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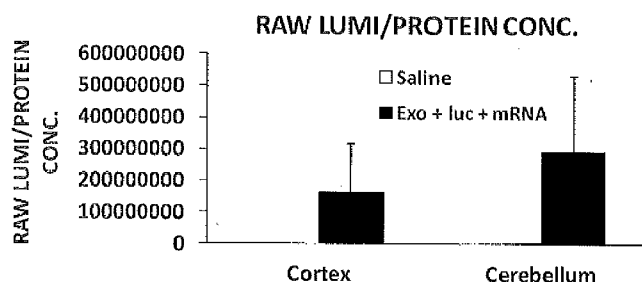
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(54) Title: METHOD FOR TREATING A CENTRAL NERVOUS SYSTEM DISORDER

A.

Figure 2



(57) Abstract: A method of treating a central nervous system disorder in a mammal is provided. The method comprises administering to the mammal a therapeutically effective amount of non-naturally occurring exosomes engineered to comprise nucleic acid encoding a functional neuropeptide and/or the neuropeptide.

METHOD FOR TREATING A CENTRAL NERVOUS SYSTEM DISORDER

Field of the Invention

[0001] The present invention generally relates to treatment of a central nervous system disorder, and more particularly relates to a method of treating a disorder such as a recessive central nervous system disorder using exosomes.

Background of the Invention

[0002] Hereditary central nervous system (CNS) disorders encompass a large group of disorders, which share a common origin of pathology based on a deficiency or dysfunction of a protein due to mutations in one or more genes encoding a protein found in the CNS. Symptoms of CNS disorders vary considerably between the individual disorders and disease severity, but generally involve conditions relating to motor impairment (spasticity, rigidity, tremor, ataxia, weakness, hypotonia), cognitive impairment (speech delay or regression, intellectual disability/dementia or regression), seizures, retinal and/or cortical blindness, sensori-neural hearing loss, speech disorders (spastic or scanning dysarthria), dysphagia, or psychiatric disorders (psychosis, depression, anxiety, bipolar disorder). These groups of disorders include genetic mutations that are either dominant or recessive in nature. Unlike autosomal dominant disorders that often have a dominant negative effect, an important consideration in both autosomal recessive and X-linked recessive disorders is that genetic replacement of the mutant allele would constitute a functional cure by restoring the normal function of the gene. Given that carriers of autosomal recessive disorders do not have clinical or functional consequences, a recovery of at least 50% of the relevant protein activity would constitute a cure. Similarly, female carriers of X-linked recessive disorders without skewed X-inactivation (> 90 %) do not have clinical or functional consequences, implying that the restoration of > 10 % of the relevant protein would constitute a cure.

[0003] One method of classifying hereditary CNS disorders is to group them into categories based on their general phenotypic characteristics. These categories may include the following groups of recessive CNS disorders: seizure disorders, spino-cerebellar ataxia (SCA), movement disorders, small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases. One example

of an X-linked recessive seizure disorder is creatine transporter deficiency 1 (CTD1), caused by mutation of the solute carrier family 6 (neurotransmitter transporter), member 8 (SLC6A8) gene. Due to the absence of a functional creatine transporter (CrT) protein, hemizygous males experience a depletion of creatine in the brain. This creatine deficiency results in symptoms such as seizures, intellectual disability and behavioural abnormalities, for which there is presently no established cure or treatment method available.

[0004] One of the greatest limitations hindering the treatment of CNS disorders is that the vast majority of potential treatments such as neurotherapeutics and small molecules, are unable to cross the blood-brain barrier and thus, cannot access the brain. Owing largely to this challenge of delivering therapeutics into the CNS and to the inherent difficulty in correcting recessive CNS disorders resulting from gene mutations, there are presently no cures available. The current mainstay of treatment is thus based on supportive care designed to treat individual symptoms of the disorders and enhance quality of life for patients. Examples of present treatments include; anti-epileptic drugs, cholinergic drugs for cognitive impairment, anti-psychotic drugs, movement disorder drugs (dopamine agonists, L-DOPA, anti-cholinergics), anti-depressants, anti-mania agents, cognitive behavioural therapy, speech therapy, bracing, anti-spasticity agents, correctional surgery (contractures), orthopedic devices (braces, orthotics), and gait assistive devices.

[0005] In addition to these supportive treatments, numerous gene therapy-based methods have been investigated in attempt to cure recessive CNS disorders, but these approaches have not adequately fulfilled the requirements of a desirable therapy due to fundamental issues such as: an inability to cross the blood-brain-barrier, high immunogenicity, toxicity, the induction of an inflammatory response, the promotion of tumorigenesis and low to nil therapeutic efficacy, among others.

[0006] Thus, there is a need to develop improved methods of treating CNS disorders.

Summary of the Invention

[0007] It has now been determined that exosomes may be effectively used as a vehicle to deliver nucleic acid encoding a protein to a mammal to treat CNS disorders, including recessive CNS disorders such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders

(such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases that result from a deficiency of a functional protein.

[0008] Thus, in one aspect of the invention, a method of treating a CNS disorder is provided comprising administering to the mammal exosomes that are genetically modified to incorporate a nucleic acid encoding a functional protein neuropeptide and/or the nue.

[0009] In another aspect, a method of increasing the amount or activity of a neuropeptide in the central nervous system of a mammal is provided, comprising administering to the mammal exosomes that are genetically modified to incorporate a nucleic acid encoding a functional neuropeptide and/or the neuropeptide.

[0010] In a further aspect, exosomes genetically engineered to incorporate nucleic acid encoding a functional neuropeptide and/or a neuropeptide are provided.

[0011] Additional aspects of the invention include aspects and variations set forth in the following lettered paragraphs:

[0012] A1. An exosome produced by a process that comprises: (a) isolating exosomes from a biological sample from an organism (autologous) or from a conditioned medium from a cultured cell (allogenic or xenogenic); and (b) introducing a modification into the exosome selected from the group consisting of:

- (i) at least one nucleic acid comprising a nucleotide sequence that encodes a functional neuropeptide or precursor thereof;
- (ii) at least one fusion product comprising a brain targeting sequence linked to an exosomal membrane marker;
- (iii) at least one nucleic acid comprising a nucleotide sequence that encodes the fusion product; and
- (iv) two or more of (i), (ii) and (iii).

[0013] A2. The exosome according to paragraph A1, wherein the isolating includes precipitation of exosomes using polyethylene glycol, and resuspension in a saccharide solution such as a trehalose solution.

[0014] A2.1 The exosome according to any one of paragraphs A1 or A2, wherein the isolating removes vesicles that are greater than 140 nm in diameter.

[0015] A3. The exosome according to paragraph A1 or A2 or A2.1, wherein the biological sample is from a mammal, or the cell is from a mammal or a mammalian cell line.

[0016] A4. The exosome according to any one of paragraphs A1 to A3, wherein the isolating removes vesicles and cellular debris less than 20 nm in diameter.

[0017] A5. An exosome that comprises a modification selected from the group consisting of:

- (i) at least one nucleic acid comprising a nucleotide sequence that encodes a functional neuropeptide or precursor thereof;
- (ii) at least one fusion product comprising a nerve targeting sequence linked to an exosomal membrane marker;
- (iii) at least one nucleic acid comprising a nucleotide sequence that encodes the fusion product; and
- (iv) two or more of (i), (ii) and (iii).

[0018] B1. The exosome according to any of paragraphs A1 – A5, having a diameter of 20-140 nm.

[0019] B2. The exosome according to any of paragraphs A1 – A5, that comprises a nucleic acid comprising a nucleotide sequence encoding a functional neuropeptide or precursor thereof, wherein the nucleic acid is present in a lumen of the exosome.

[0020] B2.1. The exosome according to paragraph B2, wherein the nucleic acid comprises a species of RNA or a species of modified RNA (modRNA, e.g. 5 methyl cytosine, or N6 methyladenine) encoding for a protein set forth in Table 1, Table 2, Table 3 and/or Table 4.

[0021] B3. The exosome according to paragraph B2 or B2.1, wherein the protein comprises one or more of the proteins set forth in Table 1, Table 2, Table 3 and/or Table 4.

[0022] B4. The exosome according to paragraph B3, wherein the protein is an enzyme.

[0023] B5. The exosome according to any one of paragraphs B2 – B2.1, wherein the nucleic acid encoding for the protein is selected from the group consisting of solute carrier family 6 (neurotransmitter transporter), member 8 (SLC6A8), guanidinoacetate N-methyltransferase (GAMT), solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1), cystatin B (CSTB), epilepsy, progressive myoclonus type 2A, Lafora disease (laforin) (EPM2A), NHL repeat containing E3 ubiquitin protein ligase 1 (NHLRC1), aristaless related homeobox (ARX), solute carrier family 25 (mitochondrial carrier: glutamate), member 22 (SLC25A22), Cdc42 guanine nucleotide exchange factor 9 (ARHGEF9), phospholipase C beta 1 (PLCB1), ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3), TBC1 domain family member 24 (TBC1D24), seizure threshold 2 homolog (mouse) (SZT2), phosphatidylinositol glycan anchor biosynthesis class A (PIGA), NECAP endocytosis associated 1 (NECAP1), dedicator of cytokinesis 7 (DOCK7), solute carrier family 13 (sodium-dependent citrate transporter), member 5 (SLC13A5), WW domain containing oxidoreductase (WWOX), alanyl-tRNA synthetase (AARS), solute carrier family 12 (potassium/chloride transporter), member 5 (SLC12A5), inosine triphosphatase (ITPA), polynucleotide kinase 3'-phosphatase (PNKP), three prime repair exonuclease 1 (TREX1), ribonuclease H2 subunit B (RNASEH2B), ribonuclease H2 subunit C (RNASEH2C), ribonuclease H2 subunit A (RNASEH2A), SAM domain and HD domain 1 (SAMHD1), adenosine deaminase, RNA-specific (ADAR), phosphomannomutase 2 (PMM2), mannose phosphate isomerase (MPI), ALG6, alpha-1,3-glucosyltransferase (ALG6), ALG3, alpha-1,3- mannosyltransferase (ALG3), dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit (DPM1), mannose-P-dolichol utilization defect 1 (MPDU1), ALG12, alpha-1,6-mannosyltransferase (ALG12), ALG8, alpha-1,3-glucosyltransferase (ALG8), ALG2, alpha-1,3/1,6-mannosyltransferase (ALG2), dolichyl-phosphate N-acetylglucosaminophosphotransferase 1 (DPAGT1), ALG1,

chitobiosyldiphosphodolichol beta-mannosyltransferase (ALG1), ALG9, alpha-1,2-mannosyltransferase (ALG9), dolichol kinase (DOLK), RFT1 homolog (RFT1), dolichyl-phosphate mannosyltransferase subunit 3 (DPM3), ALG11, alpha-1,2-mannosyltransferase (ALG11), steroid 5 alpha-reductase 3 (SRD5A3), dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic) (DDOST), ALG13, UDP-N-acetylglucosaminyltransferase subunit (ALG13), phosphoglucomutase 1 (PGM1), dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit (DPM2), STT3A, catalytic subunit of the oligosaccharyltransferase complex (STT3A), STT3B, catalytic subunit of the oligosaccharyltransferase complex (STT3B), signal sequence receptor, delta (SSR4), carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2), mannosyl-oligosaccharide glucosidase (MOGS), solute carrier family 35 member C1 (SLC35C1), UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 (B4GALT1), component of oligomeric golgi complex 7 (COG7), solute carrier family 35 member A1 (SLC35A1), component of oligomeric golgi complex 1 (COG1), component of oligomeric golgi complex 8 (COG8), component of oligomeric golgi complex 5 (COG5), component of oligomeric golgi complex 4 (COG4), transmembrane protein 165 (TMEM165), component of oligomeric golgi complex 6 (COG6), solute carrier family 39 member 8 (SLC39A8), senataxin (SETX), aprataxin (APTX), SIL1 nucleotide exchange factor (SIL1), excision repair cross-complementation group 8 (ERCC8), excision repair cross-complementation group 6 (ERCC6), saccin molecular chaperone (SACS), proteolipid protein 1 (PLP1), ATP binding cassette subfamily B member 7 (ABC7), vaccinia related kinase 1 (VRK1), exosome component 3 (EXOSC3), exosome component 8 (EXOSC8), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 2 (TSEN2), tRNA splicing endonuclease subunit 34 (TSEN34), Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS), VPS53 GARP complex subunit (VPS53), piccolo presynaptic cytomatrix protein (PCLO), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 54 (TSEN54), arginyl-tRNA synthetase 2, mitochondrial (RARS2), charged multivesicular body protein 1A (CHMP1A), adenosine monophosphate deaminase 2 (AMPD2), cleavage and polyadenylation factor I subunit 1 (CLP1), peptidase (mitochondrial processing) alpha (PMPCA), tripeptidyl peptidase I (TPP1), spectrin repeat containing, nuclear envelope 1 (SYNE1), anoctamin 10 (ANO10), spectrin beta, non-

erythrocytic 2 (SPTBN2), STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase (STUB1), glutamate ionotropic receptor delta type subunit 2 (GRID2), sorting nexin 14 (SNX14), ornithine carbamoyltransferase (OTC), argininosuccinate synthase 1 (ASS1), argininosuccinate lyase (ASL), arginase 1 (ARG1), carbamoyl-phosphate synthase 1 (CPS1), Hemochromatosis (HFE), ATPase copper transporting alpha (ATP7A), ATPase copper transporting beta (ATP7B), molybdenum cofactor synthesis 1 (MOCS1), molybdenum cofactor synthesis 2 (MOCS2), pantothenate kinase 2 (PANK2), phospholipase A2 group VI (PLA2G6), ferritin, light polypeptide (FTL), chromosome 19 open reading frame 12 (C19orf12), Coenzyme A synthase (COASY), ATPase 13A2 (ATP13A2), fatty acid 2-hydroxylase (FA2H), DDB1 and CUL4 associated factor 17 (DCAF17), fucosidase, alpha-L-1, tissue (FUCA1), kinesin family member 1A (KIF1A), fumarylacetoacetate hydrolase (fumarylacetoacetase) (FAH), tyrosine aminotransferase (TAT), 4-hydroxyphenylpyruvate dioxygenase (HPD), methylenetetrahydrofolate reductase (NAD(P)H) (MTHFR), sulfite oxidase (SUOX), phenylalanine hydroxylase (PAH), branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB), dihydrolipoamide branched chain transacylase E2 (DBT), glutaryl-CoA dehydrogenase (GCDH), methylmalonyl-CoA mutase (MUT), holocarboxylase synthetase (HLCS), propionyl-CoA carboxylase alpha subunit (PCCA), isovaleryl-CoA dehydrogenase (IVD), peroxisomal biogenesis factor 1 (PEX1), peroxisomal biogenesis factor 12 (PEX12), peroxisomal biogenesis factor 6 (PEX6), peroxisomal biogenesis factor 2 (PEX2), peroxisomal biogenesis factor 10 (PEX10), peroxisomal biogenesis factor 26 (PEX26), peroxisomal biogenesis factor 16 (PEX16), peroxisomal biogenesis factor 3 (PEX3), peroxisomal biogenesis factor 13 (PEX13), peroxisomal biogenesis factor 19 (PEX19), peroxisomal biogenesis factor 14 (PEX14), ATP binding cassette subfamily D member 1 (ABCD1), hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4), histidyl-tRNA synthetase 2 (HARS2), caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP), leucyl-tRNA synthetase 2 (LARS2) and chromosome 10 open reading frame 2 (C10orf2).

[0024] B6. The exosome according to any one of paragraphs B2 – B5, further comprising at least one fusion product comprising a brain targeting sequence linked to an exosomal membrane marker.

[0025] B7. The exosome according to any one of paragraphs A1 – A5 or B1, that comprises at least one fusion product comprising a brain targeting sequence linked to an exosomal membrane marker.

[0026] B8. The exosome according to paragraph B6 or B7, wherein the exosomal membrane marker is selected from the group consisting of CD9, CD37, CD53, CD63, CD81, CD82, CD151, an integrin, ICAM-1, CDD31, an annexin, TSG101, ALIX, lysosome-associated membrane protein 1, lysosome-associated membrane protein 2, lysosomal integral membrane protein and a fragment of any exosomal membrane marker that comprises at least one intact transmembrane domain.

[0027] B9. The exosome according to any one of paragraphs B6 – B8, wherein the brain targeting sequence is YTIWMPENPRPGTPCDIFTNSRGKRASNG (SEQ ID NO: 1) or a fragment thereof.

[0028] B10. The exosome according to any one of paragraphs B6 – B9, wherein the fusion product is a fusion protein.

[0029] B11. The exosome according to paragraph B10, further wherein the fusion protein includes a peptide linker between the brain targeting sequence and the exosomal membrane marker.

[0030] B12. The exosome according to any one of paragraphs B6-B10, wherein the fusion product includes a transmembrane domain and localizes in a membrane of the exosome.

[0031] C1. A composition comprising exosomes according to any one of paragraphs A1 – A5, and a pharmaceutically acceptable carrier.

[0032] C2. The composition according to paragraph C1, wherein the composition is substantially free of vesicles having a diameter less than 20 nm.

[0033] C3. The composition according to paragraph C1 or C2, wherein the composition is substantially free of vesicles having a diameter greater than 140 nm.

[0034] D1. A method of increasing the amount of a neuropeptide in the central nervous system of a mammal, comprising administering to the mammal an exosome according to any one of paragraphs A1 – B12, or a composition according to any one of paragraphs C1 – C3.

[0035] D2. Use of an exosome according to any one of paragraphs A1 – B12, or a composition according to any one of paragraphs C1 – C3, for increasing the amount of a neuropeptide in a mammal.

[0036] D3. A method of treating a CNS disorder in a mammal comprising administering to the mammal an exosome according to any one of paragraphs A1 – B12, or a composition according to any one of paragraphs C1 – C3.

[0037] D4. Use of an exosome according to any one of paragraphs A1 – B12, or a composition according to any one of paragraphs C1 – C3, for treating recessive disorders such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases in a mammal.

[0038] D5. The method or use according to any one of paragraphs D1 – D4, wherein the mammal is human.

[0039] D6. The method or use according to paragraph D5, wherein the human has a recessive CNS disorder selected from the group consisting of Cerebral creatine deficiency syndrome (CCDS) 1, CCDS 2, GLUT1 deficiency syndrome 1, infantile onset, severe, Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg), Epilepsy, progressive myoclonic 2A (Lafora), Epilepsy, progressive myoclonic 2B (Lafora), Epileptic encephalopathy, early infantile (EIEE) Type 1, EIEE Type 3, EIEE Type 8, EIEE Type 12, EIEE Type 15, EIEE Type 16, EIEE Type 18, Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2) / EIEE Type 20, EIEE Type 21, EIEE Type 23, EIEE Type 25, EIEE Type 28, EIEE Type 29, EIEE Type 34, EIEE Type 35, Microcephaly, seizures, and developmental delay (MCSZ), Aicardi-Goutieres syndrome (AGS) Type 1, AGS Type 2, AGS Type 3, AGS Type 4, AGS Type 5, AGS Type 6, Congenital disorder of glycosylation (CDG), Type 1a, CDG Type 1b, CDG Type 1c, CDG Type 1d, CDG Type 1e, CDG Type 1f, CDG Type 1g, CDG Type 1h, CDG Type 1i, CDG Type 1j,

CDG Type 1k, CDG Type 1l, CDG Type 1m, CDG Type 1n, CDG Type 1o, CDG Type 1p, CDG Type 1q, CDG Type 1r, CDG Type 1s, CDG Type 1t, CDG Type 1u, CDG Type 1w, CDG Type 1x, CDG Type 1y, CDG Type 1z, CDG Type IIa, CDG Type IIb, CDG Type IIc, CDG Type IId, CDG Type IIe, CDG Type IIe, CDG Type IIe, CDG Type IIg, CDG Type IIh, CDG Type Iii, CDG Type IIj, CDG Type IIk, CDG Type III, CDG Type IIn, Spinocerebellar ataxia, autosomal recessive 1, Ataxia, early-onset, with oculomotor apraxia and hypoalbuminemia, Marinesco-Sjogren syndrome, Cockayne syndrome, type A / UV-sensitive syndrome 2, Cockayne syndrome, type B / Cerebrooculofacioskeletal syndrome 1 / De Sanctis-Cacchione syndrome / UV-sensitive syndrome, Spastic ataxia, Charlevoix-Saguenay type, Pelizaeus-Merzbacher disease / Spastic paraplegia 2, X-linked, Anemia, sideroblastic, with ataxia, Pontocerebellar hypoplasia (PCH), type 1a, PCH type 1b, PCH type 1c, PCH type 2a, PCH type 2b, PCH type 2c, PCH type 2d, PCH type 2e, PCH type 3, PCH type 4, PCH type 5, PCH type 6, PCH type 8, PCH type 9, PCH type 10, Spinocerebellar ataxia, autosomal recessive (SCAR) 2 (SCAR2), SCAR7, SCAR8, SCAR10, SCAR14, SCAR16, SCAR18, SCAR20, Ornithine transcarbamylase deficiency, Citrullinemia, Argininosuccinic aciduria, Argininemia, Carbamoylphosphate synthetase I deficiency, Hemochromatosis, Menkes disease, Wilson disease, Molybdenum cofactor deficiency A, Molybdenum cofactor deficiency B, Neurodegeneration with brain iron accumulation (NBIA) 1 / HARP syndrome, NBIA 2B / Infantile neuroaxonal dystrophy 1 / Parkinson disease 14, autosomal recessive, NBIA 3, NBIA 4, NBIA 6, Kufor-Rakeb syndrome, Spastic paraplegia 35, Woodhouse-Sakati syndrome, Fucosidosis, Spastic paraplegia 30 / neuropathy, hereditary sensory type IIc / mental retardation, autosomal dominant 9, Tyrosinemia (TYRSN), type 1, TYRSN type 2, TYRSN type 3, Homocysteinemia, Sulphite oxidase deficiency, Phenylketonuria, Maple syrup urine disease (MSUD), type Ia, MSUD, type Ib, MSUD, type II, Glutaric aciduria, type I, Methylmalonic aciduria, mut(0) type, Holocarboxylase synthetase deficiency, Propionic academia, Isovaleric academia, Peroxisome biogenesis disorder 1A (Zellweger), Peroxisome biogenesis disorder 3A (Zellweger), Peroxisome biogenesis disorder 4A / 4B (Zellweger), Peroxisome biogenesis disorder 5A / 5B (Zellweger), Peroxisome biogenesis disorder 6A / 6B (Zellweger), Peroxisome biogenesis disorder 7A / 7B (Zellweger), Peroxisome biogenesis disorder 8A / 8B (Zellweger), Peroxisome biogenesis disorder 10A (Zellweger), Peroxisome biogenesis disorder 11A / 11B (Zellweger), Peroxisome biogenesis disorder 12A (Zellweger), Peroxisome

biogenesis disorder 13A (Zellweger), Adrenoleukodystrophy (ALD), Perrault syndrome (PRLT) 1, PRLTS1, PRLTS2, PRLTS3, PRLTS4 and PRLTS5.

[0040] D7. The method or use according to any one of paragraphs D1 – D4, wherein the mammal is human and has a disease set forth in Table 1, Table 2, Table 3 and/or Table 4, and the exosome comprises a nucleic acid encoding a neuropeptide as set in Table 1, Table 2, Table 3 and/or Table 4 corresponding with the disease.

[0041] These and other aspects of the invention will be described by reference to the following figures.

Brief Description of the Figures

[0042] Figure 1 graphically illustrates the *in vivo* biodistribution of exosomes over time;

[0043] Figure 2 graphically illustrates expression levels of luciferase delivered *in vivo* to the cerebellum, cerebral cortex (A) brain (whole brain), liver, lungs, heart and skeletal muscle (quadriceps, tibialis anterior and gastrocnemius) (B) as mRNA-luciferase-loaded exosomes.

[0044] Figure 3 graphically illustrates brain GAA activity in GAA KO mice (n = 5-6 per group) treated with GAA mRNA loaded exosomes or empty exosomes. Littermate wildtype (WT) mice were used as controls for both treatments. *P < 0.05. Data were analyzed using an unpaired t-test.

[0045] Figure 4 graphically illustrates GAA activity in fast-(EDL) and slow-(soleus) fiber-type skeletal muscle (A), and diaphragm and heart (B) in GAA KO mice (n = 5-6 per group) treated with GAA mRNA loaded exosomes or empty exosomes. Littermate wildtype (WT) mice were used as controls for both treatments. *P < 0.05. Data were analyzed using an unpaired t-test.

[0046] Figure 5 graphically illustrates GAA gene expression in skeletal muscle (quadriceps) and brain in GAA KO mice (n = 5-6 per group) treated with GAA mRNA loaded exosomes or empty exosomes. Littermate wildtype (WT) mice were used as controls for both treatments. *P < 0.05. Data were analyzed using an unpaired t-test.

[0047] Figure 6 graphically illustrates GAPDH gene expression in neuronal cells treated with GAPDH siRNA or scrambled RNA delivered as naked scRNA or siRNA, scRNA or siRNA transfected with lipofectamine 2000 (LF2000), scRNA- or siRNA-loaded non-targeting exosomes, scRNA- or siRNA-loaded brain targeting exosomes

Detailed Description of the Invention

[0048] A method of treating CNS disorders including recessive CNS disorders such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases in a mammal is provided in which the CNS disorder results from a nucleic acid mutation that results in a dysfunctional protein or lack of a protein. The method comprises administering to the mammal a therapeutically effective amount of exosomes engineered to incorporate nucleic acid encoding a functional target neuropeptide or the target neuropeptide.

[0049] The term “neuropeptide” is used herein to refer to proteins or peptides which function to modulate neural cell activity in the nervous system. Neuropeptides may be produced by neurons, and may include chemokines, growth factors and peptide hormones.

[0050] The term “functional” with respect to a target neuropeptide is used herein to refer to a protein product which retains innate biological activity, including but not limited to, catalytic, metabolic, regulatory, binding, transport and the like. As will be appreciated by one of skill in the art, to be functional, a target protein need not exhibit an endogenous level of biological activity, but will exhibit sufficient activity to render it useful to treat a CNS disorder such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases, e.g. at least about 10% of the biological activity of the corresponding endogenous protein, and preferably at least about 25-50% or greater of the biological activity of the corresponding endogenous protein. For autosomal recessive disorders, it is preferred that a functional protein possess at least about 25% of the biological activity of the corresponding endogenous protein, and more preferably at least about 50% or greater. For X-linked recessive disorders, it is preferred that a functional protein possess at least about 10% of the biological activity of the corresponding

endogenous protein, and preferably at least about 25% or greater of the biological activity of the corresponding endogenous protein.

[0051] The term “exosome” refers to cell-derived vesicles having a diameter of between about 20 and 140 nm, for example, a diameter of about 40-120 nm, including exosomes with a mean diameter of about 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm and/or 120 nm. Exosomes may be isolated from any suitable biological sample from a mammal, including but not limited to; whole blood, serum, plasma, urine, saliva, breast milk, cerebrospinal fluid, amniotic fluid, ascitic fluid, bone marrow and cultured mammalian cells (e.g. immature dendritic cells (wild-type or immortalized), induced and non-induced pluripotent stem cells, fibroblasts, platelets, immune cells, reticulocytes, tumor cells, mesenchymal stem cells, satellite cells, hematopoietic stem cells, pancreatic stem cells, white and beige pre-adipocytes and the like). As one of skill in the art will appreciate, cultured cell samples will be in the cell-appropriate culture media (using exosome-free serum). Exosomes include specific surface markers that distinguish them from other vesicles, including surface markers such as tetraspanins, e.g. CD9, CD37, CD44, CD53, CD63, CD81, CD82 and CD151; targeting or adhesion markers such as integrins, ICAM-1, EpCAM and CD31; membrane fusion markers such as annexins, TSG101, ALIX; and other exosome transmembrane proteins such as Rab5b, HLA-G, HSP70, LAMP2 (lysosome-associated membrane protein) and LIMP (lysosomal integral membrane protein). Exosomes may also be obtained from a non-mammalian biological sample, including cultured non-mammalian cells. As the molecular machinery involved in exosome biogenesis is believed to be evolutionarily conserved, exosomes from non-mammalian sources include surface markers which are isoforms of mammalian surface markers, such as isoforms of CD9 and CD63, which distinguish them from other cellular vesicles. As used herein, the term “mammal” is meant to encompass, without limitation, humans, domestic animals such as dogs, cats, horses, cattle, swine, sheep, goats and the like, as well as non-domesticated animals such as, but not limited to, mice, rats and rabbits. The term “non-mammal” is meant to encompass, for example, exosomes from microorganisms such as bacteria, flies, worms, plants, fruit/vegetables (e.g. corn, pomegranate) and yeast.

[0052] Exosomes may be obtained from an appropriate biological sample using a combination of isolation techniques, for example, centrifugation, filtration and ultracentrifugation methodologies, as well as PEG-based methods. In one embodiment, exosomes may be isolated

from a biological sample using a method including the steps of: i) optionally exposing the biological sample to a first centrifugation to remove cellular debris greater than about 7-10 microns in size from the sample and obtaining the supernatant following centrifugation; ii) optionally subjecting the supernatant from step i) to centrifugation to remove microvesicles and apoptotic bodies therefrom; iii) optionally microfiltering the supernatant from step ii) and collecting the microfiltered supernatant; iv) combining the microfiltered supernatant from step iii) with a polyethylene glycol solution to precipitate the exosomes and subjecting the solution to at least one round of centrifugation to obtain an exosome pellet; and v) re-suspending the exosome pellet from step iv) in a trehalose solution and conducting an optional centrifugation step to remove vesicles having a diameter of greater than 140 nm from the solution.

[0053] In accordance with an aspect of the present invention, the process of isolating exosomes from a biological sample includes a first optional step of removing undesired large cellular debris from the sample, i.e. cells, cell components, apoptotic bodies and the like greater than about 7-10 microns in size. This first step is generally conducted by centrifugation, for example, at 1000-4000x g for 10 to 60 minutes at 4 °C, preferably at 1500-2500x g, e.g. 2000x g, for a selected period of time such as 10-30 minutes, 12-28 minutes, 14-24 minutes, 15-20 minutes or 16, 17, 18 or 19 minutes. As one of skill in the art will appreciate, a suitable commercially available laboratory centrifuge, e.g. Thermo-Scientific™ or Cole-Parmer™, is employed to conduct this isolation step. To enhance exosome isolation, the resulting supernatant is subjected to an additional optional centrifugation step to further remove cellular debris and apoptotic bodies, such as debris that is at least about 7-10 microns in size, by repeating this first step of the process, i.e. centrifugation at 1000-4000x g for 10 to 60 minutes at 4 °C, preferably at 1500-2500x g, e.g. 2000x g, for the selected period of time.

[0054] Following removal of cell debris, the supernatant resulting from the first centrifugation step(s) is separated from the debris-containing pellet (by decanting or pipetting it off) and may then be subjected to an optional additional (second) centrifugation step, including spinning at 12,000-15,000x g for 30-90 minutes at 4 °C to remove intermediate-sized debris, e.g. debris that is greater than 6 microns size. In one embodiment, this centrifugation step is conducted at 14,000x g for 1 hour at 4 °C. The resulting supernatant is again separated from the debris-containing pellet.

[0055] The resulting supernatant is collected and subjected to a third optional centrifugation step, including spinning at between 40,000-60,000x g for 30-90 minutes at 4 °C to further remove impurities such as medium to small-sized microvesicles greater than 0.3 microns in size e.g. in the range of about 0.3-6 microns. In one embodiment, the centrifugation step is conducted at 50,000x g for 1 hour. The resulting supernatant is separated from the pellet for further processing.

[0056] The supernatant is then optionally filtered to remove debris, such as bacteria and larger microvesicles, having a size of about 0.22 microns or greater, e.g. using microfiltration. The filtration may be conducted by one or more passes through filters of the same size, for example, a 0.22 micron filter. Alternatively, filtration using 2 or more filters may be conducted, using filters of the same or of decreasing sizes, e.g. one or more passes through a 40-50 micron filter, one or more passes through a 20-30 micron filter, one or more passes through a 10-20 micron filter, one or more passes through a 0.22-10 micron filter, etc. Suitable filters for use in this step include the use of 0.45 and 0.22 micron filters.

[0057] The microfiltered supernatant (filtrate) may then be combined with a polyethylene glycol (PEG) solution to precipitate exosomes within the filtrate. As would be appreciated by one of skill in the art, a variety of PEG formulations may be used. Preferably, these formulations comprise PEG chain lengths having an average molecular weight of between about 400 to 20,000 daltons (e.g. 1000 to 10,000 daltons, such as 6000 daltons). Similarly, the exosome-PEG solutions may have varying final concentrations of PEG, for example, a final concentration of PEG may be between about 5-15% (such as 8%). Preferably, the filtrate is combined with an equal volume of the PEG solution, having a strength in the range of about 10-20% PEG. Salts may be added to the PEG solution to enhance the precipitation of exosomes. Preferably, a salt such as NaCl is added to the PEG solution so that the final concentration of salt in the exosome-PEG-salt solution is between about 50 to 1,000 mM (such as 500 mM). The PEG-filtrate is gently mixed and incubated under conditions suitable for exosome precipitation, e.g. incubated for 30 minutes at 4°C. Some samples may require a longer incubation period for exosome precipitation to occur.

[0058] Following incubation, the precipitated exosomes were pelleted by centrifugation, e.g. at 10,000x g for 10 min at 4°C, and the pellet was solubilized in a suitable saccharide solution,

such as a trehalose solution, that is effective to reduce aggregation of the exosomes. The saccharide is preferably solubilized in a physiological buffer, such as saline or PBS. In one embodiment, a trehalose solution of various concentrations is effective at reducing the aggregation of exosomes, such as a trehalose concentration between 10 mM to 1,000 mM (e.g. 500 mM).

[0059] To remove non-exosome extracellular vesicles (i.e. vesicles larger than 140 nm), the trehalose exosome solution may be subjected to further optional centrifugation or ultracentrifugation steps, for example, at 15,000x g - 150,000x g for 1 hr at 4°C. If ultracentrifugation is performed, exosomes will be present in both the resultant pellet and supernatant fractions, generally with a larger quantity of exosomes in the supernatant.

[0060] To enhance removal of impurities that are smaller than the exosomes, e.g. smaller than 20nm, the exosome-trehalose solution may be subjected to an optional ultrafiltration step using either a direct-flow filtration technique (such as a centrifugal spin filter) or a cross-flow filtration technique (such as a tangential flow system). As would be appreciated by one of skill in the art, filtration membranes suitable for this step may possess a molecular weight cut-off (MWCO) rating in the range of 3-500kDa and preferably between 100-300kDa.

[0061] In another embodiment, exosome isolation may include the steps of: i) exposing the biological sample to a first centrifugation to remove cellular debris greater than about 7-10 microns in size from the sample and obtaining the supernatant following centrifugation; ii) subjecting the supernatant from step i) to centrifugation to remove microvesicles and apoptotic bodies therefrom; iii) microfiltering the supernatant from step ii) and collecting the microfiltered supernatant; iv) subjecting the microfiltered supernatant from step iii) to at least one round of ultracentrifugation to obtain an exosome pellet; and v) re-suspending the exosome pellet from step iv) in a physiological solution and conducting a second ultracentrifugation in a density gradient and remove the exosome pellet fraction therefrom.

[0062] The centrifugation and filtration steps (steps i)-iii)) are as previously described.

[0063] Following the initial centrifugation and filtration steps, the exosomal solution is then subjected to ultracentrifugation to pellet exosomes and any remaining contaminating microvesicles (between 100-220 nm). This ultracentrifugation step is conducted at 110,000-

170,000x g for 1-3 hours at 4 °C, for example, 170,000x g for 3 hours. This ultracentrifugation step may optionally be repeated, e.g. 2 or more times, in order to enhance results. Any commercially available ultracentrifuge, e.g. Thermo-Scientific™ or Beckman™, may be employed to conduct this step. The exosome-containing pellet is removed from the supernatant using established techniques and re-suspended in a suitable physiological solution.

[0064] Following ultracentrifugation, the re-suspended exosome-containing pellet is subjected to density gradient separation to separate contaminating microvesicles from exosomes based on their density. Various density gradients may be used, including, for example, a sucrose gradient, a colloidal silica density gradient, an iodixanol gradient, or any other density gradient sufficient to separate exosomes from contaminating microvesicles (e.g. a density gradient that functions similar to the 1.100-1.200 g/ml sucrose fraction of a sucrose gradient). Thus, examples of density gradients include the use of a 0.25-2.5 M continuous sucrose density gradient separation, e.g. sucrose cushion centrifugation, comprising 20-50% sucrose; a colloidal silica density gradient, e.g. Percoll™ gradient separation (colloidal silica particles of 15-30 nm diameter, e.g. 30%/70% w/w in water (free of RNase and DNase), which have been coated with polyvinylpyrrolidone (PVP)); and an iodixanol gradient, e.g. 6-18% iodixanol. The resuspended exosome solution is added to the selected gradient and subjected to ultracentrifugation at a speed between 110,000-170,000x g for 1-3 hours. The resulting exosome pellet is removed and re-suspended in physiological solution.

[0065] Depending on the density gradient used, the re-suspended exosome pellet resulting from the density gradient separation may be ready for use. For example, if the density gradient used is a sucrose gradient, the appropriate sucrose fractions are collected and may be combined with other collected sucrose fractions, and the resuspended exosome pellet is ready for use, or may preferably be subjected to an ultracentrifugation wash step at a speed of 110,000-170,000x g for 1-3 hours at 4 °C. If the density gradient used is, for example, a colloidal silica (Percoll™) or a iodixanol density gradient, then the resuspended exosome pellet may be subjected to additional wash steps, e.g. subjected to one to three ultracentrifugation steps at a speed of 110,000-170,000x g for 1-3 hours each at 4 °C, to yield an essentially pure exosome-containing pellet. The pellet is removed from the supernatant and may be re-suspended in a physiologically acceptable solution for use.

[0066] As one of skill in the art will appreciate, the exosome pellet from any of the centrifugation or ultracentrifugation steps may be washed between centrifugation steps using an appropriate physiological solution, e.g. sterile PBS, sterile 0.9% saline or sterile carbohydrate-containing 0.9% saline buffer.

[0067] The present methods advantageously provide a means to obtain mammalian and non-mammalian exosomes which are useful therapeutically. In some embodiments, the methods yield exosomes which exhibit a high degree of purity, for example, at least about 50% pure, and preferably, at least about 60%, 70%, 80%, 90% or 95% or greater pure. Preferably, the exosomes are “essentially free” from cellular debris, apoptotic bodies and microvesicles having a diameter less than 20 nm or greater than 140 nm, and preferably less than 40 nm or greater than 120 nm, and which are biologically intact, e.g. not clumped or in aggregate form, and not sheared, leaky or otherwise damaged. Exosomes isolated according to the methods described herein exhibit a degree of stability, that may be evidenced by the zeta potential of a mixture/solution of such exosomes, for example, a zeta potential of at least a magnitude of ± 10 mV, e.g. ≤ -10 or $\geq +10$, and preferably, a magnitude of at least 20 mV, 30 mV, 40 mV, 50 mV, 60 mV, 70 mV, 80 mV, or greater. The term “zeta potential” refers to the electrokinetic potential of a colloidal dispersion, and the magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles (exosomes) in a dispersion. For exosomes, generally the higher the magnitude of the zeta potential, the greater the stability of the exosomes.

[0068] Moreover, high quantities of exosomes are achievable by the present isolation method. With the PEG-based method, 1 mL of serum yields about 5-10 mg of protein. With the ultracentrifugation/density gradient method, 1 mL of serum or 15-20 mL of cell culture spent media (from at least about 2×10^6 cells) yields about 100-2000 μg total protein. Thus, solutions comprising exosomes at a concentration of at least about 5 $\mu\text{g}/\mu\text{L}$, and preferably at least about 10-25 $\mu\text{g}/\mu\text{L}$, may readily be prepared due to the high exosome yields obtained by the present method. The term “about” as used herein with respect to any given value refers to a deviation from that value of up to 10%, either up to 10% greater, or up to 10% less.

[0069] Exosomes isolated in accordance with the methods herein described, beneficially retaining integrity, and exhibiting purity (being “essentially free” from entities having a diameter

less than 20 nm and or greater than 140 nm), stability and biological activity both *in vitro* and *in vivo*, have not previously been achieved. Thus, the present exosomes are uniquely useful, for example, diagnostically and/or therapeutically. They have also been determined to be non-allergenic, and thus, safe for autologous, allogenic, and xenogenic use.

[0070] For the treatment of CNS disorders, isolated exosomes are genetically engineered to incorporate exogenous nucleic acid suitable to treat the disease, for example, nucleic acid (e.g. DNA, or mRNA) encoding a functional neuropeptide, or to incorporate the neuropeptide itself. The term “loaded exosome” is used herein to refer to exosomes which have been genetically engineered to incorporate an exogenous protein, nucleic acid encoding a protein or other biological substances. The term “empty exosome” is used herein to refer to exosomes which has not been genetically engineered to incorporate an exogenous protein, nucleic acid encoding a protein or other biological substances. The term “exogenous” is used herein to refer to a nucleic acid or protein originating from a source external to the exosomes. The desired nucleic acid may be produced using known synthetic techniques and incorporated into a suitable expression vector using well established methods to form a protein-encoding expression vector which is introduced into isolated exosomes using known techniques, e.g. electroporation, transfection using cationic lipid-based transfection reagents, and the like. Similarly, the selected protein may be produced using recombinant techniques, or may be otherwise obtained, and then may be introduced directly into isolated exosomes by electroporation or other transfection methods. More particularly, electroporation applying voltages in the range of about 20-1000 V/cm may be used to introduce nucleic acid into exosomes. Transfection using cationic lipid-based transfection reagents such as, but not limited to, Lipofectamine® MessengerMAX™ Transfection Reagent, Lipofectamine® RNAiMAX Transfection Reagent, Lipofectamine® 3000 Transfection Reagent, or Lipofectamine® LTX Reagent with PLUS™ Reagent, may also be used. The amount of transfection reagent used may vary with the reagent, the sample and the cargo to be introduced. For example, using Lipofectamine® MessengerMAX™ Transfection Reagent, an amount in the range of about 0.15 µL to 10 µL may be used to load 100 ng to 2500 ng nucleic acid or protein into exosomes. Other methods may also be used to load nucleic acid or protein into exosomes including, for example, the use of cell-penetrating peptides.

[0071] Exosomes isolated in accordance with the methods herein described, which beneficially retain integrity, and exhibit a high degree of purity and stability, readily permit loading of exogenous nucleic acid in an amount of at least about 1 ng nucleic acid (e.g. mRNA) per 10 ug of exosomal protein, or at least about 30 ug protein per 10 ug of exosomal protein.

[0072] In another embodiment, a nucleic acid-encoding expression vector as above described, may be introduced directly into exosome-producing cells, e.g. autologous, allogenic, or xenogenic cells, such as immature dendritic cells (wild-type or immortalized), induced and non-induced pluripotent stem cells, fibroblasts, platelets, immune cells, reticulocytes, tumor cells, mesenchymal stem cells, satellite cells, hematopoietic stem cells, pancreatic stem cells, white and beige pre-adipocytes and the like, by electroporation or other transfection method as described above. Following a sufficient period of time, e.g. 3-7 days to achieve stable expression of the nucleic acid, exosomes incorporating the expressed nucleic acid may be isolated from the exosome-producing cells as described herein. The desired nucleic acid encoding a neuropeptide, or the neuropeptide, may be introduced into isolated exosomes, as previously described, using electroporation or other transfection methods. Introduction to the exosome of both the desired neuropeptide and nucleic acid encoding the same neuropeptide may increase delivery efficiency of the neuropeptide. In addition, introduction of a combination of neuropeptides and/or nucleic acids encoding one or more neuropeptides may be desirable to treat a CNS disorder resulting from different DNA mutations, or for the treatment of secondary pathologies such as mitochondrial dysfunction in CNS disorders associated with type 2 diabetes.

[0073] In another embodiment, prior to incorporation into exosomes nucleic acid encoding a selected protein, or incorporation of the protein, exosomes may be modified to express or incorporate a target-specific fusion product which provides targeted delivery of the exosomes to brain cells. The term "targeting exosome" is used herein to refer to said exosomes which have been modified to express or incorporate a target-specific fusion product. The term "non-targeting exosome" is used herein to refer to exosomes which have not been modified to express or incorporate any target-specific fusion product. Such a target-specific fusion product comprises a sequence that targets brain, i.e. a brain targeting sequence, linked to an exosomal membrane marker. The exosomal membrane marker of the fusion product will localize the fusion product within the membrane of the exosome to enable the targeting sequence to direct the exosome to the

intended target. Examples of exosome membrane markers include, but are not limited to: tetraspanins such as CD9, CD37, CD53, CD63, CD81, CD82 and CD151; targeting or adhesion markers such as integrins, ICAM-1 and CDD31; membrane fusion markers such as annexins, TSG101, ALIX; and other exosome transmembrane proteins such as LAMP (lysosome-associated membrane protein), e.g. LAMP 1 or 2, and LIMP (lysosomal integral membrane protein). All or a fragment of an exosomal membrane marker may be utilized in the fusion product, provided that the fragment includes a sufficient portion of the membrane marker to enable it to localize within the exosome membrane, i.e. the fragment comprises at least one intact transmembrane domain to permit localization of the membrane marker into the exosomal membrane.

[0074] The target-specific fusion product also includes a targeting sequence. In one embodiment, the targeting sequence is a brain targeting sequence, i.e. a protein or peptide sequence which facilitates the targeted delivery of the exosome to the brain. Suitable peptides for brain targeting include peptides that bind to receptors of neurons in the brain, including but not limited to, acetylcholine receptors, gamma-aminobutyric acid receptors, dopamine receptors, serotonin receptors, norepinephrine receptors and glutamate receptors. An example of a suitable brain targeting sequence is YTIWMPENPRPGTPCDIFTNSRGKCRASNG (SEQ ID NO: 1), a peptide that specifically binds to the acetylcholine receptor in neurons.

[0075] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to nerves. Examples of suitable nerve targeting proteins include, but are not limited to, myelin-associated glycoprotein, kinesin-like protein 1A, synaptobrevin, synaptosomal-associated protein 25kDa and synaptobrevin, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0076] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to cerebellum. Examples of suitable cerebellum targeting proteins include, but are not limited to, cerebellin, Munc13, LANO and CACNA1A, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0077] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to cerebrum/pyramidal cell. Examples

of suitable cerebrum/pyramidal cell targeting proteins include, but are not limited to, GLUT1, SLC1A3, cortexin, SCAMP5 and Synaptotagmin-1, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0078] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to hippocampus. Examples of suitable hippocampus targeting proteins include, but are not limited to, M1, M2, M4 muscarinic AchR and SNAP25, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0079] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to astrocytes/glia. Examples of suitable astrocyte/glia targeting proteins include, but are not limited to, GFAP and SLC1A3, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0080] In another embodiment, the brain targeting sequence comprises a protein or peptide sequence which facilitates targeted delivery of the exosome to myelin. Examples of suitable myelin targeting proteins include, but are not limited to, myelin basic protein and PMP-22, or a targeting fragment thereof, e.g. a portion of the C-terminal sequence thereof.

[0081] Exosomes incorporating a brain targeting fusion product may be produced, as described above, using recombinant technology. In this regard, an expression vector encoding the fusion product is introduced by electroporation or other transfection methods into exosome-producing cells isolated from an appropriate biological sample. As one of skill in the art will appreciate, it is also possible to produce the fusion product using recombinant techniques, and then introduce the fusion product directly into exosome-producing cells using similar techniques, e.g. electroporation, transfection using cationic lipid-based transfection reagents, and the like. Following a sufficient period of time, exosomes generated by the exosome-producing cells, and including the fusion product, may be isolated as described. The desired nucleic acid encoding the neuropeptide and/or the neuropeptide, may be introduced into isolated exosomes incorporating a brain targeting fusion product (brain targeting exosomes) as previously described, using electroporation or other transfection methods. Exosomes incorporating the brain targeting sequence and the desired nucleic acid encoding the neuropeptide and/or the neuropeptide may

exhibit increased delivery efficiency of the neuropeptide and/or nucleic acid encoding the neuropeptide.

[0082] Exosomes genetically engineered to incorporate nucleic acid encoding a neuropeptide and/or the neuropeptide, may be used to deliver the nucleic acid and/or neuropeptide to a mammal *in vivo* and across the blood brain barrier in the treatment of a CNS disorder, to upregulate the activity of the target protein and thereby treat the disease. For example, the present method may be used to treat any form of CNS disorder resulting from a recessive genetic mutation. The term “mutation” is used herein to describe any inherited or sporadic change in the nucleotide sequence or arrangement of DNA that results in a dysfunctional or absent neuropeptide including, but not limited to the following: nucleotide substitutions (e.g. missense mutations, nonsense mutations, RNA processing mutations, splice-site mutations, regulatory mutations, nucleotide transitions and nucleotide transversions), insertions or deletions of one or more nucleotides, duplications of any nucleotide sequence, repeat expansion mutations (e.g. trinucleotide repeats, etc.) and frameshift mutations. The term “recessive” is used herein to describe any X-linked recessive mutation or disorder or autosomal recessive mutation or disorder.

[0083] Examples of seizure disorders that are caused by genetic mutations and that may be treated using the present engineered exosomes are set out in Table 1 below. Table 1 identifies the disease and affected or mutated gene involved in each disease, the type of mutation, the mRNA transcript sequence information (via the NCBI (National Centre for Biotechnology Information) GenBank accession numbers) for the functional gene (which could be incorporated into the exosomes to treat a disease), and the corresponding protein sequence information for the proteins useful to treat each disease.

Table 1.

Category	Disease	Affected Gene	GenBank mRNA Accession Number	GenBank Protein Accession Number
Seizure Disorders	Cerebral creatine deficiency syndrome (CCDS) 1	solute carrier family 6 (neurotransmitter transporter), member 8 (SLC6A8)	NM_001142805.1 NM_001142806.1 NM_005629.3	NP_001136277.1 NP_001136278.1 NP_005620.1

	CCDS 2	guanidinoacetate N-methyltransferase (GAMT)	NM_000156.5 NM_138924.2	NP_000147.1 NP_620279.1
	GLUT1 deficiency syndrome 1, infantile onset, severe	solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1)	NM_006516.2	NP_006507.2
	Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg)	cystatin B (CSTB)	NM_000100.3	NP_000091.1
	Epilepsy, progressive myoclonic 2A (Lafora)	epilepsy, progressive myoclonus type 2A, Lafora disease (laforin) (EPM2A)	NM_001018041.1 NM_005670.3	NP_001018051.1 NP_005661.1
	Epilepsy, progressive myoclonic 2B (Lafora)	NHL repeat containing E3 ubiquitin protein ligase 1 (NHLRC1)	NM_198586.2	NP_940988.2
	Epileptic encephalopathy, early infantile (EIEE) Type 1	aristaless related homeobox (ARX)	NM_139058.2	NP_620689.1
	EIEE Type 3	solute carrier family 25 (mitochondrial carrier: glutamate), member 22 (SLC25A22)	NM_001191060.1 NM_001191061.1 NM_024698.5	NP_001177989.1 NP_001177990.1 NP_078974.1
	EIEE Type 8	Cdc42 guanine nucleotide exchange factor 9 (ARHGEF9)	NM_001173479.1 NM_001173480.1 NM_015185.2	NP_001166950.1 NP_001166951.1 NP_056000.1
	EIEE Type 12	phospholipase C beta 1 (PLCB1)	NM_015192.3 NM_182734.2	NP_056007.1 NP_877398.1
	EIEE Type 15	ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3)	NM_001270459.1 NM_001270460.1 NM_001270461.1 NM_001270462.1 NM_001270463.1 NM_001270464.1 NM_001270465.1 NM_001270466.1 NM_006279.3 NM_174963.3 NM_174964.2 NM_174965.2 NM_174966.2 NM_174967.2 NM_174968.3 NM_174969.2 NM_174970.2 NM_174971.3	NP_001257388.1 NP_001257389.1 NP_001257390.1 NP_001257391.1 NP_001257392.1 NP_001257393.1 NP_001257394.1 NP_001257395.1 NP_006270.1 NP_777623.2 NP_777624.1

				NP_777625.1 NP_777626.1 NP_777627.1 NP_777628.2 NP_777629.1 NP_777630.1 NP_777631.2
EIEE Type 16	TBC1 domain family member 24 (TBC1D24)	NM_001199107.1 NM_020705.2		NP_001186036.1 NP_065756.1
EIEE Type 18	seizure threshold 2 homolog (mouse) (SZT2)	NM_015284.3		NP_056099.3
Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2) / EIEE Type 20	phosphatidylinositol glycan anchor biosynthesis class A (PIGA)	NM_002641.3 NM_020473.3		NP_002632.1 NP_065206.3
EIEE Type 21	NECAP endocytosis associated 1 (NECAP1)	NM_015509.3		NP_056324.2
EIEE Type 23	dedicator of cytokinesis 7 (DOCK7)	NM_001271999.1 NM_001272000.1 NM_001272001.1 NM_001272002.1 NM_033407.3		NP_001258928.1 NP_001258929.1 NP_001258930.1 NP_001258931.1 NP_212132.2
EIEE Type 25	solute carrier family 13 (sodium-dependent citrate transporter), member 5 (SLC13A5)	NM_001143838.2 NM_001284509.1 NM_001284510.1 NM_177550.4		NP_001137310.1 NP_001271438.1 NP_001271439.1 NP_808218.1
EIEE Type 28	WW domain containing oxidoreductase (WWOX)	NM_001291997.1 NM_016373.3 NM_130791.3		NP_001278926.1 NP_057457.1 NP_570607.1
EIEE Type 29	alanyl-tRNA synthetase (AARS)	NM_001605.2		NP_001596.2
EIEE Type 34	solute carrier family 12 (potassium/chloride transporter), member 5 (SLC12A5)	NM_001134771.1 NM_020708.4		NP_001128243.1 NP_065759.1

	EIEE Type 35	inosine triphosphatase (ITPA)	NM_001267623.1 NM_033453.3 NM_181493.2	NP_001254552.1 NP_258412.1 NP_852470.1
	Microcephaly, seizures, and developmental delay (MCSZ)	polynucleotide kinase 3'-phosphatase (PNKP)	NM_007254.3	NP_009185.2
	Aicardi-Goutieres syndrome (AGS) Type 1	three prime repair exonuclease 1 (TREX1)	NM_007248.3 NM_016381.5 NM_033629.4	NP_009179.2 NP_057465.1 NP_338599.1
	AGS Type 2	ribonuclease H2 subunit B (RNASEH2B)	NM_001142279.2 NM_024570.3	NP_001135751.1 NP_078846.2
	AGS Type 3	ribonuclease H2 subunit C (RNASEH2C)	NM_032193.3	NP_115569.2
	AGS Type 4	ribonuclease H2 subunit A (RNASEH2A)	NM_006397.2	NP_006388.2
	AGS Type 5	SAM domain and HD domain 1 (SAMHD1)	NM_015474.3	NP_056289.2
	AGS Type 6	adenosine deaminase, RNA-specific (ADAR)	NM_001025107.2 NM_001111.4 NM_001193495.1 NM_015840.3 NM_015841.3	NP_001020278.1 NP_001102.2 NP_001180424.1 NP_056655.2 NP_056656.2
	Congenital disorder of glycosylation (CDG), Type 1a	phosphomannomutase 2 (PMM2)	NM_000303.2	NP_000294.1
	CDG Type 1b	mannose phosphate isomerase (MPI)	NM_001289155.1 NM_001289156.1 NM_001289157.1 NM_002435.2	NP_001276084.1 NP_001276085.1 NP_001276086.1 NP_002426.1
	CDG Type 1c	ALG6, alpha-1,3-glucosyltransferase (ALG6)	NM_013339.3	NP_037471.2
	CDG Type 1d	ALG3, alpha-1,3-mannosyltransferase (ALG3)	NM_001006941.2 NM_005787.5	NP_001006942.1 NP_005778.1
	CDG Type 1e	dolichyl-phosphate mannosyltransferase polypeptide 1,	NM_001317034.1 NM_001317035.1 NM_001317036.1 NM_003859.2	NP_001303963.1 NP_001303964.1

		catalytic subunit (DPM1)		NP_001303965.1 NP_003850.1
	CDG Type 1f	mannose-P-dolichol utilization defect 1 (MPDU1)	NM_004870.3	NP_004861.2
	CDG Type 1g	ALG12, alpha-1,6-mannosyltransferase (ALG12)	NM_024105.3	NP_077010.1
	CDG Type 1h	ALG8, alpha-1,3-glucosyltransferase (ALG8)	NM_001007027.2 NM_024079.4	NP_001007028.1 NP_076984.2
	CDG Type 1i	ALG2, alpha-1,3/1,6-mannosyltransferase (ALG2)	NM_033087.3	NP_149078.1
	CDG Type 1j	dolichyl-phosphate N-acetylglucosaminophosphotransferase 1 (DPAGT1)	NM_001382.3	NP_001373.2
	CDG Type 1k	ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase (ALG1)	NM_019109.4	NP_061982.3
	CDG Type 1l	ALG9, alpha-1,2-mannosyltransferase (ALG9)	NM_001077690.1 NM_001077691.1 NM_001077692.1 NM_024740.2	NP_001071158.1 NP_001071159.1 NP_001071160.1 NP_079016.2
	CDG Type 1m	dolichol kinase (DOLK)	NM_014908.3	NP_055723.1
	CDG Type 1n	RFT1 homolog (RFT1)	NM_052859.3	NP_443091.1
	CDG Type 1o	dolichyl-phosphate mannosyltransferase subunit 3 (DPM3)	NM_018973.3 NM_153741.1	NP_061846.2 NP_714963.1
	CDG Type 1p	ALG11, alpha-1,2-mannosyltransferase (ALG11)	NM_001004127.2	NP_001004127.2
	CDG Type 1q	steroid 5 alpha-reductase 3 (SRD5A3)	NM_024592.4	NP_078868.1
	CDG Type 1r	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic) (DDOST)	NM_005216.4	NP_005207.2
	CDG Type 1s	ALG13, UDP-N-acetylglucosaminyltransferase subunit (ALG13)	NM_001039210.3 NM_001099922.2 NM_001168385.1 NM_001257230.1 NM_001257231.1	NP_001034299.3 NP_001093392.1

			NM_001257234.1 NM_001257235.1 NM_001257237.1 NM_001257239.1 NM_001257240.1 NM_001257241.1 NM_018466.4	NP_001161857.1 NP_001244159.1 NP_001244160.1 NP_001244163.1 NP_001244164.1 NP_001244166.1 NP_001244168.1 NP_001244169.1 NP_001244170.1 NP_060936.1
	CDG Type 1t	phosphoglucosyltransferase 1 (PGM1)	NM_001172818.1 NM_001172819.1 NM_002633.2	NP_001166289.1 NP_001166290.1 NP_002624.2
	CDG Type 1u	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit (DPM2)	NM_003863.3	NP_003854.1
	CDG Type 1w	STT3A, catalytic subunit of the oligosaccharyltransferase complex (STT3A)	NM_001278503.1 NM_001278504.1 NM_152713.4	NP_001265432.1 NP_001265433.1 NP_689926.1
	CDG Type 1x	STT3B, catalytic subunit of the oligosaccharyltransferase complex (STT3B)	NM_178862.2	NP_849193.1
	CDG Type 1y	signal sequence receptor, delta (SSR4)	NM_001204526.1 NM_001204527.1 NM_006280.2	NP_001191455.1 NP_001191456.1 NP_006271.1
	CDG Type 1z	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)	NM_001306079.1 NM_004341.4	NP_001293008.1 NP_004332.2
	CDG Type IIa	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2)	NM_002408.3	NP_002399.1

	CDG Type IIb	mannosyl-oligosaccharide glucosidase (MOGS)	NM_001146158.1 NM_006302.2	NP_001139630.1 NP_006293.2
	CDG Type IIc	solute carrier family 35 member C1 (SLC35C1)	NM_001145265.1 NM_001145266.1 NM_018389.4	NP_001138737.1 NP_001138738.1 NP_060859.4
	CDG Type II d	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1 (B4GALT1)	NM_001497.3	NP_001488.2
	CDG Type IIe	component of oligomeric golgi complex 7 (COG7)	NM_153603.3	NP_705831.1
	CDG Type II f	solute carrier family 35 member A1 (SLC35A1)	NM_001168398.1 NM_006416.4	NP_001161870.1 NP_006407.1
	CDG Type II g	component of oligomeric golgi complex 1 (COG1)	NM_018714.2	NP_061184.1
	CDG Type II h	component of oligomeric golgi complex 8 (COG8)	NM_032382.4	NP_115758.3
	CDG Type II i	component of oligomeric golgi complex 5 (COG5)	NM_001161520.1 NM_006348.3 NM_181733.2	NP_001154992.1 NP_006339.3 NP_859422.2
	CDG Type II j	component of oligomeric golgi complex 4 (COG4)	NM_001195139.1 NM_015386.2	NP_001182068.1 NP_056201.2
	CDG Type II k	transmembrane protein 165 (TMEM165)	NM_018475.4	NP_060945.2
	CDG Type III	component of oligomeric golgi complex 6 (COG6)	NM_001145079.1 NM_020751.2	NP_001138551.1 NP_065802.1
	CDG Type II n	solute carrier family 39 member 8 (SLC39A8)	NM_001135146.1 NM_001135147.1 NM_001135148.1 NM_022154.5	NP_001128618.1 NP_001128619.1 NP_001128620.1 NP_071437.3
	Spinocerebellar ataxia, autosomal recessive 1	senataxin (SETX)	NM_015046.5	NP_055861.3
	Ataxia, early-onset, with oculomotor	aprataxin (APTX)	NM_001195249.1 NM_001195250.1	NP_001182178.1 NP_001182179.1

	apraxia and hypoalbuminemia		NM_001195251.1 NM_001195252.1 NM_001195254.1 NM_175069.2 NM_175073.2	NP_001182180.1 NP_001182181.1 NP_001182183.1 NP_778239.1 NP_778243.1
	Marinesco-Sjogren syndrome	SIL1 nucleotide exchange factor (SIL1)	NM_001037633.1 NM_022464.4	NP_001032722.1 NP_071909.1
	Cockayne syndrome, type A / UV-sensitive syndrome 2	excision repair cross-complementation group 8 (ERCC8)	NM_000082.3 NM_001007233.2 NM_001007234.2 NM_001290285.1	NP_000073.1 NP_001007234.1 NP_001007235.1 NP_001277214.1

[0084] Examples of SCA which are caused by genetic mutations and that may be treated using the present engineered exosomes are set out in Table 2 below. Table 2 identifies the disease and affected or mutated gene involved in each disease, the type of mutation, the mRNA transcript sequence information (via the NCBI GenBank accession numbers) for the functional gene (which could be incorporated into the exosomes to treat a disease), and the corresponding protein sequence information for the proteins useful to treat each disease.

Table 2.

Category	Disease	Affected Gene	GenBank Accession Number of Treatment mRNA	GenBank Protein Accession Number
Spinocerebellar Ataxias (SCA)	Cockayne syndrome, type B / Cerebrooculofacioskeletal syndrome 1 / De Sanctis-Cacchione syndrome / UV-sensitive syndrome	excision repair cross-complementation group 6 (ERCC6)	NM_000124.3	NP_000115.1
	Spastic ataxia, Charlevoix-Saguenay type	sacsin molecular chaperone (SACS)	NM_001278055.1 NM_014363.5	NP_001264984.1 NP_055178.3
	Pelizaeus-Merzbacher disease / Spastic paraplegia 2, X-linked	proteolipid protein 1 (PLP1)	NM_000533.4 NM_001128834.2 NM_001305004.1 NM_199478.2	NP_000524.3 NP_001122306.1 NP_001291933.1 NP_955772.1
	Anemia, sideroblastic, with ataxia	ATP binding cassette subfamily B member 7 (ABC7)	NM_001271696.1 NM_001271697.1 NM_001271698.1 NM_001271699.1	NP_001258625.1 NP_001258626.1 NP_001258627.1 NP_001258628.1

			NM_004299.4	NP_004290.2
	Pontocerebellar hypoplasia (PCH), type 1a	vaccinia related kinase 1 (VRK1)	NM_003384.2	NP_003375.1
	PCH type 1b	exosome component 3 (EXOSC3)	NM_001002269.2 NM_016042.3	NP_001002269.1 NP_057126.2
	PCH type 1c	exosome component 8 (EXOSC8)	NM_181503.2	NP_852480.1
	PCH type 2a	tRNA splicing endonuclease subunit 54 (TSEN54)	NM_207346.2	NP_997229.2
	PCH type 2b	tRNA splicing endonuclease subunit 2 (TSEN2)	NM_001145392.1 NM_001145393.1 NM_001145394.1 NM_001145395.1 NM_025265.3	NP_001138864.1 NP_001138865.1 NP_001138866.1 NP_001138867.1 NP_079541.1
	PCH type 2c	tRNA splicing endonuclease subunit 34 (TSEN34)	NM_001077446.3 NM_001282332.1 NM_001282333.1 NM_024075.4	NP_001070914.1 NP_001269261.1 NP_001269262.1 NP_076980.2
	PCH type 2d	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS)	NM_016955.3	NP_058651.3
	PCH type 2e	VPS53 GARP complex subunit (VPS53)	NM_001128159.2 NM_018289.3	NP_001121631.1 NP_060759.2
	PCH type 3	piccolo presynaptic cytomatrix protein (PCLO)	NM_014510.2 NM_033026.5	NP_055325.2 NP_149015.2
	PCH type 4	tRNA splicing endonuclease subunit 54 (TSEN54)	NM_207346.2	NP_997229.2
	PCH type 5	tRNA splicing endonuclease subunit 54 (TSEN54)	NM_207346.2	NP_997229.2
	PCH type 6	arginyl-tRNA synthetase 2, mitochondrial (RARS2)	NM_001318785.1 NM_020320.4	NP_001305714.1 NP_064716.2
	PCH type 8	charged multivesicular body protein 1A (CHMP1A)	NM_001083314.3 NM_002768.4	NP_001076783.1 NP_002759.2
	PCH type 9	adenosine monophosphate deaminase 2 (AMPD2)	NM_001257360.1 NM_001257361.1 NM_001308170.1 NM_004037.7 NM_139156.3 NM_203404.1	NP_001244289.1 NP_001244290.1 NP_001295099.1 NP_004028.3 NP_631895.1 NP_981949.1
	PCH type 10	cleavage and polyadenylation factor I subunit 1 (CLP1)	NM_001142597.1 NM_006831.2	NP_001136069.1 NP_006822.1

Spinocerebellar ataxia, autosomal recessive (SCAR) 2 (SCAR2)	peptidase (mitochondrial processing) alpha (PMPCA)	NM_001282944.1 NM_001282946.1 NM_015160.2	NP_001269873.1 NP_001269875.1 NP_055975.1
SCAR7	tripeptidyl peptidase I (TPP1)	NM_000391.3	NP_000382.3
SCAR8	spectrin repeat containing, nuclear envelope 1 (SYNE1)	NM_033071.3 NM_182961.3	NP_149062.1 NP_892006.3
SCAR10	anoctamin 10 (ANO10)	NM_001204831.1 NM_001204832.1 NM_001204833.1 NM_001204834.1 NM_018075.3	NP_001191760.1 NP_001191761.1 NP_001191762.1 NP_001191763.1 NP_060545.3
SCAR14	spectrin beta, non-erythrocytic 2 (SPTBN2)	NM_006946.2	NP_008877.1
SCAR16	STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase (STUB1)	NM_001293197.1 NM_005861.3	NP_001280126.1 NP_005852.2
SCAR18	glutamate ionotropic receptor delta type subunit 2 (GRID2)	NM_001286838.1 NM_001510.3	NP_001273767.1 NP_001501.2
SCAR20	sorting nexin 14 (SNX14)	NM_001297614.2 NM_001304479.1 NM_020468.5 NM_153816.5	NP_001284543.1 NP_001291408.1 NP_065201.1 NP_722523.1

[0085] Examples of small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) that are caused by genetic mutations and that may be treated using the present engineered exosomes are set out in Table 3 below. Table 3 identifies the disease and affected or mutated gene involved in each disease, the type of mutation, the mRNA transcript sequence information (via the NCBI GenBank accession numbers) for the functional gene (which could be incorporated into the exosomes to treat a disease), and the corresponding protein sequence information for the proteins useful to treat each disease.

Table 3.

SMALL MOLECULE DISORDERS				
Category	Disease	Affected Gene	GenBank Accession Number of Treatment mRNA	GenBank protein Accession Number

Urea Cycle Disorders	Ornithine transcarbamylase deficiency	ornithine carbamoyltransferase (OTC)	NM_000531.5	NP_000522.3
	Citrullinemia	argininosuccinate synthase 1 (ASS1)	NM_000050.4 NM_054012.3	NP_000041.2 NP_446464.1
	Argininosuccinic aciduria	argininosuccinate lyase (ASL)	NM_000048.3 NM_001024943.1 NM_001024944.1 NM_001024946.1	NP_000039.2 NP_001020114.1 NP_001020115.1 NP_001020117.1
	Argininemia	arginase 1 (ARG1)	NM_000045.3 NM_001244438.1	NP_000036.2 NP_001231367.1
	Carbamoylphosphate synthetase I deficiency	carbamoyl-phosphate synthase I (CPS1)	NM_001122633.2 NM_001122634.3 NM_001875.4	NP_001116105.1 NP_001116106.1 NP_001866.2
Metal Accumulation Disorders	Hemochromatosis	Hemochromatosis (HFE)	NM_000410.3 NM_001300749.1 NM_139003.2 NM_139004.2 NM_139006.2 NM_139007.2 NM_139008.2 NM_139009.2 NM_139010.2 NM_139011.2	NP_000401.1 NP_001287678.1 NP_620572.1 NP_620573.1 NP_620575.1 NP_620576.1 NP_620577.1 NP_620578.1 NP_620579.1 NP_620580.1
	Menkes disease	ATPase copper transporting alpha (ATP7A)	NM_000052.6 NM_001282224.1	NP_000043.4 NP_001269153.1
	Wilson disease	ATPase copper transporting beta (ATP7B)	NM_000053.3 NM_001005918.2 NM_001243182.1	NP_000044.2 NP_001005918.1 NP_001230111.1
	Molybdenum cofactor deficiency A	molybdenum cofactor synthesis 1 (MOCS1)	NM_001075098.3 NM_005943.5	NP_001068566.1 NP_005934.2
	Molybdenum cofactor deficiency B	molybdenum cofactor synthesis 2 (MOCS2)	NM_004531.4 NM_176806.3	NP_004522.1 NP_789776.1
	Neurodegeneration with brain iron accumulation (NBIA) 1 / HARP syndrome	pantothenate kinase 2 (PANK2)	NM_024960.4 NM_153638.2 NM_153640.2	NP_079236.3 NP_705902.2 NP_705904.1

	NBIA 2B / Infantile neuroaxonal dystrophy 1 / Parkinson disease 14, autosomal recessive	phospholipase A2 group VI (PLA2G6)	NM_001004426.1 NM_001199562.1 NM_003560.2	NP_001004426.1 NP_001186491.1 NP_003551.2
	NBIA 3	ferritin, light polypeptide (FTL)	NM_000146.3	NP_000137.2
	NBIA 4	chromosome 19 open reading frame 12 (C19orf12)	NM_001031726.3 NM_001256046.1 NM_001256047.1 NM_001282929.1 NM_001282930.1 NM_001282931.1 NM_031448.4	NP_001026896.2 NP_001242975.1 NP_001242976.1 NP_001269858.1 NP_001269859.1 NP_001269860.1 NP_113636.2
	NBIA 6	Coenzyme A synthase (COASY)	NM_001042529.2 NM_001042532.3 NM_025233.6	NP_001035994.1 NP_001035997.2 NP_079509.5
	Kufor-Rakeb syndrome	ATPase 13A2 (ATP13A2)	NM_001141973.2 NM_001141974.2 NM_022089.3	NP_001135445.1 NP_001135446.1 NP_071372.1
	Spastic paraplegia 35	fatty acid 2-hydroxylase (FA2H)	NM_024306.4	NP_077282.3
	Woodhouse-Sakati syndrome	DDB1 and CUL4 associated factor 17 (DCAF17)	NM_001164821.1 NM_025000.3	NP_001158293.1 NP_079276.2
	Fucosidosis	fucosidase, alpha-L-1, tissue (FUCA1)	NM_000147.4	NP_000138.2
	Spastic paraplegia 30 / neuropathy, hereditary sensory type IIc / mental retardation, autosomal dominant 9	kinesin family member 1A (KIF1A)	NM_001244008.1 NM_004321.6	NP_001230937.1 NP_004312.2
Amino Acidopathies	Tyrosinemia (TYRSN), type 1	fumarylacetoacetate hydrolase (fumarylacetoacetase) (FAH)	NM_000137.2	NP_000128.1
	TYRSN type 2	tyrosine aminotransferase (TAT)	NM_000353.2	NP_000344.1
	TYRSN type 3	4-hydroxyphenylpyruvate dioxygenase (HPD)	NM_001171993.1 NM_002150.2	NP_001165464.1 NP_002141.1
	Homocysteinemia	methylenetetrahydrofolate reductase (NAD(P)H) (MTHFR)	NM_005957.4	NP_005948.3
	Sulphite oxidase deficiency	sulfite oxidase (SUOX)	NM_000456.2 NM_001032386.1 NM_001032387.1	NP_000447.2 NP_001027558.1 NP_001027559.1
	Phenylketonuria	phenylalanine hydroxylase (PAH)	NM_000277.1	NP_000268.1
	Maple syrup urine disease (MSUD), type Ia	branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA)	NM_000709.3 NM_001164783.1	NP_000700.1 NP_001158255.1

Organic Acidopathies	MSUD, type Ib	branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB)	NM_000056.4 NM_001318975.1 NM_183050.3	NP_000047.1 NP_001305904.1 NP_898871.1
	MSUD, type II	dihydrolipoamide branched chain transacylase E2 (DBT)	NM_001918.3	NP_001909.3
	Glutaric aciduria, type I	glutaryl-CoA dehydrogenase (GCDH)	NM_000159.3 NM_013976.3	NP_000150.1 NP_039663.1
	Methylmalonic aciduria, mut(0) type	methylmalonyl-CoA mutase (MUT)	NM_000255.3	NP_000246.2
	Holocarboxylase synthetase deficiency	holocarboxylase synthetase (HLCS)	NM_000411.6 NM_001242784.1 NM_001242785.1	NP_000402.3 NP_001229713.1 NP_001229714.1
	Propionic acidemia	propionyl-CoA carboxylase alpha subunit (PCCA)	NM_000282.3 NM_001127692.2 NM_001178004.1	NP_000273.2 NP_001121164.1 NP_001171475.1
	Isovaleric acidemia	isovaleryl-CoA dehydrogenase (IVD)	NM_001159508.1 NM_002225.3	NP_001152980.1 NP_002216.2

[0086] Examples of peroxisomal diseases that are caused by genetic mutations and that may be treated using the present engineered exosomes are set out in Table 4 below. Table 4 identifies the disease and affected or mutated gene involved in each disease, the type of mutation, the mRNA transcript sequence information (via the NCBI GenBank accession numbers) for the functional gene (which could be incorporated into the exosomes to treat a disease), and the corresponding protein sequence information for the proteins useful to treat each disease.

Table 4.

Category	Disease	Affected Gene	GenBank Accession Number of Treatment mRNA	GenBank protein Accession Number
Peroxisomal Diseases	Peroxisome biogenesis disorder 1A (Zellweger)	peroxisomal biogenesis factor 1 (PEX1)	NM_000466.2 NM_001282677.1 NM_001282678.1	NP_000457.1 NP_001269606.1 NP_001269607.1
	Peroxisome biogenesis disorder 3A (Zellweger)	peroxisomal biogenesis factor 12 (PEX12)	NM_000286.2	NP_000277.1
	Peroxisome biogenesis disorder 4A / 4B (Zellweger)	peroxisomal biogenesis factor 6 (PEX6)	NM_000287.3 NM_001316313.1	NP_000278.3 NP_001303242.1
	Peroxisome biogenesis disorder 5A / 5B (Zellweger)	peroxisomal biogenesis factor 2 (PEX2)	NM_000318.2 NM_001079867.1 NM_001172086.1 NM_001172087.1	NP_000309.1 NP_001073336.1 NP_001165557.1

				NP_001165558.1
Peroxisome biogenesis disorder 6A / 6B (Zellweger)	peroxisomal biogenesis factor 10 (PEX10)	NM_002617.3 NM_153818.1		NP_002608.1 NP_722540.1
Peroxisome biogenesis disorder 7A / 7B (Zellweger)	peroxisomal biogenesis factor 26 (PEX26)	NM_001127649.2 NM_001199319.1 NM_017929.5		NP_001121121.1 NP_001186248.1 NP_060399.1
Peroxisome biogenesis disorder 8A / 8B (Zellweger)	peroxisomal biogenesis factor 16 (PEX16)	NM_004813.2 NM_057174.2		NP_004804.1 NP_476515.1
Peroxisome biogenesis disorder 10A (Zellweger)	peroxisomal biogenesis factor 3 (PEX3)	NM_003630.2		NP_003621.1
Peroxisome biogenesis disorder 11A / 11B (Zellweger)	peroxisomal biogenesis factor 13 (PEX13)	NM_002618.3		NP_002609.1
Peroxisome biogenesis disorder 12A (Zellweger)	peroxisomal biogenesis factor 19 (PEX19)	NM_001193644.1 NM_002857.3		NP_001180573.1 NP_002848.1
Peroxisome biogenesis disorder 13A (Zellweger)	peroxisomal biogenesis factor 14 (PEX14)	NM_004565.2		NP_004556.1
Adrenoleukodystrophy (ALD)	ATP binding cassette subfamily D member 1 (ABCD1)	NM_000033.3		NP_000024.2
Perrault syndrome (PRLT) 1, PRLTS1	hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4)	NM_000414.3 NM_001199291.2 NM_001199292.1 NM_001292027.1 NM_001292028.1		NP_000405.1 NP_001186220.1 NP_001186221.1 NP_001278956.1 NP_001278957.1
PRLTS2	histidyl-tRNA synthetase 2 (HARS2)	NM_001278731.1 NM_001278732.1 NM_012208.3		NP_001265660.1 NP_001265661.1 NP_036340.1
PRLTS3	caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP)	NM_006012.2		NP_006003.1
PRLTS4	leucyl-tRNA synthetase 2 (LARS2)	NM_015340.3		NP_056155.1
PRLTS5	chromosome 10 open reading frame 2 (C10orf2)	NM_001163812.1 NM_001163813.1 NM_001163814.1 NM_021830.4		NP_001157284.1 NP_001157285.1 NP_001157286.1 NP_068602.2

[0087] Thus, in one embodiment, exosomes are used to deliver to a mammal one or more proteins encoded by genes selected from the group consisting of solute carrier family 6 (neurotransmitter transporter), member 8 (SLC6A8), guanidinoacetate N-methyltransferase (GAMT), solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1), cystatin B

(CSTB), epilepsy, progressive myoclonus type 2A, Lafora disease (laforin) (EPM2A), NHL repeat containing E3 ubiquitin protein ligase 1 (NHLRC1), aristaless related homeobox (ARX), solute carrier family 25 (mitochondrial carrier: glutamate), member 22 (SLC25A22), Cdc42 guanine nucleotide exchange factor 9 (ARHGEF9), phospholipase C beta 1 (PLCB1), ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3), TBC1 domain family member 24 (TBC1D24), seizure threshold 2 homolog (mouse) (SZT2), phosphatidylinositol glycan anchor biosynthesis class A (PIGA), NECAP endocytosis associated 1 (NECAP1), dedicator of cytokinesis 7 (DOCK7), solute carrier family 13 (sodium-dependent citrate transporter), member 5 (SLC13A5), WW domain containing oxidoreductase (WWOX), alanyl-tRNA synthetase (AARS), solute carrier family 12 (potassium/chloride transporter), member 5 (SLC12A5), inosine triphosphatase (ITPA), polynucleotide kinase 3'-phosphatase (PNKP), three prime repair exonuclease 1 (TREX1), ribonuclease H2 subunit B (RNASEH2B), ribonuclease H2 subunit C (RNASEH2C), ribonuclease H2 subunit A (RNASEH2A), SAM domain and HD domain 1 (SAMHD1), adenosine deaminase, RNA-specific (ADAR), phosphomannomutase 2 (PMM2), mannose phosphate isomerase (MPI), ALG6, alpha-1,3-glucosyltransferase (ALG6), ALG3, alpha-1,3-mannosyltransferase (ALG3), dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit (DPM1), mannose-P-dolichol utilization defect 1 (MPDU1), ALG12, alpha-1,6-mannosyltransferase (ALG12), ALG8, alpha-1,3-glucosyltransferase (ALG8), ALG2, alpha-1,3/1,6-mannosyltransferase (ALG2), dolichyl-phosphate N-acetylglucosaminophosphotransferase 1 (DPAGT1), ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase (ALG1), ALG9, alpha-1,2-mannosyltransferase (ALG9), dolichol kinase (DOLK), RFT1 homolog (RFT1), dolichyl-phosphate mannosyltransferase subunit 3 (DPM3), ALG11, alpha-1,2-mannosyltransferase (ALG11), steroid 5 alpha-reductase 3 (SRD5A3), dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic) (DDOST), ALG13, UDP-N-acetylglucosaminyltransferase subunit (ALG13), phosphoglucomutase 1 (PGM1), dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit (DPM2), STT3A, catalytic subunit of the oligosaccharyltransferase complex (STT3A), STT3B, catalytic subunit of the oligosaccharyltransferase complex (STT3B), signal sequence receptor, delta (SSR4), carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2), mannosyl-oligosaccharide glucosidase (MOGS), solute carrier family 35 member C1 (SLC35C1), UDP-

Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 (B4GALT1), component of oligomeric golgi complex 7 (COG7), solute carrier family 35 member A1 (SLC35A1), component of oligomeric golgi complex 1 (COG1), component of oligomeric golgi complex 8 (COG8), component of oligomeric golgi complex 5 (COG5), component of oligomeric golgi complex 4 (COG4), transmembrane protein 165 (TMEM165), component of oligomeric golgi complex 6 (COG6), solute carrier family 39 member 8 (SLC39A8), senataxin (SETX), aprataxin (APTX), SIL1 nucleotide exchange factor (SIL1), excision repair cross-complementation group 8 (ERCC8), excision repair cross-complementation group 6 (ERCC6), sacsin molecular chaperone (SACS), proteolipid protein 1 (PLP1), ATP binding cassette subfamily B member 7 (ABC7), vaccinia related kinase 1 (VRK1), exosome component 3 (EXOSC3), exosome component 8 (EXOSC8), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 2 (TSEN2), tRNA splicing endonuclease subunit 34 (TSEN34), Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS), VPS53 GARP complex subunit (VPS53), piccolo presynaptic cytomatrix protein (PCLO), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 54 (TSEN54), arginyl-tRNA synthetase 2, mitochondrial (RARS2), charged multivesicular body protein 1A (CHMP1A), adenosine monophosphate deaminase 2 (AMPD2), cleavage and polyadenylation factor I subunit 1 (CLP1), peptidase (mitochondrial processing) alpha (PMPCA), tripeptidyl peptidase I (TPP1), spectrin repeat containing, nuclear envelope 1 (SYNE1), anoctamin 10 (ANO10), spectrin beta, non-erythrocytic 2 (SPTBN2), STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase (STUB1), glutamate ionotropic receptor delta type subunit 2 (GRID2), sorting nexin 14 (SNX14), ornithine carbamoyltransferase (OTC), argininosuccinate synthase 1 (ASS1), argininosuccinate lyase (ASL), arginase 1 (ARG1), carbamoyl-phosphate synthase 1 (CPS1), Hemochromatosis (HFE), ATPase copper transporting alpha (ATP7A), ATPase copper transporting beta (ATP7B), molybdenum cofactor synthesis 1 (MOCS1), molybdenum cofactor synthesis 2 (MOCS2), pantothenate kinase 2 (PANK2), phospholipase A2 group VI (PLA2G6), ferritin, light polypeptide (FTL), chromosome 19 open reading frame 12 (C19orf12), Coenzyme A synthase (COASY), ATPase 13A2 (ATP13A2), fatty acid 2-hydroxylase (FA2H), DDB1 and CUL4 associated factor 17 (DCAF17), fucosidase, alpha-L- 1, tissue (FUCA1), kinesin family member 1A (KIF1A), fumarylacetoacetate hydrolase (fumarylacetoacetase) (FAH), tyrosine aminotransferase (TAT), 4-hydroxyphenylpyruvate dioxygenase (HPD),

methylenetetrahydrofolate reductase (NAD(P)H) (MTHFR), sulfite oxidase (SUOX), phenylalanine hydroxylase (PAH), branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB), dihydrolipoamide branched chain transacylase E2 (DBT), glutaryl-CoA dehydrogenase (GCDH), methylmalonyl-CoA mutase (MUT), holocarboxylase synthetase (HLCS), propionyl-CoA carboxylase alpha subunit (PCCA), isovaleryl-CoA dehydrogenase (IVD), peroxisomal biogenesis factor 1 (PEX1), peroxisomal biogenesis factor 12 (PEX12), peroxisomal biogenesis factor 6 (PEX6), peroxisomal biogenesis factor 2 (PEX2), peroxisomal biogenesis factor 10 (PEX10), peroxisomal biogenesis factor 26 (PEX26), peroxisomal biogenesis factor 16 (PEX16), peroxisomal biogenesis factor 3 (PEX3), peroxisomal biogenesis factor 13 (PEX13), peroxisomal biogenesis factor 19 (PEX19), peroxisomal biogenesis factor 14 (PEX14), ATP binding cassette subfamily D member 1 (ABCD1), hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4), histidyl-tRNA synthetase 2 (HARS2), caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP), leucyl-tRNA synthetase 2 (LARS2) and chromosome 10 open reading frame 2 (C10orf2), or nucleic acid encoding one or more of these proteins.

[0088] Accordingly, the present method is useful to treat recessive CNS disorders such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases selected from the group consisting of Cerebral creatine deficiency syndrome (CCDS) 1, CCDS 2, GLUT1 deficiency syndrome 1, infantile onset, severe, Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg), Epilepsy, progressive myoclonic 2A (Lafora), Epilepsy, progressive myoclonic 2B (Lafora), Epileptic encephalopathy, early infantile (EIEE) Type 1, EIEE Type 3, EIEE Type 8, EIEE Type 12, EIEE Type 15, EIEE Type 16, EIEE Type 18, Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2) / EIEE Type 20, EIEE Type 21, EIEE Type 23, EIEE Type 25, EIEE Type 28, EIEE Type 29, EIEE Type 34, EIEE Type 35, Microcephaly, seizures, and developmental delay (MCSZ), Aicardi-Goutieres syndrome (AGS) Type 1, AGS Type 2, AGS Type 3, AGS Type 4, AGS Type 5, AGS Type 6, Congenital disorder of glycosylation (CDG), Type 1a, CDG Type 1b, CDG Type 1c, CDG Type 1d, CDG Type 1e, CDG Type 1f, CDG Type 1g, CDG Type 1h, CDG Type 1i, CDG Type 1j, CDG Type 1k, CDG Type 1l, CDG Type 1m, CDG Type 1n, CDG Type 1o, CDG Type 1p, CDG Type 1q,

CDG Type 1r, CDG Type 1s, CDG Type 1t, CDG Type 1u, CDG Type 1w, CDG Type 1x, CDG Type 1y, CDG Type 1z, CDG Type IIa, CDG Type IIb, CDG Type IIc, CDG Type IId, CDG Type IIe, CDG Type IIf, CDG Type IIg, CDG Type IIh, CDG Type Iii, CDG Type IIj, CDG Type IIk, CDG Type III, CDG Type IIIn, Spinocerebellar ataxia, autosomal recessive 1, Ataxia, early-onset, with oculomotor apraxia and hypoalbuminemia, Marinesco-Sjogren syndrome, Cockayne syndrome, type A / UV-sensitive syndrome 2, Cockayne syndrome, type B / Cerebrooculofacioskeletal syndrome 1 / De Sanctis-Cacchione syndrome / UV-sensitive syndrome, Spastic ataxia, Charlevoix-Saguenay type, Pelizaeus-Merzbacher disease / Spastic paraplegia 2, X-linked, Anemia, sideroblastic, with ataxia, Pontocerebellar hypoplasia (PCH), type 1a, PCH type 1b, PCH type 1c, PCH type 2a, PCH type 2b, PCH type 2c, PCH type 2d, PCH type 2e, PCH type 3, PCH type 4, PCH type 5, PCH type 6, PCH type 8, PCH type 9, PCH type 10, Spinocerebellar ataxia, autosomal recessive (SCAR) 2 (SCAR2), SCAR7, SCAR8, SCAR10, SCAR14, SCAR16, SCAR18, SCAR20, Ornithine transcarbamylase deficiency, Citrullinemia, Argininosuccinic aciduria, Argininemia, Carbamoylphosphate synthetase I deficiency, Hemochromatosis, Menkes disease, Wilson disease, Molybdenum cofactor deficiency A, Molybdenum cofactor deficiency B, Neurodegeneration with brain iron accumulation (NBIA) 1 / HARP syndrome, NBIA 2B / Infantile neuroaxonal dystrophy 1 / Parkinson disease 14, autosomal recessive, NBIA 3, NBIA 4, NBIA 6, Kufor-Rakeb syndrome, Spastic paraplegia 35, Woodhouse-Sakati syndrome, Fucosidosis, Spastic paraplegia 30 / neuropathy, hereditary sensory type IIc / mental retardation, autosomal dominant 9, Tyrosinemia (TYRSN), type 1, TYRSN type 2, TYRSN type 3, Homocysteinemia, Sulphite oxidase deficiency, Phenylketonuria, Maple syrup urine disease (MSUD), type Ia, MSUD, type Ib, MSUD, type II, Glutaric aciduria, type I, Methylmalonic aciduria, mut(0) type, Holocarboxylase synthetase deficiency, Propionic academia, Isovaleric academia, Peroxisome biogenesis disorder 1A (Zellweger), Peroxisome biogenesis disorder 3A (Zellweger), Peroxisome biogenesis disorder 4A / 4B (Zellweger), Peroxisome biogenesis disorder 5A / 5B (Zellweger), Peroxisome biogenesis disorder 6A / 6B (Zellweger), Peroxisome biogenesis disorder 7A / 7B (Zellweger), Peroxisome biogenesis disorder 8A / 8B (Zellweger), Peroxisome biogenesis disorder 10A (Zellweger), Peroxisome biogenesis disorder 11A / 11B (Zellweger), Peroxisome biogenesis disorder 12A (Zellweger), Peroxisome biogenesis disorder 13A (Zellweger), Adrenoleukodystrophy (ALD), Perrault syndrome (PRLT) 1, PRLTS1, PRLTS2, PRLTS3, PRLTS4, and PRLTS5. As would be appreciated by one skilled

in the art, new mutations that cause recessive CNS disorders are continually being discovered and thus, the present method may also be effective to treat CNS disorders caused by gene mutations which are yet to be identified as such.

[0089] As one of skill in the art will appreciate, the nucleic acid encoding a neuropeptide and/or the neuropeptide, for incorporation into exosomes according to the invention may be a functional native mammalian nucleic acid or protein, including for example, nucleic acid or protein from human and non-human mammals, or a functionally equivalent nucleic acid or neuropeptide. The term “functionally equivalent” refers to nucleic acid, e.g. mRNA, rRNA, tRNA, DNA, or cDNA, encoding a neuropeptide, and is meant to include any nucleic acid sequence which encodes a functional neuropeptide, including all transcript variants, variants that encode protein isoforms, variants due to degeneracy of the genetic code, artificially modified variants, and the like. Thus, nucleic acid modifications may include one or more base substitutions or alterations, addition of 5’ or 3’ protecting groups, and the like, preferably maintaining significant sequence similarity, e.g. at least about 70%, and preferably, 80%, 90%, 95% or greater. The term “functionally equivalent” is used herein also to refer to a protein which exhibits the same or similar function to the native protein (e.g. retains at least about 30% of the activity of the native protein), and includes all isoforms, variants, recombinant produced forms, and naturally-occurring or artificially modified forms, i.e. including modifications that do not adversely affect activity and which may increase cell uptake, stability, activity and/or therapeutic efficacy. Protein modifications may include, but are not limited to, one or more amino acid substitutions (for example, with a similarly charged amino acid, e.g. substitution of one amino acid with another each having non-polar side chains such as valine, leucine, alanine, isoleucine, glycine, methionine, phenylalanine, tryptophan, proline; substitution of one amino acid with another each having basic side chains such as histidine, lysine, arginine; substitution of one amino acid with another each having acidic side chains such as aspartic acid and glutamic acid; and substitution of one amino acid with another each having polar side chains such as cysteine, serine, threonine, tyrosine, asparagine, glutamine), additions or deletions; modifications to amino acid side chains, addition of a protecting group at the N- or C-terminal ends of the protein, addition of a nerve targeting sequence or targeting fragments thereof, at the N-terminal end of the protein and the like. Suitable modifications will generally maintain

at least about 70% sequence similarity with the active site and other conserved domains of the native neuropeptide, and preferably at least about 80%, 90%, 95% or greater sequence similarity.

[0090] Engineered exosomes incorporating nucleic acid encoding a neuropeptide, and/or the neuropeptide, in accordance with the invention, may be formulated for therapeutic use by combination with a pharmaceutically or physiologically acceptable carrier. The expressions "pharmaceutically acceptable" or "physiologically acceptable" means acceptable for use in the pharmaceutical and veterinary arts, i.e. not being unacceptably toxic or otherwise unsuitable for physiological use. As one of skill in the art will appreciate, the selected carrier will vary with intended utility of the exosome formulation. In one embodiment, exosomes are formulated for administration by infusion or injection, e.g. subcutaneously, intraperitoneally, intramuscularly or intravenously, and thus, are formulated as a suspension in a medical-grade, physiologically acceptable carrier, such as an aqueous solution in sterile and pyrogen-free form, optionally, buffered or made isotonic. The carrier may be distilled water (DNase- and RNase-free), a sterile carbohydrate-containing solution (e.g. sucrose or dextrose) or a sterile saline solution comprising sodium chloride and optionally buffered. Suitable sterile saline solutions may include varying concentrations of sodium chloride, for example, normal saline (0.9%), half-normal saline (0.45%), quarter-normal saline (0.22%), and solutions comprising greater amounts of sodium chloride (e.g. 3%-7%, or greater). Saline solutions may optionally include additional components, e.g. carbohydrates such as dextrose and the like. Examples of saline solutions including additional components, include Ringer's solution, e.g. lactated or acetated Ringer's solution, phosphate buffered saline (PBS), TRIS (hydroxymethyl) aminomethane hydroxymethyl) aminomethane)-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced solution (EBSS), standard saline citrate (SSC), HEPES-buffered saline (HBS) and Grey's balanced salt solution (GBSS).

[0091] In other embodiments, the present exosomes are formulated for administration by routes including, but not limited to, oral, intranasal, enteral, topical, sublingual, intra-arterial, intramedullary, intrauterine, intrathecal, inhalation, ocular, transdermal, vaginal or rectal routes, and will include appropriate carriers in each case. For oral administration, exosomes may be formulated in normal saline, complexed with food, in a capsule or in a liquid formulation with an emulsifying agent (honey, egg yolk, soy lecithin, and the like). Oral compositions may

additionally include adjuvants including sugars, such as lactose, trehalose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and derivatives thereof, including sodium carboxymethylcellulose, ethylcellulose and cellulose acetates; powdered tragacanth; malt; gelatin; talc; stearic acids; magnesium stearate; calcium sulfate; vegetable oils, such as peanut oils, cotton seed oil, sesame oil, olive oil and corn oil; polyols such as propylene glycol, glycerine, sorbitol, mannitol and polyethylene glycol; agar; alginic acids; water; isotonic saline and phosphate buffer solutions. Wetting agents, lubricants such as sodium lauryl sulfate, stabilizers, colouring agents and flavouring agents may also be present. Exosome compositions for topical application may be prepared including appropriate carriers. Creams, lotions and ointments may be prepared for topical application using an appropriate base such as a triglyceride base. Such creams, lotions and ointments may also contain a surface active agent. Aerosol formulations may also be prepared in which suitable propellant adjuvants are used. Other adjuvants may also be added to the composition regardless of how it is to be administered, for example, anti-microbial agents, anti-oxidants and other preservatives may be added to the composition to prevent microbial growth and/or degradation over prolonged storage periods.

[0092] The present engineered exosomes are useful in a method to treat a pathological condition involving a defective protein, or a condition involving lack of expression of a protein, e.g. a recessive CNS disorder such as seizure disorders, spino cerebellar ataxia (SCA), small molecule disorders (such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies) and peroxisomal diseases. The terms "treat", "treating" or "treatment" are used herein to refer to methods that favourably alter recessive CNS disorders, including those that moderate, reverse, reduce the severity of, or protect against, the progression of recessive CNS disorders. Thus, for use to treat such a disease, a therapeutically effective amount of exosomes engineered to incorporate nucleic acid encoding the functional protein, useful to treat the disease, are administered to a mammal. The term "therapeutically effective amount" is an amount of exosome required to treat the disease, while not exceeding an amount that may cause significant adverse effects. Exosome dosages that are therapeutically effective will vary on many factors including the nature of the condition to be treated as well as the particular individual being treated. Appropriate exosome dosages for use include dosages sufficient to result in an increase in the amount or activity of the target neuropeptide in the individual being treated by at least about

10%, and preferably an increase in activity of the target neuropeptide of greater than 10%, for example, at least 20%, 30%, 40%, 50% or greater. For example, in one embodiment, the dosage may be a dosage in an amount in the range of about 20 ng to about 200 mg of total exosomal protein for the delivery of RNA species such as mRNA, tRNA, rRNA, miRNA, SRP RNA, snRNA, scRNA, snoRNA, gRNA, RNase P, RNase MRP, yRNA, TERC, SLRNA, lncRNA, or piRNA. In an exemplary embodiment, a dosage of exosomes sufficient to deliver about 1 ng/kg to about 100 ug/kg of a nucleic acid (e.g. an RNA species), is administered to the mammal in the treatment of a target recessive CNS disorder. In another embodiment, the dosage may be a dosage of exosomes sufficient to deliver about 0.1 mg/kg to about 100 mg/kg of a neuropeptide is administered to the mammal in the treatment of a recessive CNS disorder. The term “about” is used herein to mean an amount that may differ somewhat from the given value, by an amount that would not be expected to significantly affect activity or outcome as appreciated by one of skill in the art, for example, a variance of from 1-10% from the given value.

[0093] As will be appreciated by one of skill in the art, exosomes comprising nucleic acid encoding the protein, for example, to treat recessive CNS disorders, may be used in conjunction with (at different times or simultaneously, either in combination or separately) one or more additional therapies to facilitate treatment, including but not limited to; anti-epileptic drugs, anti-spasticity drugs, cognitive enhancers (cholinergic drugs), movement disorder drugs (anti-cholinergic, dopamine agonists or L-DOPA), bracing or other orthopedic devices, anti-depressants, anti-psychotics, anti-oxidants (i.e., coenzyme Q10, alpha lipoic acid, vitamin E, synthetic coenzyme Q10 analogues, resveratrol, N-acetylcysteine, etc.), or creatine monohydrate.

[0094] In another embodiment, the present method of treating a CNS disorder in a mammal may include administration to the mammal of exosomes (for example, isolated as described above), genetically modified to incorporate gene-silencing systems (e.g., siRNA) to reduce the expression of a mutated gene followed by administering to the mammal exosomes genetically modified to incorporate a protein useful to treat the disorder and/or nucleic acid encoding the protein.

[0095] In another aspect of the invention, a method of treating a CNS disorder in a mammal may include administering to the mammal exosomes genetically modified to incorporate genome-

editing systems to correct the inherent primary mutation leading to the disorder. Genome editing may include gene insertions, deletions, modifications and gene silencing. Examples of nuclease genome editing systems include, but are not limited to, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) nuclease system, e.g. including a targeting gRNA and a CRISPR-associated (Cas) gene, such as CRISPR-Cas9, Transcription Activator-Like Effector Nucleases (TALEN) and mito-TALEN, ZFN Zinc-Finger Nucleases (ZFN) and aptamer-guided delivery of therapeutic nucleic acids, e.g. small interfering RNA, micro RNA, anti-microRNA, antagonist and small hairpin RNA.

[0096] In one embodiment, the exosome is genetically modified to express a CRISPR nuclease system, such as a CRISPR/Cas9 Type II genome editing system, including a Cas 9 nuclease, and a guide RNA (gRNA) comprising fusion of a targeting RNA sequence, crRNA (CRISPR RNA) and a trans-activating RNA (tracrRNA). The crRNA and tracrRNA are related to the selected Cas nuclease such that the crRNA and tracrRNA are specific for and recognized by the selected Cas nuclease.

[0097] The targeting sequence of the guide RNA (gRNA) is a strand of RNA that is homologous to a region on a target gene, i.e. a gene to be edited or silenced, associated with a CNS disorder. Target genes may be genes associated with genetic disease, including hereditary disease such as recessive CNS disorders. The targeting RNA may comprise from 10-30 nucleotides, e.g. from 15-25 nucleotides, and may comprise a GC content of about 40-80%. The CRISPR system may be utilized to disrupt expression of a gene by insertion or deletion of nucleotides to disrupt the Open Reading Frame (ORF) of a target gene, or to introduce a premature stop codon therein. Non-Homologous End Joining (NHEJ) DNA repair may be used in this instance. The CRISPR system may also be used to edit (e.g. to correct a gene mutation) by homology directed repair in which the targeting RNA includes an editing region, e.g. a region that incorporates an edit to be incorporated into the target gene, flanked by a region of homology (homologous arms) on either side thereof. The size of the editing region is not particularly restricted, and may include a single nucleotide edit, or edits of up to 100 nucleotides or more. The targeting sequence of the gRNA is selected such that it targets a site within the target gene that is proximal (e.g. within about 2-5 nucleotides or more) to a protospacer adjacent motif (PAM) located within the target gene. The PAM is recognized by the Cas nuclease and permits Cas nuclease binding. The homologous arms

will generally increase in size with the size of the editing region, for example, for edits of less than about 50 nucleotides, the homologous arms may be in the range of about 100-150 nucleotides in length, while larger editing regions may incorporate homologous arms of about 200-800 nucleotides, or more. Edits may also be introduced using CRISPR which facilitate expression of a target gene, e.g. edits which introduce a transcription factor that promote gene expression. The gRNA additionally incorporates related crRNA and a tracrRNA sequences, which interact and function to direct the Cas nuclease to the target gene and catalyze cleavage of the target gene by the Cas nuclease. As will be understood by one of skill in the art, while each of the crRNA, tracrRNA, and Cas nuclease sequences are related, these sequences may be native or mutated sequences, provided that any mutations thereof do not have an adverse impact on function. Methods for selection of suitable crRNA and tracrRNA sequences for use in gRNA are known in the art.

[0098] The Cas nuclease may, for example, be a Cas 9-based nuclease. Examples of a Cas 9 nuclease include wild-type Cas 9 (a double nickase) from *Streptococcus pyogenes* (SP), *Staphylococcus aureus* (SA), *Neisseria meningitidis* (NM), *Streptococcus thermophilus* (ST), and *Treponema denticola* (TD), as well as mutated recombinant Cas 9, e.g. mutated to function as a single nickase such as Cas9 D10A and Cas9 H840A, which may be used with 2 or more gRNAs to achieve a genome edit with increasing targeting efficiency that prevents non-specific genomic editing. Wild-type and single nickase Cas 9 may be used to edit genes, for example, that result in recessive CNS disorders, in order to correct the mutation. The mutated Cas 9 may also be a nuclease-deficient Cas (for example, incorporating both D10A and H840A to inactivate nuclease function) which binds but does not cleave and thereby silences a gene. Nuclease-deficient Cas 9 may be used to treat a recessive CNS disorder to prevent or minimize expression of a dysfunctional mutated protein, which may interfere with the activity of the desired functional protein.

[0099] The targeting RNA is an RNA strand complementary to a site on the target gene which is 3-4 nucleotides upstream of a PAM sequence recognized by the Cas nuclease. The targeting RNA does not itself include a PAM sequence. PAM sequences differ for various Cas nucleases. For example, for *Streptococcus pyogenes* (SP), the PAM sequence is NGG; for *S. aureus*, the PAM sequence is NNGRRT or NNGRR(N); for *Neisseria meningitidis*, the PAM sequence is NNNNGATT; for *Streptococcus thermophilus*, the PAM sequence is NNAGAAW;

for *Treponema denticola* (TD), the PAM sequence is NAAAAC. “N” represents any nucleotide, W = weak (A or T) and R = A or G.

[00100] For introduction into exosomes, nucleic acid encoding a nuclease genome editing system, such as a selected CRISPR nuclease system including gRNA and a Cas nuclease, may be produced using known synthetic techniques and then incorporated into the same or different expression vectors under the control of an appropriate promoter. Suitable vectors for such expression are known in the art. Alternatively, expression vectors incorporating the selected genome editing system may be obtained commercially. Expression vectors incorporating the nuclease editing system may be introduced into exosomes using electroporation or transfection using cationic lipid-based transfection reagents. Alternatively, the components of the nuclease editing system may be introduced directly into exosomes as single-stranded (ss) DNA using similar introduction techniques, e.g. gRNA of CRISPR may be introduced into exosomes as ssDNA.

[00101] In another embodiment, Class 2 CRISPR technology (such as CRISPR-spCAS9-HF) can be incorporated into exosomes and used as a gene editing system.

[00102] The use of engineered exosomes in a therapy to treat recessive CNS disorders advantageously results in delivery of nucleic acid and/or protein across the blood brain barrier to a target cell to treat genetic defects. Thus, the use of exosomes overcomes the challenges of delivery to the central nervous system.

[00103] Embodiments of the invention are described in the following examples which are not to be construed as limiting.

Example 1 – Preparation of Exosomes for Use to Treat Recessive Central Nervous System Disorders

[00104] Exosomes were isolated and loaded with mRNA encoding the creatine transporter (CrT) protein or acid alpha glucosidase (GAA) protein as follows.

[00105] Dendritic cells (DC) were isolated from mouse bone marrow progenitor cells and from human peripheral blood mononuclear cells (collected using Ficoll gradient separation of human blood). Briefly, femur and tibia were carefully harvested from mice and were flushed with

HBSS media to collect bone marrow progenitor cells. The bone marrow progenitor cells were cultured in GlutaMAX-DMEM media (Life Technologies) containing 10% FBS, 1mM sodium pyruvate, 0.5% penicillin-streptomycin, and mouse recombinant granulocyte/macrophage colony-stimulating factor (R&D Systems). For human dendritic cell isolation, blood was collected in EDTA-lavender tubes followed by dilution of blood with 4x PBS buffer (pH 7.2 and 2 mM EDTA). 40 mL of diluted cell suspension was carefully layered over 20 mL of Ficoll gradient. The gradient was centrifuged at 400x g for 60 minutes followed by collection of the interphase layer containing the mononuclear cells. The mononuclear cells were cultured in IMDM media (BD Biosciences) containing 10% FBS, 1% glutamine, 0.5% penicillin-streptomycin, and human recombinant granulocyte/macrophage colony-stimulating factor (R&D Systems). Both human and mouse dendritic cells were further purified using EasySep™ Mouse and Human Pan-DC Enrichment Kit (Stem Cell Technologies). Dendritic cells were then cultured with the aforementioned media (GlutaMAX-DMEM media for mouse DC and IMDM media for human DC). Media was pre-spun at 170,000x g for 2 hours at 37 °C for 4 days to ensure that the subsequent exosome pellet would not be contaminated with bovine microvesicles and/or exogenous exosomes.

[00106] To prepare non-targeting exosomes (not tagged with a membrane fusion product), cells were grown to about 80% confluency before exosome collection as described below. To prepare targeting exosomes (tagged with a membrane fusion product), on the third day of growth, DC were transfected with mammalian expression Lamp1-Brain Targeting Sequence (BTS) fusion plasmid 0.1-1 ug (depending on cell density) using Lipofectamine 2000 reagent (Life Technologies). The Lamp1-BTS fusion plasmid was made using Gateway® technology and vectors (Life Technologies) with amplified mouse brain cDNA that corresponds to the brain targeting sequence (YTIWMPENPRPGTPCDIFTNSRGKRASNG; SEQ ID NO: 1). The mouse LAMP1 cDNA exosome marker (NM_001317353.1) was amplified from mouse dendritic cell cDNA. The brain targeting sequence and the exosome marker were linked via PCR, and then were incorporated into a mammalian expression vector. On the fifth day, the dendritic cells were washed and combined with fresh growth media (GlutaMAX-DMEM, pre-spun at 170,000x g for 2 hours to generate media virtually free of contamination from bovine exosomes or microvesicles).

[00107] The dendritic cells were then grown to about 80% confluency in alpha minimum essential medium supplemented with ribonucleosides, deoxyribonucleosides, 4 mM L-glutamine,

1 mM sodium pyruvate, 5 ng/mL murine GM-CSF, and 20% fetal bovine serum. For conditioned media collection, cells were washed twice with sterile PBS (pH 7.4, Life Technologies) and exosome-depleted fetal bovine serum was added. Conditioned media from human and mouse immature dendritic cell culture was collected after 48 hours. The media (10 mL) was spun at 2,000x g for 15 min at 4°C to remove any cellular debris. This was followed by an optional 2000x g spin for 60 min at 4°C to further remove any contaminating non-adherent cells. The supernatant was then spun at 14,000x g for 60 min at 4°C. The resulting supernatant was spun at 50,000x g for 60 min at 4°C. The supernatant was then filtered through a 40 µm filter, followed by filtration through a 0.22 µm syringe filter (twice). The supernatant was then carefully transferred into ultracentrifuge tubes and diluted with an equal amount of sterile PBS (pH 7.4, Life Technologies). This mixture was then subjected to ultracentrifugation at 100,000x-170,000x g for 2 hours at 4°C using a fixed-angle rotor. The resulting pellet was re-suspended in PBS and re-centrifuged at 100,000x-170,000x g for 2 hours at 4°C. The pellet was resuspended carefully with 25 mL of sterile PBS (pH 7.4, Life Technologies) and then added gently on top of 4 mL of 30%/70% Percoll™ gradient cushion (made with 0.22 µm filter sterilized water) in an ultracentrifuge tube. This mixture was spun at 100,000x-170,000x g for 90 minutes at 4°C. With a syringe, the exosomal pellet-containing fraction at the gradient interface was isolated carefully, diluted in 50 mL of sterile PBS (pH 7.4, Life Technologies), followed by a final spin for 90 minutes at 100,000x-170,000x g at 4°C to obtain purified exosomes. The resulting exosomal pellet was resuspended in sterile PBS or sterile 0.9% saline for downstream use. Exosomal fraction purity was confirmed by sizing using a Beckman DelsaMax dynamic light scattering analyzer showing minimal contamination outside of the 40-120 nm size range, and by immuno-gold labelling/Western blotting using the exosome membrane markers, CD9, CD63, TSG101 and ALIX. Yield was about 1×10^9 particles around ~100 nm in size. Using the Pierce™ BCA protein quantification assay (Thermo Scientific), the yield of exosomes was estimated and found to be between 10 – 15 µg of exosomes.

[00108] Purified exosomes were suspended in 100-140 µL of pre-chilled electroporation buffer (1.5 mM potassium phosphate pH 7.2, 25 mM KCl, and 21% (vol/vol) OptiPrep for nucleic acid electroporation of targeting and non-targeting exosomes. Exosomes were then counted and sized by NanoSight nanoparticle tracking analysis (NanoSight, Ltd.). The yield of exosomes was estimated using the Pierce™ BCA protein quantification assay (Thermo Scientific).

Production of Mouse GAA mRNA

[00109] To synthesize GAA mRNA, GAA cDNA (NM_000152.4) was sub-cloned and amplified from skeletal muscle (from mouse and human). Using conventional PCR, start codon (ATG) and Kozak sequence (GCCACC) were introduced. This cDNA was then cloned into the pMRNA^{XP} plasmid using EcoRI and BamHI restriction enzyme sites followed by transformation of competent *E. coli* DH5alpha line (Life Technologies). The colony containing the vector with positive insert was amplified. The vector was isolated from these colonies (Qiagen) and T7 RNA polymerase-based *in vitro* transcription reaction was carried out. An anti-reverse cap analog (ARCA), modified nucleotides (5-Methylcytidine-5'-Triphosphate and Pseudouridine-5'-Triphosphate) and poly-A tail were incorporated into the mRNAs to enhance the stability and to reduce the immune response of host cells. DNase I digest and phosphatase treatment was carried out to remove any DNA contamination and to remove the 5' triphosphates at the end of the RNA to further reduce innate immune responses in mammalian cells, respectively. The clean-up spin columns were used to recover GAA mRNA for downstream encapsulation in engineered exosomes.

Introduction of mRNA, siRNA, or scRNA into exosomes

[00110] Electroporation mixture was prepared by carefully mixing exosomes (targeting or non-targeting) and the appropriate nucleic acid (such as Luciferase mRNA, GAA mRNA, CrT mRNA, GAPDH siRNA or scRNA) in 1:1 ratio in electroporation buffer. Electroporation was carried out in 0.4 mm electroporation cuvettes at 400 mV and 125 μ F capacitance (pulse time 14 milliseconds (ms) for mRNA and 24 ms for protein) using Gene Pulse XCell electroporation system (BioRad). After electroporation, exosomes were resuspended in 20 mL of 0.9% saline solution followed by ultracentrifugation for 2 hours at 170,000x g at 4°C. For *in vitro* exosome administration, nucleic acid-loaded targeting and non-targeting exosomes were re-suspended in 5% (wt/vol) glucose in 0.9% saline solution. Alternatively, exosomes were loaded with the appropriate nucleic acid using cationic lipid-based transfection reagents (Lipofectamine® MessengerMAX™ Transfection Reagent, Life Technologies). After transfection, exosomes were spun for 2 hours at 170,000x g at 4°C followed by re-suspension in 5% (wt/vol) glucose in 0.9% sterile saline solution.

[00111] Exosomes were determined to be loaded with luciferase mRNA by the subsequent determination of luciferase activity in various tissues to which the exosomes were delivered.

Example 2 - Exosomes can be Transported into Tissues Affected by Recessive CNS disorders

[00112] To confirm that exosomes isolated as described herein can be effectively taken up by various tissues in an *in vivo* model system, isolated exosomes were labeled using BODIPY® TE ceramide fluorescent stain (Life Technologies). BODIPY® TR ceramide is a red fluorescent stain (absorption/emission maxima ~589/617 nm), which is prepared from D-erythrosphingosine and has the same stereochemical conformation as natural biologically active sphingolipids. 10 ug of total labeled exosomes (suspended in sterile 0.9% saline) were intravenously administered to mice. Mice tissues/organs (quadriceps, heart, brain, liver, kidney, lung, inguinal white adipose tissue, brown adipose tissue, pancreas, and colon) and blood were then harvested immediately (0 min), at 10 min following injection, and at 20 minutes following injection (4 mice per group). Fluorescence was measured in serum and tissue homogenates and expressed relative to blood (plasma) to quantify the amount of labeled exosomes in various tissues/organs. At time 0 min, the majority of fluorescence was observed in blood, and over time (10 min and 20 min), an increase of fluorescence was observed in a wide range of tissues, including the brain, which is primarily affected by recessive CNS disorders (Figure 1).

[00113] To demonstrate that exosomes can be used to deliver nucleic acids and protein *in vivo*, wildtype mice were treated with luciferase mRNA loaded exosomes. Exosomes (10 ug of total exosomal protein) were loaded with 100 ng of luciferase mRNA. Loading was accomplished using a cation-based transfection reagent as previously described. 10 ug of total exosomal protein (suspended in sterile 0.9% saline) was intravenously administered to mice. Mouse cerebral cortex, cerebellum, brain, liver, lungs, heart and skeletal muscle (quadriceps, tibialis anterior and gastrocnemius) were then harvested 4 hours following injection (3 mice per group). Luciferase activity was measured using a Luciferase Assay System (Cat. No. E1500; Promega Corporation) in tissue homogenates to quantify the amount of luciferase mRNA delivered to tissues. Saline injected mice were used as a control group. Mice treated with luciferase mRNA loaded exosomes demonstrated luciferase activity in all tissues measured including whole brain, cerebral cortex and

cerebellum (Figure 2), indicating that the mRNA was both delivered across the blood-brain barrier *in vivo* and translated into a function protein.

[00114] Thus, exosomes that are loaded with nucleic acid encoding the functional protein are effective to deliver protein to the brain for the purpose of restoring protein function and treating recessive CNS disorders.

Example 3 – Treatment of an Autosomal Recessive Disorder Affecting the Central Nervous System with mRNA-loaded Exosomes *In Vivo*

[00115] To determine the efficacy of the present engineered exosomes to treat autosomal recessive and X-linked recessive CNS disorders resulting from genetic mutations, exosomes were engineered to treat Pompe Disease as a representative disorder, which is caused by a mutation in the alpha acid glucosidase (GAA) protein and results in the accumulation of glycogen within brain and muscle tissue among others.

Breeding of GAA mice

[00116] Four GAA heterozygous breeding pairs (HET; GAA^{-/+}; 6neo/6⁺) were obtained from Jackson Laboratories (Maine, USA) to generate homozygous GAA knock-outs (MUT; GAA^{-/-}; 6neo/6neo). Mice were genotyped at 1 month of age using a standard genotyping kit as per vendor's instructions (REDEExtract-N-Amp Tissue PCR Kit; Sigma Aldrich). During breeding and throughout the experimental period, animals were housed in standard cages with 12-h light/dark cycles and free access to water/rodent chow (Harlan Teklad 8640 22/5) at McMaster University's Central Animal Facility. The study was approved by McMaster University's Animal Research and Ethics Board, and the experimental procedures strictly followed guidelines published by the Canadian Council of Animal Care.

Production of Non-immunogenic GAA mRNA-loaded Exosomes for Treatment of GAA mice

[00117] To produce exosomes from mouse dendritic cells (DC), DC were harvested as described in Example 1. GAA mRNA was loaded into the exosomes using the protein loading method as in Example 1. Exosomes were loaded with an mRNA dose equivalent to delivery of 40 mg/kg GAA, ~100-150 ng mRNA, in 10 ug of total exosomal proteins. GAA mRNA-loaded

exosomes were resuspended in 0.9% saline solution for intravenous treatment. GAA and WT mice were divided into two treatment groups (n = 7 per group): empty exosome, and GAA mRNA-loaded exosome (EXO mRNA-GAA). Mice were treated once a week for 7 weeks.

[00118] Skeletal muscle (quadriceps, tibialis anterior, EDL, soleus, and diaphragm), heart, and brain were harvested from mice in all treatment groups.

GAA enzyme activity

[00119] A standard fluorometric enzyme assay, originally described by Reuser et al. (Biochem Biophys Res Commun. 1978 Jun 29;82 (4):1176-82), was used to determine acid α -glucosidase (GAA; EC 3.2.1.20) activity of treated and untreated tissue. In brief, cellular lysates were prepared by homogenizing tissue in 200 μ L mannitol buffer (70 mM sucrose, 220 mM mannitol, 10 mM HEPES, 1 mM EGTA, protease inhibitor mixture (Complete Tablets, Roche), pH 7.4). Following BCA assay (Pierce) for colorimetric determination of protein concentration, 10 μ L of each sample (in triplicate) was mixed with 20 μ L of the artificial acid α -glucosidase substrate, 4-methyl-umbelliferyl α -D-glucopyranoside, in 0.2 M sodium acetate [NaAc] buffer, pH 3.9, heated to 65 °C in a 96 well black plate. Standards were prepared from a 5 mM 4-methylumbelliferone/50% ethanol stock by serial dilution in 0.2 M NaAc buffer (pH 3.9), loaded in 10 μ L triplicates, and mixed with 20 μ L 0.2 M NaAc buffer (pH 3.9). The samples were then incubated in the dark for 1 h at 37.5 °C and the reaction was terminated by adding 200 μ L of 0.5 M sodium carbonate (pH 10.7). The release of the product, 4-methylumbelliferone, from the substrate is proportional to acid α -glucosidase activity (nmol/mg protein/hr), and the resulting fluorescence was read at 360 nm excitation/460 nm emission with a monochromator-based microplate detection system (Tecan).

GAA mRNA-loaded exosomes therapy restores GAA activity and GAA mRNA in all tissues tested relative to wild-type mice

[00120] Treatment of GAA KO mice with GAA mRNA-loaded exosomes restored GAA activity in the GAA KO brain depicting that GAA mRNA-loaded exosomes could not only cross the blood-brain barrier, but that GAA mRNA delivered via exosomes did induce functional changes in neuronal tissue (Figure 3). Empty exosomes and GAA mRNA-loaded exosomes were

given to wild-type mice (WT) as controls. GAA activity was also restored in fast- (EDL) and slow- (soleus) fiber-type skeletal muscle, diaphragm, and heart, as compared to that in GAA KO mice treated with empty exosomes (Figures 4A and 4B). It should be noted that the mice were harvested four days after the last intravenous bolus of GAA mRNA-loaded exosomes and the GAA activity was maintained in all tissues tested. GAA mRNA expression was similarly maintained in brain and skeletal muscle (quadriceps femoris) in GAA KO mice treated with GAA mRNA-loaded exosomes (Figure 5).

[00121] There appear to be physiological alterations in mRNA stabilizing proteins and/or the miRNA network in GAA KO mice only (where GAA protein activity is negligible) that prevents degradation of the GAA mRNA delivered, compared to WT mice where mRNA levels do not increase significantly (since they have optimal GAA activity). Interestingly, tissues from WT mice treated with GAA mRNA-loaded exosomes did not show an up-regulation/overexpression of GAA activity indicating that there are inherent pathways that protect against an abnormal increase in GAA activity above physiological levels. From the perspective of treatment safety, this indicates the potential to avoid non-specific effects of very high levels of GAA activity (such as levels achieved by strategies like adeno-associated virus-mediated GAA induction) using exosomal protein or nucleic acid delivery. Thus, exosomes loaded with mRNA encoding a functional protein were delivered into the central nervous system and subsequently restored the amount of said functional protein to wildtype levels, indicating the efficacy of the disclosed method for treating recessive genetic CNS disorders.

Example 4 - Biengineering Targeting Exosomes can Enhance Exosome Delivery to Targeted Tissues

[00122] Primary mouse neuronal cells were isolated from C57Bl/6 mice using standard protocols. To test the efficiency of *in vitro* delivery of potential therapeutic agents (siRNA, proteins, mRNA, DNA, or small molecules), siRNA targeting a house-keeping gene GAPDH (abundantly expressed in almost all cell types) was used as proof of principle. Mouse primary neurons were treated with GAPDH siRNA or scRNA (scrambled RNA; control for siRNA) using several delivery methods. Delivery methods include 100 ng of naked scRNA or siRNA, 100 ng of scRNA or siRNA transfected with Lipofectamine 2000 (LF2000), scRNA- or siRNA-loaded non-

targeting exosomes, scRNA-loaded skeletal muscle targeting exosomes and siRNA loaded brain targeting exosomes (100 ng of scRNA or siRNA packaged in 10 ug of total exosomal protein in 0.9% sterile saline). GAPDH qPCR was carried out to assess down-regulation of GAPDH mRNA. scRNA and GAPDH siRNA were purchased from Sigma.

[00123] Treatment of neuronal cells with GAPDH siRNA loaded non-targeting exosomes or GAPDH siRNA-loaded brain targeting exosomes showed the most down-regulation of GAPDH compared to conventional GAPDH siRNA transfected with LF2000 (Figure 6).

Example 5 - Treatment of Perrault Syndrome with mRNA-loaded Exosomes

[00124] To determine the efficacy of the present engineered exosomes to treat the peroxisomal disorder type of CNS disorders resulting from genetic mutations, exosomes were engineered to treat a representative recessive CNS disease: Perrault syndrome.

[00125] Human primary dermal fibroblasts are isolated from skin biopsies of healthy subjects (referred to as Control/CON) and patients with Perrault syndrome (with HSD17B4 mutations) (n = 3 age/sex-matched per group). Fibroblasts are treated with HSD17B4 mRNA at a dose of about 100-150 ng of mRNA, 10 ug (total exosomal protein) of empty exosomes (exosome control), or exosomal HSD17B4 mRNA in dose equivalent to delivery of 40 mg/kg HSD17B4, about 100-150 ng mRNA, in 10 ug of total exosomal protein) for 48 hours in pre-spun growth media devoid of bovine microvesicles and exosomes.

[00126] A D-bifunctional protein enzyme activity assay is performed, while Western blotting is used to measure D-bifunctional protein content *in vitro*.

[00127] Primary fibroblasts are expected to show partial to complete rescue of D-bifunctional protein enzyme activity and protein content when treated with exosomal D-bifunctional protein mRNA.

Example 6 – Treatment of Creatine Transporter Deficiency 1 *In Vivo* with mRNA-loaded Exosomes

[00128] To determine the efficacy of the present engineered exosomes to treat autosomal recessive and X-linked recessive CNS disorders resulting from genetic mutations, exosomes are engineered to treat a representative X-linked recessive CNS disease: creatine transporter deficiency 1 (CTD1).

[00129] Exosomes were isolated and loaded with mRNA encoding the creatine transporter (CrT) protein as described in Example 2.

[00130] CrT deficient mice (a mouse model of human CTD1) were obtained from the laboratory of R. Skelton. Male CrT^{-/y} (CrT deficient) mice and CrT^{+/y} (normal CrT protein activity or WT) mice were used. All animals were housed three to five per cage in a 12-h light/dark cycle and were fed ad libitum (Harlan-Teklad 8640 22/5 rodent diet) after weaning. The study was approved by the McMaster University Animal Research and Ethics Board under the global Animal Utilization Protocol # 12-03-09, and the experimental protocol strictly followed guidelines put forth by Canadian Council of Animal Care.

[00131] A 7-week intravenous treatment of CrT deficient mice with CrT mRNA-loaded exosomes is conducted. Exosomes are loaded with an mRNA dose equivalent to delivery of ~100-150 ng mRNA, in 10 ug of total exosomal proteins, isolated and prepared as described in Example 1.

[00132] Immunohistochemistry using CrT antibody is carried out to measure CrT protein localization, while Western blotting is used to measure CrT content in vitro. Quantitative real-time PCR is also carried out to quantify CrT copy number.

[00133] Treatment CrT deficient with *CrT* mRNA-loaded exosomes is expected to restore CrT content in brain, skeletal muscle and heart, to those levels seen in WT mice treated with empty exosomes or naked *CrT* mRNA. Empty exosomes and *CrT* mRNA-loaded exosomes are given to WT mice as controls.

Example 7 – Exosome Isolation using PEG-based method

[00134] Exosomes were isolated from various human and other mammalian biological samples as follows.

[00135] Blood samples were collected from healthy human subjects using red top serum collection tubes (e.g. BD, Ref #367812) and blue top plasma collection tubes containing sodium citrate (e.g. BD, Ref #369714) for serum and plasma isolations, respectively. For serum isolation, blood was allowed to clot for 1 hour at room temperature followed by centrifugation at 2,000x g for 15 min at 4°C. For plasma isolation, blood was spun down immediately after collection at 2,000x g for 15 min at 4°C. Plasma and serum was similarly collected from C57Bl/6J mice and Sprague Dawley rats. Exosomes were then isolated from these samples, as well as from bovine whole milk (Natrell fine-filtered 3.25% milk) and cells in culture (e.g. CHO cells). From this point onwards, all exosome sources were treated the same.

[00136] Serum, plasma and milk were spun at 2000x g for 15 min at 4°C. The supernatant from the first centrifugation was spun at 2000x g for 60 min at 4°C to pellet debris. The supernatant was then spun at 15,000x g for 60 min at 4°C. The resulting supernatant was then filtered through a 45 µm filter (Millipore, cat. # SLHV033RS), followed by filtration through a 0.22 µm syringe filter (Millipore, cat. # SLGP0334B). The centrifugation and filtering steps have been determined to be optional steps. The filtered supernatant was then added to an equal volume of 16% PEG 6000 (Sigma, cat. # 81253) and 500mM NaCl in PBS (Bioshop, cat. # SOD002), mixed by inversion or gentle pipetting and incubated for 30 min at 4°C. The filtrate-PEG (8 %) solution was then spun at 10,000x g for 10 min at 4°C to pellet the exosomes. The supernatant was discarded and the pellet was solubilized in 600uL of 0.5M trehalose (Sigma, cat. # T0167) in PBS by gentle pipetting or on a mechanical plate rocker for 30 min at 4°C. Exosomes were further purified by applying the exosome-containing solution to centrifugation between 15,000x g - 150,000x g for 1 hr at 4°C. The resulting supernatant containing purified exosomes was then collected.

[00137] A BCA assay (Pierce™) was used to determine exosome yield of between 5-10mg of exosomal protein per 1mL of serum used. Transmission electron microscopy was performed on exosome solutions confirming the isolation of exosomes in the size range of 20-140 nm in

diameter. The size distribution profile of exosomes isolated using the present PEG- based method was then measured using a Beckman DelsaMax dynamic light scattering analyzer, showing that the majority of particles in these solutions were within the 20-140 nm size range with minimal contamination outside of this exosome size range. Exosomal purity was further exemplified by performing Western blots with the canonical exosome markers CD9, CD63, CD81 and TSG101. Both the supernatant and pellet fractions of exosome solutions isolated from mouse serum and plasma samples using the PEG- based isolation method (and a final ultracentrifugation step) demonstrated robust expression of these markers confirming the presence of exosomes. The purity of exosomes was also determined by performing a Ponceau S stain, a widely used indicator for the presence of protein bands during Western blotting.

[00138] Relevant portions of references referred to herein are incorporated by reference.

[00139] While the present application has been described with reference to examples, it is to be understood that the scope of the claims should not be limited by the embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

CLAIMS

1. A method of treating a central nervous system (CNS) disorder in a mammal comprising administering to the mammal exosomes that are genetically modified to incorporate a nucleic acid encoding a functional protein neuropeptide and/or the neuropeptide.
2. The method of claim 1, wherein the exosomes are essentially free from particles having a diameter less than 20 nm or greater than 140 nm.
3. The method of claim 1, wherein the CNS disorder is a recessive disorder selected from seizure disorders, spino cerebellar ataxia (SCA), a small molecule disorder such as urea cycle disorders, metal accumulation disorders, amino acidopathies and organic acidopathies, and peroxisomal diseases.
4. The method of claim 3, wherein the recessive CNS disorder selected from the group consisting of Cerebral creatine deficiency syndrome (CCDS) 1, CCDS 2, GLUT1 deficiency syndrome 1, infantile onset, severe, Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg), Epilepsy, progressive myoclonic 2A (Lafora), Epilepsy, progressive myoclonic 2B (Lafora), Epileptic encephalopathy, early infantile (EIEE) Type 1, EIEE Type 3, EIEE Type 8, EIEE Type 12, EIEE Type 15, EIEE Type 16, EIEE Type 18, Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2) / EIEE Type 20, EIEE Type 21, EIEE Type 23, EIEE Type 25, EIEE Type 28, EIEE Type 29, EIEE Type 34, EIEE Type 35, Microcephaly, seizures, and developmental delay (MCSZ), Aicardi-Goutieres syndrome (AGS) Type 1, AGS Type 2, AGS Type 3, AGS Type 4, AGS Type 5, AGS Type 6, Congenital disorder of glycosylation (CDG), Type 1a, CDG Type 1b, CDG Type 1c, CDG Type 1d, CDG Type 1e, CDG Type 1f, CDG Type 1g, CDG Type 1h, CDG Type 1i, CDG Type 1j, CDG Type 1k, CDG Type 1l, CDG Type 1m, CDG Type 1n, CDG Type 1o, CDG Type 1p, CDG Type 1q, CDG Type 1r, CDG Type 1s, CDG Type 1t, CDG Type 1u, CDG Type 1w, CDG Type 1x, CDG Type 1y, CDG Type 1z, CDG Type IIa, CDG Type IIb, CDG Type IIc, CDG Type IId, CDG Type IIe, CDG Type IIf, CDG Type IIg, CDG Type IIh, CDG Type Iii, CDG Type IIj, CDG Type IIk, CDG Type III, CDG Type IIn, Spinocerebellar ataxia, autosomal recessive 1, Ataxia, early-onset, with oculomotor apraxia and

hypoalbuminemia, Marinesco-Sjogren syndrome, Cockayne syndrome, type A / UV-sensitive syndrome 2, Cockayne syndrome, type B / Cerebrooculofacioskeletal syndrome 1 / De Sanctis-Cacchione syndrome / UV-sensitive syndrome, Spastic ataxia, Charlevoix-Saguenay type, Pelizaeus-Merzbacher disease / Spastic paraplegia 2, X-linked, Anemia, sideroblastic, with ataxia, Pontocerebellar hypoplasia (PCH), type 1a, PCH type 1b, PCH type 1c, PCH type 2a, PCH type 2b, PCH type 2c, PCH type 2d, PCH type 2e, PCH type 3, PCH type 4, PCH type 5, PCH type 6, PCH type 8, PCH type 9, PCH type 10, Spinocerebellar ataxia, autosomal recessive (SCAR) 2 (SCAR2), SCAR7, SCAR8, SCAR10, SCAR14, SCAR16, SCAR18, SCAR20, Ornithine transcarbamylase deficiency, Citrullinemia, Argininosuccinic aciduria, Argininemia, Carbamoylphosphate synthetase I deficiency, Hemochromatosis, Menkes disease, Wilson disease, Molybdenum cofactor deficiency A, Molybdenum cofactor deficiency B, Neurodegeneration with brain iron accumulation (NBIA) 1 / HARP syndrome, NBIA 2B / Infantile neuroaxonal dystrophy 1 / Parkinson disease 14, autosomal recessive, NBIA 3, NBIA 4, NBIA 6, Kufor-Rakeb syndrome, Spastic paraplegia 35, Woodhouse-Sakati syndrome, Fucosidosis, Spastic paraplegia 30 / neuropathy, hereditary sensory type IIc / mental retardation, autosomal dominant 9, Tyrosinemia (TYRSN), type 1, TYRSN type 2, TYRSN type 3, Homocysteinemia, Sulphite oxidase deficiency, Phenylketonuria, Maple syrup urine disease (MSUD), type Ia, MSUD, type Ib, MSUD, type II, Glutaric aciduria, type I, Methylmalonic aciduria, mut(0) type, Holocarboxylase synthetase deficiency, Propionic academia, Isovaleric academia, Peroxisome biogenesis disorder 1A (Zellweger), Peroxisome biogenesis disorder 3A (Zellweger), Peroxisome biogenesis disorder 4A / 4B (Zellweger), Peroxisome biogenesis disorder 5A / 5B (Zellweger), Peroxisome biogenesis disorder 6A / 6B (Zellweger), Peroxisome biogenesis disorder 7A / 7B (Zellweger), Peroxisome biogenesis disorder 8A / 8B (Zellweger), Peroxisome biogenesis disorder 10A (Zellweger), Peroxisome biogenesis disorder 11A / 11B (Zellweger), Peroxisome biogenesis disorder 12A (Zellweger), Peroxisome biogenesis disorder 13A (Zellweger), Adrenoleukodystrophy (ALD), Perrault syndrome (PRLT) 1, PRLTS1, PRLTS2, PRLTS3, PRLTS4 and PRLTS5.

5. The method of claim 1, wherein the neuropeptide or nucleic acid is exogenous.

6. The method of claim 1, wherein the recessive CNS disorder is creatine transporter deficiency 1 (CTD1) and the exosomes are genetically modified to incorporate creatine transporter mRNA.
7. The method of claim 1, wherein the CNS disorder is a peroxisomal disorder.
8. The method of claim 1, wherein the peroxisomal disorder is Perrault syndrome and the exosomes are genetically modified to incorporate D-bifunctional protein.
9. A method of increasing the amount or activity of a neuropeptide in the central nervous system of a mammal, comprising administering to the mammal exosomes that are genetically modified to incorporate a nucleic acid encoding a functional neuropeptide and/or the neuropeptide.
10. The method of claim 9, wherein the exosomes are genetically modified to incorporate a protein selected from the group consisting of solute carrier family 6 (neurotransmitter transporter), member 8 (SLC6A8), guanidinoacetate N-methyltransferase (GAMT), solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1), cystatin B (CSTB), epilepsy, progressive myoclonus type 2A, Lafora disease (laforin) (EPM2A), NHL repeat containing E3 ubiquitin protein ligase 1 (NHLRC1), aristaless related homeobox (ARX), solute carrier family 25 (mitochondrial carrier: glutamate), member 22 (SLC25A22), Cdc42 guanine nucleotide exchange factor 9 (ARHGEF9), phospholipase C beta 1 (PLCB1), ST3 beta-galactoside alpha-2,3-sialyltransferase 3 (ST3GAL3), TBC1 domain family member 24 (TBC1D24), seizure threshold 2 homolog (mouse) (SZT2), phosphatidylinositol glycan anchor biosynthesis class A (PIGA), NECAP endocytosis associated 1 (NECAP1), dedicator of cytokinesis 7 (DOCK7), solute carrier family 13 (sodium-dependent citrate transporter), member 5 (SLC13A5), WW domain containing oxidoreductase (WWOX), alanyl-tRNA synthetase (AARS), solute carrier family 12 (potassium/chloride transporter), member 5 (SLC12A5), inosine triphosphatase (ITPA), polynucleotide kinase 3'-phosphatase (PNKP), three prime repair exonuclease 1 (TREX1), ribonuclease H2 subunit B (RNASEH2B), ribonuclease H2 subunit C (RNASEH2C), ribonuclease H2 subunit A (RNASEH2A), SAM domain and HD domain 1 (SAMHD1), adenosine deaminase, RNA-specific (ADAR), phosphomannomutase 2 (PMM2), mannose phosphate isomerase (MPI), ALG6, alpha-1,3-glucosyltransferase (ALG6), ALG3, alpha-1,3- mannosyltransferase (ALG3),

dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit (DPM1), mannose-P-dolichol utilization defect 1 (MPDU1), ALG12, alpha-1,6-mannosyltransferase (ALG12), ALG8, alpha-1,3-glucosyltransferase (ALG8), ALG2, alpha-1,3/1,6-mannosyltransferase (ALG2), dolichyl-phosphate N-acetylglucosaminophosphotransferase 1 (DPAGT1), ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase (ALG1), ALG9, alpha-1,2-mannosyltransferase (ALG9), dolichol kinase (DOLK), RFT1 homolog (RFT1), dolichyl-phosphate mannosyltransferase subunit 3 (DPM3), ALG11, alpha-1,2-mannosyltransferase (ALG11), steroid 5 alpha-reductase 3 (SRD5A3), dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic) (DDOST), ALG13, UDP-N-acetylglucosaminyltransferase subunit (ALG13), phosphoglucomutase 1 (PGM1), dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit (DPM2), STT3A, catalytic subunit of the oligosaccharyltransferase complex (STT3A), STT3B, catalytic subunit of the oligosaccharyltransferase complex (STT3B), signal sequence receptor, delta (SSR4), carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT2), mannosyl-oligosaccharide glucosidase (MOGS), solute carrier family 35 member C1 (SLC35C1), UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 (B4GALT1), component of oligomeric golgi complex 7 (COG7), solute carrier family 35 member A1 (SLC35A1), component of oligomeric golgi complex 1 (COG1), component of oligomeric golgi complex 8 (COG8), component of oligomeric golgi complex 5 (COG5), component of oligomeric golgi complex 4 (COG4), transmembrane protein 165 (TMEM165), component of oligomeric golgi complex 6 (COG6), solute carrier family 39 member 8 (SLC39A8), senataxin (SETX), aprataxin (APTX), SIL1 nucleotide exchange factor (SIL1), excision repair cross-complementation group 8 (ERCC8), excision repair cross-complementation group 6 (ERCC6), saccin molecular chaperone (SACS), proteolipid protein 1 (PLP1), ATP binding cassette subfamily B member 7 (ABC7), vaccinia related kinase 1 (VRK1), exosome component 3 (EXOSC3), exosome component 8 (EXOSC8), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 2 (TSEN2), tRNA splicing endonuclease subunit 34 (TSEN34), Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS), VPS53 GARP complex subunit (VPS53), piccolo presynaptic cytomatrix protein (PCLO), tRNA splicing endonuclease subunit 54 (TSEN54), tRNA splicing endonuclease subunit 54 (TSEN54), arginyl-tRNA synthetase 2,

mitochondrial (RARS2), charged multivesicular body protein 1A (CHMP1A), adenosine monophosphate deaminase 2 (AMPD2), cleavage and polyadenylation factor I subunit 1 (CLP1), peptidase (mitochondrial processing) alpha (PMPCA), tripeptidyl peptidase I (TPP1), spectrin repeat containing, nuclear envelope 1 (SYNE1), anoctamin 10 (ANO10), spectrin beta, non-erythrocytic 2 (SPTBN2), STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase (STUB1), glutamate ionotropic receptor delta type subunit 2 (GRID2), sorting nexin 14 (SNX14), ornithine carbamoyltransferase (OTC), argininosuccinate synthase 1 (ASS1), argininosuccinate lyase (ASL), arginase 1 (ARG1), carbamoyl-phosphate synthase 1 (CPS1), Hemochromatosis (HFE), ATPase copper transporting alpha (ATP7A), ATPase copper transporting beta (ATP7B), molybdenum cofactor synthesis 1 (MOCS1), molybdenum cofactor synthesis 2 (MOCS2), pantothenate kinase 2 (PANK2), phospholipase A2 group VI (PLA2G6), ferritin, light polypeptide (FTL), chromosome 19 open reading frame 12 (C19orf12), Coenzyme A synthase (COASY), ATPase 13A2 (ATP13A2), fatty acid 2-hydroxylase (FA2H), DDB1 and CUL4 associated factor 17 (DCAF17), fucosidase, alpha-L- 1, tissue (FUCA1), kinesin family member 1A (KIF1A), fumarylacetoacetate hydrolase (fumarylacetoacetase) (FAH), tyrosine aminotransferase (TAT), 4-hydroxyphenylpyruvate dioxygenase (HPD), methylenetetrahydrofolate reductase (NAD(P)H) (MTHFR), sulfite oxidase (SUOX), phenylalanine hydroxylase (PAH), branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA), branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB), dihydrolipoamide branched chain transacylase E2 (DBT), glutaryl-CoA dehydrogenase (GCDH), methylmalonyl-CoA mutase (MUT), holocarboxylase synthetase (HLCS), propionyl-CoA carboxylase alpha subunit (PCCA), isovaleryl-CoA dehydrogenase (IVD), peroxisomal biogenesis factor 1 (PEX1), peroxisomal biogenesis factor 12 (PEX12), peroxisomal biogenesis factor 6 (PEX6), peroxisomal biogenesis factor 2 (PEX2), peroxisomal biogenesis factor 10 (PEX10), peroxisomal biogenesis factor 26 (PEX26), peroxisomal biogenesis factor 16 (PEX16), peroxisomal biogenesis factor 3 (PEX3), peroxisomal biogenesis factor 13 (PEX13), peroxisomal biogenesis factor 19 (PEX19), peroxisomal biogenesis factor 14 (PEX14), ATP binding cassette subfamily D member 1 (ABCD1), hydroxysteroid (17-beta) dehydrogenase 4 (HSD17B4), histidyl-tRNA synthetase 2 (HARS2), caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP), leucyl-tRNA synthetase 2 (LARS2) and chromosome 10 open reading frame 2 (C10orf2), or a nucleic acid encoding one or more of these proteins.

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Figure 1

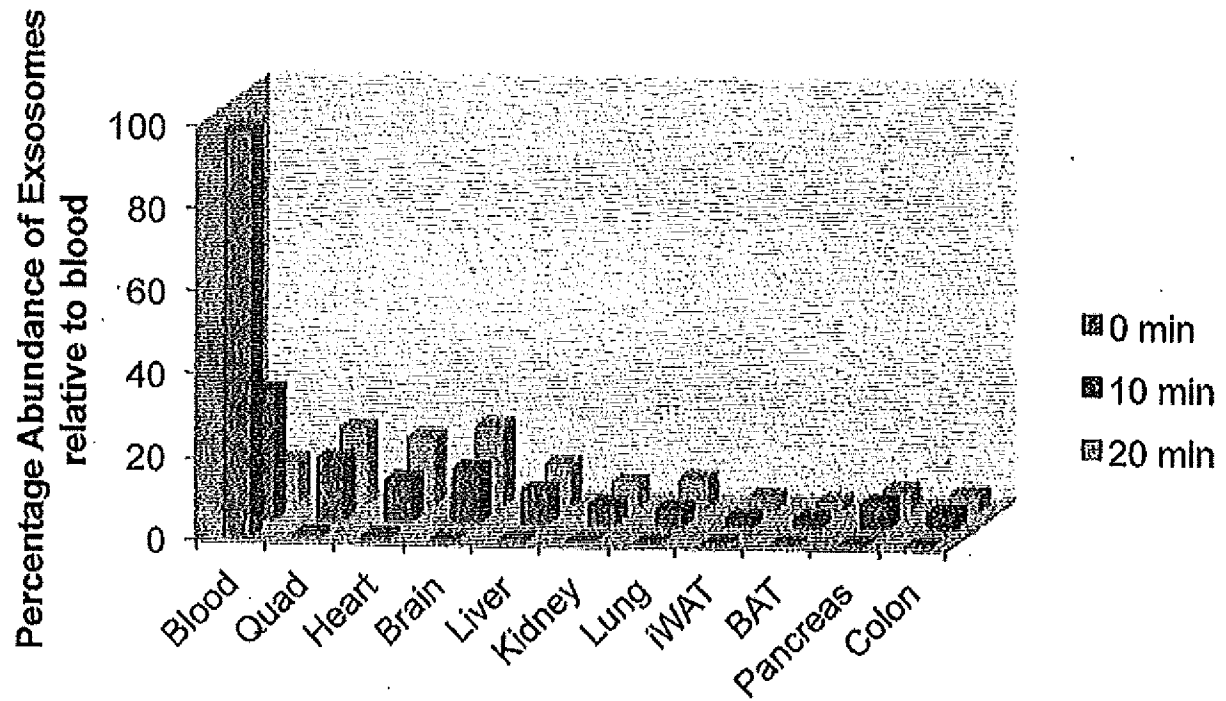
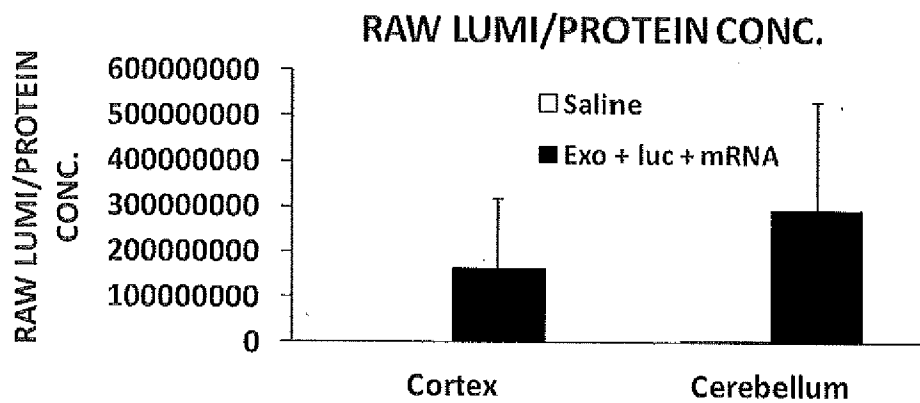


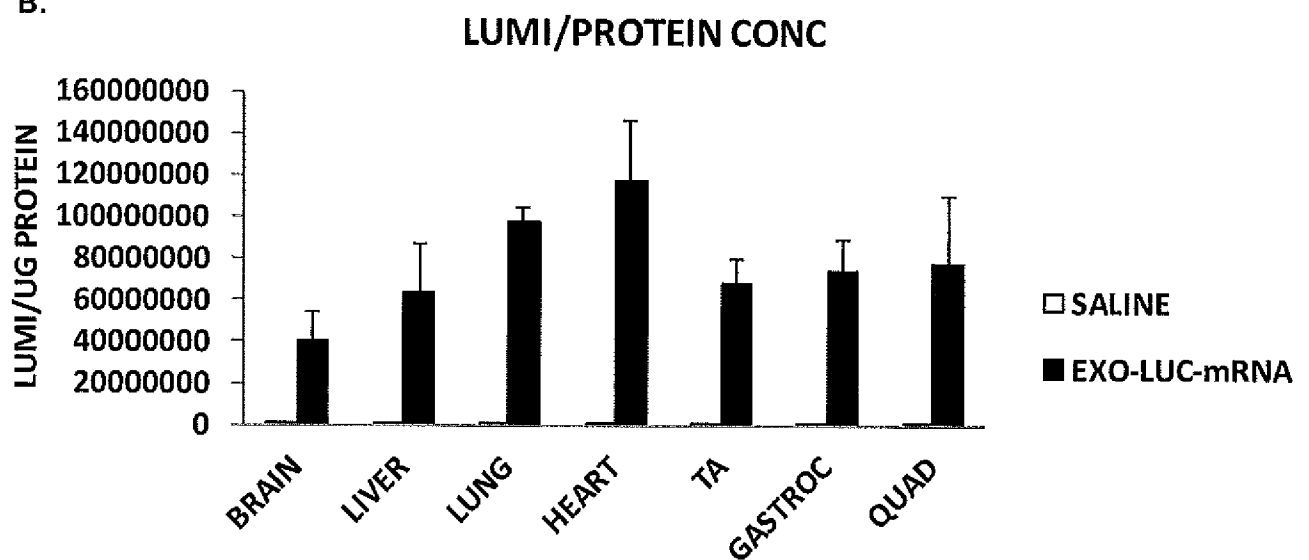
Figure 2

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A.



B.



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Figure 3

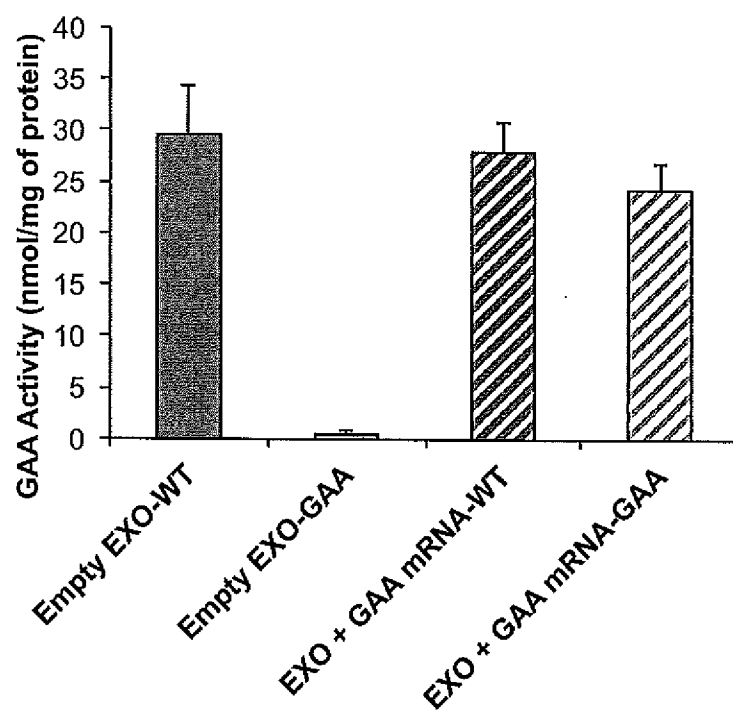
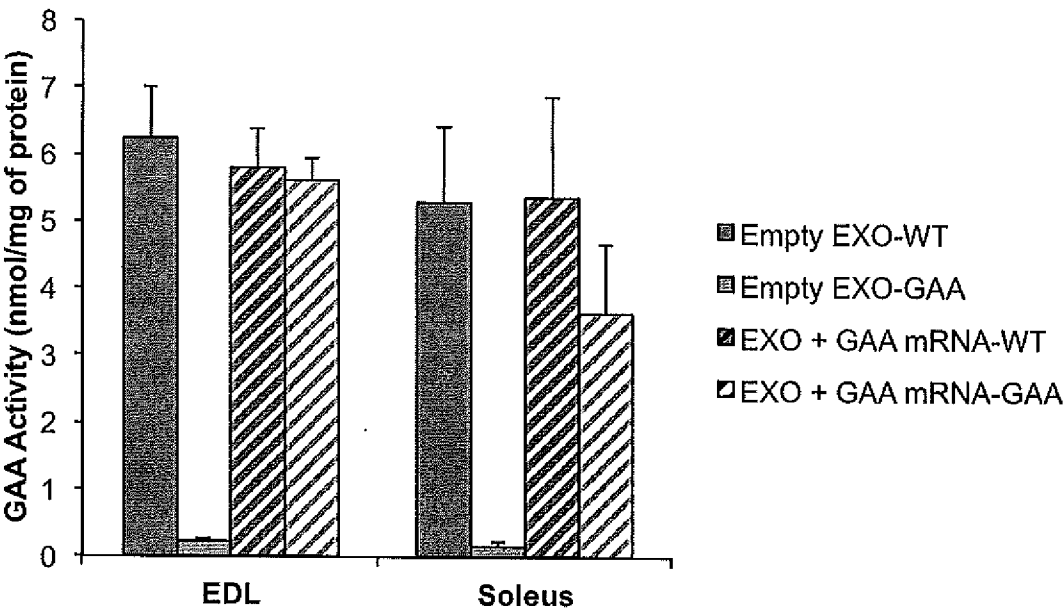


Figure 4

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A.



B.

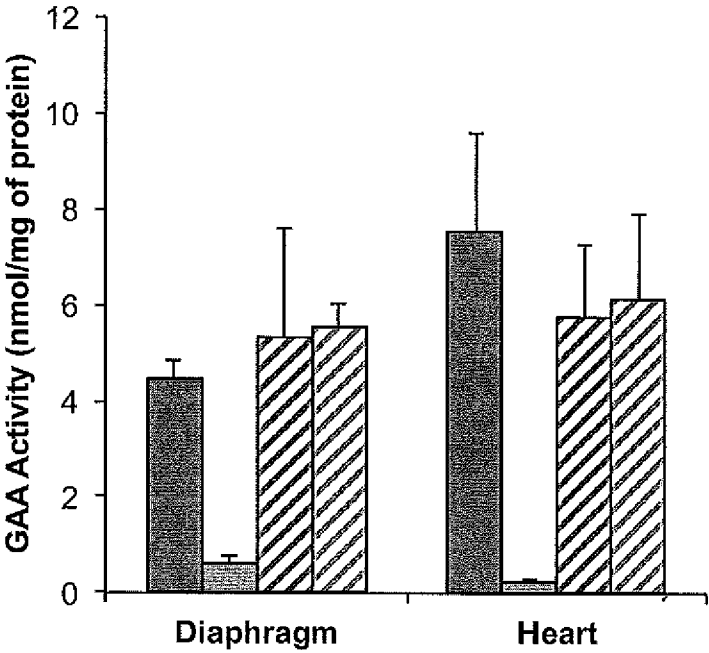


Figure 5

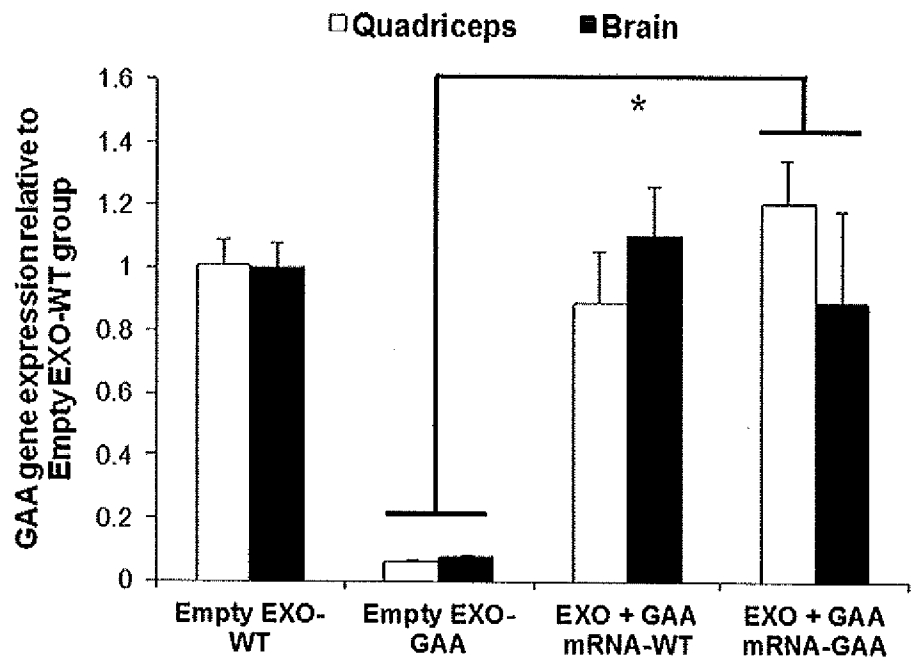
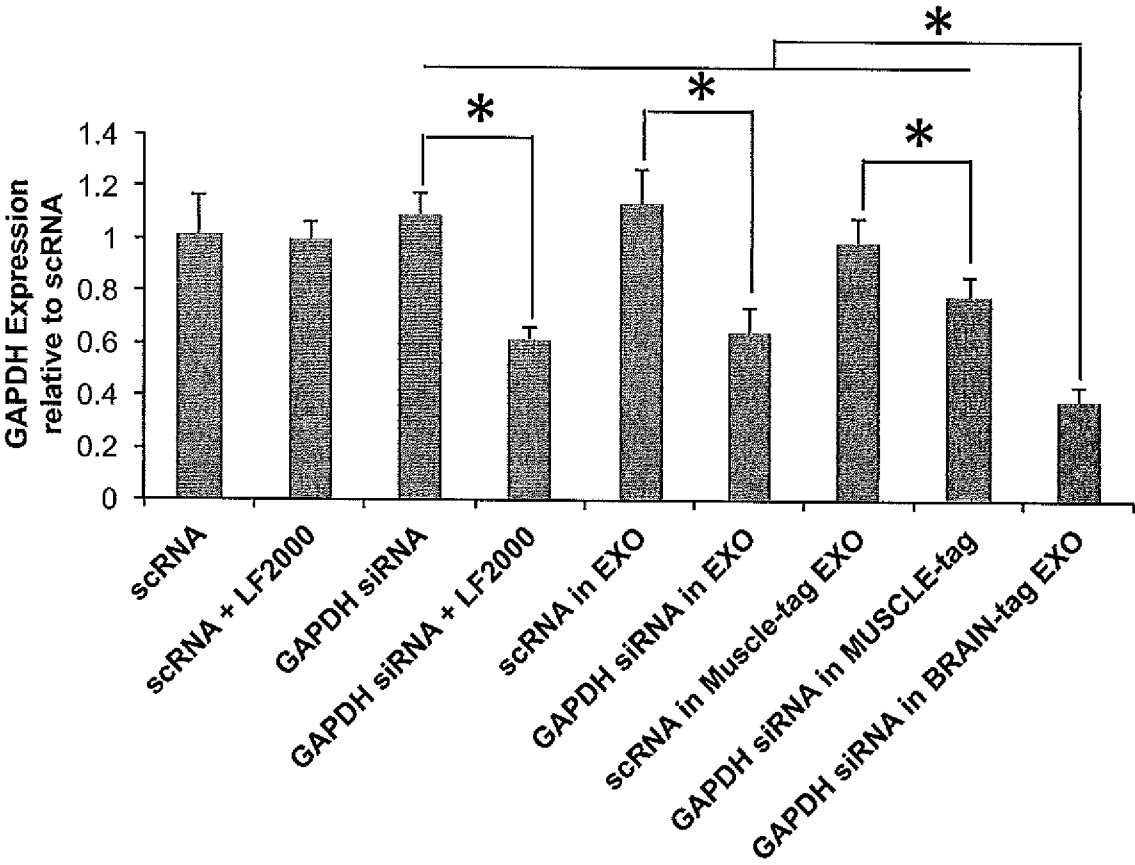


Figure 6

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050296

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 9/51** (2006.01), **A61K 35/12** (2015.01), **A61K 48/00** (2006.01), **A61P 25/00** (2006.01),
A61P 3/00 (2006.01), **C12N 5/10** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 9/51 (2006.01), **A61K 35/12** (2015.01), **A61K 48/00** (2006.01), **A61P 25/00** (2006.01),
A61P 3/00 (2006.01), **C12N 5/10** (2006.01) (more IPCs on the last page)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent database, Scopus, STN, Google

Keywords: exosomes, gene therapy, central nervous system (CNS), neuropeptide, menkes disease, creatine transporter deficiency, member 8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOUGHLIN et al., "Exosomes and the Emerging Field of Exosome-Based Gene Therapy". Current Gene Therapy, 2012, Vol. 12, pp. 262-274, ISSN 1875-5631 [online] [retrieved on 30 May 2017 (30-05-2017)]. Retrieved from the Internet: < http://www.eurekaselect.com/101143/article >	1-10
Y	WO2010119256 (SEOW, Y. et al.) 21 October 2010 (21-10-2010)	1-10
Y	WO2007126386 (LOTVALL, J.O. et al.) 8 November 2007 (08-11-2007)	1-10

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
01 June 2017 (01-06-2017)

Date of mailing of the international search report
13 June 2017 (13-06-2017)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
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50 Victoria Street
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Facsimile No.: 819-953-2476

Authorized officer

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050296

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DONSANTE et al., " <i>ATP7A Gene Addition to the Choroid Plexus Results in Long-term Rescue of the Lethal Copper Transport Defect in a Menkes Disease Mouse Model</i> ". The American Society of Gene & Cell Therapy, December 2011 (12-2011), Vol. 19(12), pp. 2114-2123, ISSN 1525-0024 [online] [retrieved on 31 May 2017 (31-05-2017)]. Retrieved from the Internet: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3242653/ >	1-10
Y	SMITH et al., " <i>Phase I/II Trial of Adeno-Associated Virus-Mediated Alpha-Glucosidase Gene Therapy to the Diaphragm for Chronic Respiratory Failure in Pompe Disease: Initial Safety and Ventilatory Outcomes</i> ". Human Gene Therapy, June 2013 (06-2013), Vol. 24, pp. 630-640, ISSN 1557-7422 [online] [retrieved on 31 May 2017 (31-05-2017)]. Retrieved from the Internet: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3689178/ >	1-10
Y	WO2011/058364 A1 (LYGATE, C. et al.) 19 May 2011 (19-05-2011)	1-10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050296**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 1-10
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-10 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 1-10.
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2017/050296

Patent Document Cited in Search Report	Publication Date	Patent Family Members	Publication Date
WO2010119256 A1	21 October 2010 (21-10-2010)	CN 102497887 A EP2419144 A1 GB0906692 DO GB0906693 DO JP2012524052 A JP5713325 B2 US2013053426 A1	13 June 2012 (13-06-2012) 22 February 2012 (22-02-2012) 03 June 2009 (03-06-2009) 03 June 2009 (03-06-2009) 11 October 2012 (11-10-2012) 07 May 2015 (07-05-2015) 28 February 2013 (28-02-2013)
WO2007126386 A1	08 November 2007 (08-11-2007)	CN101432432 A EP2010663 A1 EP2010663 A4 EP2010663 B1 EP2905339 A1 ES2539760 T1 US2007298118 A1 US9085778 B2 US2015290343 A1	13 May 2009 (13-05-2009) 07 January 2009 (07-01-2009) 17 November 2010 (17-11-2010) 18 March 2015 (18-03-2015) 12 August 2015 (12-08-2015) 03 July 2015 (03-07-2015) 27 December 2007 (27-12-2007) 21 July 2015 (21-07-2015) 15 October 2015 (15-10-2015)
WO2011/058364 A1	19 May 2011 (19-05-2011)	GB0919837 WO2011058364 EP2498769 US2012276012 JP2013510839	30 December 2009 (30-12-2009) 19 May 2011 (19-05-2011) 19 September 2012 (19-09-2012) 01 November 2012 (01-11-2012) 28 March 2013 (28-03-2013)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2017/050296

C12N 15/12 (2006.01), *C12N 15/52* (2006.01), *C12N 15/85* (2006.01), *C12N 15/88* (2006.01)