

CLAIMS

1. A nucleic acid construct comprising a transgene encoding:
 - i. a gamma butyric acid (GABA) transporter protein 1 (GAT-1) comprising SEQ ID NO: 18, 19, ~~20~~; or
 - ii. a sequence having at least 95% or 96% or 97% or 98% or 99% or 99.5% sequence identity to SEQ ID NO: 18 or, 19 ~~or 20~~ and retaining functionality as GAT-1; or
 - iii. a naturally-occurring variant comprising, with reference to SEQ ID NO: 18, one or more mutations, preferably 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; or Val578Ile;

wherein the nucleic acid construct further comprises a promoter operably linked to said transgene, wherein said promoter preferably comprises:

- a. SEQ ID NO: 1, or preferably SEQ ID NO: 1 operably linked in a 5' to 3' orientation to SEQ ID NO: 2; or
- b. SEQ ID NO: 3; or
- c. SEQ ID NO: 4; or
- d. SEQ ID NO: 5 or SEQ ID NO: 35 or SEQ ID NO: 6, or preferably SEQ ID NO: 35 operably linked in a 5' to 3' orientation to SEQ ID NO: 6; or

e. SEQ ID NO: 7; or preferably SEQ ID NO: 7 operably linked in a 5' to 3' orientation to SEQ ID NO: 34; or

f. SEQ ID NO: 8; or

g. SEQ ID NO: 9; or

h. SEQ ID NO: 10; or

i. SEQ ID NO: 11, or SEQ ID NO: 11 operably linked in a 5' to 3' orientation to SEQ ID NO: 12 or preferably SEQ ID NO: 11 operably linked in a 5' to 3' orientation to SEQ ID NO: 12, wherein SEQ ID NO: 12 is operably linked in a 5' to 3' orientation to SEQ ID NO: 13; or

j. SEQ ID NO: 14; and.

wherein the construct optionally comprises a polyadenylation signal sequence, preferably a polyadenylation signal sequence comprising SEQ ID NO: 17.

2. The nucleic acid construct according to claim 1, wherein the transgene is a solute carrier family 6 member 1 (SLC6A1) gene, wherein the transgene preferably comprises:

i. SEQ ID NO: 15, 26, 27, 28 or 29, preferably SEQ ID NO: 15

ii. or a sequence having at least 95% or 96% or 97% or 98% or 99% or 99.5% sequence identity to SEQ ID NO: 15, 26, 27, 28 or 29.

- ~~3. The nucleic acid construct according to any one of claims 1 or 2, further comprising a promoter operably linked to said transgene, wherein said promoter preferably comprises:~~

~~a. SEQ ID NO: 1, or preferably SEQ ID NO: 1 operably linked in a 5' to 3' orientation to SEQ ID NO: 2; or~~

~~b. SEQ ID NO: 3; or~~

~~c. SEQ ID NO: 4; or~~

~~d. SEQ ID NO: 5 or SEQ ID NO: 35 or SEQ ID NO: 6, or preferably SEQ ID NO: 35 operably linked in a 5' to 3' orientation to SEQ ID NO: 6; or~~

e. ~~SEQ ID NO: 7; or preferably SEQ ID NO: 7 operably linked in a 5' to 3' orientation to SEQ ID NO: 34; or~~

f. ~~SEQ ID NO: 8; or~~

g. ~~SEQ ID NO: 9; or~~

h. ~~SEQ ID NO: 10; or~~

i. ~~SEQ ID NO: 11, or SEQ ID NO: 11 operably linked in a 5' to 3' orientation to SEQ ID NO: 12 or preferably SEQ ID NO: 11 operably linked in a 5' to 3' orientation to SEQ ID NO: 12, wherein SEQ ID NO: 12 is operably linked in a 5' to 3' orientation to SEQ ID NO: 13; or~~

j. ~~SEQ ID NO: 14.~~

4. ~~The nucleic acid construct according to any one of the preceding claims, wherein the construct comprises a polyadenylation signal sequence, preferably a polyadenylation signal sequence comprising SEQ ID NO: 17.~~

35. A viral vector comprising the nucleic acid construct according to any one of the preceding claims, wherein the viral vector further comprises inverted terminal repeat (ITR) at 5' and/or 3' flanking said nucleic acid construct, preferably a 5'ITR and a 3'ITR; wherein preferably the 5'ITR and/or the 3'ITR comprises the ITR of a natural adeno-associated virus (AAV), such as AAV2; wherein more preferably the 5'ITR comprises SEQ ID NO: 22 and/or the 3'ITR comprises SEQ ID NO: 23.

~~6. The viral vector according to claim 5, wherein the 5'ITR and/or the 3'ITR comprises the ITR of a natural adeno-associated virus (AAV), such as AAV2.~~

~~7. The viral vector according to any one of claims 5 or 6, wherein the 5'ITR comprises SEQ ID NO: 22 and/or the 3'ITR comprises SEQ ID NO: 23.~~

48. A viral particle comprising a nucleic acid construct according to any one of claims ~~1 to 4~~ 2 or a viral vector according to ~~any one of claims 35 to 7.~~

59. The viral particle according to claim 48, wherein the viral particle comprises at least a VP1 capsid protein from an AAV, wherein said capsid protein preferably comprises AAV2, AAV5, AAV6, AAV8, AAV9 (such as comprising SEQ ID NO: 25), AAV10, AAV-true type (AAVtt) or combinations thereof; wherein preferably the capsid protein is from AAVtt and

preferably comprises SEQ ID NO: 24 or it is at least 98.5%, preferably 99% or 99.5% identical to SEQ ID NO: 24.

~~10. The viral particle according to claim 9, wherein the capsid protein is from AAVtt and preferably comprises SEQ ID NO: 24 or it is at least 98.5%, preferably 99% or 99.5% identical to SEQ ID NO: 24.~~

611. A viral vector comprising a nucleic acid construct comprising a transgene encoding:

i. a gamma butyric acid (GABA) transporter protein 1 (GAT-1) comprising SEQ ID NO: 18, 19, ~~20~~; or

ii. a sequence having at least 95% or 96% or 97% or 98% or 99% or 99.5% sequence identity to SEQ ID NO: 18 or, 19 ~~or 20~~ and retaining functionality as GAT-1; or

iii. a naturally-occurring variant comprising, with reference to SEQ ID NO: 18, one or more mutations, preferably 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; or Val578Ile;

wherein said viral vector further comprises a promoter operably-linked to said transgene, wherein said promoter preferably comprises SEQ ID NO: 4 or SEQ ID NO: 14; wherein the nucleic acid construct comprised in said viral vector comprises a polyadenylation signal sequence; and wherein said viral vector further comprises inverted terminal repeat (ITR) at 5' and/or 3' flanking said nucleic acid construct, preferably a 5'ITR and a 3'ITR; and wherein optionally the polyadenylation signal sequence comprising SEQ ID NO: 17.

~~712~~. A viral vector comprising a nucleic acid construct comprising a transgene which is a solute carrier family 6 member 1 (SLC6A1) gene, wherein the transgene preferably comprises:

- i. SEQ ID NO: 15, 26, 27, 28 or 29, more preferably SEQ ID NO: 15
- ii. or a sequence having at least 95% or 96% or 97% or 98% or 99% or 99.5% sequence identity to SEQ ID NO: 15, 26, 27, 28 or 29.

wherein said viral vector further comprises a promoter operably linked to said transgene, wherein said promoter preferably comprises SEQ ID NO: 4 or SEQ ID NO: 14; wherein the nucleic acid construct comprised in said viral vector comprises a polyadenylation signal sequence and wherein said viral vector further comprises inverted terminal repeat (ITR) at 5' and/or 3' flanking said nucleic acid construct, preferably a 5'ITR and a 3'ITR; and wherein optionally the polyadenylation signal sequence comprising SEQ ID NO: 17.

~~813~~. The viral vector according to any one of claims ~~611~~ or ~~712~~, wherein said transgene encodes a gamma butyric acid (GABA) transporter protein 1 (GAT-1) comprising SEQ ID NO: 18.

~~14. The viral vector according to any one of claims 11 to 13, wherein the polyadenylation signal sequence comprising SEQ ID NO: 17.~~

~~915~~. A viral particle comprising the viral vector according to any one of claims ~~11 to 146~~ to 8; wherein the viral particle optionally comprises at least a VP1 capsid protein from an AAV, wherein said capsid protein preferably comprises AAV2, AAV5, AAV6, AAV8, AAV9 (such as comprising SEQ ID NO: 25), AAV10, AAV-true type (AAVtt) or combinations thereof; wherein preferably the capsid protein is from AAV9 and preferably comprising SEQ ID NO: 25 or AAVtt and preferably comprises SEQ ID NO: 24 or it is at least 98.5%, preferably 99% or 99.5% identical to SEQ ID NO: 24.

~~16. The viral particle according to claim 15, wherein the viral particle comprises at least a VP1 capsid protein from an AAV, wherein said capsid protein preferably comprises AAV2, AAV5, AAV6, AAV8, AAV9 (such as comprising SEQ ID NO: 25), AAV10, AAV-true type (AAVtt) or combinations thereof.~~

~~17. The viral particle according to claim 16, wherein the capsid protein is from AAV9 and preferably comprising SEQ ID NO: 25 or AAVtt and preferably comprises SEQ ID NO: 24 or it is at least 98.5%, preferably 99% or 99.5% identical to SEQ ID NO: 24.~~

~~1018~~. A plasmid comprising the nucleic acid construct according to any one of claims 1 ~~or 2 to 4~~ or the viral vector according to any one of claims ~~3, 6 to 8-5 to 7 or 11 to 14~~.

~~1119~~. A host cell for producing a viral particle according to any one of claims ~~4 or 5 8 to 10 or 15 to 17~~; wherein the host cell preferably comprises:

~~a. a nucleic acid construct according to any one of claims 1 or 2 or the viral vector according to any one of claims 3, 6 to 8;~~

~~b. a nucleic acid construct, preferably a plasmid, encoding AAV rep and/or cap genes which does not carry the ITR sequences; and, optionally a nucleic acid construct, for example a plasmid or virus, comprising viral helper genes.~~

~~20. The host cell according to claim 19, wherein the host cell comprises:~~

~~a. a nucleic acid construct according to any one of claims 1 to 4 or the viral vector according to any one of claims 5 to 7 or 11 to 14;~~

~~b. a nucleic acid construct, preferably a plasmid, encoding AAV rep and/or cap genes which does not carry the ITR sequences; and, optionally~~

~~c. a nucleic acid construct, for example a plasmid or virus, comprising viral helper genes.~~

~~1221~~. A method of producing a viral particle according to any one of claims ~~4 or 5 or 98 to 10 or 15 to 17~~, the method comprising the step of:

~~a. culturing a host cell according to claim 11 any one of claims 18 or 19 in a culture medium; and~~

~~b. harvesting the viral particles from the host cell culture media and/or inside the host cells.~~

~~1322~~. A pharmaceutical composition comprising a nucleic acid construct according to any one of claims 1 ~~or 2 to 4~~ or the viral vector according to any one of claims ~~5 to 7 or 11 to 14~~, ~~6 to 8~~ or a viral particle according to any one of claims ~~8 to 10 or 15 to 17~~ ~~4 or 5 or 9~~, in combination with one or more pharmaceutical acceptable excipient, diluent or carrier.

~~1423~~. The viral particles according to any one of claims ~~8 to 10 or 15 to 17~~, ~~4, 5 or 9~~ for use in therapy.

~~1524~~. The viral particles according to any one of claims ~~8 to 10 or 15 to 17~~, ~~4, 5 or 9~~ for use in the treatment and/or prevention of a disease characterised by SLC6A1 haploinsufficiency, wherein the disease preferably comprises single-gene epilepsies accompanied by cognitive,

motor behavioural comorbidities, early onset developmental and epileptic encephalopathy, epileptic encephalopathy, childhood onset Epilepsy Syndromes, myoclonic atonic epilepsy (MAE), MEA-like and other epilepsy indications such as Lennox Gastaut Syndrome as well as autism spectrum disorder and schizophrenia or diseases associated with impaired GABA uptake or combinations thereof.

1625. The viral particle for use according to any one of claims 23 or 24, wherein the use is for restoring GAT-1 function and/or decreasing seizure frequency.

1726. The viral particle for use according to any one of claims ~~8 to 10 or 15 to 174~~, 5 or 9, wherein said disease is associated with at least one mutation in a patient which leads to a pathological GAT-1 variant, wherein said pathological GAT-1 variants comprises a mutation or combinations of mutations; wherein said mutation preferably comprises, with reference to SEQ ID NO: 18, 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, V342M, A357V, G362R, L366V, A367T, F385L, G393S, S456R, S459R, M487T, V511L, G550R or combination thereof.

~~27. The viral particle for use according to claim 26, wherein said mutation comprises, with reference to SEQ ID NO: 18, 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, V342M, A357V, G362R, L366V, A367T, F385L, G393S, S456R, S459R, M487T, V511L, G550R or combination thereof.~~

1828. Use of the viral particles according to any one of claims 4 or 5 or 9 in the manufacture of a medicament ~~A method offor~~ treating and/or preventing a disease characterised by SLC6A1 haploinsufficiency, wherein the disease preferably comprises single-gene epilepsies accompanied by cognitive, motor behavioural comorbidities, early onset developmental and epileptic encephalopathy, epileptic encephalopathy, childhood onset Epilepsy Syndromes, myoclonic atonic epilepsy (MAE), MEA-like and other epilepsy indications such as Lennox Gastaut Syndrome as well as autism spectrum disorder and schizophrenia or diseases associated with impaired GABA uptake or combinations thereof, with or without autism and/or schizophrenia, ~~the method comprising administering to a subject in need thereof of viral particles according to any one of claims 8 to 10 or 15 to 17.~~

1929. The usemethod according to claim 1828, wherein the medicament restores method is ~~for restoring~~ GAT-1 function and/or decreasing seizure frequency.

2030. The ~~use method~~ according to any one of claims ~~1828~~ or ~~1929~~, wherein said disease is associated with at least one mutation in a patient which leads to a pathological GAT-1 variant, wherein said pathological GAT-1 variants comprises a mutation or combinations of mutations; wherein the mutation optionally-

~~31. The method according to claim 30 wherein said mutation~~ comprises, with reference to SEQ ID NO: 18, 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, G550R or combinations thereof.