizures may be determined by administering a dose of 1µl of a 1.35E13 gc/ml dose of the rAAV into each hemisphere intra-thalamically or intra-striatally into STXBP1 knock out mice, measuring the number of spike wave discharges (SWDs) by EEG 16 weeks after administration, and comparing this to the number of SWDs in mice which had not received the rAAV particle.

Without wishing to be bound by theory, the present inventors predicted that major disease symptoms (such as seizures) caused by genetic disorders associated with impaired GABA uptake originated in the thalamus and cortical areas. Surprisingly, it was found that rAAV particles comprising an SLC6A1 transgene delivered to the thalamus or striatum of a subject

were able to transduce cells throughout broader brain regions and promote increased expression of GAT-1. In particular, high levels of expression of the transgene product are seen in the striatum, thalamus, hippocampus, isocortex and frontal cortex when administering rAAV to either the thalamus or striatum. The inventors also surprisingly found that administration of rAAV particles comprising an SLC6A1 transgene to the thalamus or striatum of a mouse model of a genetic disorder associated with impaired GABA uptake led to a decrease in disease symptoms including seizures.

In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 10%. In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 20%. In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 30%. In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 50%. In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 70%. In some embodiments, administration of an rAAV particle of the invention to a subject leads to a decrease in the number of seizures by at least 90%.

In some embodiments, the subject is a mammal. In some embodiments, the subject is a primate. In a preferred embodiment, the subject is a human.

Without wishing to be bound by theory, it is believed that, while ICV is a suitable route of administration for neonatal animals, it results in poor brain coverage in juvenile animals, as shown by the inventors in the present application. In some embodiments, the subject is not a neonatal subject. In some embodiments, the subject is a juvenile. In some embodiments, the subject is at a post symptomatic age, i.e. the subject is treated after symptoms have appeared. In some embodiments, the subject has already been treated with anti-epileptic drugs or other neuromodulatory treatments.

The SLC6A1 gene therapy described herein may be administered in combination with anti-epileptic drugs or other neuromodulatory treatments.

The terms "*therapeutic amount*" or "*therapeutically effective amount*" typically refer to the amount or the dose of a compound that is sufficient to exhibit a positive pharmacologic and/or physiologic effect on a disease and therefore to treat a disease, upon administration to a subject. For example, a therapeutic amount or therapeutically effective amount of rAAV particle may refer to an amount of rAAV particles which is sufficient to cause a decrease in the number of seizures in a subject suffering from an SLC6A1 genetic disorder associated with early onset developmental and epileptic encephalopathy as described above.

Recombinant adeno-associated viral particle

The term "*viral particle*" relates to a typically replication-defective virus particle comprising (i) at least a portion of a viral genome (ii) a capsid and optionally, (iii) a lipidic envelope surrounding the capsid. The term "*viral particle*" includes recombinant adeno-associated viral (AAV) particles.

The term "*viral genome*" refers to the nucleic acid part of the viral particle disclosed herein, which may be packaged in a capsid.

In the present invention, the viral particle is a recombinant adeno-associated viral particle (rAAV). The terms rAAV and AAV are used interchangeably herein, unless the context indicates otherwise.

The genomic organization of all known AAV serotypes is very similar. The genome of AAV (*i.e.* the vector genome) is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VP1, -2 and -3) form the capsid. The terminal 145 nt are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild type (wt) AAV infection in mammalian cells the Rep genes (*i.e.* encoding Rep78 and Rep52 proteins) are expressed from the P5 promoter and the P19 promoter, respectively, and both Rep proteins have a function in the replication of the vector genome. A

splicing event in the Rep ORF results in the expression of four Rep proteins (i.e. Rep78, Rep68, Rep52 and Rep40). However, it has been shown that the unspliced mRNA, encoding Rep78 and Rep52 proteins, in mammalian cells are sufficient for AAV vector production. Also in insect cells the Rep78 and Rep52 proteins suffice for AAV vector production.

AAV capsid

AAV capsids are generally formed from three proteins, VP1, VP2 and VP3. The amino acid sequence of VP1 comprises the sequence of VP2. The amino acid sequence of VP2 comprises the sequence of VP3. Differences among the capsid protein sequences of the various AAV serotypes result in the use of different cell surface receptors for cell entry. In combination with alternative intracellular processing pathways, this gives rise to distinct tissue tropisms for each AAV serotype.

In some embodiments of the invention, the AAV capsid is an AAV2, AAV5, AAV6, AAV8, AAV9, AAV10 or AAVTT capsid. In particular, the AAV capsid may be an AAV9 capsid. In particular, the AAV capsid may be an AAVTT capsid.

The terms "*AAV true type*", "*AAVTT*", "*AAV-TT*" and "*AAVtt*" are used interchangeably herein and refer to a capsid as defined in WO2015/121501 and Tordo J. et al., 2018, both incorporated herein by reference. The amino acid sequence of the AAVTT capsid is given in SEQ ID NO: 21. SEQ ID NO: 21 represents the amino acid sequence of the AAVTT VP1 protein, and comprises within it the amino acid sequences of the AAVTT VP2 and VP3 proteins. For the purposes of the present invention, the rAAV particle will be considered to comprise an AAVTT capsid if it comprises at least an AAVTT VP3, VP2 or VP1 protein. Optionally, the rAAV particle will be considered to comprise an AAVTT capsid if it comprises at least an AAVTT VP1 protein. Optionally, the rAAV particle will be considered to comprise an AAVTT capsid if it comprises an AAVTT VP1 protein, an AAVTT VP2 protein and an AAVTT VP3 protein.

In one embodiment, an AAVTT VP1 capsid protein comprises at least one amino acid substitution with respect to the wild-type AAV2 VP1 capsid protein at a position corresponding to one or more of the following positions in an AAV2 protein sequence (NCBI

reference sequence: YP_680426.1): 125, 151, 162, 205, 312, 457, 492, 499, 533, 546, 548, 585, 588 and/or 593.

In one embodiment, an AAVTT VP1 capsid protein comprises one or more of the following amino acid substitutions with respect to a wild-type AAV2 VP1 capsid protein (NCBI Reference sequence: YP_680426.1): V125I, V151A, A162S, T205S, N312S, Q457M, S492A, E499D, F533Y, G546D, E548G, R585S, R588T and/or A593S.

In one embodiment, an AAVTT VP1 capsid protein comprises four or more mutations with respect to the wild type AAV2 VP1 capsid protein at the positions 457, 492, 499 and 533.

In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 96% identity to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 97% identity to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 98% identity to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 99% identity to the amino acid sequence of SEQ ID NO: 21.

An exemplary sequence of an AAV9 capsid is given in SEQ ID NO: 22. SEQ ID NO: 22 represents the amino acid sequence of the AAV9 VP1 protein, and comprises within it the amino acid sequences of the AAV9 VP2 and VP3 proteins. For the purposes of the present invention, the rAAV particle will be considered to comprise an AAV9 capsid if it comprises at least an AAV9 VP3, VP2 or VP1 protein. Optionally, the rAAV particle will be considered to comprise an AAV9 capsid if it comprises at least an AAV9 VP1 protein. Optionally, the rAAV particle will be considered to comprise an AAV9 capsid if it comprises an AAV9 VP1 protein, an AAV9 VP2 protein and an AAV9 VP3 protein.

In a preferred embodiment, the AAV capsid comprises or consists of the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 96% identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 97% identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 98% identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the AAV capsid comprises or consists of an amino acid sequence having at least 99% identity to the amino acid sequence of SEQ ID NO: 22.

In a preferred embodiment, the AAV capsid comprises or consists of the amino acid sequence of SEQ ID NO: 22.

Viral genome

The term "*viral genome*" refers to the nucleic acid sequence packaged inside an rAAV capsid which forms an rAAV particle. Such a viral genome contains at least one AAV inverted terminal repeat sequences (ITRs). In the embodiments of the invention described herein, a viral genome contains, at a minimum, a transgene (i.e. a gene different from the gene encoding viral proteins), and at least one AAV ITR. A viral genome may also comprise one or more regulatory sequences that direct, assist and/or control expression of said transgene.

Inverted terminal repeats (ITRs)

The term "*inverted terminal repeat*" (ITR) refers to a nucleotide sequence located at the 5'-end (5'ITR) and a nucleotide sequence located at the 3'end (3'ITR) of a viral genome. An ITR comprises palindromic sequences and can fold over to form T-shaped hairpin structures that function as primers during initiation of DNA replication. ITRs also play a role in viral genome integration into the host genome, rescue from the host genome, and encapsidation of the viral genome into mature virions. The ITRs are required in cis for the vector genome replication and its packaging into the viral particles.

In one embodiment of the invention, the viral genome comprises at least one ITR. In some embodiments of the invention, the ITRs are AAV2 ITRs.

In some embodiments, the 5' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 9. In other embodiments, the 5' ITR comprises or consists of a nucleic acid sequence having at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 9.

In some embodiments, the 3' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 10. In other embodiments, the 3' ITR comprises or consists of a nucleic acid sequence having at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 10.

In one embodiment, the 5' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 9 and the 3' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 10.

Transgene

The term "*transgene*" refers to the nucleic acid sequence (typically encoding a protein) to be expressed in a subject once administered to the subject via the rAAV particles according to the invention, wherein said sequence is not an AAV-derived sequence. The transgene is typically of the same origin as the subject to be treated with the rAAV particle. The term "*a transgene*" should be construed as comprising one or more transgenes.

In one embodiment of the present invention, the transgene encodes a GAT-1 polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1), for example a polypeptide comprising an amino acid sequence of SEQ ID NO: 1-3;

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of

Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

In one embodiment, the transgene comprises or consists of a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the nucleic acid sequence of any one of SEQ ID NO: 4-8.

In a further embodiment, the transgene comprises or consists of the nucleic acid sequence of any one of SEQ ID NO: 4-8. In a preferred embodiment, the transgene comprises or consists of the nucleic acid sequence of SEQ ID NO: 4. In another preferred embodiment, the transgene comprises or consists of SEQ ID NO: 5.

Regulatory sequences

The term "*regulatory sequence*" refers to one or more nucleic acid sequences that direct and/or are involved in the expression of a gene (herein of the transgene). Typically, said one or more regulatory sequences are specific, meaning that they are selected to drive, assist and/or control the expression of the transgene in a target tissue, e.g. central nervous system.

In some embodiments, the one or more regulatory sequences are selected from:
a) one or more transcription initiation sequences (such as a promoter),
b) one or more translation initiation sequences,
c) one or more mRNA stability sequences,
d) one or more polyadenylation sequences,
e) one or more secretory sequences,
f) one or more enhancer sequences,
g) one or more introns,
h) one or more TATA boxes,
i) one or more microRNA targeted sequences,
j) one or more polylinker sequences facilitating the insertion of a DNA fragment within a vector,
k) one or more splicing signal sequences,
l) one or more transcription termination sequences (such as polyadenylation sequences), or
m) a combination thereof.

As used herein, the term "*promoter*" refers to a regulatory element that directs the transcription of a transgene to which it is operably linked. A promoter can regulate both rate and efficiency of transcription of an operably linked transgene. A promoter may also be operably linked to other regulatory sequences which enhance ("*enhancer sequences*") or repress ("*repressor sequences*") promoter-dependent transcription of a transgene. An enhancer sequence may be an intron. These regulatory sequences may also include, without limitations, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter, including e.g. attenuators, enhancer sequences, and silencers. The promoter is generally located near the transcription start site of the transgene to which it is operably linked, on the same strand and upstream of the DNA sequence (towards the 5' region of the sense strand). As used herein, the term "*operably linked*" refers to a

linkage of elements in a functional relationship. A transgene is "*operably linked*" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or transcription regulatory sequence is operably linked to a transgene if it affects the transcription of the transgene. Optionally, the promoter or transcription regulatory sequence is 5' of the transgene. Optionally, the promoter is immediately 5' of the transgene, or it is separated from the transgene by another sequence such as an intron.

Preferably, the one or more regulatory sequences according to the invention comprised in the viral genome are specifically selected to drive the expression of the transgene in the central nervous system (CNS). The one or more regulatory sequences may comprise a neuronal promoter. Accordingly, the viral genome may comprise any regulatory sequences such as any of those listed above, either alone or in any combinations of two or more sequences, any combinations of three or more sequences, any combinations of four or more sequences, any combinations of five or more sequences and so forth. In another embodiment, the one or more regulatory sequences comprise or consist of a promoter and a transcription termination sequence. In a further embodiment, the one or more regulatory sequences comprise or consist of a promoter, an enhancer (such as one or more introns) and a polyadenylation site. Generally, the viral genome comprises regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence that are required for expression of the transgene. Thus, in specific embodiments, the viral genome comprises at least (i) a transgene under the control of (ii) a promoter and (iii) a 3' untranslated region that usually contains a polyadenylation sequence/site and/or transcription termination sequence.

In one embodiment, the promoter is a CAG 1.6kb promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 11 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 11. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 11. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 11.

In a further embodiment, the promoter is a UbC promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 12 or a nucleic acid sequence having at

least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 12. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 12. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 12.

In a further embodiment, the promoter is a PGK promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 13 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 13. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 13. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 13.

In a further embodiment, the promoter is a EF1a promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 14 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 14. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 14. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 14.

In a further embodiment, the promoter is a MECP2 promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 15 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 15. Optionally, the promoter comprises anucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 15. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 15.

In a further embodiment, the promoter is a hNSE promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 16 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid

sequence of SEQ ID NO: 16. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 16. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 16.

In a further embodiment, the promoter is a hSYN promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 17 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 17. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 17. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 17.

In a further embodiment, the promoter is a CamKII promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 18 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 18. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 18. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 18.

In a further embodiment, the promoter is a hDLX promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 19 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 19. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 19. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 19.

In a preferred embodiment, the hDLX promoter is operably linked in a 5' to 3' orientation to an intron. In some embodiments, the intron comprises or consists of a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to the

nucleic acid sequence of SEQ ID NO: 25 or 26. In a preferred embodiment, the intron comprises or consists of SEQ ID NO: 25 or 26.

In a further embodiment, the promoter is a hSLC6A1 promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 20 or a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 20. Optionally, the promoter comprises a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 20. Optionally, the promoter comprises a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 20.

In some embodiments, the MECP2 promoter is operably linked in a 5' to 3' orientation to a MECP2 intron. In some embodiments, the MECP2 intron is a natural intron taken from a naturally occurring stretch of the MECP2 gene. In a preferred embodiment, the MECP2 intron is a synthetic intron, constructed by combining disparate sequences derived from the MECP2 gene.

In some embodiments, the MECP2 intron comprises or consists of a nucleic acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 24. In a preferred embodiment, the MECP2 intron comprises or consists of the nucleic acid sequence of SEQ ID NO: 24.

It has been found that promoters derived from the endogenous SLC6A1 promoter (hSLC6A1) and the hDLX promoter are highly effective for driving CNS targeted expression of transgenes in a gene therapy setting. Such promoters have been observed to provide higher SLC6A1 expression and transduction efficiency than equivalent promoters comprising alternative CNS specific promoters.

Polyadenylation sequence

As used herein, the term "*polyadenylation sequence*" (or polyA sequence) refers to a specific recognition sequence within the 3' untranslated region (3' UTR) of the gene, which is

transcribed into a precursor mRNA molecule and guides the termination of gene transcription. The polyA sequence acts as a signal for the endonucleolytic cleavage of the newly formed precursor mRNA at its 3'-end, and for the addition of a polyA tail to this 3'-end. This is known as polyadenylation. A polyA tail is an RNA stretch consisting only of adenine bases. The polyA tail is important for nuclear export, translation, and stability of mRNA. In the context of the invention, the polyadenylation sequence is a recognition sequence that can direct polyadenylation of mammalian genes and/or viral genes, in mammalian cells. In a further embodiment, the polyadenylation sequence comprises or consists of the nucleic acid sequence of SEQ ID NO: 23. In some embodiments, the polyadenylation sequence comprises or consists of a sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 23.

Administration of rAAV

rAAV particles may be administered by injection into the brain of the subject. In the present invention, the rAAV particles are administered to the thalamus or striatum of the subject.

The thalamus is a grey matter structure located near the centre of the brain. The thalamus is connected to the cerebral cortex in all directions. The thalamus is made up of a series of nuclei which are responsible for the relay of the different sensory signals.

Specifically, these relay nuclei can be divided into lateral, medial and anterior nuclei. In particular, the lateral nuclei include the ventral posterolateral nucleus, ventral posteromedial nucleus, lateral geniculate nucleus, medial geniculate nucleus, ventral lateral nucleus, ventral anterior nucleus, pulvinar, lateral dorsal nucleus, lateral posterior nucleus, ventral medial nucleus. The medial nuclei include the mediodorsal nucleus and ventro medial nucleus. The anterior thalamic nuclei include the anterior nucleus. In particular, the anterior thalamic nuclei, ventromedial and ventral anterior nuclei have many connections to the frontal cortex.

In some embodiments of the present invention, the rAAV particle is administered to the thalamus. In some embodiments of the present invention, the rAAV particle is administered to an anterior thalamic nucleus, ventromedial nucleus or ventral anterior nucleus.

The striatum is a cluster of neurons in the subcortical basal ganglia of the forebrain. In primates, the striatum is divided into the ventral striatum, consisting of the nucleus accumbens and the olfactory tubercle, and the dorsal striatum, consisting of the caudate nucleus and the putamen. The striatum is thought to play a role in regulating voluntary movement.

The putamen is connected to the substantia nigra, the globus pallidus, the claustrum, and the thalamus, as well as many regions of the cerebral cortex.

In some embodiments of the present invention, the rAAV particle is administered to the striatum. In some embodiments of the present invention, the rAAV particle is administered to the dorsal striatum. In an embodiment, the rAAV particle is administered to the putamen. In some embodiments, the rAAV particle is not administered via intracerebroventricular administration.

Advantageously, when the rAAV particle is administered to the thalamus or striatum, it is able to spread to the cortical and subcortical regions of the brain after administration. The examples of the present application demonstrate that intra-thalamic and intra-striatal administration of rAAV particles lead to a broad distribution of the SLC6A1 transgene product in different brain areas including major areas of the cortex and mid brain regions.

Administration of rAAV particles to the thalamus or striatum of a subject advantageously leads to an increase in SLC6A1 expression throughout the brain compared to other routes of administration, e.g. intracerebroventricular delivery. In particular, administration of rAAV particles to the thalamus or striatum results in an increase in SLC6A1 expression in the frontal cortex compared to other routes of administration.

Without wishing to be bound by theory, the inventors believe that the numerous connections between the thalamus, striatum, and cortex allow for the AAV to spread from the thalamus or striatum to the cortex in order to promote expression of SLC6A1.

Dosage

A suitable dosage (i.e. therapeutic amount) of the rAAV particle according to the present invention may be determined by a skilled practitioner. The selected dose will depend upon a variety of pharmacokinetic factors including the size of the brain, time of administration, the rate of spreading of the rAAV particle, the rate of expression of the transgene, the frequency of administration, the optional presence of other drugs, compounds and/or materials used in combination with the particular rAAV particles, the age, sex, weight, condition, general health and prior medical history of the patient being treated.

A suitable total dose of rAAV particles to be administered according to the present invention as a whole is at least $1x10^6$ vg of rAAV particles. Preferably, a suitable total dose of rAAV particles to be administered according to the present invention as a whole is in the range of about $1x10^6$ to about $1x10^{20}$, in the range of about $1x10^8$ to about $1x10^{18}$, in the range of about $1x10^{10}$ to about $1x10^{16}$, or in the range of about $1x10^{12}$ to about $1x10^{14}$ vg of rAAV particles. A total dose should be considered to be the sum total of rAAV particles administered over a defined period, e.g. over 6 weeks or less, 4 weeks or less, 2 weeks or less or 1 week or less.

In the context of the invention as a whole, the total dose of rAAV particles can be administered to the subject either as one single dose or as a multidose (e.g. two administrations or more, three administrations or more, four administrations or more, six administrations or more and the like). When a multidose is administered, they can be administered simultaneously or sequentially. They can target a particular area of the brain in one hemisphere only or in each of the two hemispheres. They are preferably administered to the thalamus, in particular a thalamic nucleus, in each of the two hemispheres to allow a homogeneous distribution of the particles and/or homogeneous expression of the transgene comprised in the particles. When particles are administered in the two hemispheres, each one of the hemispheres may receive one or more doses. When the thalamus, particularly a thalamic nucleus, of each hemisphere receives more than one dose, the doses are typically administered at different but yet still intra-thalamic administration sites (such as two administration sites within the thalamus if two doses are administered, three administration sites if three doses are administered, four administration sites if four doses are administered) to improve even more the homogeneous distribution of the particles/protein to be expressed by the transgene incorporated in the particle). For example, for a total dose of rAAV particles to be administered, two equal doses of rAAV particles can be administered each in

the thalamus, and in particular a thalamic nucleus, of the right hemisphere and in the thalamus, and in particular a thalamic nucleus, of the left hemisphere, so that equal doses equivalent to half of the total dose is administered per each hemisphere. Alternatively, the total dose can be split as desired across the thalamus, and in particular a thalamic nucleus, of each hemisphere.

Doses may also be administered bilaterally to the striatum, in particular the putamen, of the subject. When particles are administered in the two hemispheres, each one of the hemispheres may receive one or more doses. When the striatum, in particular the putamen, of each hemisphere receives more than one dose, the doses are typically administered at different but yet still intra-striatal administration sites (such as two administration sites within the striatum if two doses are administered, three administration sites if three doses are administered, four administration sites if four doses are administered) to improve even more the homogeneous distribution of the particles/protein to be expressed by the transgene incorporated in the particle). For example, for a total dose of rAAV particles to be administered, two equal doses of rAAV particles can be administered each in the striatum, in particular the putamen, of the right hemisphere and in the striatum, and in particular the putamen, of the left hemisphere, so that equal doses equivalent to half of the total dose is administered per each hemisphere. Alternatively, the total dose can be split as desired across the striatum, and in particular the putamen, of each hemisphere.

Solute carrier family 6 member 1 (SLC6A1)

SLC6A1 encodes GAT-1, a member of the gamma-amino butyric acid (GABA) transporter family expressed in the central nervous system (Brder S. and Gather U. 2012. Br J Pharmacol 167: 256-278). The SLC6A1 gene was first cloned in 1990 (Guastella J. et al. 1990. Science 249: 1303-1306) and belongs to a family of 20 paralogs. The proteins encoded by 13 of these genes exhibit above 80% sequence identity and six of them are able to transport GABA with different degrees of substrate specificity.

The GAT-1 protein is composed by 12 transmembrane domains that come together to form a single chain transporter. The primary function of GABA transporters is to lower the

concentration of GABA in the extracellular space (Scimemi A. 2014. Front Cell Neurosci 8). This task is accomplished by coupling the translocation of GABA across the cell membrane with the dissipation of the electrochemical gradient for sodium and chloride (Figure 1). By moving these ions across the membrane in fixed ratio with GABA (1 GABA: 2 Na+: 1 Cl'), GAT-1 generates a stoichiometric current (Lester H.A. et al. 1994. Annual Review of Pharmacology and Toxicology 34: 219-249). At rest, in the pre-synaptic terminal of GABAergic neurons, the driving force for sodium and chloride forces these ions to move from the extracellular space towards the cell cytoplasm, thus carrying GABA in the same direction. The translocation of GABA across the membrane is relatively rapid, allowing GABA to be removed from the extracellular space within few milliseconds after its release (Isaacson et al. 1993. Neuron 10: 165-175). In addition to regulating the transport of GABA, GAT-1 also behaves as an ion channel, and generates two ionic currents that are not stoichiometrically coupled to the movement of GABA across the membrane. The first is a sodium inward current activated by GABA binding to GAT-1 (Risse et al. 1996. J Physiol 490: 691-702). The second is a leak current that can be detected even in the absence of GABA and is mediated, in vitro, by alkali ions like lithium and caesium (MacAulay et al. 2002. J Physiol (Lend) 544: 447-458). Last, in the absence of GABA, GAT-1 generates sodium-dependent capacitive currents (Mager et al.
1993. Neuron 10: 177-188). Through the coordinated activation of these currents, GAT-1 activation can generate a local shunt (i.e. a change in membrane resistance) or membrane depolarization.

The SLC6A1 gene is located in the short arm of chromosome 3 (GRCh38 genomic coordinates: 3:10,992,733-11,039,248 10,992,748-11,039,247) between the SLC6A11 gene (encoding another type of GABA transporter) and the HRH1 gene (encoding the histamine receptor H1). The SLC6A1 gene is approximately 46.5 Kilobase (Kb) long and comprises 18 exons (https://www.ncbi.nlm.nih.gov/gene/6529). There are five major variants leading to 3 splice isoforms (a, b and c) of human GAT-1 that differ from one another for alternative use of exons three to five. The transcript ENST00000287766 corresponding to the coding sequence portion CDS is the longest isoform of human SLC6A1 and is considered canonical (Hunt et al. 2018) and comprises SEQ ID NO: 4. Thus, most genetic variants are mapped into this sequence. Known genetic variants comprise variants 2 comprising SEQ ID NO: 5, variant

3 comprising SEQ ID NO: 6, variant 4 comprising SEQ ID NO: 7 and variant 5 comprising SEQ ID NO: 8.

In particular, the rAAV particle according to the present invention comprises viral genome comprising a transgene encoding GAT-1, preferably encoding human GAT-1, wherein the transgene comprises SEQ ID NO: 4, 5, 6, 7 or 8, for example SEQ ID NO: 4.

As used herein, the term "GAT-1" refers to gamma butyric acid (GABA) transporter protein 1 (GAT-1) (also called GABA transporter 1; MAE; GAT1; GABATR; GABATHG (Uniprot code: P30531). GAT-1 protein is composed by 12 transmembrane domains that come together to form a single chain transporter. The five splice variants of human SLC6A1 leads to three splice isoforms of GAT-1, isoform a comprising SEQ ID NO: 1 (which is considered the canonical sequence), encoded by splice variants 1 or 2, comprising SEQ ID NO: 4 and 5, respectively; isoform b, comprising SEQ ID NO: 2, encoded by splice variant 3 comprising SEQ ID NO: 6; and isoform c, comprising SEQ ID NO: 3, encoded by splice variants 4 or 5, comprising SEQ ID NO: 7 and 8, respectively. As used herein, the term GAT-1 polypeptide refers to all variants and isoforms of GAT-1 described herein (unless specified otherwise).

In some embodiments, the GAT-1 polypeptide comprises or consists of an amino acid sequence having at least 95% identity, at least 96%, at least 97%, at least 98% or at least 99% identity to the amino acid sequence of SEQ ID NO: 1-3.

In one embodiment, the GAT-1 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1-3. In a preferred embodiment, the GAT-1 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1.

The activity of the GAT-1 polypeptide can be measured by expressing the polypeptide in a GAT-1-deficient cell and measuring GABA uptake into the cell and comparing uptake to a cell expressing a GAT-1 polypeptide having the amino acid sequence of SEQ ID NO: 1.

Pharmaceutical composition

The rAAV particles according to the present invention may be comprised in a pharmaceutical composition along with one or more carriers and/or excipients, which are optionally

pharmaceutically acceptable carriers and/or excipients. The term "*pharmaceutically acceptable*" means approved by a regulatory agency or recognized pharmacopeia such as European Pharmacopeia, for use in animals and/or humans. The term "*carrier*" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like as long as they are physiologically compatible and are suitable for administration to the central nervous system of a subject in the context of the present invention. Examples of carriers include water, saline, phosphate buffered saline, buffers and the like, as well as combinations thereof. The term "*excipient*" refers to a diluent, adjuvant, carrier, or vehicle with which the therapeutic agent is administered.

Any suitable pharmaceutically acceptable carrier, diluent or excipient can be used in the preparation of a pharmaceutical composition (See e.g., Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro (Editor) Mack Publishing Company, April 1997). Pharmaceutical compositions are typically sterile and stable under the conditions of manufacture and storage. Pharmaceutical compositions may be formulated as solutions (e.g. saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluids), microemulsions, liposomes, or other ordered structure suitable to accommodate a high product concentration (e.g. microparticles or nanoparticles). The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the require particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, or salts such as sodium chloride in the composition.

rAAV production

The rAAV particles according to the present invention may be produced by means of conventional methods and protocols. Briefly, rAAV particles can be produced in a host cell, more particularly in a specific virus-producing cell (packaging cell), which is transfected with the appropriate viral genome to be packaged, in the presence of a helper vector or virus or other DNA constructs. The term "*packaging cells*" as used herein refers to a cell or cell line

which may be transfected with a viral genome of the disclosure, through an appropriate plasmid, and provides in trans all the missing functions which are required for the complete replication and packaging of an rAAV particle.

Typically, a process of producing rAAV particles comprises the following steps: a) culturing a packaging cell comprising a viral genome in a culture medium; b) harvesting the rAAV particles from the cell culture supernatant and/or inside the cells; c) purifying the rAAV particles, typically via at least affinity chromatography and/or ion chromatography; and d) optionally formulating the rAAV particles to obtain a pharmaceutical composition.

Conventional methods can be used to produce rAAV particles, which involve transient cell co-transfection with a nucleic acid construct or expression vector (e.g. a plasmid) carrying the viral genome; a second nucleic acid construct (e.g. an AAV helper plasmid) that encodes rep and cap genes, but does not carry ITR sequences; and a third nucleic acid construct (e.g. a plasmid) providing the adenoviral functions necessary for AAV replication.

Viral genes necessary for AAV replication are referred to as viral helper genes. Typically, said genes necessary for AAV replication are adenoviral helper genes, such as E1 A, E1B, E2a, E4, or VA RNAs. In one embodiment, the adenoviral helper genes are of the Ad5 or Ad2 serotype. Production of AAV particles may alternatively be carried out by infection of insect cells with a combination of recombinant baculoviruses (Urabe et al. Hum. Gene Ther. 2002; 13: 1935- 1943). SF9 cells are co-infected with two or three baculovirus vectors respectively expressing AAV rep, AAV cap and the AAV vector to be packaged. The recombinant baculovirus vectors provide the viral helper gene functions required for virus replication and/or packaging. Smith et al 2009 (Molecular Therapy, vol.17, no.11, pp 1888-1896) describes a dual baculovirus expression system for large-scale production of AAV particles in insect cells.

Suitable culture media are known to a person skilled in the art. The ingredients that make up a culture medium may vary depending on the type of cell to be cultured. In addition to nutrient composition, osmolarity and pH are considered important parameters of culture media. The cell growth medium comprises a number of ingredients well known by the person skilled in the art, including amino acids, vitamins, organic and inorganic salts,sources of carbohydrate,

lipids, trace elements (to name a few, CuS04, FeS04, Fe(N03)3, ZnS04), each ingredient being present in an amount which supports the cultivation of a cell in vitro (i.e., survival and growth of cells). Ingredients may also include auxiliary substances, such as buffer substances (for example sodium bicarbonate, Hepes, Tris or similarly performing buffers), oxidation stabilisers, stabilisers to counteract mechanical stress, protease inhibitors, animal growth factors, plant hydrolysates, anti-clumping agents, anti-foaming agents. Characteristics and compositions of cell growth media vary depending on the particular cellular requirements. Examples of commercially available cell growth media include: MEM (Minimum Essential Medium), BME (Basal Medium Eagle), DMEM (Dulbecco's modified Eagle's Medium), Iscoves DMEM (Iscove's modification of Dulbecco's Medium), GMEM, RPMI 1640, Leibovitz L-15, McCoy's, Medium 199, Ham (Ham's Media) F10 and derivatives, Ham F12, DMEM/F12.

Further guidance for the construction and production of viral vectors for use according to the disclosure can be found in Viral Vectors for Gene Therapy, Methods and Protocols. Series: Methods in Molecular Biology, Vol. 737. Merten and Al-Rubeai (Eds.); 2011 Humana Press (Springer); Gene Therapy. M. Giacca. 2010 Springer-Verlag; Heilbronn R. and Weger S. Viral Vectors for Gene Transfer: Current Status of Gene Therapeutics. In: Drug Delivery, Handbook of Experimental Pharmacology 197; M. Schäfer-Korting (Ed.). 2010 Springer-Verlag; pp. 143-170; Adeno-Associated Virus: Methods and Protocols. R.O. Snyder and P. Mouillier (Eds). 2011 Humana Press (Springer); Bünning H. et al. Recent developments in adeno-associated virus technology. J. Gene Med. 2008; 10:717-733; Adenovirus: Methods and Protocols. M. Chillon and A. Bosch (Eds.); Third Edition. 2014 Humana Press (Springer).

SEQ ID NO	Sequence Name	Sequence
1	GAT-1 protein sequence isoform a	MATNGSKVADGQISTEVSEAPVANDKPKTLVVKVQKKAADLPDRDTWKGRFDFLM SCVGYAIGLGNVWRFPYLCGKNGGGAFLIPYFLTLIFAGVPLFLLECSLGQYTSIGG LGVWKLAPMFKGVGLAAAVLSFWLNIYYIVIISWAIYYLYNSFTTTLPWKQCDNPW NTDRCFSNYSMVNTTNMTSAVVEFWERNMHQMTDGLDKPGQIRWPLAITLAIAWI LVYFCIWKGVGWTGKVVYFSATYPYIMLIILFFRGVTLPGAKEGILFYITPNFRKLSD SEVWLDAATQIFFSYGLGLGSLIALGSYNSFHNNVYRDSIIVCCINSCTSMFAGFVIFSI VGFMAHVTKRSIADVAASGPGLAFLAYPEAVTQLPISPLWAILFFSMLLMLGIDSQFC TVEGFITALVDEYPRLLRNRRELFIAAVCIISYLIGLSNITQGGIYVFKLFDYYSASGMS LLFLVFFECVSISWFYGVNRFYDNIQEMVGSRPCIWWKLCWSFFTPIIVAGVFIFSAV QMTPLTMGNYVFPKWGQGVGWLMALSSMVLIPGYMAYMFLTLKGSLKQRIQVMV QPSEDIVRPENGPEQPQAGSSTSKEAYI

2	GAT-1 protein sequence isoform b	MFKGVGLAAAVLSFWLNIYYIVIISWAIYYLYNSFTTTLPWKQCDNPWNTDRCFS NYSMVNTTNMTSAVVEFWERNMHQMTDGLDKPGQIRWPLAITLAIAWILVYFC IWKGVGWTGKVVYFSATYPYIMLIILFFRGVTLPGAKEGILFYITPNFRKLSDSEVW LDAATQIFFSYGLGLGSLIALGSYNSFHNNVYRDSIIVCCINSCTSMFAGFVIFSIVGF MAHVTKRSIADVAASGPGLAFLAYPEAVTQLPISPLWAILFFSMLLMLGIDSQFCT VEGFITALVDEYPRLLRNRRELFIAAVCIISYLIGLSNITQGGIYVFKLFDYYSASGMSL LFLVFFECVSISWFYGVNRFYDNIQEMVGSRPCIWWKLCWSFFTPIIVAGVFIFSA VQMTPLTMGNYVFPKWGQGVGWLMALSSMVLIPGYMAYMFLTLKGSLKQRIQ VMVQPSEDIVRPENGPEQPQAGSSTSKEAYI
3	GAT-1 protein sequence isoform c	MVNTTNMTSAVVEFWERNMHQMTDGLDKPGQIRWPLAITLAIAWILVYFCIWKGV GWTGKVVYFSATYPYIMLIILFFRGVTLPGAKEGILFYITPNFRKLSDSEVWLDAATQ IFFSYGLGLGSLIALGSYNSFHNNVYRDSIIVCCINSCTSMFAGFVIFSIVGFMAHVTK RSIADVAASGPGLAFLAYPEAVTQLPISPLWAILFFSMLLMLGIDSQFCTVEGFITALV DEYPRLLRNRRELFIAAVCIISYLIGLSNITQGGIYVFKLFDYYSASGMSLLFLVFFECV SISWFYGVNRFYDNIQEMVGSRPCIWWKLCWSFFTPIIVAGVFIFSAVQMTPLTMGN YVFPKWGQGVGWLMALSSMVLIPGYMAYMFLTLKGSLKQRIQVMVQPSEDIVRPE NGPEQPQAGSSTSKEAYI
4	SLC6A1 splice variant ORF region	atggcgaccaacggcagcaaggtggccgacgggcagatctccaccgaggtcagcgaggcccctgt ggccaatgacaagcccaaaaccttggtggtcaaggtgcagaagaaggcggcagacctccccgacc gggacacgtggaagggccgcttcgacttcctcatgtcctgtgtgggctatgccatcggcctgggcaa cgtctggaggttcccctatctctgcgggaaaaatggtgggggagccttcctgatcccctatttcctga cactcatctttgcgggggtcccactcttcctgctggagtgctccctgggccagtacacctccatcggg gggctaggggtatggaagctggctcctatgttcaagggcgtgggccttgcggctgctgtgctatcatt ctggctgaacatctactacatcgtcatcatctcctgggccatttactacctgtacaactccttcacca cgacactgccgtggaaacagtgcgacaaccccctggaacacagaccgctgcttctccaactacagca tggtcaacactaccaacatgaccagcgctgtggtggagttctgggagcgcaacatgcatcagatga cggacgggctggataagccaggtcagatccgctggccactggccatcacgctggccatcgcctgga tccttgtgtatttctgtatctggaagggtgttggctggactggaaaggtggtctacttttcagccacat acccctacatcatgctgatcatcctgttcttccgtggagtgacgctgcccggggccaaggagggcat cctcttctacatcacacccaacttccgcaagctgtctgactccgaggtgtggctggatgcggcaacc cagatcttcttctcatacgggctgggcctggggtccctgatcgctctcgggagctacaactctttcca caacaatgtctacagggactccatcatcgtctgctgcatcaattcgtgcaccagcatgttcgcagga ttcgtcatcttctccatcgtgggcttcatggcccatgtcaccaagaggtccattgctgatgtggcggc ctcaggccccgggctggcgttcctggcatacccagaggcggtgacccagctgcctatctccccactc tgggccatcctcttcttctccatgctgttgatgctgggcattgacagccagttctgcactgtggagggc ttcatcacagccctggtggatgagtaccccaggctcctccgcaaccgcagagagctcttcattgctg ctgtctgcatcatctcctacctgatcggtctctctaacatcactcaggggggtatttatgtcttcaaac tctttgactactactctgccagtggcatgagcctgctgttcctcgtgttctttgaatgtgtctctatttc ctggttttacggtgtcaaccgattctatgacaatatccaagagatggttggatccaggccctgcatct ggtggaaactctgctggtctttcttcacaccaatcattgtggcgggcgtgttcattttcagtgctgtgc agatgacgccactcaccatgggaaactatgttttccccaagtggggccagggtgtgggctggctgat ggctctgtcttccatggtcctcatccccgggtacatggcctacatgttcctcaccttaaagggctccc tgaagcagcgcatccaagtcatggtccagcccagcgaagacatcgttcgcccagagaatggtcctg agcagccccaggcgggcagctccaccagcaaggaggcctacatctag
5	SLC6A1 splice variant	gccctcggaagaccgagacagcggagaggttgcgggtgagctgcgctgagcccaggagccgagga gtcgggagcgcagtagcgctgagcccgagcccgagcggccccgcgtcccgagcgcatcggagcgg ccgagccgcccggatgcagcgcctgtcccgggcagcgcagccccggccgcaggatctcacccagg

	(isoform a)	gtggcagaaggaggccttctggagctgacccacccccgacgaccatcagggtgcccttgagccgca aaactgctgtccacgtggaccgggggtgacatcgcacgtccatctgccaggacccctgcgtccaaa ttccgagacatggcgaccaacggcagcaaggtggccgacgggcagatctccaccgaggtcagcga ggcccctgtggccaatgacaagcccaaaaccttggtggtcaaggtgcagaagaaggcggcagacct ccccgaccgggacacgtggaagggccgcttcgacttcctcatgtcctgtgtgggctatgccatcggc ctgggcaacgtctggaggttcccctatctctgcgggaaaaatggtgggggagccttcctgatcccct atttcctgacactcatctttgcgggggtcccactcttcctgctggagtgctccctgggccagtacacc tccatcgggggctaggggtatggaagctggctcctatgttcaagggcgtgggccttgcggctgctgt gctatcattctggctgaacatctactacatcgtcatcatctcctgggccatttactacctgtacaact ccttcaccacgacactgccgtggaaacagtgcgacaacccctggaacacagaccgctgcttctcca actacagcatggtcaacactaccaacatgaccagcgctgtggtggagttctgggagcgcaacatgc atcagatgacggacgggctggataagccaggtcagatccgctggccactggccatcacgctggcca tcgcctggatccttgtgtatttctgtatctggaagggtgttggctggactggaaaggtggtctacttttt cagccacataccctacatcatgctgatcatcctgttcttccgtggagtgacgctgcccggggccaa ggagggcatcctcttctacatcacacccaacttccgcaagctgtctgactccgaggtgtggctggat gcggcaacccagatcttcttctcatacgggctgggcctggggtccctgatcgctctcgggagctaca actctttccacaacaatgtctacagggactccatcatcgtctgctgcatcaattcgtgcaccagcatg ttcgcaggattcgtcatcttctccatcgtgggcttcatggcccatgtcaccaagaggtccattgctga tgtggcggcctcaggccccgggctggcgttcctggcatacccagaggcggtgacccagctgcctatc tccccactctgggccatcctcttcttctccatgctgttgatgctgggcattgacagccagttctgcact gtggagggcttcatcacagccctggtggatgagtaccccaggctcctccgcaaccgcagagagctct tcattgctgctgtctgcatcatctcctacctgatcggtctctctaacatcactcaggggggtatttatg tcttcaaactctttgactactactctgccagtggcatgagcctgctgttcctcgtgttctttgaatgtgt ctctatttcctggttttacggtgtcaaccgattctatgacaatatccaagagatggttggatccaggc cctgcatctggtggaaactctgctggtctttcttcacaccaatcattgtggcgggcgtgttcattttca gtgctgtgcagatgacgccactcaccatgggaaactatgttttccccaagtggggccagggtgtggg ctggctgatggctctgtcttccatggtcctcatccccgggtacatggcctacatgttcctcaccttaaa gggctccctgaagcagcgcatccaagtcatggtccagcccagcgaagacatcgttcgcccagagaa tggtcctgagcagccccaggcgggcagctccaccagcaaggaggcctacatctagggtgggggcca ctcaccgacccgacactctcacccccgacctggctgagtgcgaccaccacttgatgtctgaggata ccttccatctcaacctacctcgagtggcgagtccagacaccatcaccacgcagagaggggaggtgg gaggacagttagaccctgggtgggccctgccgtgggcaaggataccggtggcttctggcacctgg cgggctggtgacctttttaatccaggccccatcagcatcccacgatcggccttggtaaccgccgcgg tagatcatttttatcccgccagggagtgtgatgcaggaagaccacatgcgctcctggcttttaaacct gttcctgactgttctcttactgccgaaaccttgactgttatctcggactttgcaggagttcctttccct ccgaacgctgctccatgcacaggaaaagggcattttgtacaatggggacttcccgggaacgcttgct cttaagtaccagaagccggcggagctctggctttcgtgtttttggttttctccttcccaaggcagctgg attgaaaaaacaaaacaaaacaaaaaaacccaggggcgtcagtcgatattcccagggccgcttct cctgcagtctgtggagcgtccttgtccccgccgccggaatgaatgagcattctgcagcccgatgtcc ctgtcccctcctcgccgggccattctgattggacctggcccagtgcaatctgtccagacaagccctg cttgctggaaaactgccacaagcacaattgatctcttttatcgccattccaggggcctcaggtccta ctggggaaacttcctataccggagctccagtttctcttaagctgcccaatttcacagagtacaaaat agttgtaggggaaatcaaggtgaaggatctgtccgacagtcaagacggatccacagtaatctttcgg tctccttaaactaccaccctcgctgccacccacccccaagctgctgccgcctcaccttccttgaaattt ctcagcgggagtctcctcactgccactaaaatccacccagcccactaactgaggagctagtgttaat ccagagaaccccccgcaatgtgcttccgagattcagactgcttcattgggaagtatgatttgttcctt tctggaattgggctccgtggtggcggcggcacttcaagcaaagacagtttcttgcaagctccagtag ctccgcgtgtctcatttgccaggaagatgggttcccacgtagcaaatcgtacattgtgccctgtagct ccttagctagttagctcacaagccgtgttttatgactaatccttaataactatggtaaataactgtga

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20	Endogenous hSLC6A1 promoter	agaatccctcaaacctcaagaactgagagaagggtgtctggggctcctgccaccatccctgtttccc ttttaagtaatctgtttccccatctgtccatccatacacacagccacttgtgtctccatgaccaaccg ctggcagtggaagggtgtccttcccacccccactcttacacacactcccagctggtacccagagcct ggtcaccccaggccaggcctgtgtttccaggtgtaacgggcagcagacgctgccctaggactagag cagggaggggcacgggcccacccccaacccacagcgacccacagagggcgaaaagaggacgacc gcagagagaaacggaaaggacaggccaacggaagcagtactgcaaggctggaaggagaaaagcc aggaggggagtgcttgctgtgaaagacagggagacagagaccaagacggacaggcagacaggctg gtgacccaggatgaggccggaaagagccatcaaaggaaggagaaggaaggagagagattggagc gggacggcggggcaggcgagggaaggaggggtggggagagggagggaggaagagaggggagaa agagggaggaagagaggggagaaggagggagaagagagcgggagaatgcgagaggaaagaagg gagaggggaggcgtagaaggggagaggaggtgaagggaaaaggagagagcctgctggcggcgaa gctgcaagaggcagctgcggagggagcgcgcggcgggcctgggggagcgctgggcgggggcgggc ggtgcgggcagggctatacccgagctgggcgggctccgggcgccgcgggccctgccctccccctcc atccctccggactcgctcccccctcctctcccttccccgcgaccctccgcccgccctcggaagaccg agacagcggagaggttgcgggtgagctgcgctgagcccaggagccgaggagtcgggagcgcagta gcgctgagcccgagcccgagcggccccgcgtcccgagcgcatcggagcggccgagccgcccggat gcagcgcctgtcccgggcagcgcagccccggccgcaggatctcacccagggtggcagaaggaggc cttctggagctgacccacccccgacgaccatcagggtgaggcaactccaaggtcctactctctttct gtgcctgttacccaccccgtcctcctagggtgcccttgagccgcaaaactgctgtccacgtggaccg ggggtgacatcgcacgtccatctgccaggacccctgcgtccaaattccgagac
21	AAVTT capsid	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGP FNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSF

		GGNLGRAVFQAKKRILEPLGLVEEPVKTAPGKKRPVEHSPAEPDSSSGTGKSGQQP ARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMASGSGAPMADNNEGADG VGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFG YSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNDGT TTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQ AVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYL YYLSRTNTPSGTTTMSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTAADNNN SDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKYFPQSGVLIFGKQDSGKTNV DIEKVMITDEEEIRTTNPVATEQYGSVSTNLQSGNTQAATSDVNTQGVLPGMVWQ DRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAA KFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYS EPRPIGTRYLTRNL
22	AAV9 capsid	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLG PGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTS FGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQ PAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADG VGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYF GYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNG VKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGS QAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQY LYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNV DADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVW QDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFN KDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL
23	polyA sequence	gatccagacatgataagatacattgatgagtttggacaaaccacaactagaatgcagtgaaaaaaa tgctttatttgtgaaatttgtgatgctattgctttatttgtaaccattataagctgcaataaacaagtt
24	MECP2 intron	gcgctccctcctctcggagagagggctgtggtaaaacccgtccggaaattggccgccgctgccgcc accgccgccgccgccgccgcgccgagcggaggaggaggaggaggcgaggaggagagactgtgagt gggaccgccaaggccgcgggcggggacccttgctgggggcgggtaggggcgggacgtggcgcgg gaggggcccgcggggtcgggcgacacggctggcggttggcgtccctcctctctaccctccccctccc tctgccgccggtggtggctttctccactcgtctcccgcaatcgcgagcgacggttctcagcgcgatct ccctggagccaccttcgattgacgccctcccgctgcccgccccatctgtgcgcatcctaggccccag ctgtgcaagcgcccttgtcgtctgggcttcgccagttggggctgcgcgcgctcctgcccttcttgggg ctttgggcctcggcactgtcgcgcgcccgcggtcccggcctctccctggatcgcgctgtcccttctc cctcgcgcgcccccactcccgttacttgctccccccctcacacacacagactggcgcgcgtgcgcagt ccatctcccgttgggagagtgcgccacaagggctcctgagctcttacccccatctctgggttttgctc cctcctcctcctctcccattccgtgacttttgcccccactgcaagcgagtcggtccatcagctccat tccccacttggcaggaacaagttgagggttattgtccacccacaaaaaggactagacattttgttcc taggtcccacaactcatcataaagagttggttgtagttctcatcaggaaccgtgggcaagggactgt gcgttcctcagcactcgaagctcttccgtgagaccttgcccgcagggtgctctggttctttggggttg ctgtgctgtggcttcggaatttgagcgtcttcccaccctccctcccctcccttcgccagcgttctgtct acaagaaagaataggcaggtgtccttggatatcgtagttgctaatcgcctatacactgttctattaca cctttctgctaaggatagggtttttggttttggttttggttttgttccccaccctccagtttggtttagtt ttggttttggcatttagggtttttttgggggggagtaatatcttgtggtaaagacccatctgacccaaga tacctttttttctcatactggaaccctaggcagcagttgctatttccctgagttagcaatagttttacag

		tattttgaggccttttgtccataattctcacggaatccctcagggatcagattagctgctgttgggatc aggaaattgggttacaccgctgaaatctcttgctggggcccttgttttgaattggaaagtcaggaggc tggaacgaaggctcacaagttaacagtgccagctgctcttccagaagccctggattcagtcccacc aatccatcgcgggtcacaaccatctgtaacttcagtcccaaggggtccgaagccctcttctggctttg ccctattattttatttatcttatctgtttttgtcttgtcatctggcaagcccagggggccattgggtgca acttataaactgacttctgtatcttaagaagccaaccatacagtgcttacattccagaaaaaaaatc tgccactttaacagcactagaactagggtttagagaagtatcataaaggtcaaatatctttgaccaa tatcaccagcaacctaaagctgttaagaaatctttgggccccagcttgacccaaggatacagtatcc tagggaagttaccaaaatcagagatagtatgcagcagccaggggtctcatgtgtggcactcaagct cacctatactcactactgtgcagacagctgtgttctctgtaatacttacatatttgtttaatacttcag ggaggaaaagtcagaagaccaggatctccagggcctca
25	pB globin	ctgggcataaaagtcagggcagagccatctattgcttacatttgcttct
26	hDLX intron	gtaagtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagacagaga agactcttgcgtttctgataggcacctattggtcttactgacatccactttgcctttctctccacag
27	GAT-1 peptide	VADGQISTEVSEAPVASDKPK

EXAMPLES

The sequences used in the Examples are as follows: The AAV9 capsid used has the amino acid sequence of SEQ ID NO: 22, the AAVTT capsid has the amino acid sequence of SEQ ID NO: 21, the Endo promoter has the nucleotide sequence of SEQ ID NO: 20, the SLC6A1 transgene encodes a GAT-1 protein having the amino acid sequence of SEQ ID NO: 1, the polyA sequence used has the nucleotide sequence of SEQ ID NO: 23, and the ITRs used have the nucleotide sequences of SEQ ID NOs: 9 and 10. For example, the AAVTT-Endo-SLC6A1-HA construct comprises:

(i) an AAVTT capsid having the amino acid sequence of SEQ ID NO: 21;

(ii) an Endo promoter having the nucleotide sequence of SEQ ID NO: 20,

(iii) an SLC6A1 transgene encoding a GAT-1 protein having the amino acid sequence of SEQ ID NO: 1;

(iv) a 5' ITR and 3' ITR having the nucleotide sequences of SEQ ID NOs: 9 and 10 respectively; and

(v) a polyA sequence having the nucleotide sequence of SEQ ID NO: 23.

The AAV9-Endo-SLC6A1-HA construct comprises:

(i) an AAV9 capsid having the amino acid sequence of SEQ ID NO: 22;

(ii) an Endo promoter having the nucleotide sequence of SEQ ID NO: 20,

(iii) an SLC6A1 transgene encoding a GAT-1 protein having the amino acid sequence of SEQ ID NO: 1;
(iv) a 5' ITR and 3' ITR having the nucleotide sequences of SEQ ID NOs: 9 and 10 respectively; and
(v) a polyA sequence having the nucleotide sequence of SEQ ID NO: 23.

EXAMPLE 1: Biodistribution with multiple RoAs

PND24-28, male heterozygous SLC6A1S295L/+ mice were injected with either the AAV9-Endo-SLC6A1-HA (1.23E+13 vgc/ml) or AAVTT-Endo-SLC6A1-HA (1.32E+13 vgc/ml) vector (n=3-6 mice/ condition). Seven different conditions were tested, including 5 different routes of administration (intracerebroventricular, ectorhinal cortex, striatum, thalamus and a dual injection in thalamus and cortex, as described in Table 1). Mice were injected unilaterally in the right hemisphere and injection volumes between 0.5 - 1 µL and 2 - 4µl were applied for intraparenchymal and ICV routes, respectively. The AAV was administered at a fixed infusion rate of 200nl/ min through a 25-µl Hamilton with a 32 gauge and 30 degrees angle needle. At the end of injection, a waiting period of 5-min was introduced to restrict back flow of the vector along the injection tract. One animal injected at the ectorhinal cortex (1 out of 6) did not recover from anesthesia after surgery.

During the 4 weeks after injection, clinical signs, adverse effects, body weight and mortality was assessed in all animal groups (see Table 1). Body weight differences were monitored once a week in order to assess the overall health status of the mice. There were no significant differences in the body gain weights in the different groups injected with the different viral vectors up and until the last evaluation. None of the mice injected showed any signs of morbidity.

Table 1: Overview of experimental conditions

Targeted brain region	Age	Viral vector	Sample size	Volume

ICV	PND24 - 28	AAV9-Endo-SLC6A1-HA	5	2 µL
ICV	PND24 - 28	AAV9-Endo-SLC6A1-HA	5	4 µL
Thalamus	PND24 - 28	AAV9-Endo-SLC6A1-HA	5	1 µL
Thalamus + cortex	PND24 - 28	AAV9-Endo-SLC6A1-HA	5	2* 0.5 µL
Ectorhinal cortex	PND24 - 28	AAV9-Endo-SLC6A1-HA	6	1 µL
Striatum	PND24 - 28	AAV9-Endo-SLC6A1-HA	5	1 µL
Striatum	PND24 - 28	AAVTT-Endo-SLC6A1-HA	3	1 µL

At 4 weeks post-injection, the animal was perfused intracardially with PBS under isoflurane anesthesia, in accordance with European Committee Council directive (2010/63/EU). The brain was collected and fixated with 4% paraformaldehyde for 3h at room temperature. Afterwards the brain was transferred to a 15% sucrose solution in PBS containing 0.01% sodium azide.

Sectioning of the mouse brains and immunohistochemistry were carried out as follows. Free-floating coronal sections (fixed-frozen; 40 µm-thick) were obtained using a cryostat microtome and permeabilized 15 min in Tris buffered saline (TBS) containing 0.3% Triton X-100 (TBS-T). Then, sections were incubated overnight at room temperature with the primary antibody diluted in TBS-T (anti-hemagglutinin (HA) tag; 1:50,000; #3724, Cell Signaling). After three washes of 5 min in TBS, they were incubated for one hour at room temperature with secondary antibody and 4′,6-diamidino-2-phenylindole (DAPI, 300 nM) prepared in TBS-T, rinsed 3 times 5 min in TBS, and finally mounted on glass slides using Fluoromount mounting medium (Thermo Fisher Scientific). Whole slide imaging was performed using an Axioscan Z1 slide scanner (Zeiss) with 20x objective.

Image analysis was carried out with VisioPharm software (VisioPharm, Hørsholm, Denmark) as described previously (Albert et al. 2019). In brief, the regions of interest (ROI) were delineated manually, and automatic quantification of the immunoreactive signal was performed using a linear Bayesian algorithm, providing a value of signal area (marker area in μm^2). Then, a % marker coverage was calculated (i.e. ratio between the immunoreactivity signal area in μm^2 and the area of the ROI in μm^2 multiplicated by 100). Percent HA marker coverage in each brain section was quantified throughout the whole brain (from Bregma +3.08 to -6.48 mm; n=24 sections per animal) using well-defined landmarks based on a mouse brain atlas (Paxinos, Franklin, and Ebscohost 2019) (see Figure 2). All quantifications were done blindly until the end of statistical analysis.

EXAMPLE 2:

PND24-28, male heterozygous SLC6A1$^{S295L/+}$ mice were bilaterally injected in the thalamus with either the AAV9-Endo-hSLC6A1-HA (1.23E+13 vgc/ml) or AAVTT-Endo-hSLC6A1-HA (1.32E+13 vgc/ml) vector (Table 2). A group of mice was, simultaneous to the AAV9-Endo-SLC6A1-HA administration procedure, implanted with cortical EEG electrodes and a subcutaneous EEG transmitter to allow longitudinal EEG monitoring following treatment (see further, see Table 2). One additional group of mice was injected with vehicle-PBS (containing 0.001% PF-68). A volume of 1µL per injection was infused at a rate of 200nl/min. One animal from the vehicle-PBS group (1 out of 20) did not recover from the isoflurane surgery and a total of 4 animals reached humane endpoints in the critical 5-day period post-surgery, to which end animals from the following groups were euthanized: AAV9-ENDO-hSLC6A1-HA longitudinal EEG (2 out of 21) and AAVTT-ENDO-hSLC6A1-HA (2 out of 20). No post-surgical issues were encountered in the animals from the AAV9-ENDO-hSLC6A1-HA group (0 out of 21).

During the 5 weeks after injection, clinical signs, adverse effects, body weight and mortality was assessed in all animal groups (vehicle-PBS, AAV9-ENDO-hSLC6A1-HA, AAVTT-ENDO-hSLC6A1-HA). Body weight differences were monitored once a week in order to assess the overall health status of the mice. There were no significant differences in the body gain weights in the different groups injected with the different viral vectors up and until the last evaluation. None of the mice injected with vehicle-PBS, AAV9-ENDO-hSLC6A1-HA or

AAVTT-ENDO-hSLC6A1 -HA showed any signs of morbidity outside of the critical 5-day post-surgery phase.

Six weeks after injections, *in vivo* wireless EEG (electroencephalogram) video-telemetry recordings were performed for 1 week to evaluate seizure occurrence. SLC6A1+/S295L mice were surgically implanted with subcutaneous telemetry transmitter and cortical EEG electrodes 5 weeks after injections. Surgery was performed under sterile/aseptic conditions. Anaesthetized mice (Isoflurane in oxygen- Induction: 5 % at 2 l/min, maintenance 2.5 - 1.5 % at 1.5 l/min) were placed in a stereotaxic frame with heating pad, holes were drilled on the skull surface of the prefrontal cortex (over bregma) for the recording electrode and on the skull surface of the cerebellum (behind the lambda) for the reference electrode. Thereafter, an Open Source Instruments (OSI) A3028S2 ECoG transmitter was implanted subcutaneously over the dorsum with the attached wires extending subcutaneously up to the cranium where the recording and reference electrodes were positioned through each hole approximately 0.5 mm into the brain parenchyma. Each electrode was secured in place with a screw (Plastics One). The whole assembly was held in place with cyanoacrylate and dental cement forming a small, circular headpiece and the dorsum was closed with nylon absorbable suture material. Post-operative medication and pain management included a second and third Carprofen dose (10mg/kg) 24 hours and 48 hours following the pre-surgery dose. After the surgery, mice were recovering in warm-chamber for 2-3h. For in vivo wireless EEG video-telemetry recordings, mice were group housed (3-5 mice/cage). Mice cages were placed in Faraday enclosures to facilitate recordings. Welfare monitoring of implanted mice was conducted once per day for 2 weeks. Mice were weighed daily for 5 days, thereafter weekly. All recordings were carried in a purposely designed recording room with temperature and humidity control in order to decrease ambient interference and improve the reception of the transmitting signals. Signals were radio transmitted from the implanted transmitter to the antennas placed inside the Faraday enclosures. EEG signal from one recording channel was digitized at 256 Hz (Band-pass filter: 0.3-80 Hz). Spike wave discharges (SWDs), typical of absence seizures, were analysed with an in-house automated seizure detection software. SWDs detection algorithm was based on event duration analysis (> 2 s), band frequency analysis (5-9 Hz) and identification of specific fundamental harmonic frequencies. Each SWD detected by the algorithm was confirmed by at least one experienced observer in a blinded fashion. A period of high SWD occurrence (5 hours from 1pm to 6pm), was initially observed in the transgenic line SLC6A1+/S295L non-injected with the viral

vectors. Consequently, EEG analysis was performed for 24h or during this shortened period for the different viral vector and control groups. A total of 8 animals were excluded from the analysis due to a technical issue with the EEG electrodes and telemetry device in the following groups: AAV9-ENDO-hSLC6A1-HA longitudinal EEG (7 out of 19) and AAVTT-ENDO-hSLC6A1-HA (1 out of 18). An additional 2 animals were also removed from the analysis in the group AAV9-ENDO-hSLC6A1-HA (2 out of 21); one displayed technical artefacts in the EEG and the other one encountered an issue with the EEG telemetry device. Also, 2 animals were excluded from the vehicle-PBS group (2 out of 19); one animal reached humane endpoints in the critical 5-day period post-surgery, to which the animal was euthanized and one animal due to a technical issue with the EEG telemetry device. The difference between groups was analyzed using a negative binomial regression model on the cumulative number of seizures across the 7-day recording period (***$p<0.001$).

At 7 weeks post-injection, the animals were sacrificed following the same methodology as described in Example 1. The brain was collected, divided longitudinally in the two respective hemispheres and processed as follows: the right-sided hemisphere was dissected and submitted for biochemical analysis while the left-sided hemisphere was either processed for IHC (as described in Example 1) or for histopathological analysis. A pathological safety assessment was carried out in half of the animals divided across the different treatment groups. Selected tissues for analysis (hemi-brain together with the spinal cord, dorsal root ganglia, liver, kidney, spleen, thymus and eyes) were fixed in 10% neutral buffered formalin, embedded in paraffin, processed to wax blocks, sectioned at approximately 5uM thickness and stained with Hematoxylin and Eosin (H&E).

Biochemical analysis comprised of DNA/RNA extraction from the caudal cortex and protein extraction from the medial frontal cortex, lateral frontal cortex and thalamus/hypothalamus. Total RNA was extracted from specific CNS brain regions of mice injected with the various AAV vectors using Mag-Bind Total RNA 96 kit (Omega, M6731) on a KingFisher Flex (ThermoFisher). Subsequently, ± 500 ng of total RNA from each sample was subjected to reverse transcription (RT) using a High Capacity cDNA RT Kit + RNase Inhibitor (ThermoFisher cat n°4374966). Next, a cDNA amount corresponding to 100 ng of total RNA was amplified by quantitative qPCR on an CFX Opus 384 Real-Time PCR System (Biorad, cat n° 12011452) or CFX384 Touch Real-Time PCR Detection System (Biorad, cat n° 1855484).

To quantify mRNA levels, human and mouse SLC6A1 custom made TaqMan assays were design, SLC6A1_Hs: assay ID: Hs01104473_g1 with an amplicon size of 80, Slc6a1_Mm: assay ID: Mm01183569_m1 with an amplicon size of 84. The SLC6A1 mRNA levels were normalized to mRNA levels of two selected housekeeping genes, Bcl2l13_Mm: assay ID: Mm00463355_M1 (Inventoried) with an amplicon size of 62 bp and Brap_Mm: assay ID: Mm00518493_M1 (Inventoried) with an amplicon size of 82 bp. RNA samples were amplified with and without reverse transcriptase to exclude DNA amplification. ΔCt was calculated by subtracting the Ct of the control gene from the Ct of the gene of interest for each tissue (heart, gastrocnemius and quadriceps). The ΔCt of the control tissue sample was subtracted from the ΔCt of the corresponding experimental tissue sample and the results were graphically represented as ΔΔCt for each tissue of different treated groups. Genomic DNA was extracted from specific CNS brain regions of mice injected with the various AAV vectors using Mag-Bind® HDQ Blood DNA & Tissue 96 Kit (Omega, M6399) on a KingFisher Flex (ThermoFisher), then 10 to 40 ng of DNA was analyzed on a CFX Opus 384 Real-Time PCR System (Biorad, cat n° 12011452) or CFX384 Touch Real-Time PCR Detection System (Biorad, cat n° 1855484) using a Taqman assay targeting SV40pA (TaqMan Custom assay, ThermoFisher) and ValidPrime® Mouse – Probe assay – 1000 rxns (TATAA Biocenter, ordered at Tebu-bio, ref. A106P10) as control. To generate standard curves, known copy numbers of the corresponding vector plasmid was used. DNA copy number was calculated relative to the standard curve and expressed as copy per cell or per mg of tissue.

The levels of the HA-hGAT1 in lateral frontal cortex and thalamus/hypothalamus were measured using a ligand binging assay run on the MSD platform. Briefly, biotinylated anti- HA tag antibody was used as a capture reagent to bind the target protein to the streptavidin coated MSD plate. A commercially available Rabbit monoclonal antibody to GABA Transporter 1 / GAT 1 was used as the detection antibody. The levels of mouse GAT1 proteins in the thalamus/hypothalamus and lateral frontal cortex was measured using an LC-MS method. Briefly, the brain tissue was homogenised and separated into a crude membrane fraction. After lysis the samples were digested with trypsin/Lys-C and the digested peptides measured via LC-MS, specifically targeting the mouse specific GAT-1 peptide VADGQISTEVSEAPVASDKPK (SEQ ID NO: 27). Normalisation was performed against the stable isotope labelled equivalent peptide (Figure 4).

DNA viral genome copies was identified in all injected groups, without observing a significant difference between the injected groups (AAV9 vs AAV9 LT vs AAVTT). Interestingly, levels of viral genome are widely distributed across the animals. There is no significant difference of human slc6a1 mRNA levels between the injected groups (AAV9 vs AAV9 LT vs AAVTT) (Figure 5).

The range and standard deviation of the levels of the HA-hGAT1 in the thalamus, in the AAVTT and AAV9 data sets are comparable despite the geometric mean being slightly higher in the AAVTT group. This data indicates that the thalamic exposure of HA-hGAT1 is comparable between the two capsids. In the cortex region, a lower geometric mean and range was observed in the AAV9 dataset, suggesting lower cortical exposure in the AAV9 treated group as compared to the AAVTT treated group. However, this was not statistically significant.

Treatment with AAV9-ENDO-hSLC6A1-HA did not impact the endogenous mGAT1 levels in comparison to vehicle treatment (Figure 6).

Table 2: Overview of the experimental conditions in Example 2.

Genotype	**Age at treatment**	**Delivery Route**	**Viral Vector**	**Titer**	**Sample size**	**In Life Assessment**	**Seizure monitoring**
HET (KI)	Postnatal day 24-28 PND24-28	Intra-thalamic bilaterally injected 1µl/ hemisphere	**AAV9-ENDO-HA-hSLC6A1**	1.23E+13 GC/mL	21	Clinical signs, adverse effects, body weight and mortality during 7 weeks post-injection	*In vivo* wireless EEG video-telemetry recordings in week 1, week 3 and week 6 post-injection
			AAV9-ENDO-HA-hSLC6A1	1.23E+13 GC/mL	21		*In vivo* wireless EEG video-telemetry recordings; 6-7

							weeks post-injection
			AAVTT-ENDO-HA-hSLC6A1	1.32E+13 GC/mL	20		
			Vehicle-PBS (Sterile Phosphate-buffered saline 1X)	--	20		

As illustrated in Figure 7A, the average number of SWDs per day recorded over 7 consecutive days during the peak hours of SWD occurrence was significantly reduced by 84% and 97% in SLC6A1$^{+/S295L}$ mice injected with either AAV9-ENDO-hSLC6A1-HA or AAVTT-ENDO-hSLC6A1-HA, respectively, compared to the control group. In addition, Figure 7B demonstrates the time course of SWD reduction at 1, 3 and 6 weeks after AAV9-ENDO-hSLC6A1-HA injection. Notably, quantification of the HA signal throughout the brain indicates that AAVTT results in a larger rostrocaudal brain coverage compared to AAV9 (Figure 8), which could contribute to the increased reduction in SWDs from the AAVTT-ENDO-hSLC6A1-HA compared to AAV9-ENDO-hSLC6A1-HA treated group.

EXAMPLE 3: NCD3948: NHP biodistribution study

A single dose bilateral intra-thalamic pilot study of AAVTT-CAG-NLS-mCherry followed by a 4-week observation period in Cynomolgus monkey to evaluate the thalamus as a route of administration.

The biodistribution of the adeno associated virus (AAVTT) and reporter transgene product (mCherry) was assessed after a single bilateral infusion to the dorsomedial nuclei (DMN) of the Cynomolgus monkey thalamus 4-week post dosing.

The study design and conditions are described in Table 3. The construct will be named AAVTT-mCherry through the document.

Table 3: Study design table

Group	Test item[a]	Dosing sites	Dose conc. (vg/ml)	Dose level (vg/animal)	Dose vol. (µL)	Infusion[b] rate (µL/h)	Animal N & tag
1	AAVTT-CAG-NLS-Cherry	2 (1 per thalamus hemisphere)	1E13	2.4E12	100 (per site)	120, 300	2 males UCG280/1001 & UGC281/1002

[a] CAG: CMV early enhancer/chicken β actin promoter; NLS: nuclear localization signal.

[b] the injection pattern followed a 2-step infusion rate: 120 µL/hour for the first 5 minutes of infusion (total 10 µL infused), followed by 300 µL/hour to complete the dosing for the site.

Surgical procedure. The animals were fasted overnight (at least 8 hours) prior to the surgical/dosing procedures. Lidocaine 10 mg/mL on the epiglottis may be used to aid intubation. Anaesthesia (isoflurane/oxygen by inhalation) was maintained during the surgery.

The surgical sites were shaved and aseptically prepared. The head was secured in the head fixation frame and the Clearpoint frame base placed on each hemisphere. An MRI (T1) with contrast agent (Gadobenate dimeglumine, 0.2 mL/kg, 0.1 mmol/kg) was performed before dosing to plan the surgical trajectories. The base and tower were secured onto the head, and a 0.4-0.5 cm hole made through the skull using a manual drill and the SmartFlow® cannula (NGS-NC-05) was inserted.

The test article at a concentration of 1E13 vg/ml was infused into the thalamus at a rate 120, 300 µL/hour for 5 min (total of 10 µL infused), followed by a rate 300 µL/hour to complete the dosing for the site using the Clearpoint® system. The canula was left in place for at least

10 minutes before stepwise removed over approximately 1 minute. During the course of the study the animals were examined for clinical signs with neurological assessment performed prior to dosing, at week 1, and prior to necropsy.

Blood collection. During in life blood samples (1 ml) were collected via the femoral vein (day 3, 4 and 28), and serum derived was kept frozen (-70°C) for viral genome (vg) copies number quantification by PCR.

CSF collection. CSF samples (1 ml) were collected from the cisterna magna (prior to surgery, day 4 and day 28), centrifuged and the supernatant maintained frozen (-70°C) for vg copy number quantification by PCR.

Tissue collection

Brain. After necropsy and following transcardial perfusion with RNAse-free PBS, the brain was removed, and cut coronally into 4-mm thick slabs. 19 punch biopsies (3 mm) per animal were collected from the right brain hemisphere for vg copy number determination by PCR. The right remaining tissue and the slab from the left side were fixed by immersion for 72 to 96 hours at room temperature in methanol-free 4% PFA prepared in 1X PBS, then transferred to 1X PBS and kept at 2 to 8°C until further processed. The 4 mm coronal brain slabs were cut in 40 µm sections, and 68 sections equally spaced from rostral to caudal direction were stained for mCherry, Haematoxylin & Eosin (H&E), and Iba1, using standard procedure for immunohistochemistry analysis.

Peripheral tissues including liver, heart, spleen, testes, kidney, lung, adrenal gland, cervical lymph node, phrenic and peroneal nerve, and spinal cord with the DRGs (C2, T4, L5 and S1) were also collected (8 mm punch biopsy). The locations and number of brain punches collected are listed in the Table 4.

Vector genomes copies were quantified in tissue punches using a qualified duplex droplet digital PCR assay (ddPCR) to detect the delivered mCherry transgene. A reference assay to detect the single copy per haploid genome gene, CAPZA3 was used to normalize the data to the genomic DNA (*g*DNA). In tissue, data is reported as vector genome copies per µg *g*DNA, in biofluids (CSF/blood) as vector genome copies per mL of biofluid. The detection lower

limits of quantification (LLoQ) of the assays were 100vg/µg *g*DNA, 1.33E3vg/ml blood and 1E3vg/ml CSF for tissue, blood, and CSF, respectively.

Immunostaining for immunohistochemistry analysis

mCherry immunohistochemistry using diaminobenzidine tetrahydrochloride (DAB), sections were stained with the primary antibodies to mCherry (rat M11217, ThermoScientific) followed by a biotinylated secondary antibody (rat IgG BA-9401, Vector labs). An avidin-biotin complex (Vector Lab's ABC solution, VECTASTAIN® Elite ABC, Vector, Burlingame, CA) was applied and treated with DAB and hydrogen peroxide to create a visible reaction product. The sections were mounted on gelatine coated glass slides, dehydrated, and cleared. Sections were finally cover-slipped with Permount mounting media. Each slide was laser etched with the block number and the stain.

H&E, Iba1 immunohistochemistry ten coronal thick sections of whole brain (rostral to caudal including the injection site in the thalamus) were stained with H&E and Iba1. Sections from the cervical, thoracic, lumbar, and sacral dorsal root ganglion (DRG) as well as cervical, thoracic and lumbar spinal cord were stained with H&E. Slides from the lumbar and sacral DRG were also stained for Iba1 (microglial IHC marker). To characterize changes in the injection tract, IHC to stain to neuronal nuclei (NeuN) and cleave caspase 3 (CC3) were done.

mCherry area coverage was quantified along the rostro-caudal axis of the brain using 60-70 stained sections per animal. The immunoreactive signal of each marker was quantified on each brain section as previously described (Albert *et al* 2019), providing a value of marker area in μm^2. Results were reported as a percentage of marker occupancy, which corresponds to the ratio of the immunoreactive area divided by the area of the region of interest (in this case, the entire brain section) multiplied by 100.

Percentage of mCherry expressing cells was quantified along the rostro-caudal axis of the brain. The number of mCherry expressing cells and total cells (based on thionine light counterstaining) was quantified on each brain section using Visiopharm deep-learning U-Net algorithm. Deep-learning network was trained using manual annotations drawn on 11 regions of interest. Results were reported as a percentage of transfected cells (ratio of mCherry cells

positive divided by total cell number based on mCherry and thionine light respectively and expressed as percentage).

RESULTS

NHP biodistribution assessment by vg copy number

Vector genomes (vg) were detected across all brain regions for both dosed animals as shown in Figure 9. The highest vector genome copies (vgc) were detected at the injection site, the thalamus, (1E8vg/µg *g*DNA). The vgc across all other brain areas ranged from 1E5 to 5E6vg/µg *g*DNA with values in the prefrontal, temporal cortex, and other cortical areas of approx. 3E6vg/µg *g*DNA (for individual tissue values refer to Table 5). Low vgc numbers were detected in the tissues from spinal cord and DRG (Figure 10A). In peripheral tissues only spleen showed a low detectable vgc count (1E4vg/µg *g*DNA), in all other tissues (heart, liver and testes) the counts were below the limit of quantification (Figure 10B).

Vgc were determined in blood and CSF at day 4 after dose and at day 28. At day 4 the CSF vgc count (1.45 & 2.27E7vg copies/ml CSF) were higher than blood (1.5 & 7.7E5vg copies/ml blood), and the vgc for both biofluids were below the limit of quantification at day 28, the terminal collection timepoint (Figure10C).

NHP biodistribution by immunohistochemistry assessment of mCherry protein

The mCherry reporter transgene expression determined by immunohistochemistry followed a similar biodistribution pattern as the vgc, with efficient transduction of cells and expression of the mCherry protein in the thalamic parenchyma, as well as across brain areas with thalamic projections such as the dorsal cerebral cortex, basal ganglia nuclei, caudate, deep cerebellar nuclei (DCN), and specific nuclei of the midbrain, pons, and medulla (Figure 11).

No mCherry expression signal was observed in the ventral cerebral cortical areas, cerebellar cortex and hippocampus, areas where a low vg copy number was detected. Additionally, mCherry-positive cells were observed in the posterior part of the caudate nucleus and along the needle tract in the cortex, likely transfected following reflux of the AAV during the injection procedure.

In the cerebral cortex mCherry-expressing cells were predominantly localized in the dorsal areas across the anteroposterior extent of the cortex (Figure 12), with mCherry signal observed in deep cortical layers, V and VI layers, as anticipated from corticothalamic connections (Figure 13).

mCherry protein expression on thalamic projections following intra-thalamic administration of AAVTT is shown in Figure 14. Immunohistochemical analysis is shown for the primary motor cortex, ventral anterior thalamic nucleus and reticular thalamus, caudate nucleus, and globus pallidus.

Quantification of percentage of cells transduced (mCherry positive cells number over total cell number) along the rostro-caudal axis of the brain indicated that the highest percentage of cell transduction was observed in the thalamus, close to the injection site, which values ranging from 7 to 13% of the cells transduced, as expected by the high vg copy number determined in the area (Figure 15).

NHP pathology analysis

Mild microscopic changes were observed bilaterally along the injection tract, and within the thalamus and surrounding regions. Changes were characterized mainly by mild gliosis, PV cuffing, gliosis, occasional apoptotic bodies, and pigment eosinophilic spheroids (axonal degeneration) and were predominantly located within the thalamus with limited extension into surrounding areas. Similar changes, with occasional mononuclear inflammatory cells (MIC) and eosinophils, were noted in deeper (more caudal) sections of the thalamus. In more anterior regions of the brain – predominantly the caudate and putamen - minimal levels of PV cuffing, gliosis and pigment were noted. Some cavitation caused by the Clearpoint catheter tracts were noted but were within normal limits (see Table 6).

Additional Neuronal Nuclei (NeuN) and cleaved caspase 3 (CC3) IHC staining confirmed that there were no significant changes in NeuN or CC3 staining beyond the site of injection which correlated with the H&E evaluation of the slides.

The changes were within the normal expectations for an intra-parenchymal injection of an AAVTT GT product and considered acceptable.

CONCLUSIONS

From the above results it can be concluded that delivery of AAVTT-mCherry in the dorsomedial nucleus of the thalamus resulted in efficient cell transduction as determined by both the vg copy number and mCherry reporter expression in the thalamic nuclei, as well as in regions of the brain that are synaptically connected to the thalamus and are considered target areas for epilepsy (e.g. prefrontal, temporal and cingulated cortex) achieving vg copy numbers higher than 1E6vg/µg *g*DNA at the dose tested (2.4E12vg/animal). The route of administration, dorsomedial nuclei of the thalamus, and the delivery conditions used, induced changes that are normally expected for an intra-parenchymal deliver of an AAV based GT product, and were considered acceptable.

Table 4: Brain biopsies

Region	**Biopsy numbers per region/animal**
Prefrontal cortex	1
Premotor cortex	2
Cingulate cortex	1
Temporal cortex	1
Somatosensory cortex	1
Parietal cortex	1
Visual cortex	1
Caudate nucleus	1
Putamen	1
Amygdala	1
Hypothalamus	1
Hippocampus	1
Globus pallidus	1

Thalamus	1
Midbrain	1
Pons	1
Medulla	1
Cerebellum	1

Table 5: vg copy number determined across brain tissues and biofluids

			Accepted values	
Tissue	**Animal ID**	**Reason for re-analysis**	**copy mCherry/ug gDNA**	**copy mCherry/diploid genome**
Amygdala(S6Amy)	1002	droplets failed	2.65.E+05	1.710
Cervical DRG (DRG-C2)	1001	droplets failed	3.14.E+02	0.002
Caudate nucleus (S5CN)	1001	>ULOQ, re-run	2.63.E+06	16.978
Caudate nucleus (S5CN)	1002	>ULOQ, re-run	3.23.E+06	20.869
Cingulate cortex (S5A8)	1001	>ULOQ, re-run	2.08.E+06	13.412
Cingulate cortex (S5A8)	1002	>ULOQ, re-run	1.51.E+06	9.777
Globus pallidus (S6GP)	1002	>ULOQ, re-run	1.64.E+06	10.583
Midbrain (S10MID)	1002	>ULOQ, re-run	2.03.E+06	13.107
Parietal cortex (S8PC)	1001	>ULOQ, re-run	1.25.E+06	8.054
Prefrontal Cortex (S3DPFC)	1001	>ULOQ, re-run	2.44.E+06	15.758
Prefrontal Cortex (S3DPFC)	1002	>ULOQ, re-run	4.13.E+06	26.688
Premotor cortex (S5PC-1)	1001	>ULOQ, re-run	2.91.E+06	18.779
Premotor cortex (S5PC-2)	1001	>ULOQ, re-run	4.59.E+06	29.635
Premotor cortex (S6MC-1)	1002	>ULOQ, re-run	1.04.E+06	6.749
Premotor cortex (S6MC-2)	1002	>ULOQ, re-run	1.48.E+06	9.576
Putamen (S5PU)	1001	>ULOQ, re-run	3.35.E+06	21.669
Putamen (S5PU)	1002	>ULOQ, re-run	2.88.E+06	18.597
Somatosensory cortex (S6SS)	1001	>ULOQ, re-run	3.00.E+06	19.405
Somatosensory cortex (S6SS)	1002	>ULOQ, re-run	1.46.E+06	9.415
Temporal cortex (S5TC)	1001	>ULOQ, re-run	1.38.E+06	8.915
Thalamus (S7TH)	1001	>ULOQ, re-run	6.15.E+07	397.277
Thalamus (S7TH)	1002	>ULOQ, re-run	1.02.E+08	660.342
Biofluid			**copy mCherry/mL fluid**	
CSF Day 4	1001	>ULOQ, re-run	1.45.E+07	
CSF Day 4	1002	>ULOQ, re-run	2.27.E+07	

Table 6: Pathology analysis of individual brains

Approximate section level based off human anatomy											
Animal No.	Section Level	Prefrontal Cortex	Dorsal Prefrontal Cortex	Prefrontal Cortex	Striatum Caudate-Putamen	Striatum - Caudate - Putamen - (Ant. Thalamus)	Globus Pallidus-Thalamus-Amygdala	Thalamus-Hippocampus	Thalamus-Midbrain	Hindbrain-Cb DCN	Hindbrain-Cb
	Section No.	6	11	16	21	26	31	36	46	52	59
280 (1001)	Increased Iba1 staining	0	0	0	1 (Caudate-Putamen)	2 (Internal capsule, Caudate - Putamen & Cortex)	3 Cortex, Internal capsule, Injection Tract)	2.5 (Thalamus)	1 (Mininges, Choroid Plexus)	0	1 (DCN)
	H&E	NAD	NAD	NAD	Pigment (1) CP; Gliosis (1) CP	Pigment (1) CP; Gliosis (1) CP	Inj Tract Cavitation, Gliosis/ MIC with occasional eosinophils & surrounding area of Apoptotic bodies, Pigment, Eosinophilic spheroids (2) (Thalamus); PV Cuffing with neuropil gliosis & occasional eosinophilic spheroids (2) (Cortex/ Thalamus); Nn vacuolation (1)	PV Cuffing with neuropil gliosis/MIC with occasional eosinophils and pigment (2) (Thalamus); Nn vacuolation (1) (Thalamus); Apoptotic bodies (1) (Thalamus)	NAD	NAD	NAD

							(Thalamus)				
IHC	Increased CC3 staining					1 (increased single cell staining of PV inflammatory cells with fewer cells in a gradient around injection tracts)	1 (increased single cell staining of PV inflammatory cells with fewer cells in a gradient around injection tracts)				
Animal No.	Section No. 7	7	12	17	21	27	31	36	45	51	57
281 (1002)	Increased Iba1 staining	0	0	1 (Caudate)	1.5 Caudate-Putamen/Thalamus	2 (Caudate-Putamen, Thalamus)	2.5 (Caudate-Putamen, Thalamus, Inj Tract)	1.5 (Thalamus)	0	1 (DCN)	0
	H&E	NAD	NAD	NAD	NAD	PV cuffing & Pigment (1) Thalamus	Inj Tract cavitation, gliosis/MIC, haemorrhage/pigment & surrounding area of Apoptotic bodies, Pigment, Eosinophilic spheroids (2.5) (Thalamus); PV cuffing	PV Cuffing with neurophil gliosis/MIC with occasional eosinophils and pigment (1) (Thalamus); Nn vacuolation (1) (Thalamus)	NAD	NAD	NAD

							with neuropil gliosis (2) (Thalamus); Nn vacuolation (1) (Thalamus)				
IHC	Increased CC3 staining					0					
IHC	Decreased NeuN staining					0	0				
CP=caudate-putamen	MIC=mononuclear cell infiltrate	PV=perivascular	Nn=neuronal			Dark grey boxes indicate approximate level of NeuN and CC3 stains	Blank boxes indicate no slide				

EXAMPLE 4: POM5: Dose response study

To evaluate the dose-response relationship of selected viral vectors, PND24-28, male heterozygous SLC6A1$^{S295L/+}$ mice were bilaterally injected in the thalamus with the AAVTT-Endo-hSLC6A1-HA at three different dose levels (referred herein as “MAX”, “MID” and “LOW” dose levels). Respective titers for the MAX, MID and LOW dose were 1.32E+13 vgc/ml, 7.92E+12 vgc/ml, 2.64E+12 vgc/ml. One cohort of animals was injected with the AAVTT-Endo-hSLC6A1 at a single dose level (MAX, 1.32E+13 vgc/ml) to confirm functionality of the vector in the absence of a tag. Additionally, one group of mice was injected with vehicle-PBS (containing 0.001% PF-68), serving as a negative control. A volume of 1µL per injection was infused at 200nl/min.

Two animals from the vehicle-PBS group (2 out of 19) did not recover from anesthesia post-surgery. No issues were encountered in the animals from the AAVTT-ENDO-hSLC6A1-HA MAX dose group (N = 18 mice), AAVTT-ENDO-hSLC6A1-HA MID dose group (N = 17 mice), AAVTT-ENDO-hSLC6A1 LOW dose group (N = 18 mice) and AAVTT-ENDO-hSLC6A1 non-tagged MAX dose (N = 18 mice). A technical error was observed with an injector, causing the vector to not be correctly infused, to that end a total of 8 animals were excluded from further analysis. Excluded mice belonged to the following groups: 1 each from the vehicle-PBS, the AAVTT-ENDO-hSLC6A1-HA MID dose and the AAVTT-ENDO-hSLC6A1-HA LOW dose groups, 2 from the AAVTT-ENDO-hSLC6A1-HA MAX dose group and 3 from the AAVTT-ENDO-hSLC6A1 non-tagged MAX dose group.

In this study (cohort A), the dams of the injected offspring were flagged with a risk of parvovirus contamination. To that end, mice were subjected to repeated PCR testing and serological analysis. Results from two PCR analyses confirmed that all mice tested negative for parvovirus. Additionally, two serological tests – one at 6 weeks and at 11 weeks of age – were conducted to assess whether mice had experienced any prior parvovirus infections. Only if animals tested positive in both serological tests, it was considered to have undergone an active viral infection and was subsequently excluded from the study. Notably, mice with positive serology at 6 weeks but not at 11 weeks were suspected to have maternal antibodies and thus were not infected themselves with the parvovirus. A total of 5 of the remaining mice tested positive during the first serological test, but not during the second serology test and thus were not excluded from the final analysis. Unfortunately, a complication at the tail level was encountered in a subset of animals due to a human error during tail vein blood sampling. Progressive tail necrosis led to a humane endpoint in 29 mice, to which animals were euthanized: vehicle-PBS group (6 mice), AAVTT-ENDO-hSLC6A1-HA MAX dose group (5 mice), AAVTT-ENDO-hSLC6A1-HA MID dose group (5 mice), AAVTT-ENDO-hSLC6A1-HA LOW dose group (6 mice) and AAVTT-ENDO-hSLC6A1 non-tagged MAX dose (7 mice). Besides the aforementioned issue, none of the injected mice showed any signs of morbidity related to the treatment during the 7 weeks post-injection.

This resulted in the following final sample sizes per group: vehicle-PBS group (N = 11 mice), AAVTT-ENDO-hSLC6A1-HA MAX dose group (N = 13 mice), AAVTT-ENDO-hSLC6A1-HA MID dose group (N = 12 mice), AAVTT-ENDO-hSLC6A1-HA LOW dose group (N = 12 mice) and AAVTT-ENDO-hSLC6A1 non-tagged MAX dose (N = 11 mice).

Table 7: Overview of the experimental conditions, cohort A.

Genotype	**Age at treatment**	**Delivery Route**	**Viral Vector**	**Titer**	**Final sample size**	**In Life Assessment**	**Seizure monitoring**
HET (KI)	Postnatal day 24-28 PND24-28	Intra-thalamic bilaterally injected 1µl/ hemisphere	**AAVTT-ENDO-HA-hSLC6A1**	1.32 E+13GC /mL	13	Clinical signs, adverse effects, body weight and mortality during 7 weeks post-injection	*In vivo* wireless EEG video-telemetry recordings; 6-7 weeks post-injection
			AAVTT-ENDO-HA-hSLC6A1	7.92 E+12GC /mL	12		
			AAVTT-ENDO-HA-hSLC6A1	2.64 E+12GC /mL	12		
			AAVTT-ENDO-hSLC6A1	1.32 E+13GC /mL	11		
			Vehicle-PBS (Sterile Phosphate-buffered saline 1X)	--	11		

As sample sizes were lower than initially planned, an additional cohort of animals was subjected to the same experimental design (cohort B) to obtain sufficient statistical power. To this end, an additional 9 animals were added to the vehicle-PBS, 11 to the AAVTT-ENDO-

hSLC6A1 non-tagged MAX dose, 9 to the AAVTT-ENDO-hSLC6A1-HA MAX dose group, 8 to the AAVTT-ENDO-hSLC6A1-HA MID dose group and 8 to the AAVTT-ENDO-hSLC6A1-HA LOW dose group.

Three animals from the vehicle-PBS group (3 out of 9) were excluded, as one animal did not recover from anesthesia and two other animals were euthanized as they reached humane endpoints after surgery. Two animals from the AAVTT-ENDO-hSLC6A1 non-tagged MAX dose group (2 out of 11) were excluded, due to non-recovery from anesthesia and due to euthanasia due to a humane endpoint after surgery. One animal from the AAVTT-ENDO-hSLC6A1-HA MID dose group (1 out of 8) was euthanized due to a humane endpoint after surgery.

One animal treated with AAVTT-ENDO-hSLC6A1-HA at MAX dose level showed signs of distress, evidenced by a body weight reduction exceeding 20%, . Additionally, a suspected convulsion was visually observed,thus reaching a humane endpoint for the animal and triggering subsequent euthanasia. The animal was subjected to intra-cardiac perfusion and tissue sampling as reported below, to allow further tissue analysis. Finally, none of the mice injected with AAVTT-ENDO-hSLC6A1-HA at LOW dose showed any signs of morbidity.

Table 8: Study cohort B

Genotype	**Age at treatment**	**Delivery Route**	**Viral Vector**	**Titer**	**Final sample size**	**In Life Assessment**	**Seizure monitoring**
HET (KI)	Postnatal day 24-28 PND24-28	Intra-thalamic bilaterally injected 1µl/ hemisphere	**AAVTT-ENDO-HA-hSLC6A1**	1.32 E+13GC /mL	8	Clinical signs, adverse effects, body weight and mortality during 7 weeks post-injection	*In vivo* wireless EEG video-telemetry recordings; 6-7 weeks post-injection
			AAVTT-ENDO-HA-hSLC6A1	7.92 E+12GC /mL	7		
			AAVTT-ENDO-	2.64 E+1	8		

			HA-hSLC6A1	2GC/mL			
			AAVTT-ENDO-hSLC6A1	1.32 E+13GC/mL	9		
			Vehicle-PBS (Sterile Phosphate-buffered saline 1X)	--	6		

During the 7 weeks after injection, clinical signs, adverse effects, body weight and mortality was assessed in all animal groups (vehicle-PBS, AAVTT-ENDO-hSLC6A1-HA at MAX, MID and LOW dose, AAVTT-ENDO-hSLC6A1 at MAX dose). There were no significant differences in the body weight gains in the different groups injected with the different viral vectors up and until the last evaluation for mice from either cohort A or B.

Six weeks after injections, *in vivo* wireless EEG (electroencephalogram) video-telemetry recordings were performed for 1 week to evaluate seizure occurrence. As reported in Example 2: SLC6A1+/S295L mice were surgically implanted with subcutaneous telemetry transmitter and cortical EEG electrodes 5 weeks after injections. Consequently, EEG analysis was performed for the different viral vector and control groups, focusing on SWDs and convulsion analysis.

In cohort A, a total of 16 animals were excluded from the EEG analysis, of which 15 due to a software related issue in the following groups: AAVTT-ENDO-hSLC6A1-HA MAX dose(4 out of 13), AAVTT-ENDO-hSLC6A1-HA LOW dose group (2 out of 12), AAVTT-ENDO-hSLC6A1 non-tagged MAX dose group (2 out of 11), vehicle-PBS group (3 out of 11). An additional 5 animals were also removed from the analysis in the AAVTT-ENDO-hSLC6A1-HA MID dose group (5 out of 12); 4 due to an issue with the EEG software in the following groups and 1 other encountered an issue with the EEG telemetry device. In cohort B, 6 mice

were excluded from the EEG analysis due to technical artefacts on the EEG signal: 1 from the vehicle-PBS group, 2 from the AAVTT-ENDO-hSLC6A1-HA LOW dose group, 1 from the AAVTT-ENDO-hSLC6A1-HA MID dose group and 2 from the AAVTT-ENDO-hSLC6A1 non-tagged MAX dose group.

The Likelihood Ratio Test statistical test demonstrated that there was no significant batch effect, ensuring the data from both cohorts can be combined during analysis.

At 7 weeks post-injection, the animals were sacrificed following the same methodology as described in Example 2. The brain was collected, divided longitudinally in the two respective hemispheres and processed as follows: the right hemisphere was dissected and submitted for biochemical analysis while the left hemisphere was either processed for IHC or for autoradiography.

Biochemical analysis comprised of DNA/RNA and protein extraction from the isocortex and protein extraction from the thalamus/hypothalamus were performed as described in Example 2. Total GAT-1 expression was assessed in the isocortex using a radioligand binding assay, while human GAT-1 overexpression was analysed in isocortex and thalamus/hypothalamus using MSD for HA-tag detection.

As illustrated in Figure 17, the average number of SWDs per day recorded over 7 consecutive days was noticeably reduced in SLC6A1$^{+/S295L}$ mice administered all dose levels of AAVTT-ENDO-hSLC6A1-HA and AAVTT-ENDO-hSLC6A1. Statistical analysis of the data revealed significant reductions in SWDs at all dose levels administered compared to the negative control (Figure 18). For SLC6A1$^{+/S295L}$ mice treated with 5.28e9vg, 1.58e10, and 2.64e10vg AAVTT -hSLC6A1-HA, there is a 75.4%, 86.0%, and 93.4% decrease, respectively, in SWDs observed and for 2.64e10vg AAVTT-hSLC6A1 a 98.6% decrease.

For DNA viral genome copies, in POM5 cohorts A and B, no significant difference between the MID and MAX group were observed, however there is a clear difference between the LOW and MAX group due to the difference in AAV dose administered. Although human SLC6A1 mRNA levels in the LOW dose are lower than the MID and MAX doses, there is no significant difference between the dose groups with HA tagged viral vector (Figure 19).

In the combined POM5 cohorts (A and B), the levels of protein expression correlated well with the dose levels, with the highest amount of protein expressed in the high dose group

(MAX) and the lowest levels expressed in the LOW dose group (Figure 20). This trend was the same in both the isocortex and thalamus regions. HA-GAT1 protein levels were found to be significantly increased in AAVtt-HA-hslc6a1 MAX dose and AAVtt-HA-hslc6a1 MID dose groups compared to AAVtt-HA-hslc6a1 LOW dose, in both isocortex and thalamus/hypothalamus tissue.

ASPECTS

1. A method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus or striatum of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(iv) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(v) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion

of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(vi) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

2. A recombinant adeno-associated virus (rAAV) particle for use in a method of treating a disease, the method comprising administering the rAAV particle to a subject, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus or striatum of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(iv) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(v) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr;

Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(vi) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

3. A pharmaceutical composition comprising a recombinant adeno-associated virus (rAAV) particle and one or more carriers and/or excipients for use in a method of treating a disease, the method comprising administering the rAAV particle to a subject in need thereof, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the pharmaceutical composition to the thalamus or striatum of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(iv) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(v) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn;

Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(vi) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

4. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the disease is a genetic disorder associated with impaired GABA uptake.

5. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the disease comprises a single-gene epilepsy accompanied by cognitive, motor behavioural comorbidities, early onset developmental and epileptic encephalopathy, epileptic encephalopathy, childhood onset Epilepsy Syndromes, myoclonic atonic epilepsy (MAE), an MEA-like indication or an other epilepsy indication, optionally wherein the other epilepsy indication is Lennox Gastaut Syndrome, autism spectrum disorder and/or schizophrenia.

6. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the disease is characterised by SLC6A1 haploinsufficiency.

7. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the disease is associated with at least one mutation in a patient which leads to a pathological GAT-1 variant.

8. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 7, wherein the pathological GAT-1 variant comprises one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from R44W, R44Q, R50L, D52E, D52V, F53S, S56F, G63S, N66D, G75R, G79R, G79V, F92S, G94E, G105S, Q106R, G112V, Y140C, C173Y, G232V, F270S, R277H, A288V, S295L, G297R, A305T, G307R, V323I, A334P, A367T, V342M, A357V, G362R, L366V, F385L, G393S, S456R, S459R, M487T, V511L, and G550R.

9. A method of delivering a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle to the central nervous system of a subject, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus or striatum of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(iv) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(v) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr;

Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile;

(vi) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

10. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises or consists of an amino acid sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1-3.

11. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises or consists of an amino acid sequence having at least 98% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1-3.

12. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 1-3.

13. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide has at least 50% of the activity of the GAT-1 having an amino acid sequence of SEQ ID NO: 1.

14. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide has at least 90% of the activity of the GAT-1 having an amino acid sequence of SEQ ID NO: 1.

15. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 13 or aspect 14, wherein the activity is measured by expressing the GAT-1 polypeptide in a cell not expressing a GABA transporter and measuring uptake of GABA into the cell.

16. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises an amino acid sequence at least 85% identical to at least 500 amino acids of any one of SEQ ID NO: 1-3.

17. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises an amino acid sequence at least 90% identical to at least 550 amino acids of any one of SEQ ID NO: 1-3.

18. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the GAT-1 polypeptide comprises an amino acid sequence at least 95% identical to at least 575 amino acids of any one of SEQ ID NO: 1-3.

19. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the transgene is a solute carrier family 6 member 1 (SLC6A1) gene.

20. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the transgene comprises or consists of a nucleic acid sequence having at least 95% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 4-8.

21. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the transgene comprises or consists of a nucleic acid sequence having at least 98% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 4-8.

22. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the transgene comprises or consists of the nucleic acid sequence of any one of SEQ ID NOs: 4-8.

23. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle is administered to the thalamus.

24. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle is administered to a thalamic nucleus.

25. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle spreads from the thalamus to the cortical and subcortical regions after administration.

26. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle spreads from the thalamus to the frontal cortex after administration.

27. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 1-22, wherein the rAAV particle is administered to the striatum.

28. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the subject is a mammal.

29. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 28, wherein the subject is a primate.

30. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 28 or aspect 29, wherein the subject is a human.

31. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein a total dose comprising at least $1x10^6$ viral genomes (vg) of rAAV particle is administered.

32. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein a total dose comprising at most $1x10^{20}$, at most $1x10^{18}$, at most $1x10^{15}$, or at most $1x10^{12}$ viral genomes (vg) of rAAV particle is administered.

33. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle is administered in two doses, preferably wherein one dose is administered to the right hemisphere of the subject's brain and one dose is administered to the left hemisphere of the subject's brain.

34. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein:

a) the 5' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 9, or a sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 9; and/or

b) the 3' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 10, or a sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 10.

35. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the one or more regulatory sequences direct, assist and/or control expression of the transgene.

36. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the one or more regulatory sequences comprises at least one regulatory sequence selected from:

a) one or more transcription initiation sequences (such as a promoter);
b) one or more translation initiation sequences;
c) one or more mRNA stability sequences;
d) one or more polyadenylation sequences;
e) one or more secretory sequences;
f) one or more enhancer sequences;
g) one or more introns;
h) one or more TATA boxes;
i) one or more microRNA targeted sequences;
j) one or more polylinker sequences facilitating the insertion of a DNA fragment within a vector;
k) one or more splicing signal sequences;
l) one or more transcription termination sequences (such as polyadenylation sequences); or
m) a combination thereof.

37. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the one or more regulatory sequences comprises a promoter.

38. The method, rAAV particle for use, or pharmaceutical composition for use according aspect 37, wherein the promoter comprises:

a) a CAG 1.6kb promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 11 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 11;
b) a UbC promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 12 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 12;
c) a PGK promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 13 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 13;

d) an EF1a promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 14 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 14;

e) a MECP2 promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 15 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 15;

f) an hNSE promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 16 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 16;

g) an hSYN promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 17 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 17;

h) a CAMKII promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 18 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 18;

i) an hDLX promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 19 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 19; or

j) an endogenous hSLC6A1 promoter, optionally wherein the promoter comprises the nucleic acid sequence of SEQ ID NO: 20 or a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 20.

39. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 38, wherein the promoter is an endogenous hSLC6A1 promoter and wherein the promoter comprises or consists of a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 20.

40. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 38 or aspect 39, wherein the promoter is an endogenous hSLC6A1 promoter and wherein the promoter comprises or consists of a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 20.

41. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 38-40, wherein the promoter is an endogenous hSLC6A1 promoter and wherein the promoter comprises or consists of a nucleic acid sequence of SEQ ID NO: 20.

42. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 38, wherein the promoter is an hDLX promoter and wherein the promoter comprises or consists of a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 19.

43. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 38 or aspect 42, wherein the promoter is an hDLX promoter and wherein the promoter comprises or consists of a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 19.

44. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 38 or 42-43, wherein the promoter is an hDLX promoter and wherein the promoter comprises or consists of a nucleic acid sequence of SEQ ID NO: 19.

45. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 42-44, wherein the hDLX promoter is operably linked in a 5' to 3' orientation to an intron.

46. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 45, wherein the intron comprises or consists of a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 25 or 26.

47. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 45, wherein the intron comprises or consists of a nucleic acid sequence having at least 98% identity to the nucleic acid sequence of SEQ ID NO: 25 or 26.

48. The method, rAAV particle for use, or pharmaceutical composition for use according to aspect 45, wherein the intron comprises or consists of a nucleic acid sequence having the nucleic acid sequence of SEQ ID NO: 25 or 26.

49. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the AAV capsid is an AAV2, AAV5, AAV6, AAV8, AAV9, AAV10 or AAVTT capsid.

50. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the AAV capsid is an AAV true type (AAVTT) capsid.

51. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 21.

52. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 98% identity to the amino acid sequence of SEQ ID NO: 21.

53. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the AAV capsid comprises or consists of the amino acid sequence of SEQ ID NO: 21.

54. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 1-49, wherein the AAV capsid is an AAV9 capsid.

55. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 1-49 or 54, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 22.

56. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of aspects 1-49 or 54-55, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 98% identity to the amino acid sequence of SEQ ID NO: 22.

57. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the aspects 1-49 or 54-56, wherein the AAV capsid comprises or consists of the amino acid sequence of SEQ ID NO: 22.

58. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures.

59. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 10%.

60. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 20%.

61. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 30%.

62. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 50%.

63. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 70%.

64. A method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid comprising or consisting of the amino acid sequence of SEQ ID NO: 21; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) comprising or consisting of the nucleic acid sequence of SEQ ID NO: 9 and a 3' ITR comprising or consisting of the nucleic acid sequence of SEQ ID NO: 10;

ii) a transgene encoding a gamma butyric acid (GABA) transporter protein 1 (GAT-1) polypeptide, wherein the GAT-1 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1; and

iii) an endogenous hSCL6A1 promoter comprising or consisting of the nucleic acid sequence of SEQ ID NO: 20, wherein the endogenous hSCL6A1 promoter controls expression of the transgene;

wherein the method comprises administering the rAAV particle to the thalamus of the subject.

65. The method, rAAV particle for use, or pharmaceutical composition for use according to any one of the preceding aspects, wherein the rAAV particle or pharmaceutical composition is not administered intracerebroventricularly.

CLAIMS

1. A method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) or a fragment thereof and a 3' ITR or a fragment thereof;

ii) a transgene; and

iii) one or more regulatory sequences;

wherein the method comprises administering the rAAV particle to the thalamus of the subject, and wherein the transgene encodes a GAT-1 polypeptide which is:

(i) a gamma butyric acid (GABA) transporter protein 1 (GAT-1);

(ii) a variant of GAT-1 comprising one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from the group consisting of Ala2Thr; Asp165Tyr; Arg277Ser; Ile434Met; Arg579His; Gly5Ser; Arg172Cys; Arg277Cys; Ser470Cys; Pro580Ser; Asp10Asn; Arg172His; Arg277Pro; Ile471Val; Pro587Ala; Gly11Arg; Phe174Tyr; Ser280Cys; Gly476Ser; Ala589Val; Ile13Thr; Ser178Asn; Asn310Ser; Arg479Gln; Ile599Val; Glu16Lys; Asn181Asp; Tyr317His; Lys497Asn; Glu19Gly; Asn181Lys; Ile321Val; Phe502Tyr; Pro21Thr; Arg195His; Ser328Leu; Ile506Val; Lys33Glu; Met197Leu; Met332Val; Ala509Val; Val34Leu; Asp202Glu; Val337Ile; Thr520Met; Asp40Asn; Lys206Glu; His347Arg; Gly535Val; deletion of Met1; stop codon after Glu411; Asp43Glu; Arg211Cys; Ala354Val; Leu547Phe; Lys76Asn; Ile220Val; Leu375Met; Met552Ile; Asn77Asp; Ile220Asn; Ile377Val; Met555Val; Ile84Phe; Ala221Thr; Ile405Val; Thr558Asn; Phe87Leu; Val240Ala; Val409Met; Arg566His; Ile91Val; Phe242Val; Leu415Ile; Gln572Arg; Val142Ile; Tyr246Cys; Arg417Cys; Pro573Thr; Thr156Asn; Arg257Cys; Arg417His; Pro573Ser; Thr158Pro; Arg257His; Arg419Cys; Ser574Asn; Asp165Asn; Thr260Met; Arg419His; and Val578Ile; or

(iii) a variant or fragment of (i) or (ii) comprising an amino acid sequence having at least 80% sequence identity to a fragment of at least 450 amino acids of the amino acid sequence of any one of SEQ ID NOs: 1-3.

2. The method according to claim 1, wherein the disease is a genetic disorder associated with impaired GABA uptake.

3. The method according to claim 1 or claim 2, wherein the disease comprises a single-gene epilepsy accompanied by cognitive, motor behavioural comorbidities, early onset developmental and epileptic encephalopathy, epileptic encephalopathy, childhood onset Epilepsy Syndromes, myoclonic atonic epilepsy (MAE), an MEA-like indication or an other epilepsy indication, optionally wherein the other epilepsy indication is Lennox Gastaut Syndrome, autism spectrum disorder and/or schizophrenia.

4. The method according to any one of the preceding claims, wherein the disease is characterised by SLC6A1 haploinsufficiency.

5. The method according to any one of the preceding claims, wherein the disease is associated with at least one mutation in a patient which leads to a pathological GAT-1 variant.

6. The method according to claim 5, wherein the pathological GAT-1 variant comprises one or more mutations corresponding to mutations in SEQ ID NO: 1 selected from R44W, R44Q, R50L, D52E, D52V, F53S, S56F, G63S, N66D, G75R, G79R, G79V, F92S, G94E, G105S, Q106R, G112V, Y140C, C173Y, G232V, F270S, R277H, A288V, S295L, G297R, A305T, G307R, V323I, A334P, A367T, V342M, A357V, G362R, L366V, F385L, G393S, S456R, S459R, M487T, V511L, and G550R.

7. The method according to any one of the preceding claims, wherein the GAT-1 polypeptide comprises or consists of an amino acid sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1-3.

8. The method according to any one of the preceding claims, wherein the GAT-1 polypeptide has at least 50% of the activity of the GAT-1 having an amino acid sequence of SEQ ID NO: 1.

9. The method according to claim 8, wherein the activity is measured by expressing the GAT-1 polypeptide in a cell not expressing a GABA transporter and measuring uptake of GABA into the cell.

10. The method according to any one of the preceding claims, wherein the GAT-1 polypeptide comprises an amino acid sequence at least 85% identical to at least 500 amino acids of any one of SEQ ID NO: 1-3.

11. The method according to any one of the preceding claims, wherein the transgene is a solute carrier family 6 member 1 (SLC6A1) gene.

12. The method according to any one of the preceding claims, wherein the transgene comprises or consists of a nucleic acid sequence having at least 95% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 4-8.

13. The method according to any one of the preceding claims, wherein the rAAV particle is administered to a thalamic nucleus.

14. The method according to any one of the preceding claims, wherein the rAAV particle spreads from the thalamus to the cortical and subcortical regions after administration.

15. The method according to any one of the preceding claims, wherein the rAAV particle spreads from the thalamus to the frontal cortex after administration.

16. The method according to any one of the preceding claims, wherein the rAAV particle is administered in two doses, preferably wherein one dose is administered to the right hemisphere of the subject's brain and one dose is administered to the left hemisphere of the subject's brain.

17. The method according to any one of the preceding claims, wherein:

a) the 5' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 9, or a sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 9; and/or

b) the 3' ITR comprises or consists of the nucleic acid sequence of SEQ ID NO: 10, or a sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the nucleic acid sequence of SEQ ID NO: 10.

18. The method according to any one of the preceding claims, wherein the one or more regulatory sequences comprises a promoter, wherein the promoter is an endogenous

hSLC6A1 promoter and wherein the promoter comprises or consists of a nucleic acid sequence having at least 95% identity to the nucleic acid sequence of SEQ ID NO: 20.

19. The method according to any one of the preceding claims, wherein the AAV capsid is an AAV true type (AAVTT) capsid.

20. The method according to any one of the preceding claims, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 21.

21. The method according to any one of claims 1 to 18, wherein the AAV capsid is an AAV9 capsid.

22. The method according to any one of claims 1-18 or 21, wherein the AAV capsid comprises or consists of an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 22.

23. The method according to any one of the preceding claims, wherein administration of the rAAV particle leads to a decrease in the number of seizures.

24. The method according to any one of the preceding claims, wherein administration of the rAAV particle leads to a decrease in the number of seizures by at least 10%, at least 20%, at least 30%, at least 50%, or at least 70%.

25. A method of treating a disease in a subject, the method comprising administering to the subject a therapeutic amount of a recombinant adeno-associated virus (rAAV) particle, wherein the rAAV particle comprises:

a) an AAV capsid comprising or consisting of the amino acid sequence of SEQ ID NO: 21; and

b) a viral genome packaged therein, wherein the viral genome comprises:

i) a 5' inverted terminal repeat (ITR) comprising or consisting of the nucleic acid sequence of SEQ ID NO: 9 and a 3' ITR comprising or consisting of the nucleic acid sequence of SEQ ID NO: 10;

ii) a transgene encoding a gamma butyric acid (GABA) transporter protein 1 (GAT-1) polypeptide, wherein the GAT-1 polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 1; and

iii) an endogenous hSCL6A1 promoter comprising or consisting of the nucleic acid sequence of SEQ ID NO: 20, wherein the endogenous hSCL6A1 promoter controls expression of the transgene;

wherein the method comprises administering the rAAV particle to the thalamus of the subject.