CLAIMS

What is claimed is:

~~1. An isolated nucleic acid encoding aspartoacyltransferase (ASPA) comprising a nucleic acid sequence at least about 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:2.~~

~~2. A~~

1. An isolated or modified nucleic acid encoding aspartoacyltransferase (ASPA) comprising a nucleic acid sequence at least about 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:2.

2.

~~3. A~~ A vector genome comprising ~~a~~the modified nucleic acid ~~encoding aspartoacyltransferase (ASPA) comprising a nucleic acid sequence at least about 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:2.~~

~~4. The vector genome~~ of claim ~~3~~1.

3. The vector genome of claim 2, wherein the vector genome is a recombinant adeno-associated virus (rAAV) vector genome~~.~~

~~5. The vector genome of claim 3 or 4, wherein the vector genome is,~~ self-complementary, or both.

~~6~~4. A recombinant adeno-associated virus (rAAV) vector comprising ~~a vector genome comprising a modified nucleic acid comprising a nucleic acid sequence at least about 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the nucleic acid sequence~~the vector genome of any one of ~~SEQ ID NO:2~~claims 2 and 3, and a capsid selected from the group consisting of a capsid of Olig001, Olig002, Olig003, AAV1, AAV2, AAV3, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7,

AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh10, AAVrh74, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAVhu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, AAV2i8, AAV2G9, AAV2i8G9, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAV-DJ, AAV-DJ/8, AAV-DJ/9 and AAV-LK03.

~~7~~5. The rAAV vector of claim ~~6,~~ 4, comprising one or more of the following features:

_____wherein the capsid is an Olig001, an Olig002 or an Olig003 capsid~~.~~;

~~8. The rAAV vector of claim 6 or 7,~~ _____wherein the capsid is an Olig001 capsid comprising a viral protein 1(VP1) and wherein the VP1 comprises an amino acid sequence at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14~~.~~;

~~9. The rAAV vector of any one of claims 6-8,~~ ____wherein the capsid is an Oligo001 capsid comprising a VP1 and wherein the VP1 comprises the amino acid sequence of SEQ ID NO:14~~.~~;

~~10. The rAAV vector of any one of claim 6-9,~~ _____wherein the vector genome is self-complementary~~.~~;_____

~~11. The rAAV vector of any one of claims 6-10,~~ ___wherein the vector genome further comprises at least one element selected from the group consisting of at least one AAV inverted terminal repeat (ITR) sequence, an enhancer, a promoter, an exon, an intron, and a poly-adenylation (polyA) signal sequence~~.~~;

~~12. The rAAV vector of any one of claims 6-11,~~ wherein the vector genome further comprises at least one element selected from the group consisting of at least one AAV2 ITR, a cytomegalovirus (CMV) enhancer, a hybrid form of the CBA promoter (CBh promoter), a chicken B-actin (CBA) exon, a CBA intron, an minute virus of mice (MVM) intron and a bovine growth hormove (BGH) polyA~~.~~; and

~~13. The rAAV vector of any one of claims 6-12,~~ ___wherein the vector genome further comprises a least one element selected from the group consisting of at least one ITR comprising the nucleic acid sequence of SEQ ID NO:5, SEQ ID NO:12 or SEQ ID NO:19, an enhancer comprising the nucleic acid sequence of SEQ ID NO:6 or SEQ ID NO:17, a promoter comprising the nucleic acid sequence of SEQ ID NO:7, an exon comprising the nucleic acid sequence of SEQ ID NO:8 or SEQ ID NO:18, an intron comprising the nucleic acid sequence of SEQ ID NO:9, an intron comprising the nucleic acid sequence of SEQ ID NO:10 and a polyA comprising the nucleic acid sequence of SEQ ID NO:11.

~~14~~6. An rAAV vector comprising a vector genome comprising from 5' to 3':

a) an AAV inverted terminal repeat (ITR) comprising the nucleic acid sequence of SEQ ID NO:5, SEQ ID NO:12 or SEQ ID NO:19;

b) an enhancer comprising the nucleic acid sequence of SEQ ID NO:6 or SEQ ID NO:17;

c) a promoter comprising the nucleic acid sequence of SEQ ID NO:7;

d) an exon comprising the nucleic acid sequence of SEQ ID NO:8 or SEQ ID NO:18;

e) an intron comprising the nucleic acid sequence of SEQ ID NO:9;

f) an intron comprising the nucleic acid sequence of SEQ ID NO:10;

g) a modified nucleic acid encoding aspartoacyltransferase (ASPA) comprising the nucleic acid sequence of SEQ ID NO:2

h) a polyA comprising the nucleic acid sequence of SEQ ID NO:11; and

i) an AAV ITR comprising the nucleic acid sequence of SEQ ID NO:5, SEQ ID NO:12 or SEQ ID NO:19.

~~15~~7. The rAAV vector of claim ~~14,~~ 6, comprising one or more of the following features:

_____wherein the nucleic acid is self-complementary~~.~~ and

~~16. The rAAV vector of claim 14 or 15,~~ ___wherein the vector comprises an Olig001 capsid comprising a viral protein 1 (VP1) and wherein the VP1 comprises the amino acid sequence of SEQ ID NO:14.

~~17. An rAAV vector comprising an Olig001 capsid comprising a viral protein 1 (VP1) and wherein the VP1 comprises the amino acid sequence of SEQ ID NO:14 and a self-complementary nucleic acid comprising from 5' to 3':~~

~~a) an AAV2 inverted terminal repeat (ITR) comprising the nucleic acid sequence of SEQ ID NO:5, SEQ ID NO:12 or SEQ ID NO:19;~~

~~b) a CMV enhancer comprising the nucleic acid sequence of SEQ ID NO:6 or SEQ ID NO:17;~~

~~c) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:7;~~

~~d) a CBA exon 1 comprising the nucleic acid sequence of SEQ ID NO:8 or SEQ ID NO:18;~~

~~e) a CBA intron 1 comprising the nucleic acid sequence of SEQ ID NO:9;~~

~~f) an MMV intron comprising the nucleic acid sequence of SEQ ID NO:10;~~

~~g) a modified nucleic acid encoding aspartoacyltransferase (ASPA) comprising the nucleic acid sequence of SEQ ID NO:2;~~

~~h) a BGH polyA comprising the nucleic acid sequence of SEQ ID NO:11; and~~

~~i) an AAV2 ITR comprising the nucleic acid sequence of SEQ ID NO:5, SEQ ID NO:12 or SEQ ID NO:19.~~

~~18.~~ 8. A pharmaceutical composition comprising the rAAV vector of any one of claims ~~6-17~~4-7.

~~19. A method of treating and/or preventing a disease, disorder or condition associated with deficiency or dysfunction of ASPA, the method comprising administering a therapeutically effective amount of the rAAV vector of any one of claims 6-17, or the pharmaceutical composition of claim 17.~~

~~20. The method of claim 19, wherein the disease, disorder or condition associated with deficiency or dysfunction of ASPA is Canavan disease.~~

~~21. The method of claim 19 or 20, wherein the rAAV vector is administered directly to the brain and/or central nervous system.~~

~~22. The method of any one of claims 19-21, wherein the rAAV vector is administered to a region of the central nervous system selected from the group consisting of brain parenchyma, spinal canal, subarachnoid space, a ventricle of the brain, cisterna magna and a combination thereof.~~

~~23. The method of any one of claims 19-22, wherein the rAAV vector is administered by a method selected from the group consisting of intraparenchymal administration, intrathecal administration, intracerebroventricular administration, intracisternal magna administration and a combination thereof.~~

~~24~~9. A host cell comprising the isolated nucleic acid ~~of claim 1,~~or the modified nucleic acid of claim ~~2~~1, the vector genome of any one of claims 2-3 ~~5~~ or the rAAV vector of any one of claims ~~6-17~~4-7.

~~25~~10. The host cell of claim ~~24, 9~~, comprising one or more of the following features:

_____wherein the cell is selected from the group consisting of VERO, WI38, MRC5, A549, HEK293, B-50 or any other HeLa cell, HepG2, Saos-2, HuH7, and HT1080~~.~~;

~~26. The host cell of claim 25,~~ _____wherein the cell is a HEK293 cell adapted to growth in suspension culture~~.~~;

~~27. The host cell of claim 26,~~ _____wherein the cell is a HEK293 cell having American Type Culture Collection (ATCC) No. PTA 13274~~.~~; and

~~28. The host cell of any one of claims 25-27,~~ _____wherein the cell comprises at least one nucleic acid encoding at least one protein selected from the group consisting of an AAV rep

protein, an AAV capsid (Cap) protein, an adenovirus (Ad) early region 1A (E1a) protein, an Ad E1b protein, an Ad E2a protein, an Ad E4 protein and a viral associated (VA) RNA.

~~29~~11. A kit for the treatment of Canavan disease (CD), comprising a therapeutically effective amount of an isolated nucleic acid ~~of claim 1,~~or a modified nucleic acid of claim ~~2~~1, a vector genome of any one of claims 2-3~~-5~~, an rAAV vector of any one of claims ~~6-17~~4-7, or a pharmaceutical composition of claim ~~18.~~8, and optionally,

~~30. The kit of claim 29,~~ ___wherein the kit further comprises a label or insert including instructions for using one or more of the kit components.

~~31~~12. An isolated nucleic acid ~~of claim 1,~~or a modified nucleic acid of claim ~~2~~1, a vector of any one of claims 2-3~~-5~~, an rAAV vector of any one of claims ~~6-17~~4-7 or a pharmaceutical composition of claim ~~18~~8 for use in treating or preventing a disease, disorder or condition associated with deficiency or dysfunction of ASPA.

~~32~~

13. The isolated nucleic acid, modified nucleic acid, vector genome, rAAV vector, or pharmaceutical composition for use of claim ~~31~~12, wherein the disease, disorder or condition is Canavan disease.

~~33~~14. Use of an isolated nucleic acid ~~of claim 1,~~or a modified nucleic acid of claim ~~2~~1, a vector of any one of claims 2-3~~-5~~, or an rAAV vector of any one of claims ~~6-17~~4-7 in the manufacture of a medicament for treating and/ or preventing a disease, disorder of condition associated with deficiency or dysfunction of ASPA.

~~34~~15. The use of claim ~~33~~14, wherein the disease, disorder or condition is Canavan disease.

~~35~~16. A method of determining biodistribution of a transgene in the brain of a subject wherein the transgene is expressed from an rAAV vector comprising an Olig001 capsid, the method comprising

a) administration of the rAAV vector to the subject by intracrebroventricular (ICV) injection or by intraparenchymal (IP) injection;

b) fixation of the brain;

c) electrophoretic clearing of the brain;

d) 3D microscopic imaging of a brain tissue section;

e) quantification of transgene expression.

~~36~~17. The method of claim ~~35,~~ 16, comprising one or more of the following features:

_____wherein the transgene encodes a marker and wherein the marker is green fluorescent protein (GFP~~).~~);

~~37. The method of claim 35,~~ _____wherein the transgene encodes ASPA~~.~~;

~~38. The method of any one of claims 35-37,~~ wherein the transgene expression correlates with rAAV vector transduction efficiency~~.~~;

~~39. The method of any one of claims 35-38,~~ further comprising step (f) comprising evaluation of cell-type tropism by assessment of cell morphology and spatial location determination~~.~~; and

~~40. The method of any one of claims 35-39,~~ further comprising volumetric rendering of transgene expression.