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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF ATPASE-MEDIATED DISEASES

(57) Abstract: The present disclosure provides nucleic acid expression cassettes, vectors, compositions and methods for the treatment of ATPase-mediated diseases in a subject.

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COMPOSITIONS AND METHODS FOR THE TREATMENT OF ATPASE-MEDIATED DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Patent Application Serial Number 62/847,416, filed May 14, 2019, the contents of which is hereby incorporated by reference in its entirety.

FEDERAL FUNDING LEGEND

[002] This invention was made with government support under Grant No. UL1TR002553 awarded by the National Center for Advancing Translational Sciences. The Federal Government has certain rights to this invention

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED ELECTRONICALLY

[003] This application contains a Sequence Listing submitted as an electronic text file named "20-489-WO_SequenceListing_ST25.txt", having a size in bytes of 90 kb, and created on May 14, 2020. The information contained in this electronic file is hereby incorporated by reference in its entirety.

BACKGROUND

Field

[004] The present disclosure provides nucleic acid expression cassettes, vectors comprising a nucleic acid encoding ATPases, such as ATP1A3, and compositions thereof, and gene therapy methods for the treatment of ATPase-mediated diseases in a subject.

Description of the Related Art

[005] Alternating Hemiplegia of Childhood (AHC) is a devastating neurological disorder that manifests with severe developmental impairments, episodes of hemiplegias, dystonias, epilepsy, behavioral problems, and often also regression. AHC causes severe morbidity and increased mortality estimated at 3.2/1000 patients/year and at least 4.5% by the age of 29 years. (Panagiotakaki *et. al.* (2015) *Orphanet J Rare Dis.* 10: 123). It affects approximately 1 in 1,000,000 children. About 80% of the cases are caused by *ATP1A3* mutations with the D801N mutation being the most common of those (~40% of patients).

AHC is an example of an ATPase-related disease caused by ATPase mutations and the most common of the disorders caused by *ATP1A3* mutations. ATPase-related diseases also encompass disorders with secondary ATPase deficiency resulting from other causes such as neurodegenerative disease like Alzheimer's and Parkinson's diseases.

[006] ATP1A3 is the principal subunit of the Na/K-ATPase protein that is expressed in neurons, while ATP1A2 is expressed in glia. ATP1A3 is an enzyme, a signal transduction factor and, most importantly, a pump that consumes 50% of the energy of the brain. (Holm *et al.* (2016) *Biochem Biophys Acta*. 1857(11): 1807-1828). Primary Na/K-ATPase dysfunction due to *ATP1A3* mutations results in AHC and in other neurogenetic disorders. In addition, secondary dysfunction of this pump, resulting from other common neurological disorders, contributes to the pathophysiology of these disorders that include epilepsy, stroke, hypoglycemia, acute ataxia, dystonia and to neuronal death in neurodegenerative diseases including Alzheimer's and Parkinson's diseases. *ATP1A3* disease causing mutations, including D801N and I810N, result in profound abnormalities in neuronal excitability. (Hunanyan *et al.* (2015) *Epilepsia* 56(1): 82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468; Helseth *et al.* (2018) *Neurobiol Dis*. 119: 100-112). Despite normal levels of ATP1A3 protein expression, these mutations result in reduced ATPase enzyme activity in HeLa cells and COS cells (down to about 54% of normal) as well as in mouse brain (down to about 58% of normal). (Heinzen *et al.* (2012) *Nat Genet*. 44(9): 1030-1034; Clapcote *et al.* (2009) *Proc Natl Acad Sci USA*. 106(33): 14085-14090; Kirshenbaum *et al.* (2013) *PLoS One*. 8(3): e60141).

[007] Currently, the only available therapy for AHC is the calcium channel blocker flunarizine, which causes partial reduction in the hemiplegia spells but has no effect on the other usually very severe manifestations of the disease. Accordingly, there is a need for novel and effective therapies for the treatment of ATP1A3-mediated diseases, including AHC. There is also an acute need for effective therapies to treat other disorders resulting from either secondary deficiencies of other ATPase activities or from mutations of other ATPases, such as mutations of ATP1A2 resulting in severe epileptic encephalopathy.

BRIEF SUMMARY OF THE DISCLOSURE

[008] The present disclosure provides, in part, nucleic acid expression cassettes, vectors, pharmaceutical compositions, kits, and methods for the treatment of ATPase-mediated diseases (*e.g.*, AHC) in a subject.

[009] One aspect of the disclosure provides a nucleic acid expression cassette comprising a nucleic acid sequence encoding an ATPase. In some embodiments, the ATPase is ATP1A1, ATP1A2, ATP1A3, ATP2C1, ATP6A1, ATP6V1B1, ATP6V0A4, ATP7A, ATP7B, or ATP11C. In some embodiments, the ATPase is ATP1A3.

[0010] In some embodiments of the disclosure, the nucleic acid sequence encoding an ATPase comprises the sequence set forth in any of SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07, or a fragment, isoform, or homologue thereof, or a sequence having at least 50%-90% identity to the sequence set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07.

[0011] In some embodiments of the disclosure, the nucleic acid expression cassette further comprises a nucleotide sequence encoding ATP1A3 that is codon-optimized to reduce CpG methylation sites and for mammalian expression.

[0012] In some embodiments of the disclosure, the nucleic acid expression cassette comprises a nucleic acid sequence encoding ATP1A3 that is operably linked to a promoter and a polyadenylation sequence. In some embodiments of the disclosure, the promoter is a tissue-specific promoter (e.g., a neuron-specific promoter or a heart-specific promoter). In some embodiments of the disclosure, the neuron-specific promoter is selected from the group consisting of synapsin 1, calcium/calmodulin-dependent protein kinase II, tubulin alpha 1, neuron-specific enolase, and platelet-derived growth factor beta chain promoters.

[0013] In some embodiments of the disclosure, the promoter is a human synapsin promoter, which can comprise the proximal region of the synapson 1 promoter (-422 to -22). In some embodiments of the disclosure, the human synapsin promoter comprises the nucleic acid sequence set forth in any of SEQ ID NO:04, SEQ ID NO:05, or SEQ ID NO:09.

[0014] In some embodiments of the disclosure, the promoter is a constitutively active promoter, such as the human β-actin, human elongation factor-1α, chicken β-actin combined with cytomegalovirus early enhancer, cytomegalovirus (CMV), simian virus 40, or herpes simplex virus thymidine kinase.

[0015] In some embodiments of the disclosure, the nucleic acid expression cassette comprises a transcriptional termination signal, such as the bovine growth hormone polyadenylation signal (BGHpA), Simian virus 40 polyadenylation signal (SV40pA), or a synthetic polyadenylation signal.

[0016] Another aspect of the present disclosure provides a vector comprising a nucleic acid sequence encoding an ATPase. In some embodiments, the ATPase is ATP1A1, ATP1A2, ATP1A3, ATP2C1, ATP6A1, ATP6V1B1, ATP6V0A4, ATP7A, ATP7B, or ATP11C. In other embodiments, the ATPase is ATP1A3.

[0017] In some embodiments, the vector is a viral vector or non-viral vector. In some embodiments, the vector is a recombinant viral vector.

[0018] In some embodiments, the viral vector is selected from the group consisting of an adenovirus vector, an adeno-associated virus vector, a herpes simplex virus vector, a retrovirus vector, a lentivirus vector, and alphavirus vector, a flavivirus vector, a rhabdovirus vector, a measles virus vector, a Newcastle disease viral vector, a poxvirus vector, or a picornavirus vector.

[0019] In some embodiments of the disclosure, the adenovirus vector is an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVrh74, AAV8, AAV9, AAV10, AAV11, AAV12 and AAV13. In other embodiments of the disclosure, the adenovirus vector is an AAV serotype selected from the group consisting of AAV1, AAV8, or AAV9. In yet other embodiments of the disclosure, the adenovirus vector is AAV9.

[0020] In some embodiments of the disclosure, the vector comprises the AAV9-hSyn-ATP1A3-p2A-mCherry (pBK828) vector.

[0021] In some embodiments of the disclosure, the nucleic acid sequence encoding an ATPase is contained in a nucleic acid expression cassette within the vector.

[0022] In some embodiments of the disclosure, the vector further comprises one or more of the following elements: (a) an inverted terminal repeat sequence (ITR); (b) a promoter; (c) a transcription terminator; and (d) a flanking inverted terminal repeat sequence (ITR).

[0023] In some embodiments of the disclosure, the vector comprises a tissue specific promoter, such as a neuron-specific promoter, muscle-specific promoter, liver-specific promoter, or cardiac-specific promoter. In some embodiments of the disclosure, the vector comprises a promoter that is derived from the human synapsin promoter, which can have a nucleotide sequence set forth in any of SEQ ID NO:04, SEQ ID NO:05, or SEQ ID NO:09.

[0024] In some embodiments of the disclosure, the vector comprises a constitutively active promoter, such as a human β-actin, human elongation factor-1α, chicken β-actin combined with cytomegalovirus early enhancer, cytomegalovirus (CMV), simian virus 40, and herpes simplex virus thymidine kinase.

[0025] In some embodiments of the disclosure, the vector comprises a transcription terminator, such as the bovine growth hormone polyadenylation signal (BGHpA), Simian virus 40 polyadenylation signal (SV40pA), or a synthetic polyadenylation signal.

[0026] Yet another aspect of the disclosure provides a composition comprising a vector comprising a nucleic acid sequence encoding an ATPase.

[0027] Yet another aspect of the disclosure provides a pharmaceutical composition comprising a vector comprising a nucleic acid sequence encoding an ATPase and a pharmaceutically acceptable carrier and/or excipient.

[0028] Yet another aspect of the disclosure provides a method of treating or preventing an ATPase-mediated disease in a subject, the method comprising administering to the subject a therapeutically effective amount of the nucleic acid expression cassettes, vectors, or compositions thereof described herein, such that the ATPase-mediated disease in the subject is treated or prevented.

[0029] In some embodiments of the disclosure, the nucleic acid expression cassettes, vectors, or compositions thereof is administered by a route selected from the group consisting of intramuscular injection, systemically, parenterally by injection, infusion or implantation, intracerebraoventricular, intra-cisterna magna, intrahippocampal, and intrathecal.

[0030] In some embodiments of the disclosure, the subject is a human.

[0031] In some embodiments of the disclosure, the subject has an ATP1A3 protein mutation selected from the group consisting of an E815K mutation, a D801N mutation, an I180N mutation, a R756C mutation, or a V589F mutation.

[0032] In some embodiments of the disclosure, the ATPase-mediated disease is selected from the group consisting of rapid-onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (AHC), epileptic encephalopathy (EE), cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss (CAPOS) syndrome, fever induced paroxysmal weakness and encephalopathy (FIPWE), recurrent episodes of cerebellar ataxia (RECA), early-onset schizophrenia Dystonia Dysmorphism of the face Encephalopathy MRI abnormalities without hemiplegia (D-DEMO) syndrome and Childhood Rapid Onset ataxia (CROA). In other embodiments, the ATPase-mediated disease is AHC.

[0033] Yet another aspect of the disclosure provides a use of the nucleic acid expression cassettes, vectors, or compositions thereof for the preparation of a medicament for the treatment or prevention of an ATPase-mediated disease (e.g., AHC).

[0034] Yet another aspect of the disclosure provides a kit for the treatment and/or prevention of an ATPase-mediated disease in a subject, the kit comprising a composition of the disclosure and instructions for use.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **FIG. 1** is a schematic of a representative isolated nucleic acid molecule comprising the following elements: 5' ITR, hSyn promoter, *ATP1A3* transgene, cMyc tag, FLAG tag, p2A peptide, mCherry, poly A (pA), and 3' ITR that can be used in the nucleic acid expression cassettes, vectors, and compositions described herein.

[0036] **FIG. 2** is a construct map of the AAV vector comprising pBK828 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA, active vector).

[0037] **FIG. 3** is a construct map of the AAV vector comprising pBK292 (control vector).

[0038] **FIG. 4** is a bar graph showing the effect of AAV-ATP1A3 on ATPase activity in the hippocampus of adult wild type (WT) mice injected with AAV-ATP1A3-cherry (n=2) virus into the hippocampus as compared to those injected with AAV-cherry (n=2) in the presence and absence of 3 mM ouabain (specific inhibitor of Na/K-ATPase) in accordance with one embodiment of the present disclosure. AAV injected 1 µL/per hemisphere, intra-parenchymal, into each dorsal hippocampus ($4x10^{13}$ vg/mL, total 2 µL per mouse).

[0039] **FIG. 5** is a graph showing the effect of unilateral intracerebroventricular (ICV) injection of active vector (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA, referred to as AAV9-Tr, dose 5 µL of $4x10^{13}$vg/mL) into the lateral ventricle at P10 on ipsilateral hippocampus ouabain-sensitive ATPase activity (nmol Pi/mg protein/min) of P40 WT mice as compared to control vector lacking the transgene (AAV9-CTL, n=3 in each group).

[0040] **FIGS. 6A-6B** are epifluorescence images showing AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA transduction in adult $Mashl^{+/-}$ mice 10 days after intrahippocampal injection of $20x10^{10}$ vg (V=5 µL of $4x10^{13}$ vg/mL) mouse. **FIG. 6A** is an image of neurons in CA1 hippocampal pyramidal layer. **FIG. 6B** is an image of stratum oriens interneuron of the same mouse.

[0041] **FIGS. 7A-7B** are confocal images showing mCherry expression of AAV9-ATP1A3-mCherry-FLAG ($4x10^{13}$ vg/mL; V=5 µL) after 4 weeks of ICV injection in the right lateral ventricle at P10 in accordance with one embodiment of the present disclosure. **FIG. 7A** shows the CA1 ventral hippocampus. **FIG. 7B** shows cerebellar Purkinje cells and deep nucleus (insert). Images stained with DAPI (blue). Red color is mCherry. Scale bar is 50 µm. n=3 mice.

[0042] **FIG. 8** is a graph showing the effect of AAV-ATP1A3-Cherry and AAV-cherry on beam test in $Mashl^{+/-}$ mice. Unilateral intrahippocampal injection of $20x10^{10}$ vg (volume 5

µl) of active vector into the hippocampus was performed on 2-3 month old mice. Test performed 3 weeks post-injection.

[0043] **FIGS. 9A-9F** are confocal images showing vector transduction as assessed by FLAG-tag green fluorescence, mCherry red fluorescence and DAPI (stains nuclei) in blue. Injections were performed at P10 with sacrifice at P40. **FIG. 9A** is an image showing hippocampal CA3 region vector transduction (unilateral ICV injection of $20x10^{10}$ vg). **FIG. 9B** is an image showing higher magnification image taken from CA3 region, box in FIG. 9A. **FIG. 9C** is an image showing expression in hippocampal CA1 region after the same ICV dose injected similarly. **FIG. 9D** is an image showing higher magnification image, also taken from CA1 region, after the same ICV dose injected similarly. **FIG. 9E** is an image showing trypan blue, 0.4%, staining of cerebellum two hours after intra-cisterna magna (ICM) injection showing robust staining of the cerebellum. **FIG. 9F** is an image showing cerebellar vector transduction after ICM injection of AAV9-ATP1A3-mcherrry ($15x10^{10}$ vg). Robust signal, indicating robust viral vector mediated expression, is seen in cerebellar Purkinje cells but not in deeper areas. Scale bar in **FIG. 9A, FIG. 9B,** and **FIG. 9C** is 10, in **FIG. 9B** is 5 µm and **FIG. 9E** is 20 µm.

[0044] **FIGS. 10A-10H** show expression of reporter genes at P40 (confocal merged images in all, FLAG, green; mCherry, red; DAPI, staining nuclei as blue): high in hippocampus (A, B) and in cerebellum close to ICM injection site (E). It is lower in cortex (C,D), and very low if any in cerebellum distant from ICM injection site (F), brainstem (G) and thalamus (H) following vector injected ICM and bilateral ICV of $9x10^{10}$ in each (total $27x10^{10}$ vg/mouse) at P10. **FIG. 10A** is an image of expression of reporter genes at P40 in hippocampal CA1 region. **FIG. 10B** is a high power image of the image in FIG. 10A. **FIG. 10C** is an image of expression of reporter genes in the sensorimotor cortex. **FIG. 10D** is a magnification of the image in FIG. 10C. **FIG. 10E** is an image of expression of reporter genes in the cerebellum close to the ICM injection site. **FIG. 10F** is an image of expression of reporter genes in the cerebellum distant from the ICM injection site. **FIG. 10G** is an image of expression of reporter genes in the midbrain. **FIG. 10H** is an image of expression of reporter genes in the thalamus. Scale bars=100 µm (A, C), 10 µm (B, D) and 500 µm (E-H).

[0045] **FIG. 11** is a graph showing a comparison of cold water induced hemiplegia among the following groups of mice treated via ICM and ICV injections: WT uninjected mice (naïve), WT mice treated with control vector (WT CTRL), WT treated with active vector (WT Treatment), $Mashl^{+/-}$ mice treated with control vector (Het CTRL), and $Mashl^{+/-}$ treated with active vector (Het Treatment). Mice were injected ICM and bilateral ICV in each

lateral ventricle at P10 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x10^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site. Comparison groups were WT naïve (n=10), WT-CTRL group (n=10), WT-treatment (n=4), Het-CTRL (n=10), Het-treatment (n=9). These were compared at P40.

[0046] **FIG. 12** is a graph showing a comparison of the duration of cold water induced dystonia between Mashl$^{+/-}$ mice treated with control vector (Het CTRL) and Mashl$^{+/-}$ treated with active vector via ICM and ICV injection (Het Treatment). Mice were injected ICM and bilateral ICV in each lateral ventricle at P10 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x0^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site, as per [0045]) and were compared at P40.

[0047] **FIG. 13** is a graph showing a comparison of cold-water induced seizures between Mashl$^{+/-}$ mice treated with control vector (Het CTRL) Mashl$^{+/-}$ treated with active vector via ICM and ICV injection (Het Treatment). Mice were injected ICM and bilateral ICV in each lateral ventricle at P10 and sacrificed at P40 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40-polyA $22.5x0^{10}$ vg/animal, $7.5x10^{10}$vg in each site, as per [0045] and were compared at P40.

[0048] **FIG. 14** is a graph showing a comparison of the time to cross a balance beam among the following groups of mice treated via ICM and ICV injections: WT uninjected mice (naïve), WT mice treated with control vector (WT CTRL), WT treated with active vector (WT Treatment), Mashl$^{+/-}$ mice treated with control vector (Het CTRL), and Mashl$^{+/-}$ treated with active vector (Het Treatment). Mice were injected ICM and bilateral ICV in each lateral ventricle at P10 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x0^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site, as per [0045] and were compared at P40.

[0049] **FIG. 15** is a graph showing the Kaplan-Meier survival curve comparing the treatment and control groups demonstrating a major positive effect on survival in mice injected with active vector at P10 with the above ICM and bilateral ICV active and control vector injection regimen described in [0045] above (p=0.009). The groups were WT mice treated with control vector (WT CTRL), WT treated with active vector (WT Treatment), Mashl$^{+/-}$ mice treated with control vector (Het CTRL), and Mashl$^{+/-}$ treated with active vector (Het Treatment). Mice were injected ICM and bilateral ICV in each lateral ventricle at P10 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x0^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site, [as per 0045]) and were followed into adulthood through P90.

[0050] **FIG. 16** is Western blot of 3 mice WT-Tr (cerebellum), WT-CTL (cerebellum) and HET-Tr (hippocampus). The FLAG band is the top band and β actin is the bottom band. Mice were injected ICM and bilateral ICV in each lateral ventricle at P10 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x0^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site, 9-10/group) and were sacrificed for Western blot after P90.

[0051] **FIG. 17** is a graph showing comparison of Western blot densitometry results of the treatment and control groups in the hippocampus with combined ICM and bilateral ICV injections at P10 and sacrificed after P90 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA $22.5x0^{10}$ vg/animal, or with control vector without *ATP1A3* transgene, $7.5x10^{10}$vg in each site). WT CTRL, n=2 mice; WT treated with active vector (WT Treatment), n=2 mice; $Mashl^{+/-}$ CTRL (HeT CTRL), n=2 mice; $Mashl^{+/-}$ treated with active vector (Het Treatment), n=3 mice.

DETAILED DESCRIPTION

[0052] For the purposes of promoting an understanding of the principles of the present disclosure, reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the disclosure is thereby intended, such alteration and further modifications of the disclosure as illustrated herein, being contemplated as would normally occur to one skilled in the art to which the disclosure relates.

[0053] As used in the specification, articles “a” and “an” are used herein to refer to one or to more than one (i.e., at least one) of the grammatical object of the article. By way of example, “an element” means at least one element and can include more than one element.

[0054] “About” is used to provide flexibility to a numerical range endpoint by providing that a given value may be “slightly above” or “slightly below” the endpoint without affecting the desired result. The term “about” in association with a numerical value means that the numerical value can vary plus or minus by 5% or less of the numerical value.

[0055] Throughout this specification, unless the context requires otherwise, the word “comprise” and “include” and variations (e.g., “comprises,” “comprising,” “includes,” “including”) will be understood to imply the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other integer or step or group of integers or steps.

[0056] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations where interpreted in the alternative ("or").

[0057] As used herein, the transitional phrase "consisting essentially of" (and grammatical variants) is to be interpreted as encompassing the recited materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

[0058] Moreover, the present disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0059] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise-Indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure.

[0060] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0061] **Gene Therapy of ATPase-Mediated Diseases**

[0062] The inventors have developed a knock-in mouse model of the D801N mutation (referred to herein as Mashlool, Mashl, or Mashl$^{+/-}$) that faithfully reproduces the human condition including a response to flunarizine, which is similar to what is observed in humans. In support of using gene therapy to treat genetic ATP1A3 disease is that in the mouse model carrying the, albeit different, I810N *ATP1A3* mutation, behavioral abnormalities were rescued by injection of bacterial artificial chromosome (BAC) containing the wild type *ATP1A3* gene into pronuclei of fertilized oocytes. This behavioral improvement was associated with a 16 % increase in brain-specific Na^+, K^+-ATPase activity. An alternative approach is to use gene therapy. This approach has proven to be a promising mode of therapy

for rare and severe neurogenetic disorders in mouse models and in humans. Gene therapy that may prove to be effective in AHC may have implications on therapy of other neurological disorders of secondary ATPase deficiency such as Alzheimer's disease and Parkinson's disease because in these disorders ATP1A3 dysfunction has been shown to be responsible for neuronal death and degeneration. It has been shown in the above I810N model (Clapcote *et al.* (2006) *Proc Natl Acad Sci USA,* 106(33): 14085-14090) as well as in the D801N model (240±19, in wild type and 134±25 nmol Pi/min/mg protein, in Mashl mice, n=3 in each group, p= 0.002) that mutations of *ATP1A3* result in about a 40% reduction in ATPase activity. They also cause abnormal hippocampal firing and behavioral abnormalities in mutant mice (Clapcote *et al.* (2006) *Proc Natl Acad Sci USA,* 106(33): 14085-14090; Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468; Masoud *et al.* (2017) *Curr Treat Options Neurol.* 19(2):8; Heinzen *et al.* (2012) *Nat Genet.* 44(9):1030-1034; Helseth *et. al.* (2018) *Neurobiol Dis.* 119:100-112; Holm *et al.* (2016) *Biochim Biophys Acta.* 1857(11):1807-1828; Ikeda *et al.* (2017) *Brain Res.* 1666: 27–37).

[0063] Hippocampus is one of the brain regions relevant to the AHC phenotype as human patients (as well as mice) with α-subunit mutations develop seizures of temporal lobe origin and have memory deficits. Another region relevant to the AHC phenotype is the cerebral cortex. Both humans and mice with AHC mutations also manifest neocortical onset seizures, behavioral impairments and cognitive deficits. In addition, basal ganglia, cerebellum and thalamus are involved due to the dystonia, movement control abnormalities and ataxia. The cerebellum is a region of particular interest. This is because patients with AHC invariably have ataxia, often have cerebellar hypometabolism on PET scans and cerebellar atrophy on MRIs and because the cerebellum is involved in the generation of dystonia (Severino *et al.* (2020) *J Neurol.* 2020 May;267(5):1300-1311; Ghusayni *et al.* (2020) *European J Ped Neurol;* Feb 13:S1090-3798(20)30032-5; Isaksen *et al.* (2017) *PLoS Genet.* 13(5): e1006763). The results described herein in the Examples section support the therapeutic effect of gene therapy in the D801N mouse model similar to studies in other neurogenetic disorders. The results described herein is the first demonstration of an effective gene therapy of any ATPase deficiency constituting gene therapy that targets an enzyme, a pump, and a signal transduction factor.

[0064] As used herein, the term "ATPase-mediated disease" refers to those diseases and/or disorders characterized by mutations in a gene encoding an ATPase. The term

"ATPase" refers to a class of enzymes that catalyze the hydrolysis of phosphate bonds in an adenosine triphosphate (ATP) to form adenosine diphosphate (ADP) or the inverse reaction.

[0065] As used herein, the term "ATP1A3-mediated disease" refers to those diseases and/or disorders characterized by mutations in the *ATP1A3* gene or by dysfunction of the ATP1A3 protein pump.

[0066] As used herein, the term "*ATP1A3*" refers to the ATPase Na+/K+ Transporting subunit alpha 3 [Homo Sapiens (human)] gene, in which the protein encoded by this gene belongs to the family of P-type cation transport ATPases, and to the subfamily of Na^+/K^+ -ATPases. The Na^+/K^+-ATPase is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of Na^+ and K^+ ions across the plasma membrane. According to the present disclosure, *ATP1A3* polynucleotides are provided that function along or in combination with additional nucleic acid sequence(s) to encode the ATP1A3 protein.

[0067] As used herein, the term "*ATP1A3* polynucleotide" is any nucleic acid polymer that encodes an ATP1A3 protein and when present in a vector, plasmid, or translatable construct, expresses such ATP1A3 protein in a cell, tissue, organ or organism. *ATP1A3* polynucleotides include precursor molecules, which are expressed inside the cell. *ATP1A3* polynucleotides or the processed forms thereof can be contained in a plasmid, vector, genome, or other nucleic acid expression vector for delivery into a cell. In some embodiments, the *ATP1A3* polynucleotides are designed as components of AAV viral genomes and packaged in AAV viral particles, which are processed within the cell to express the wild-type ATP1A3 protein.

[0068] As used herein, the term "wild-type ATP1A3 protein" can be the ATP1A3 protein having the UniProtKB No. P13637, or any of the naturally occurring isoforms or variants encoded by the *ATP1A3* gene, including any multiple alternatively spliced transcript variants encoding transcript variants encoding different isoforms of ATP1A3. A wild-type ATP1A3 protein can also include an ATP1A3 protein sequence that has at least 70%, 75%, 80%, 85%, or 90% sequence identity to a naturally occurring ATP1A3 protein sequence that retains the same or similar function to the naturally occurring APT1A3 protein.

[0069] Mutations in the *ATP1A3* gene refer to an alteration in the *ATP1A3* polynucleotide sequence as compared to the wild-type *ATP1A3* polynucleotide sequence, resulting in the expression of an ATP1A3 protein mutant.

[0070] As used herein, the term "ATP1A3 protein mutant" refers to an ATP1A3 protein having an amino acid sequence in which at least one amino acid residue in a wild-type

ATP1A3 protein is lost, substituted, or added. Examples of ATP1A3 protein mutants that can be associated with an ATP1A3-mediated disease include, but are not limited to the following mutations: E815K, D801N, G947R, I180N, R756C, V589F, E818K, T613M, E277K, D923N, R756H, V589F, F913del, S137F, S137Y, Q140L, D220N, I274N, I274T, V322D, C333F, T335P, G358C, G358V, I363N, T370N, L371P, S684F, G706R, G755A, G755C, G755S, L757P, I758S, T771I, T771N, S772R, N773I, N773S, F780L, D801E, D801Y, L802P, T804I, D805E, D805H, M806K, 806R, P808L, I810F, I810N, I810S, S811P, L839P, G867D, D923N, C927F, C927Y, A955D, and D992Y and any other disease-causing ATP1A3 protein mutants. In some embodiments, the ATP1A3 protein mutant comprises a D801N mutation.

[0071] Mutations in the *ATP1A3* gene can cause a variety of neurological diseases, movement disorders and epilepsies. Examples of ATP1A3-mediated diseases include, but are not limited to, rapid-onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (AHC), epileptic encephalopathy (EE), cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss (CAPOS) syndrome, fever induced paroxysmal weakness and encephalopathy (FIPWE, also called RECA or Relapsing Encephalopathy with Cerebellar Ataxia), recurrent episodes of cerebellar ataxia (RECA), early-onset schizophrenia, Dystonia Dysmorphism of the face Encephalopathy MRI abnormalities without hemiplegia (D-DEMO) syndrome and Childhood Rapid Onset ataxia (CROA). In some embodiments, this could include other diseases that are likely to be described in the future as being caused by other ATP1A3 mutations or caused by abnormalities in ATP1A3 protein structure or function. In some embodiments, the ATP1A3-mediated disease comprises Alternating Hemiplegia of Childhood (AHC), epilepsy, stroke, hypoglycemia acute ataxia, dystonia and neuronal death in neurodegenerative diseases including Alzheimer's and Parkinson's diseases. In some embodiments, the use of a vector comprising an *ATP1A3* transgene (e.g., AAV9-specific promoter-ATP1A3-FLAG-p2a-Cherry-SV40polyA) or a related construct can include therapy of disorders resulting from mutations of other ATPases that may benefit from the ATP1A3 vector that is targeted at the specific tissue of that disorder. An example of that can include kidney diseases caused by decreased function of the V-ATPase due to mutations in the ATP6V1B1 or ATP6V0A4 ATPase genes.

[0072] In some embodiments, the nucleic acid expression cassettes, vectors or related construct of the disclosure can include ATPase-mediated diseases resulting from mutations of other *ATPase* genes leading to other ATPase deficiencies. In such cases, the transgene can be the relevant *ATPase* for that disease and the promoter can be one that is capable of driving

expression of the *ATPase* gene in a relevant organ system or cell. Examples of ATPase-mediated diseases include immunodeficiency disorders caused by ATP6A1 mutations, liver disease caused by ATP7A or ATP7B mutations. Another example is central nervous system disorders such as hemiplegic migraine or epileptic encephalopathy that result from ATP1A2 mutations, or peripheral neuropathy and hypomagnesemia/intellectual disability syndrome caused by ATP1A1 mutations. Other examples include mutations to the *ATPase* genes that cause muscle disease such as *Myosin* and *ATP2A1* genes or mutations causing hematologic diseases such as mutations in *ATP11C* and *ATP2C1* mutations that cause skin disease.

[0073] In some embodiments, the use of the nucleic acid expression cassettes, active vectors or related constructs can include disorders resulting from other disease states leading to symptoms caused by dysfunction of any ATPase. In such cases, the transgene can be the relevant ATPase for that disease and the promoter can be one that is capable of driving expression of the *ATPase* gene in a pertinent organ system or cell. Examples of such diseases would be *VMA21* gene mutations resulting in secondary V-ATPase misassembly and dysfunction of the V-ATPase as seen in congenital disorder of glycosylation with autophagic liver disease and *WDR72* gene mutations that results in abnormal trafficking of kidney V-ATPase and secondary kidney disease. Another includes inflammatory bowel disease with secondary abnormal ATPase function due to chronic mucosal inflammation resulting in gastrointestinal symptoms.

[0074] **Nucleic Acid Expression Cassettes**

[0075] The present disclosure provides, in part, a nucleic acid expression cassette comprising, consisting of, or consisting essentially of a nucleic acid sequence encoding an ATPase (e.g., ATP1A3).

[0076] As used herein, the term "nucleic acid expression cassette" refers to an isolated nucleic acid molecule that includes one or more transcriptional control elements (e.g., promoters, enhancers, and/or regulatory elements, polyadenylation sequences, and introns) that direct gene expression in one or more desired cell types, tissues or organs. A nucleic acid expression cassette can contain a transgene, although it is also envisaged that a nucleic acid expression cassette directs expression of an endogenous gene in a cell into which the nucleic acid sequence is inserted.

[0077] As used herein, the term "transgene" refers to exogenous nucleic acid sequences that encode a polypeptide to be expressed in a cell into which the transgene is introduced. A transgene can include a heterologous nucleic acid sequence that is not naturally found in the cell into which it has been introduced, a nucleic acid sequence that is a mutant form of a

nucleic acid sequence naturally found in the cell into which it has been introduced, or a nucleic acid sequence that is the same as a naturally occurring nucleic in the cell into which it has been introduced. A transgene can include genes from the same organism into which it is introduced or from a different organism.

[0078] A transgene of the disclosure can include, but is not limited to, *ATP1A1*, *ATP1A2*, *ATP1A3*, *ATP1A4*, *ATP1B1*, *ATP1B2*, *ATP1B3*, *ATP1B4*, *ATP2A1*, *ATP2A2*, *ATP2A3*, *ATP2B1*, *ATP2B2*, *ATP2B3*, *ATP2B4*, *ATP2C1*, *ATP2C2*, *ATP3*, *ATP4*, *ATP5A1*, *ATP5B*, *ATP5C1*, *ATP5C2*, *ATP5D*, *ATP5E*, *ATP5F1*, *ATP5G1*, *ATP5G2*, *ATP5G3*, *ATP5H*, *ATP5I*, *ATP5J*, *ATP5J2*, *ATP5L*, *ATP5L2*, *ATP5O*, *ATP5S*, *ATP6A1*, *ATP6AP1*, *ATP6AP2*, *ATP6V1A*, *ATP6V1B1*, *ATP6V1B2*, *ATP6V1C1*, *ATP6V1C2*, *ATP6V1D*, *ATP6V1E1*, *ATP6V1E2*, *ATP6V1F*, *ATP6V1G1*, *ATP6V1G2*, *ATP6V1G3*, *ATP6V1H*, *ATP6V0A1*, *ATP6V0A2*, *ATP6V0A4*, *ATP6V0B*, *ATP6V0C*, *ATP6V0D1*, *ATP6V0D2*, *ATP6V0E*, *ATP11C*, *ATP7A*, *ATP7B*, *ATP8A1*, *ATP8B1*, *ATP8B2*, *ATP8B3*, *ATP8B4*, *ATP9A*, *ATP9B*, *ATP10A*, *ATP10B*, *ATP10D*, *ATP11A*, *ATP11B*, *ATP11C*, *ATP12A*, *ATP13A1*, *ATP13A2*, *ATP13A3*, *ATP13A4*, *ATP13A5*, *VMA21*, *V-ATPase*, or *WDR72* or any gene encoding an ATPase. In some embodiments, the transgene is a nucleic acid sequence encoding ATP1A3.

[0079] The term "nucleic acid sequence," "nucleic acid molecule," "polynucleotide," and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides that may have various lengths, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Nucleic acid molecules can be deoxyribonucleic acid (DNA) orribonucleic acid (RNA) fragments generated, for example, by a polymerase chain reaction (PCR) or by *in vitro* translation, and fragments generated by any one or more of ligation, scission, endonuclease action, or exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (e.g., α-enantiomeric forms of naturally-occurring nucleotides), or a combination thereof. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, morpholino, or the like. Nucleic acid molecules can be either single stranded or double stranded (e.g., ssDNA, dsDNA, ssRNA, or dsRNA).

[0080] The term "nucleotide" refers to sequences with conventional nucleotide bases, sugar residues and internucleotide phosphate linkages, but also to those that contain

modifications of any or all of these moieties. The term "nucleotide" as used herein includes those moieties that contain not only the natively found purine and pyrimidine bases adenine (A), guanine (G), thymine (T), cytosine (C), and uracil (U), but also modified or analogous forms thereof. Polynucleotides include RNA and DNA sequences of more than one nucleotide in a single chain. Modified RNA or modified DNA, as used herein, refers to a nucleic acid molecule in which one or more of the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are different from that which occurs in nature.

[0081] As used herein, the term "isolated" nucleic acid molecule (e.g., an isolated DNA, isolated cDNA, or an isolated vector genome) means a nucleic acid molecule separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the nucleic acid.

[0082] Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

[0083] In some embodiments, the nucleic acid sequence encoding ATP1A3 is provided in ensemble.org (HGNC ID: HGNC:801, encoding the ATP1A3 protein corresponding to UniProtKB identifier P13637) as set forth in SEQ ID NO:01, and any fragments, isoforms, and/or homologues thereof. In another embodiment, the nucleic acid sequence encoding ATP1A3 comprises a cDNA nucleic acid sequence as set forth in SEQ ID NO:02, and any fragments, isoforms, or homologues thereof. In another embodiment, the nucleic acid sequence encoding ATP1A3 comprises a nucleic acid sequence as set forth in SEQ ID NO:07, and any fragments, isoforms, or homologues thereof.

[0084] In other embodiments, the nucleic acid sequence encoding ATP1A3 can be any of the nucleic acid sequences set forth in the following GenBank Accession Numbers: BC009282.2, BC015566.2, AK295078.1, AK296557.1, AK295833.1, AK316069.1, BC013763.1, KJ896471.1, JF432325.1, KR710324.1, KR710323.1, or KR710322.1 or NCBI Accession Numbers: NM_152296.5, XM_016934231.2, NM_001256213.1, XM_004060817.3, NM_001256214.2, or AK223569.1. In other embodiments, the nucleic acid sequence encodes a wild-type ATP1A3 protein having the amino acid sequence set forth in SEQ ID NO:03 (NCBI Reference Sequence: P13637-1) or the amino acid sequence set forth in SEQ ID NO:06, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:03 or SEQ ID NO:06.

[0085] Table 1 provides exemplary sequences that are related to the transgenes of the present disclosure.

[0086] Table 1: Nucleic Acid and Amino Acid Sequences

Representative Nucleic acid sequence encoding ATP1A3	ATGGGGGACAAGAAAGATGACAAGGACTCACCCAAGAAGAACAAGGGCAAGGAGCGCCGGGAC CTGGATGACCTCAAGAAGGAGGTGGCTATGACAGAGCACAAGATGTCAGTGGAAGAGGTCTGC CGGAAATACAACACAGACTGTGTGCAGGGTTTGACCCACAGCAAAGCCCAGGAGATCCTGGCC CGGGATGGGCCTAACGCACTCACGCCACCGCCTACCACCCCAGAGTGGGTCAAGTTTTGCCGG CAGCTCTTCGGGGGCTTCTCCATCCTGCTGTGGATCGGGGCTATCCTCTGCTTCCTGGCCTAC GGTATCCAGGCGGGCACCGAGGACGACCCCTCTGGTGACAACCTGTACCTGGGCATCGTGCTG GCGGCCGTGGTGATCATCACTGGCTGCTTCTCCTACTACCAGGAGGCCAAGAGCTCCAAGATC ATGGAGTCCTTCAAGAACATGGTGCCCCAGCAAGCCCTGGTGATCCGGGAAGGTGAGAAGATG CAGGTGAACGCTGAGGAGGTGGTGGTCGGGGACCTGGTGGAGATCAAGGGTGGAGACCGAGTG CCAGCTGACCTGCGGATCATCTCAGCCCACGGCTGCAAGGTGGACAACTCCTCCCTGACTGGC GAATCCGAGCCCCAGACTCGCTCTCCCGACTGCACGCACGACAACCCCTTGGAGACTCGGAAC ATCACCTTCTTTTCCACCAACTGTGTGGAAGGCACGGCTCGGGGCGTGGTGGTGGCCACGGGC GACCGCACTGTCATGGGCCGTATCGCCACCCTGGCATCAGGGCTGGAGGTGGGCAAGACGCCC ATCGCCATCGAGATTGAGCACTTCATCCAGCTCATCACCGGCGTGGCTGTCTTCCTGGGTGTC TCCTTCTTCATCCTCTCCCTCATTCTCGGATACACCTGGCTTGAGGCTGTCATCTTCCTCATC GGCATCATCGTGGCCAATGTCCCAGAGGGTCTGCTGGCCACTGTCACTGTGTGTCTGACGCTG ACCGCCAAGCGCATGGCCCGGAAGAACTGCCTGGTGAAGAACCTGGAGGCTGTAGAAACCCTG GGCTCCACGTCCACCATCTGCTCAGATAAGACAGGGACCCTCACTCAGAACCGCATGACAGTC GCCCACATGTGGTTTGACAACCAGATCCACGAGGCTGACACCACTGAGGACCAGTCAGGGACC TCATTTGACAAGAGTTCGCACACCTGGGTGGCCCTGTCTCACATCGCTGGGCTCTGCAATCGC GCTGTCTTCAAGGGTGGTCAGGACAACATCCCTGTGCTCAAGAGGGATGTGGCTGGGGATGCG TCTGAGTCTGCCCTGCTCAAGTGCATCGAGCTGTCCTCTGGCTCCGTGAAGCTGATGCGTGAA CGCAACAAGAAAGTGGCTGAGATTCCCTTCAATTCCACCAACAAATACCAGCTCTCCATCCAT GAGACCGAGGACCCCAACGACAACCGATACCTGCTGGTGATGAAGGGTGCCCCCGAGCGCATC CTGGACCGCTGCTCCACCATCCTGCTACAGGGCAAGGAGCAGCCTCTGGACGAGGAAATGAAG GAGGCCTTCCAGAATGCCTACCTTGAGCTCGGTGGCCTGGGCGAGCGCGTGCTTGGTTTCTGC CATTATTACCTGCCCGAGGAGCAGTTCCCCAAGGGCTTTGCCTTCGACTGTGATGACGTGAAC TTCACCACGGACAACCTCTGCTTTGTGGGCCTCATGTCCATGATCGACCCACCCCGGGCAGCC GTCCCTGACGCGGTGGGCAAGTGTCGCAGCGCAGGCATCAAGGTCATCATGGTCACCGGCGAT CACCCCATCACGGCCAAGGCCATTGCCAAGGGTGTGGGCATCATCTCTGAGGGCAACGAGACT GTGGAGGACATCGCCGCCCGGCTCAACATTCCCGTCAGCCAGGTTAACCCCCGGGATGCCAAG GCCTGCGTGATCCACGGCACCGACCTCAAGGACTTCACCTCCGAGCAAATCGACGAGATCCTG CAGAATCACACCGAGATCGTCTTCGCCCGCACATCCCCCCAGCAGAAGCTCATCATTGTGGAG GGCTGTCAGAGACAGGGTGCAATTGTGGCTGTGACCGGGGATGGTGTGAACGACTCCCCCGCT CTGAAGAAGGCCGACATTGGGGTGGCCATGGGCATCGCTGGCTCTGACGTCTCCAAGCAGGCA GCTGACATGATCCTGCTGGACGACAACTTTGCCTCCATCGTCACAGGGGTGGAGGAGGGCCGC CTGATCTTCGACAACCTAAAGAAGTCCATTGCCTACACCCTGACCAGCAATATCCCGGAGATC ACGCCCTTCCTGCTGTTCATCATGGCCAACATCCCGCTGCCCCTGGGCACCATCACCATCCTC TGCATCGATCTGGGCACTGACATGGTCCCTGCCATCTCACTGGCGTACGAGGCTGCCGAAAGC GACATCATGAAGAGACAGCCCAGGAACCCGCGGACGGACAAATTGGTCAATGAGAGACTCATC AGCATGGCCTACGGGCAGATTGGAATGATCCAGGCTCTCGGTGGCTTCTTCTCTTACTTTGTG ATCCTGGCAGAAAATGGCTTCTTGCCCGGCAACCTGGTGGGCATCCGGCTGAACTGGGATGAC CGCACCGTCAATGACCTGGAAGACAGTTACGGGCAGCAGTGGACATACGAGCAGAGGAAGGTG GTGGAGTTCACCTGCCACACGGCCTTCTTTGTGAGCATCGTTGTCGTCCAGTGGGCCGATCTG ATCATCTGCAAGACCCGGAGGAACTCGGTCTTCCAGCAGGGCATGAAGAACAAGATCCTGATC TTCGGGCTGTTTGAGGAGACGGCCCTGGCTGCCTTCCTGTCCTACTGCCCCGGCATGGACGTG GCCCTGCGCATGTACCCTCTCAAGCCCAGCTGGTGGTTCTGTGCCTTCCCCTACAGTTTCCTC ATCTTCGTCTACGACGAAATCCGCAAACTCATCCTGCGCAGGAACCCAGGGGGTTGGGTGGAG AAGGAAA (SEQ ID NO:07)
Representative nucleic acid sequence	CGCGCGCACCTACCGAGGCGCGGGCGCTGCAGAGGCTCCCAGCCCAAGCCTGAGCCTGAGCCC GCCCCGAGGTCCCCGCCCCGCCCGCCTGGCTCTCTCGCCGCGGAGCCGCCAAGATGGGGGACA AGAAAGATGACAAGGACTCACCCAAGAAGAACAAGGGCAAGGAGCGCCGGGACCTGGATGACC

encoding ATP1A3	TCAAGAAGGAGGTGGCTATGACAGAGCACAAGATGTCAGTGGAAGAGGTCTGCCGGAAATACA ACACAGACTGTGTGCAGGGTTTGACCCACAGCAAAGCCCAGGAGATCCTGGCCCGGGATGGGC CTAACGCACTCACGCCACCGCCTACCACCCCAGAGTGGGTCAAGTTTTGCCGGCAGCTCTTCG GGGGCTTCTCCATCCTGCTGTGGATCGGGGCTATCCTCTGCTTCCTGGCCTACGGTATCCAGG CGGGCACCGAGGACGACCCCTCTGGTGACAACCTGTACCTGGGCATCGTGCTGGCGGCCGTGG TGATCATCACTGGCTGCTTCTCCTACTACCAGGAGGCCAAGAGCTCCAAGATCATGGAGTCCT TCAAGAACATGGTGCCCCAGCAAGCCCTGGTGATCCGGGAAGGTGAGAAGATGCAGGTGAACG CTGAGGAGGTGGTGGTCGGGGACCTGGTGGAGATCAAGGGTGGAGACCGAGTGCCAGCTGACC TGCGGATCATCTCAGCCCACGGCTGCAAGGTGGACAACTCCTCCCTGACTGGCGAATCCGAGC CCCAGACTCGCTCTCCCGACTGCACGCACGACAACCCCTTGGAGACTCGGAACATCACCTTCT TTTCCACCAACTGTGTGGAAGGCACGGCTCGGGGCGTGGTGGTGGCCACGGGCGACCGCACTG TCATGGGCCGTATCGCCACCCTGGCATCAGGGCTGGAGGTGGGCAAGACGCCCATCGCCATCG AGATTGAGCACTTCATCCAGCTCATCACCGGCGTGGCTGTCTTCCTGGGTGTCTCCTTCTTCA TCCTCTCCCTCATTCTCGGATACACCTGGCTTGAGGCTGTCATCTTCCTCATCGGCATCATCG TGGCCAATGTCCCAGAGGGTCTGCTGGCCACTGTCACTGTGTGTCTGACGCTGACCGCCAAGC GCATGGCCCGGAAGAACTGCCTGGTGAAGAACCTGGAGGCTGTAGAAACCCTGGGCTCCACGT CCACCATCTGCTCAGATAAGACAGGGACCCTCACTCAGAACCGCATGACAGTCGCCCACATGT GGTTTGACAACCAGATCCACGAGGCTGACACCACTGAGGACCAGTCAGGGACCTCATTTGACA AGAGTTCGCACACCTGGGTGGCCCTGTCTCACATCGCTGGGCTCTGCAATCGCGCTGTCTTCA AGGGTGGTCAGGACAACATCCCTGTGCTCAAGAGGGATGTGGCTGGGGATGCGTCTGAGTCTG CCCTGCTCAAGTGCATCGAGCTGTCCTCTGGCTCCGTGAAGCTGATGCGTGAACGCAACAAGA AAGTGGCTGAGATTCCCTTCAATTCCACCAACAAATACCAGCTCTCCATCCATGAGACCGAGG ACCCCAACGACAACCGATACCTGCTGGTGATGAAGGGTGCCCCCGAGCGCATCCTGGACCGCT GCTCCACCATCCTGCTACAGGGCAAGGAGCAGCCTCTGGACGAGGAAATGAAGGAGGCCTTCC AGAATGCCTACCTTGAGCTCGGTGGCCTGGGCGAGCGCGTGCTTGGTTTCTGCCATTATTACC TGCCCGAGGAGCAGTTCCCCAAGGGCTTTGCCTTCGACTGTGATGACGTGAACTTCACCACGG ACAACCTCTGCTTTGTGGGCCTCATGTCCATGATCGACCCACCCCGGGCAGCCGTCCCTGACG CGGTGGGCAAGTGTCGCAGCGCAGGCATCAAGGTCATCATGGTCACCGGCGATCACCCCATCA CGGCCAAGGCCATTGCCAAGGGTGTGGGCATCATCTCTGAGGGCAACGAGACTGTGGAGGACA TCGCCGCCCGGCTCAACATTCCCGTCAGCCAGGTTAACCCCCGGGATGCCAAGGCCTGCGTGA TCCACGGCACCGACCTCAAGGACTTCACCTCCGAGCAAATCGACGAGATCCTGCAGAATCACA CCGAGATCGTCTTCGCCCGCACATCCCCCCAGCAGAAGCTCATCATTGTGGAGGGCTGTCAGA GACAGGGTGCAATTGTGGCTGTGACCGGGGATGGTGTGAACGACTCCCCCGCTCTGAAGAAGG CCGACATTGGGGTGGCCATGGGCATCGCTGGCTCTGACGTCTCCAAGCAGGCAGCTGACATGA TCCTGCTGGACGACAACTTTGCCTCCATCGTCACAGGGGTGGAGGAGGGCCGCCTGATCTTCG ACAACCTAAAGAAGTCCATTGCCTACACCCTGACCAGCAATATCCCGGAGATCACGCCCTTCC TGCTGTTCATCATGGCCAACATCCCGCTGCCCCTGGGCACCATCACCATCCTCTGCATCGATC TGGGCACTGACATGGTCCCTGCCATCTCACTGGCGTACGAGGCTGCCGAAAGCGACATCATGA AGAGACAGCCCAGGAACCCGCGGACGGACAAATTGGTCAATGAGAGACTCATCAGCATGGCCT ACGGGCAGATTGGAATGATCCAGGCTCTCGGTGGCTTCTTCTCTTACTTTGTGATCCTGGCAG AAAATGGCTTCTTGCCCGGCAACCTGGTGGGCATCCGGCTGAACTGGGATGACCGCACCGTCA ATGACCTGGAAGACAGTTACGGGCAGCAGTGGACATACGAGCAGAGGAAGGTGGTGGAGTTCA CCTGCCACACGGCCTTCTTTGTGAGCATCGTTGTCGTCCAGTGGGCCGATCTGATCATCTGCA AGACCCGGAGGAACTCGGTCTTCCAGCAGGGCATGAAGAACAAGATCCTGATCTTCGGGCTGT TTGAGGAGACGGCCCTGGCTGCCTTCCTGTCCTACTGCCCCGGCATGGACGTGGCCCTGCGCA TGTACCCTCTCAAGCCCAGCTGGTGGTTCTGTGCCTTCCCCTACAGTTTCCTCATCTTCGTCT ACGACGAAATCCGCAAACTCATCCTGCGCAGGAACCCAGGGGGTTGGGTGGAGAAGGAAACCT ACTACTGACCTCAGCCCCACCACATCGCCCATCTCTTCCCCGTCCCGCAGGCCCAGGACCGCC CCTGTCAGTCCCCCCAATTTTGTATTCTGGGGGGAGGAGCCCTCTCTTCCTGTGGCCCCACCT TGGCCCCCACCCCCTCCACTATCTCCTGCCGCCCCCACTCTGGCTGGCTTCTCTCCCCTGCCC CAAACCTCTCTCCTCTCTCTTTTCTGTGTCAGTTTCTCTCCCTCTCCTCACCCCTCTATCCAT TCCTCCCGCCCCAGCCACCTCCCTGGGCTCTTTTTTTACTCCCCTTCAGCCCCCCGGCTGATGC CATCTCTGGTTCTGGACAATTATCAAATATATCAGTGGGGAGAGAGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:02)
Representative amino acid sequence for ATP1A3	MGDKKDDKDS PKKNKGKERR DLDDLKKEVA MTEHKMSVEE VCRKYNTDCV QGLTHSKAQE ILARDGPNAL TPPPTTPEWV KFCRQLFGGF SILLWIGAIL CFLAYGIQAG TEDDPSGDNL YLGIVLAAVV IITGCFSYYQ EAKSSKIMES FKNMVPQQAL VIREGEKMQV NAEEVVVGDL VEIKGGDRVP ADLRIISAHG CKVDNSSLTG ESEPQTRSPD CTHDNPLETR NITFFSTNCV EGTARGVVVA TGDRTVMGRI ATLASGLEVG KTPIAIEIEH FIQLITGVAV FLGVSFFILS LILGYTWLEA VIFLIGIIVA NVPEGLLATV TVCLTLTAKR MARKNCLVKN

	LEAVETLGST STICSDKTGT LTQNRMTVAH MWFDNQIHEA DTTEDQSGTS FDKSSHTWVA LSHIAGLCNR AVFKGGQDNI PVLKRDVAGD ASESALLKCI ELSSGSVKLM RERNKKVAEI PFNSTNKYQL SIHETEDPND NRYLLVMKGA PERILDRCST ILLQGKEQPL DEEMKEAFQN AYLELGGLGE RVLGFCHYYL PEEQFPKGFA FDCDDVNFTT DNLCFVGLMS MIDPPRAAVP DAVGKCRSAG IKVIMVTGDH PITAKAIAKG VGIISEGNET VEDIAARLNI PVSQVNPRDA KACVIHGTDL KDFTSEQIDE ILQNHTEIVF ARTSPQQKLI IVEGCQRQGA IVAVTGDGVN DSPALKKADI GVAMGIAGSD VSKQAADMIL LDDNFASIVT GVEEGRLIFD NLKKSIAYTL TSNIPEITPF LLFIMANIPL PLGTITILCI DLGTDMVPAI SLAYEAAESD IMKRQPRNPR TDKLVNERLI SMAYGQIGMI QALGGFFSYF VILAENGFLP GNLVGIRLNW DDRTVNDLED SYGQQWTYEQ RKVVEFTCHT AFFVSIVVVQ WADLIICKTR RNSVFQQGMK NKILIFGLFE ETALAAFLSY CPGMDVALRM YPLKPSWWFC AFPYSFLIFV YDEIRKLILR RNPGGWVEKE TYY (SEQ ID NO:03)
Representative amino acid sequence for ATP1A3	MGDKKDDKDSPKKNKGKERRDLDDLKKEVAMTEHKMSVEEVCRKYNTDCVQGLTHSKAQEILA RDGPNALTPPPTTPEWVKFCRQLFGGFSILLWIGAILCFLAYGIQAGTEDDPSGDNLYLGIVL AAVVIITGCFSYYQEAKSSKIMESFKNMVPQQALVIREGEKMQVNAEEVVVGDLVEIKGGDRV PADLRIISAHGCKVDNSSLTGESEPQTRSPDCTHDNPLETRNITFFSTNCVEGTARGVVVATG DRTVMGRIATLASGLEVGKTPIAIEIEHFIQLITGVAVFLGVSFFILSLILGYTWLEAVIFLI GIIVANVPEGLLATVTVCLTLTAKRMARKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTV AHMWFDNQIHEADTTEDQSGTSFDKSSHTWVALSHIAGLCNRAVFKGGQDNIPVLKRDVAGDA SESALLKCIELSSGSVKLMRERNKKVAEIPFNSTNKYQLSIHETEDPNDNRYLLVMKGAPERI LDRCSTILLQGKEQPLDEEMKEAFQNAYLELGGLGERVLGFCHYYLPEEQFPKGFAFDCDDVN FTTDNLCFVGLMSMIDPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKGVGIISEGNET VEDIAARLNIPVSQVNPRDAKACVIHGTDLKDFTSEQIDEILQNHTEIVFARTSPQQKLIIVE GCQRQGAIVAVTGDGVNDSPALKKADIGVAMGIAGSDVSKQAADMILLDDNFASIVTGVEEGR LIFDNLKKSIAYTLTSNIPEITPFLLFIMANIPLPLGTITILCIDLGTDMVPAISLAYEAAES DIMKRQPRNPRTDKLVNERLISMAYGQIGMIQALGGFFSYFVILAENGFLPGNLVGIRLNWDD RTVNDLEDSYGQQWTYEQRKVVEFTCHTAFFVSIVVVQWADLIICKTRRNSVFQQGMKNKILI FGLFEETALAAFLSYCPGMDVALRMYPLKPSWWFCAFPYSFLIFVYDEIRKLILRRNPGGWVE KE (SEQ ID NO:06)

[0087] As provided herein and in accordance with one embodiment of the present disclosure, the nucleic acid sequence encoding ATP1A3 can comprise a sequence which has a sequence identity to any of SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. The nucleic acid sequence encoding ATP1A3 can have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. The nucleic acid sequence encoding ATP1A3 can have 1-10%, 10-20%, 30-40%, 50-60%, 50-70%, 50-80%, 50-90%, 50-99%, 50-100%, 60-70%, 60-80%, 60-90%, 60-99%, 60-100%, 70-80%, 70-90%, 70-99%, 70-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-100%, 90-95%, 90-99%, or 90-100% to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. As a non-limiting example, the nucleic acid sequence encoding ATP1A3 can comprise a sequence which has 80% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. As another non-limiting example, the nucleic acid sequence encoding ATP1A3 can comprise a sequence which has 85% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. As another non-limiting example, the nucleic acid

sequence encoding ATP1A3 can comprise a sequence which has 90% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. As another non-limiting example, the nucleic acid sequence encoding ATP1A3 can comprise a sequence which has 95% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07. As another non-limiting example, the nucleic acid sequence encoding ATP1A3 can comprise a sequence which has 99% identity to the nucleic acid sequences set forth in SEQ ID NO:01, SEQ ID NO:02, or SEQ ID NO:07.

[0088] In another embodiment, the expression cassette comprises, consists of, or consists essentially of a transgene that encodes mRNA that can be translated into an amino sequence encoding the protein for ATP1A3. In some embodiment, the nucleic acid sequence encoding ATP1A3 is a cDNA sequence that has the sequence set forth in SEQ ID NO:02, or fragments, isoforms, or homologues thereof.

[0089] The term "sequence identity" refers to the number of identical or similar residues (i.e., nucleotide bases or amino acid) on a comparison between a test and reference nucleotide or amino acid sequence. Sequence identity can be determined by sequence alignment of nucleic acid to identify regions of similarity or identity. As described herein, sequence identity is generally determined by alignment to identify identical residues. Matches, mismatches, and gaps can be identified between compared sequences. Alternatively, sequence identity can be determined without taking into account gaps as the number of identical positions/length of the total aligned sequence x 100. In one non-limiting embodiment, the term "at least 90% sequence identity to" refers to percent identities from 90 to 100%, relative to the reference nucleotide or amino acid sequence. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplary purposes a test and reference oligonucleotide or length of 100 nucleotides are compared, no more than 10% (*i.e.*, 10 out of 100) of the nucleotides in the test oligonucleotide differ from those of the reference oligonucleotide. Differences are defined as nucleic acid or amino acid substitutions, insertions, or deletions.

[0090] In some embodiments, the expression cassette comprises a nucleotide sequence encoding ATP1A3 that is codon-optimized to reduce CpG methylation sites and for mammalian expression (e.g., human cell expression). In other embodiments, the nucleic acid expression cassette does not contain codon optimized nucleic acid sequences.

[0091] The term "codon optimized" relates to the alteration of codons in nucleic acid molecules to reflect the typical codon usage of the host organism (e.g., mammals such as humans) without altering the polypeptide encoded by the DNA, to improve expression. Many

methods and software tools for codon optimization have been reported previously. See, for example, genomes.urv.es/OPTIMIZER/; Puigbò *et al.*, *Nucleic Acids Res.* (2007) (Web Server issue): W126–W131; Chin *et al.* (2014) *Bioinformatics*, 30(15):2210-2; Fuglsang, (2003) *Protein Expr Purif.*, 31(2):247-9; Narum *et al.*, (2001) *Infect. Immun.*, 69(12):7250-7253, Outchkourov *et al.*, (2002) *Protein Expr. Purif*, 24(1):18-24, Humphreys *et al.*, (2000) *Protein Expr. Purif.*, 20(2):252-64.

[0092] Those of ordinary skill in the art will appreciate that the nucleic acid expression cassette comprising a nucleic acid encoding ATP1A3 can contain transcription/translation control signals or secretory signal sequences, which can be included in the nucleic acid expression cassette or by a vector backbone. For example, specific initiation signals can be required for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which can include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

[0093] A variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter can be tissue-specific or ubiquitous and can be constitutive or inducible, depending on the pattern of the gene expression desired. The promoter can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0094] The promoter can be chosen so that it will function in the target cell(s) of interest. Tissue-specific promoters refer to promoters that have activity in only certain cell types. The use of a tissue-specific promoter in a nucleic acid expression cassette can restrict unwanted transgene expression in the unaffected tissues as well as facilitate persistent transgene expression by escaping from transgene induced host immune responses. Tissue specific promoters include, but are not limited to, neuron-specific promoters, muscle-specific promoters, liver-specific promoters, skeletal muscle-specific promoters, and heart-specific promoters.

[0095] Neuron-specific promoters include, but are not limited to, the synapsin I (SYN) promoter, the calcium/calmodulin-dependent protein kinase II promoter, the tubulin alpha I promoter, the neuron-specific enolase promoter, and the platelet-derived growth factor beta chain promoter. In some embodiments, the neuron-specific promoter is a human synapsin promoter. In other embodiments, the neuron-specific promoter is the human synapsin promoter has the nucleic acid sequence that is set forth in SEQ ID NO:04 or SEQ ID NO:09 human promoter sequence or the mouse promoter sequence set forth in SEQ ID

NO:08. In other embodiments, the human synapsin promoter comprises the proximal region of the synapson 1 promoter (-422 to -22). In some embodiments, the proximal region of the synapsin 1 promoter (-422 to -22) has the nucleic acid sequence that is set forth in SEQ ID NO:05.

[0096] Liver-specific promoters include, but are not limited to, the α1-microglobulin/bikunin enhancer/thyroid hormone-binding globulin promoter, the human albumin (hALB) promoter, the thyroid hormone-binding globulin promoter, the α-1-anti-trypsin promoter, the bovine albumin (bAlb) promoter, the murine albumin (mAlb) promoter, the human a1-antitrypsin (hAAT) promoter, the ApoEhAAT promoter composed of the ApoE enhancer and the hAAT promoter, the transthyretin (TTR) promoter, the liver fatty acid binding protein promoter, the hepatitis B virus (HBV) promoter, the DC172 promoter consisting of the hAAT promoter and the a1-microglobulin enhancer, the DC190 promoter containing the human albumin promoter and the prothrombin enhancer, and other natural and synthetic liver-specific promoters.

[0097] Muscle specific promoters include, but are not limited to, the MHCK7 promoter, the muscle creatine kinase (MCK) promoter/enhancer, the slow isoform of troponin I (TnIS) promoter, the MYODI promoter, the MYLK2 promoter, the SPc5-12 promoter, the desmin (Des) promoter, the unc45b promoter, and other natural and synthetic muscle-specific promoters.

[0098] Skeletal muscle-specific promoters include, but are not limited to, the HSA promoter, the human α-skeletal actin promoter.

[0099] Heart-specific promoters include, but art not limited to, the MYH6 promoter, the TNNI3 promoter, the cardiac troponin C (cTnC) promoter, the alpha-myosin heavy chain (α-MHC) promoter, myosin light chain 2 (MLC-2), and the MYBPC3 promoter.

[00100] Constitutive promoters refer to promoters that allow for continual transcription of its associated gene. Constitutive promoters are always active and can be used to express genes in a wide range of cells and tissues, including, but not limited to, the liver, kidney, skeletal muscle, cardiac muscle, smooth muscle, diaphragm muscle, brain, spinal cord, endothelial cells, intestinal cells, pulmonary cells (e.g., smooth muscle or epithelium), peritoneal epithelial cells and fibroblasts.

[00101] Constitutive promoters include, but are not limited to, a CMV major immediate-early enhancer/chicken beta-actin promoter, a cytomegalovirus (CMV) major immediate-early promoter, an Elongation Factor 1-α (EF1-α) promoter, a simian vacuolating virus 40 (SV40) promoter, an AmpR promoter, a PγK promoter, a human ubiquitin C gene (Ubc)

promoter, a MFG promoter, a human beta actin promoter, a CAG promoter, a EGR1 promoter, a FerH promoter, a FerL promoter, a GRP78 promoter, a GRP94 promoter, a HSP70 promoter, a β-kin promoter, a murine phosphoglycerate kinase (mPGK) or human PGK (hPGK) promoter, a ROSA promoter, human Ubiquitin B promoter, a Rous sarcoma virus promoter, or any other natural or synthetic ubiquitous promoters. In some embodiments, the constitutively active promoter is selected from the group consisting of human β-actin, human elongation factor-1α, chicken β-actin combined with cytomegalovirus early enhancer, cytomegalovirus (CMV), simian virus 40, or herpes simplex virus thymidine kinase.

[00102] Inducible promoters refer to promoters that can be regulated by positive or negative control. Factors that can regulate an inducible promoter include, but are not limited to, chemical agents (e.g., the metallothionein promoter or a hormone inducible promoter), temperature, and light.

[00103] The tissue-specific promoters can be operably linked to one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) enhancer elements (e.g., a neuron-specific promoter fused to a cytomegalovirus enhancer) or combined to form a tandem promoter (e.g., neuron-specific/constitutive tandem promoter). When two or more tissue-specific promoters are present, the isolated nucleic acid can be targeted to two or more different tissues at the same time.

[00104] An enhancer element is a nucleic acid sequence that functions to enhance transcription.

[00105] In some embodiments, the expression cassette comprises the ATP1A3 transgene sequence operably linked to a promoter and a polyadenylation sequence.

[00106] In other aspects, the nucleic acid expression cassette according to the present disclosure further comprises a transcriptional termination signal. A transcriptional termination signal is a nucleic acid sequence that marks the end of a gene during transcription. Examples of a transcriptional termination signal include, but are not limited to, bovine growth hormone polyadenylation signal (BGHpA), Simian virus 40 polyadenylation signal (Sv40 PolyA), and a synthetic polyadenylation signal. A polyadenylation sequence can comprise the nucleic acid sequence AATAAA. In some embodiments, a Sv40 PolyA has the sequence set forth in SEQ ID NO:17, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:17.

[00107] As used herein, the term "intron" refers to nucleic acid sequences that can enhance transgene expression. An intron can also be a part of the nucleic acid expression cassette or positioned downstream or upstream of the expression cassette in the expression vector.

Introns can include, but are not limited to, the SV40 intron, EF-1alpha gene intron 1, or the MVM intron. In some embodiments, the nucleic acid expression cassettes do not contain an intron.

[00108] As used herein, the terms "enhance" and "enhancement" with respect to nucleic acid expression or polypeptide production, refers to an increase and/or prolongation of steady-state levels of the indicated nucleic acid or polypeptide, e.g., by at least about 2%, 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 2-fold, 2.5-fold, 3-fold, 5-fold, 10-fold, 15-fold, 20-fold, 30-fold, 50-fold, 100-fold or more.

[00109] In some embodiments, the nucleic acid sequence encoding ATP1A3 is used as a "donor" template for homologous recombination with a mutant ATP1A3 gene in diseased cells. Further embodiments according to the present disclosure include the co-administration of the nucleic acid sequence with gene editing nucleases selected from the group consisting of zinc finger nucleases, TALENS, RNA-guided nucleases such as CRISPR/Cas98, and other programmable endonucleases.

[00110] **Vectors**

[00111] Another aspect of the present disclosure provides a vector comprising, consisting of, or consisting essentially of a nucleic acid sequence encoding ATP1A3. In some embodiments, the vector comprises, consists, or consists essentially of a nucleic acid expression cassette comprising a nucleic acid sequence encoding ATP1A3.

[00112] It will be apparent to those skilled in the art that any suitable vector can be used to deliver the isolated nucleic acids of the disclosure to the target cell(s) or subject of interest. The choice of delivery vector can be made based on a number of factors known in the art, including age and species of the target host, *in vitro* vs. *in vivo* delivery, level and persistence of expression desired, intended purpose (e.g., for therapy or enzyme production), the target cell or organ, route of delivery, size of the isolated nucleic acid, safety concerns, and the like.

[00113] Suitable vectors that are known in the art and that can be used to deliver, and optionally, express the isolated nucleic acids of the disclosure (e.g., viral and non-viral vectors), including, virus vectors (e.g., retrovirus, adenovirus, AAV, lentiviruses, or herpes simplex virus), lipid vectors, poly-lysine vectors, synthetic polyamino polymer vectors that are used with nucleic acid molecules, such as a plasmid, and the like. In some embodiments, the non-viral vector can be a polymer based vector (e.g., polyethyleimine (PEI), chitosan, poly (DL-Lactide) (PLA), or poly (DL-lactidie-co-glycoside) (PLGA), dendrimers, polymethacrylate) a peptide based vector, a lipid nanoparticle, a solid lipid nanoparticle, or a cationic lipid based vector.

[00114] Protocols for producing recombinant viral vectors and for using viral vectors for nucleic acid delivery can be found in *Current Protocols in Molecular Biology*; Ausubel, F. M. *et al.* (eds.) Greene Publishing Associates; (1989) and other standard laboratory manuals (e.g., Vectors for Gene Therapy, In: *Current Protocols in Human Genetics*, John Wiley and Sons, Inc.; 1997).

[00115] "Recombinant" is used herein to refer to new combinations of genetic material as a result of genetic engineering. For instance, a recombinant organism (e.g., bacteria) can be an organism that contains different genetic material from either of its parents as a result of genetic modification, recombinant DNA can be a form of artificial DNA, a recombinant protein or enzyme can be an artificially produced and purified form of the protein or enzyme, and a recombinant virus can be a virus formed by recombining genetic material.

[00116] In some embodiments, the nucleic acid expression cassettes and/or transgenes (*e.g.*, *ATP1A3* and variants thereof) can be incorporated into a recombinant viral vector.

[00117] As used herein, the term "viral vector" refers to a virus (e.g., AAV) particle that functions as a nucleic acid delivery vehicle, and which comprises the vector genome (e.g., viral DNA) packaged within a virion. Alternatively, in some contexts, the term "vector" is used to refer to the vector genome/viral DNA alone.

[00118] Any suitable recombinant viral vector suitable for gene therapy is suitable for use in the compositions and methods according to the present disclosure. Examples of such viral vectors include, but are not limited to vectors derived from: Adenoviridae; Birnaviridae; Bunyaviridae; Caliciviridae, Capillovirus group; Carlavirus group; Carmovirus virus group; Group Caulimovirus; Closterovirus Group; Commelina yellow mottle virus group; Comovirus virus group; Coronaviridae; PM2 phage group; Corcicoviridae; Group Cryptic virus; group Cryptovirus; Cucumovirus virus group family ([PHgr]6 phage group; Cysioviridae; Group Carnation ringspot; Dianthovirus virus group; Group Broad bean wilt; Fabavirus virus group; Filoviridae; Flaviviridae; Furovirus group; Group Germinivirus; Group Giardiavirus; Hepadnaviridae; Herpesviridae; Hordeivirus virus group; Illarvirus virus group; Inoviridae; Iridoviridae; Leviviridae; Lipothrixviridae; Luteovirus group; Marafivirus virus group; Maize chlorotic dwarf virus group; icroviridae; Myoviridae; Necrovirus group; Nepovirus virus group; Nodaviridae; Orthomyxoviridae; Papovaviridae; Paramyxoviridae; Parsnip yellow fleck virus group; Partitiviridae; Parvoviridae; Pea enation mosaic virus group; Phycodnaviridae; Picornaviridae; Plasmaviridae; Prodoviridae; Polydnaviridae; Potexvirus group; Potyvirus; Poxyiridae; Reoviridae; Retroviridae; Rhabdoviridae; Group Rhizidiovirus; Siphoviridae; Sobemovirus group; SSV 1-Type Phages; Tectiviridae;

Tenuivirus; Tetraviridae; Group Tobamovirus; Group Tobravirus; Togaviridae; Group Tombusvirus; Group Torovirus; Totiviridae; Group Tymovirus; and plant virus satellites.

[00119] In some embodiments, the recombinant viral vector is selected from the group consisting of adenoviruses, Adeno-associated viruses (AAV) (e.g., AAV serotypes and genetically modified AAV variants), a herpes simplex viruses (*e.g.*, e.g., HSV-1, HSV), a retrovirus vector (e.g., MMSV, MSCV), a lentivirus vector (HIV-1, HIV-2), and alphavirus vector (e.g., SFV, SIN, VEE, M1), a flavivirus vector (e.g., Kunjin, West Nile, Dengue virus), a rhabdovirus vector (e.g., Rabies, VSV), a measles virus vector (e.g., MV-Edm), a Newcastle disease virus vector, a poxvirus vector (VV), or a picornavirus vector (e.g., Coxsackievirus). The recombinant viral vector of the present disclosure includes any type of viral vector that is capable of packaging and delivering the *ATP1A3* transgene or viral vectors that can be designed engineered and generated by methods known in the art.

[00120] In some embodiments, the delivery vector is an adenovirus vector. The term "adenovirus" as used herein encompasses all adenoviruses, including the *Mastadenovirus* and *Aviadenovirus* genera.

[00121] The various regions of the adenovirus genome have been mapped and are understood by those skilled in the art. The genomic sequences of the various Ad serotypes, as well as the nucleotide sequence of the particular coding regions of the Ad genome, are known in the art and may be accessed from GenBank and NCBI (see, e.g., GenBank Accession Nos. J0917, M73260, X73487, AF108105, L19443, NC 003266 and NCBI Accession Nos. NC 001405, NC 001460, NC 002067, NC 00454).

[00122] A recombinant adenovirus (rAd) vector genome can comprise the adenovirus terminal repeat sequences and packaging signal. An "adenovirus particle" or "recombinant adenovirus particle" comprises an adenovirus vector genome or recombinant adenovirus vector genome, respectively, packaged within an adenovirus capsid. Generally, the adenovirus vector genome is most stable at sizes of about 28 kb to 38 kb (approximately 75% to 105% of the native genome size). In the case of an adenovirus vector containing large deletions and a relatively small transgene, "stutter DNA" can be used to maintain the total size of the vector within the desired range by methods known in the art.

[00123] The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 (Ad5) or other strains of adenovirus (e.g., Ad2, Ad3, Ad7, etc.) are known to those skilled in the art.

[00124] In some embodiments, the viral vector comprises a recombinant Adeno-Associated Viruses (AAV). AAV are parvoviruses and have small icosahedral virions and can contain a single stranded DNA molecule about 4.7 kb (e.g., about 4.5 kb, 4.6 kb, 4.8 kb, 4.9 kb, or 5.0 kb) or less in size. The viruses contain either the sense or antisense strand of the DNA molecule and either strand is incorporated into the virion. Two open reading frames encode a series of Rep and Cap polypeptides. Rep polypeptides (e.g., Rep50, Rep52, Rep68 and Rep78) are involved in replication, rescue and integration of the AAV genome, although significant activity may be observed in the absence of all four Rep polypeptides. The Cap proteins (e.g., VP1, VP2, VP3) form the virion capsid. Flanking the rep and cap open reading frames at the 5′ and 3′ ends of the genome are inverted terminal repeats (ITRs). Typically, in recombinant AAV (rAAV) vectors, the entire rep and cap coding regions are excised and replaced with a transgene of interest.

[00125] Recombinant AAV vectors generally require only the terminal repeat(s) (TR(s)) in *cis* to generate virus. All other viral sequences are dispensable and may be supplied in *trans*. Typically, the rAAV vector genome will only retain the one or more TR sequence so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences may be provided in *trans* (e.g., from a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). In embodiments of the present disclosure, the rAAV vector genome comprises at least one terminal repeat (TR) sequence (e.g., AAV TR sequence), optionally two TRs (e.g., two AAV TRs), which typically will be at the 5' and 3' ends of the vector genome and flank the heterologous nucleic acid sequence, but need not be contiguous thereto. The TRs can be the same or different from each other.

[00126] The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (i.e., mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV TR or a non-AAV TR. For example, a non-AAV TR sequence such as those of other parvoviruses (e.g., canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or any other suitable virus sequence *(e.g.* , the SV40 hairpin that serves as the origin of SV40 replication) can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR can be partially or completely synthetic, such as the "double-D sequence."

[00127] An "AAV terminal repeat" or "AAV TR" may be from any AAV, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 or any other AAV now known or later

discovered. An AAV terminal repeat need not have the native terminal repeat sequence (e.g., a native AAV TR sequence may be altered by insertion, deletion, truncation and/or missense mutations), as long as the terminal repeat mediates the desired functions, e.g., replication, virus packaging, integration, and/or provirus rescue, and the like. In some embodiments, the vector comprises flanking ITRs derived from the AAV2 genome. The ITRs of the present disclosure can have a sequence set forth in SEQ ID NO:10 or SEQ ID NO:18, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:10 or SEQ ID NO:18.

[00128] Wild-type AAV can integrate their DNA into non-dividing cells, and exhibit a high frequency of stable integration into human chromosome 19. A rAAV vector genome will typically comprise the AAV terminal repeat sequences and packaging signal.

[00129] An "AAV particle" or "rAAV particle" comprises an AAV vector genome or rAAV vector genome, respectively, packaged within an AAV capsid. The AAV rep/cap genes can be expressed on a single plasmid. The AAV rep and/or cap sequences may be provided by any viral or non-viral vector. For example, the rep/cap sequences may be provided by a hybrid adenovirus or herpesvirus vector (e.g., inserted into the Ela or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV cap and rep genes. One advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (i.e., are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," *see* Margolski (1992) *Curr. Top. Microbiol. Immun.* 158:67). The AAV rep and cap genes may be from any AAV serotype for which recombinant virus can be derived and may be from a different AAV serotype than the rAAV genome ITRs.

[00130] However, the rAAV vector itself need not contain AAV genes encoding the capsid (cap) and Rep proteins. In particular embodiments of the disclosure, the rep and/or cap genes are deleted from the AAV genome. In a representative embodiment, the rAAV vector retains only the terminal AAV sequences (ITRs) necessary for integration, excision, and replication.

[00131] Sources for the AAV capsid genes can include naturally isolated serotypes, including but not limited to, AAV1, AAV2, AAV3 (including 3a and 3b), AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV13, AAVrh39, AAVrh43, AAVcy.7, as well as bovine AAV, caprine AAV, canine AAV, equine AAV, ovine AAV, avian AAV, primate AAV, non-primate AAV, and any other virus

classified by the International Committee on Taxonomy of Viruses (ICTV) as an AAV. In particular embodiments, the AAV capsids are chimeras either created by capsid evolution or by rational capsid engineering from the naturally isolated AAV variants to capture desirable serotype features such as enhanced or specific tissue tropism and host immune response escape, including but not limited to AAV-DJ, AAV-HAE1, AAV-HAE2, AAVM41, AAV-1829, AAV2 Y/F, AAV2 T/V, AAV2i8, AAV2.5, AAV9.45, AAV9.61, AAV-B1, AAV-AS, AAV9.45A-String *(e.g.,* AAV9.45-AS), AAV9.45Angiopep, AAV9.47-Angiopep, and AAV9.47-AS., AAV-PHP.B, AAV-PHP.eB, and AAV-PHP.S.

[00132] Accordingly, when referring herein to a specific AAV capsid protein (e.g., an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV1 or AAV12 capsid protein) it is intended to encompass the native capsid protein as well as capsid proteins that have alterations other than the modifications of the invention. Such alterations include substitutions, insertions and/or deletions.

[00133] In some embodiments, the recombinant AAV vector is selected from the group consisting of AAV1, AAV8, or AAV9. In certain embodiments, the recombinant AAV vector comprises AAV9 due to its ability to easily cross the blood-brain barrier.

[00134] In some embodiments, the recombinant viral vectors (e.g., rAAV) according to the present disclosure generally comprise, consist of, or consist essentially of one or more of the following elements: (1) an Inverted Terminal Repeat sequence (ITR); (2) a promoter (e.g., a neuron-specific promoter); (3) a transgene (e.g., a nucleic acid sequence encoding ATP1A3, a fragment thereof, an isoform thereof, or a homologue thereof); (4) a transcription terminator (e.g., a polyadenylation signal); and (5) a flanking Inverted Terminal Repeat sequence (ITR).

[00135] In some embodiments, the recombinant viral vector can comprise a linker sequence. The term "linker sequence" as used herein refers to a nucleic acid sequence that encodes a short polypeptide sequence. A linker sequence can comprise at least 6 nucleotide sequences, at least 15 nucleotides, 27 nucleotides, or at least 30 nucleotides. In some embodiments, the linker sequence has 6 to 27 nucleotides. In other embodiments, the linker sequence has 6 nucleotides, 15 nucleotides, and/or 27 nucleotides. A linker sequence can be used to connect various encoded elements in the vector constructs. For example, a transgene and Myc tag can be operably linked via a linker, or a Myc tag and FLAG can be operably linked via a linker or a FLAG tag and mCherry tag can be operably linked via a linker. Alternatively, the vector elements can be directly linked (e.g., not via a linker). Exemplary linker sequences are shown in Table 2:

[00136] Table 2: Representative Linker Sequences

Linker Sequence (27 nucleotides)	ACCTACTACACGCGTACGCGGCCGCTC (SEQ ID NO:11)
Linker Sequence (15 nucleotides)	GCAGCAAATGATATCCTG (SEQ ID NO:13)
Linker Sequence (6 nucleotides)	GGATTC (SEQ ID NO:15)

[00137] In some embodiments, the vectors according to the present disclosure can comprise fluorescent protein tags (e.g., mCherry, sfGFP, and mKikGR) and/or epitope tags (e.g., HA, Myc, FLAG).

[00138] In some embodiments, a mCherry tag can be encoded by the nucleic acid sequence set forth in SEQ ID NO:16, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:16.

[00139] In some embodiments, a Myc tag can be encoded by the nucleic acid sequence set forth in SEQ ID NO:12, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:12.

[00140] In some embodiments, a FLAG tag can be encoded by the nucleic acid sequence set forth in SEQ ID NO:14, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:14.

[00141] In other embodiments, the vectors can optionally comprise a 2A self-cleaving peptide (2A peptide), which is a class of peptides that can be about 18–22 amino acids in length and can induce the cleaving of a recombinant protein in a cell. Examples of 2A peptides include, but are not limited to, P2A, E2A, F2A and T2A. In some embodiments, the 2A peptide can be P2A. In some embodiments, P2A can be encoded by the nucleic acid sequence set forth in SEQ ID NO:22, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:22.

[00142] In some embodiments, a 2A peptide can be combined with an internal ribosome entry site (IRES) element, which can make it possible to generate four separated peptides within a single transcript. The location for IRES elements can be at the 5'UTR, but can also occur elsewhere in the nucleic acid sequence.

[00143] In some embodiments, the vectors of the present disclosure optionally comprise an intron. In other embodiments, the vectors of the present disclosure do not contain an intron.

[00144] In one embodiment, the recombinant AAV vector comprises a nucleotide sequence encoding ATP1A3.

[00145] In some embodiments, the active vector comprises a construct as shown in **FIG. 1**.

[00146] In some embodiments, the active vector comprises AAV9-pBK828-ATP1A3-cherry (also referred to herein as AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA or AAV9-ATP1A3-Cherry or active vector) (**FIG. 2**). An AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA vector of the present disclosure can comprise an ITR, a human Syn promoter, an *ATP1A3* open reading frame (ORF), one or more epitope tags (e.g., a Myc tag, or a FLAG tag), a P2A sequence, one or more fluorescence tags (e.g., an mCherry tag), a Sv40 PolyA tail, and a flanking ITR (**FIG. 1**). The AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA vector can also comprise one or more linker sequences in between the elements.

[00147] In some embodiments, the AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA vector can comprise the following elements operably linked in order of 5' to 3': an AAV-ITR, a human Syn promoter, an *ATP1A3* transgene, a linker sequence, a Myc tag, a linker sequence, a FLAG tag, a linker sequence, a p2A sequence, a linker sequence, a mCherry tag, a Sv40 PolyA sequence, and an AAV-ITR.

[00148] The active vector can comprise the nucleic acid sequence set forth in SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a sequence having at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21.

[00149] In another embodiment, the recombinant AAV control vector can comprise AAV9-hSyn-mCherry-pBK292-9 (**FIG. 3**). This vector does not contain a transgene can be used as a control vector to the AAV9-pBK828-ATP1A3-cherry vector.

[00150] **Table 3: Nucleic Acid Constructs**

Nucleic Acid construct containing the following elements in order of 5' to 3': AAV-ITR, hSyn promoter, ATP1A3, linker, Myc tag , linker, Flag tag, linker,	TCCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGTCCCGGGC GTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCTGCGCAGAGAGGGAGT GGCCAACTCCATCACTAGGGGTTCCTAGTGCAAGTGGGTTTTTAGGACCAGGATGAGGC GGGGTGGGGGTGCCTACCTGACGACCGACCCCGGACCCACTGGACAAGCACCCAACCCC CATTCCCCAAATTGCGCATCCCCTAATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGA GGCGCGTGCGCACTGCCAAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCG GCGCGCGCCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCC GACAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCCAGCCGG ACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCCATCTGCGCTGCGGC GCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAAGCGGAGGAGTCGTGTCGTGCC TGAGAGCGCAGTCGAGAAGGTACCGAGGAGATCTGCCGCCATGGGGGACAAGAAAGATG ACAAGGACTCACCCAAGAAGAACAAGGGCAAGGAGCGCCGGGACCTGGATGACCTCAAG AAGGAGGTGGCTATGACAGAGCACAAGATGTCAGTGGAAGAGGTCTGCCGGAAATACAA CACAGACTGTGTGCAGGGTTTGACCCACAGCAAAGCCCAGGAGATCCTGGCCCGGGATG

mCherry, Sv40 PolyA, AAV-ITR	GGCCTAACGCACTCACGCCACCGCCTACCACCCCAGAGTGGGTCAAGTTTTGCCGGCAG CTCTTCGGGGGCTTCTCCATCCTGCTGTGGATCGGGGCTATCCTCTGCTTCCTGGCCTA CGGTATCCAGGCGGGCACCGAGGACGACCCCTCTGGTGACAACCTGTACCTGGGCATCG TGCTGGCGGCCGTGGTGATCATCACTGGCTGCTTCTCCTACTACCAGGAGGCCAAGAGC TCCAAGATCATGGAGTCCTTCAAGAACATGGTGCCCCAGCAAGCCCTGGTGATCCGGGA AGGTGAGAAGATGCAGGTGAACGCTGAGGAGGTGGTGGTCGGGGACCTGGTGGAGATCA AGGGTGGAGACCGAGTGCCAGCTGACCTGCGGATCATCTCAGCCCACGGCTGCAAGGTG GACAACTCCTCCCTGACTGGCGAATCCGAGCCCCAGACTCGCTCTCCCGACTGCACGCA CGACAACCCCTTGGAGACTCGGAACATCACCTTCTTTTCCACCAACTGTGTGGAAGGCA CGGCTCGGGGCGTGGTGGTGGCCACGGGCGACCGCACTGTCATGGGCCGTATCGCCACC CTGGCATCAGGGCTGGAGGTGGGCAAGACGCCCATCGCCATCGAGATTGAGCACTTCAT CCAGCTCATCACCGGCGTGGCTGTCTTCCTGGGTGTCTCCTTCTTCATCCTCTCCCTCA TTCTCGGATACACCTGGCTTGAGGCTGTCATCTTCCTCATCGGCATCATCGTGGCCAAT GTCCCAGAGGGTCTGCTGGCCACTGTCACTGTGTGTCTGACGCTGACCGCCAAGCGCAT GGCCCGGAAGAACTGCCTGGTGAAGAACCTGGAGGCTGTAGAAACCCTGGGCTCCACGT CCACCATCTGCTCAGATAAGACAGGGACCCTCACTCAGAACCGCATGACAGTCGCCCAC ATGTGGTTTGACAACCAGATCCACGAGGCTGACACCACTGAGGACCAGTCAGGGACCTC ATTTGACAAGAGTTCGCACACCTGGGTGGCCCTGTCTCACATCGCTGGGCTCTGCAATC GCGCTGTCTTCAAGGGTGGTCAGGACAACATCCCTGTGCTCAAGAGGGATGTGGCTGGG GATGCGTCTGAGTCTGCCCTGCTCAAGTGCATCGAGCTGTCCTCTGGCTCCGTGAAGCT GATGCGTGAACGCAACAAGAAAGTGGCTGAGATTCCCTTCAATTCCACCAACAAATACC AGCTCTCCATCCATGAGACCGAGGACCCCAACGACAACCGATACCTGCTGGTGATGAAG GGTGCCCCCGAGCGCATCCTGGACCGCTGCTCCACCATCCTGCTACAGGGCAAGGAGCA GCCTCTGGACGAGGAAATGAAGGAGGCCTTCCAGAATGCCTACCTTGAGCTCGGTGGCC TGGGCGAGCGCGTGCTTGGTTTCTGCCATTATTACCTGCCCGAGGAGCAGTTCCCCAAG GGCTTTGCCTTCGACTGTGATGACGTGAACTTCACCACGGACAACCTCTGCTTTGTGGG CCTCATGTCCATGATCGACCCACCCCGGGCAGCCGTCCCTGACGCGGTGGGCAAGTGTC GCAGCGCAGGCATCAAGGTCATCATGGTCACCGGCGATCACCCCATCACGGCCAAGGCC ATTGCCAAGGGTGTGGGCATCATCTCTGAGGGCAACGAGACTGTGGAGGACATCGCCGC CCGGCTCAACATTCCCGTCAGCCAGGTTAACCCCCGGGATGCCAAGGCCTGCGTGATCC ACGGCACCGACCTCAAGGACTTCACCTCCGAGCAAATCGACGAGATCCTGCAGAATCAC ACCGAGATCGTCTTCGCCCGCACATCCCCCCAGCAGAAGCTCATCATTGTGGAGGGCTG TCAGAGACAGGGTGCAATTGTGGCTGTGACCGGGGATGGTGTGAACGACTCCCCCGCTC TGAAGAAGGCCGACATTGGGGTGGCCATGGGCATCGCTGGCTCTGACGTCTCCAAGCAG GCAGCTGACATGATCCTGCTGGACGACAACTTTGCCTCCATCGTCACAGGGGTGGAGGA GGGCCGCCTGATCTTCGACAACCTAAAGAAGTCCATTGCCTACACCCTGACCAGCAATA TCCCGGAGATCACGCCCTTCCTGCTGTTCATCATGGCCAACATCCCGCTGCCCCTGGGC ACCATCACCATCCTCTGCATCGATCTGGGCACTGACATGGTCCCTGCCATCTCACTGGC GTACGAGGCTGCCGAAAGCGACATCATGAAGAGACAGCCCAGGAACCCGCGGACGGACA AATTGGTCAATGAGAGACTCATCAGCATGGCCTACGGGCAGATTGGAATGATCCAGGCT CTCGGTGGCTTCTTCTCTTACTTTGTGATCCTGGCAGAAAATGGCTTCTTGCCCGGCAA CCTGGTGGGCATCCGGCTGAACTGGGATGACCGCACCGTCAATGACCTGGAAGACAGTT ACGGGCAGCAGTGGACATACGAGCAGAGGAAGGTGGTGGAGTTCACCTGCCACACGGCC TTCTTTGTGAGCATCGTTGTCGTCCAGTGGGCCGATCTGATCATCTGCAAGACCCGGAG GAACTCGGTCTTCCAGCAGGGCATGAAGAACAAGATCCTGATCTTCGGGCTGTTTGAGG AGACGGCCCTGGCTGCCTTCCTGTCCTACTGCCCCGGCATGGACGTGGCCCTGCGCATG TACCCTCTCAAGCCCAGCTGGTGGTTCTGTGCCTTCCCCTACAGTTTCCTCATCTTCGT CTACGACGAAATCCGCAAACTCATCCTGCGCAGGAACCCAGGGGGTTGGGTGGAGAAGG AAAACCTACTACACGCGTACGCGGCCGCTCGAGCAGAAACTCATCTCAGAAGAGGATCT GGCAGCAAATGATATCCTGGATTACAAGGATGACGACGATAAAGGATTCGTGAGCAAGG GCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAG GGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGG ACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGAC ATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAA CTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGT TCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAG AAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCT GAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGG TCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAAC ATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACG

	CGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAATAAGATACAT TGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAA TTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAGGAAC CCCTAGTGATTGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGG GTCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGTCGAGCG AGCGCGCAGCTGCCTGCAGG (SEQ ID NO:19)
Nucleic Acid construct containing the following elements in order of 5' to 3': AAV-ITR, hSyn promoter , ATP1A3, linker, Myc tag , linker, Flag tag, linker, p2a, linker, mCherry, Sv40 PolyA, AAV-ITR	TCCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGTCCCGGGC GTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCTGCGCAGAGAGGGAGT GGCCAACTCCATCACTAGGGGTTCCTAGTGCAAGTGGGTTTTTAGGACCAGGATGAGGC GGGGTGGGGGTGCCTACCTGACGACCGACCCCGGACCCACTGGACAAGCACCCAACCCC CATTCCCCAAATTGCGCATCCCCTAATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGA GGCGCGTGCGCACTGCCAAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCG GCGCGCGCCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCC GACAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCCAGCCGG ACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCCATCTGCGCTGCGGC GCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAAGCGGAGGAGTCGTGTCGTGCC TGAGAGCGCAGTCGAGAAGGTACCGAGGAGATCTGCCGCCATGGGGGACAAGAAAGATG ACAAGGACTCACCCAAGAAGAACAAGGGCAAGGAGCGCCGGGACCTGGATGACCTCAAG AAGGAGGTGGCTATGACAGAGCACAAGATGTCAGTGGAAGAGGTCTGCCGGAAATACAA CACAGACTGTGTGCAGGGTTTGACCCACAGCAAAGCCCAGGAGATCCTGGCCCGGGATG GGCCTAACGCACTCACGCCACCGCCTACCACCCCAGAGTGGGTCAAGTTTTGCCGGCAG CTCTTCGGGGGCTTCTCCATCCTGCTGTGGATCGGGGCTATCCTCTGCTTCCTGGCCTA CGGTATCCAGGCGGGCACCGAGGACGACCCCTCTGGTGACAACCTGTACCTGGGCATCG TGCTGGCGGCCGTGGTGATCATCACTGGCTGCTTCTCCTACTACCAGGAGGCCAAGAGC TCCAAGATCATGGAGTCCTTCAAGAACATGGTGCCCCAGCAAGCCCTGGTGATCCGGGA AGGTGAGAAGATGCAGGTGAACGCTGAGGAGGTGGTGGTCGGGGACCTGGTGGAGATCA AGGGTGGAGACCGAGTGCCAGCTGACCTGCGGATCATCTCAGCCCACGGCTGCAAGGTG GACAACTCCTCCCTGACTGGCGAATCCGAGCCCCAGACTCGCTCTCCCGACTGCACGCA CGACAACCCCTTGGAGACTCGGAACATCACCTTCTTTTCCACCAACTGTGTGGAAGGCA CGGCTCGGGGCGTGGTGGTGGCCACGGGCGACCGCACTGTCATGGGCCGTATCGCCACC CTGGCATCAGGGCTGGAGGTGGGCAAGACGCCCATCGCCATCGAGATTGAGCACTTCAT CCAGCTCATCACCGGCGTGGCTGTCTTCCTGGGTGTCTCCTTCTTCATCCTCTCCCTCA TTCTCGGATACACCTGGCTTGAGGCTGTCATCTTCCTCATCGGCATCATCGTGGCCAAT GTCCCAGAGGGTCTGCTGGCCACTGTCACTGTGTGTCTGACGCTGACCGCCAAGCGCAT GGCCCGGAAGAACTGCCTGGTGAAGAACCTGGAGGCTGTAGAAACCCTGGGCTCCACGT CCACCATCTGCTCAGATAAGACAGGGACCCTCACTCAGAACCGCATGACAGTCGCCCAC ATGTGGTTTGACAACCAGATCCACGAGGCTGACACCACTGAGGACCAGTCAGGGACCTC ATTTGACAAGAGTTCGCACACCTGGGTGGCCCTGTCTCACATCGCTGGGCTCTGCAATC GCGCTGTCTTCAAGGGTGGTCAGGACAACATCCCTGTGCTCAAGAGGGATGTGGCTGGG GATGCGTCTGAGTCTGCCCTGCTCAAGTGCATCGAGCTGTCCTCTGGCTCCGTGAAGCT GATGCGTGAACGCAACAAGAAAGTGGCTGAGATTCCCTTCAATTCCACCAACAAATACC AGCTCTCCATCCATGAGACCGAGGACCCCAACGACAACCGATACCTGCTGGTGATGAAG GGTGCCCCCGAGCGCATCCTGGACCGCTGCTCCACCATCCTGCTACAGGGCAAGGAGCA GCCTCTGGACGAGGAAATGAAGGAGGCCTTCCAGAATGCCTACCTTGAGCTCGGTGGCC TGGGCGAGCGCGTGCTTGGTTTCTGCCATTATTACCTGCCCGAGGAGCAGTTCCCCAAG GGCTTTGCCTTCGACTGTGATGACGTGAACTTCACCACGGACAACCTCTGCTTTGTGGG CCTCATGTCCATGATCGACCCACCCCGGGCAGCCGTCCCTGACGCGGTGGGCAAGTGTC GCAGCGCAGGCATCAAGGTCATCATGGTCACCGGCGATCACCCCATCACGGCCAAGGCC ATTGCCAAGGGTGTGGGCATCATCTCTGAGGGCAACGAGACTGTGGAGGACATCGCCGC CCGGCTCAACATTCCCGTCAGCCAGGTTAACCCCCGGGATGCCAAGGCCTGCGTGATCC ACGGCACCGACCTCAAGGACTTCACCTCCGAGCAAATCGACGAGATCCTGCAGAATCAC ACCGAGATCGTCTTCGCCCGCACATCCCCCCAGCAGAAGCTCATCATTGTGGAGGGCTG TCAGAGACAGGGTGCAATTGTGGCTGTGACCGGGGATGGTGTGAACGACTCCCCCGCTC TGAAGAAGGCCGACATTGGGGTGGCCATGGGCATCGCTGGCTCTGACGTCTCCAAGCAG GCAGCTGACATGATCCTGCTGGACGACAACTTTGCCTCCATCGTCACAGGGGTGGAGGA GGGCCGCCTGATCTTCGACAACCTAAAGAAGTCCATTGCCTACACCCTGACCAGCAATA TCCCGGAGATCACGCCCTTCCTGCTGTTCATCATGGCCAACATCCCGCTGCCCCTGGGC ACCATCACCATCCTCTGCATCGATCTGGGCACTGACATGGTCCCTGCCATCTCACTGGC GTACGAGGCTGCCGAAAGCGACATCATGAAGAGACAGCCCAGGAACCCGCGGACGGACA AATTGGTCAATGAGAGACTCATCAGCATGGCCTACGGGCAGATTGGAATGATCCAGGCT CTCGGTGGCTTCTTCTCTTACTTTGTGATCCTGGCAGAAAATGGCTTCTTGCCCGGCAA

	CCTGGTGGGCATCCGGCTGAACTGGGATGACCGCACCGTCAATGACCTGGAAGACAGTT ACGGGCAGCAGTGGACATACGAGCAGAGGAAGGTGGTGGAGTTCACCTGCCACACGGCC TTCTTTGTGAGCATCGTTGTCGTCCAGTGGGCCGATCTGATCATCTGCAAGACCCGGAG GAACTCGGTCTTCCAGCAGGGCATGAAGAACAAGATCCTGATCTTCGGGCTGTTTGAGG AGACGGCCCTGGCTGCCTTCCTGTCCTACTGCCCCGGCATGGACGTGGCCCTGCGCATG TACCCTCTCAAGCCCAGCTGGTGGTTCTGTGCCTTCCCCTACAGTTTCCTCATCTTCGT CTACGACGAAATCCGCAAACTCATCCTGCGCAGGAACCCAGGGGGTTGGGTGGAGAAGG AAAACCTACTACACGCGTACGCGGCCGCTCGAGCAGAAACTCATCTCAGAAGAGGATCT GGCAGCAAATGATATCCTGGATTACAAGGATGACGACGATAAAGGATTCGCCACGAACT TCTCTCTGTTAAAGCAAGCAGGAGACGTGGAAGAAAACCCCGGTCCCGGATTCGTGAGC AAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACAT GGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCT ACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCC TGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGC CGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGA TGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAAT GCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCG CCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCT GAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGT CAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACG AACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAATAAGAT ACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGT GAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAG GAACCCCTAGTGATTGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG CCGGGTCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGTCG AGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:20)
Nucleic Acid construct containing the following elements in order of 5’ to 3’: hSyn promoter and ATP1A3	AGTGCAAGTGGGTTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGCCTACCTGACGACC GACCCCGGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATTGCGCATCCCCTAA TCAGAGAGGGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCACTGCCAAGCTTCAG CACCGCGGACAGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCCACCGCCGCCTCAGCAC TGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGACAAACTCCCCTTCCCGGCCACCTT GGTCGCGTCCGCGCCGCCGCCGGCCCCAGCCGGACCGCACCACGCGAGGCGCGAGATAG GGGGGCACGGGCGCGACCCATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTC TGCGGTGGGCAAGCGGAGGAGTCGTGTCGTGCCTGAGAGCGCAGTCGAGAAGGTACCGA GGAGATCTGCCGCCATGGGGGACAAGAAAGATGACAAGGACTCACCCAAGAAGAACAAG GGCAAGGAGCGCCGGGACCTGGATGACCTCAAGAAGGAGGTGGCTATGACAGAGCACAA GATGTCAGTGGAAGAGGTCTGCCGGAAATACAACACAGACTGTGTGCAGGGTTTGACCC ACAGCAAAGCCCAGGAGATCCTGGCCCGGGATGGGCCTAACGCACTCACGCCACCGCCT ACCACCCCAGAGTGGGTCAAGTTTTGCCGGCAGCTCTTCGGGGGCTTCTCCATCCTGCT GTGGATCGGGGCTATCCTCTGCTTCCTGGCCTACGGTATCCAGGCGGGCACCGAGGACG ACCCCTCTGGTGACAACCTGTACCTGGGCATCGTGCTGGCGGCCGTGGTGATCATCACT GGCTGCTTCTCCTACTACCAGGAGGCCAAGAGCTCCAAGATCATGGAGTCCTTCAAGAA CATGGTGCCCCAGCAAGCCCTGGTGATCCGGGAAGGTGAGAAGATGCAGGTGAACGCTG AGGAGGTGGTGGTCGGGGACCTGGTGGAGATCAAGGGTGGAGACCGAGTGCCAGCTGAC CTGCGGATCATCTCAGCCCACGGCTGCAAGGTGGACAACTCCTCCCTGACTGGCGAATC CGAGCCCCAGACTCGCTCTCCCGACTGCACGCACGACAACCCCTTGGAGACTCGGAACA TCACCTTCTTTTCCACCAACTGTGTGGAAGGCACGGCTCGGGGCGTGGTGGTGGCCACG GGCGACCGCACTGTCATGGGCCGTATCGCCACCCTGGCATCAGGGCTGGAGGTGGGCAA GACGCCCATCGCCATCGAGATTGAGCACTTCATCCAGCTCATCACCGGCGTGGCTGTCT TCCTGGGTGTCTCCTTCTTCATCCTCTCCCTCATTCTCGGATACACCTGGCTTGAGGCT GTCATCTTCCTCATCGGCATCATCGTGGCCAATGTCCCAGAGGGTCTGCTGGCCACTGT CACTGTGTGTCTGACGCTGACCGCCAAGCGCATGGCCCGGAAGAACTGCCTGGTGAAGA ACCTGGAGGCTGTAGAAACCCTGGGCTCCACGTCCACCATCTGCTCAGATAAGACAGGG ACCCTCACTCAGAACCGCATGACAGTCGCCCACATGTGGTTTGACAACCAGATCCACGA GGCTGACACCACTGAGGACCAGTCAGGGACCTCATTTGACAAGAGTTCGCACACCTGGG TGGCCCTGTCTCACATCGCTGGGCTCTGCAATCGCGCTGTCTTCAAGGGTGGTCAGGAC AACATCCCTGTGCTCAAGAGGGATGTGGCTGGGGATGCGTCTGAGTCTGCCCTGCTCAA GTGCATCGAGCTGTCCTCTGGCTCCGTGAAGCTGATGCGTGAACGCAACAAGAAAGTGG CTGAGATTCCCTTCAATTCCACCAACAAATACCAGCTCTCCATCCATGAGACCGAGGAC CCCAACGACAACCGATACCTGCTGGTGATGAAGGGTGCCCCCGAGCGCATCCTGGACCG

	CTGCTCCACCATCCTGCTACAGGGCAAGGAGCAGCCTCTGGACGAGGAAATGAAGGAGG CCTTCCAGAATGCCTACCTTGAGCTCGGTGGCCTGGGCGAGCGCGTGCTTGGTTTCTGC CATTATTACCTGCCCGAGGAGCAGTTCCCCAAGGGCTTTGCCTTCGACTGTGATGACGT GAACTTCACCACGGACAACCTCTGCTTTGTGGGCCTCATGTCCATGATCGACCCACCCC GGGCAGCCGTCCCTGACGCGGTGGGCAAGTGTCGCAGCGCAGGCATCAAGGTCATCATG GTCACCGGCGATCACCCCATCACGGCCAAGGCCATTGCCAAGGGTGTGGGCATCATCTC TGAGGGCAACGAGACTGTGGAGGACATCGCCGCCCGGCTCAACATTCCCGTCAGCCAGG TTAACCCCCGGGATGCCAAGGCCTGCGTGATCCACGGCACCGACCTCAAGGACTTCACC TCCGAGCAAATCGACGAGATCCTGCAGAATCACACCGAGATCGTCTTCGCCCGCACATC CCCCCAGCAGAAGCTCATCATTGTGGAGGGCTGTCAGAGACAGGGTGCAATTGTGGCTG TGACCGGGGATGGTGTGAACGACTCCCCCGCTCTGAAGAAGGCCGACATTGGGGTGGCC ATGGGCATCGCTGGCTCTGACGTCTCCAAGCAGGCAGCTGACATGATCCTGCTGGACGA CAACTTTGCCTCCATCGTCACAGGGGTGGAGGAGGGCCGCCTGATCTTCGACAACCTAA AGAAGTCCATTGCCTACACCCTGACCAGCAATATCCCGGAGATCACGCCCTTCCTGCTG TTCATCATGGCCAACATCCCGCTGCCCCTGGGCACCATCACCATCCTCTGCATCGATCT GGGCACTGACATGGTCCCTGCCATCTCACTGGCGTACGAGGCTGCCGAAAGCGACATCA TGAAGAGACAGCCCAGGAACCCGCGGACGGACAAATTGGTCAATGAGAGACTCATCAGC ATGGCCTACGGGCAGATTGGAATGATCCAGGCTCTCGGTGGCTTCTTCTCTTACTTTGT GATCCTGGCAGAAAATGGCTTCTTGCCCGGCAACCTGGTGGGCATCCGGCTGAACTGGG ATGACCGCACCGTCAATGACCTGGAAGACAGTTACGGGCAGCAGTGGACATACGAGCAG AGGAAGGTGGTGGAGTTCACCTGCCACACGGCCTTCTTTGTGAGCATCGTTGTCGTCCA GTGGGCCGATCTGATCATCTGCAAGACCCGGAGGAACTCGGTCTTCCAGCAGGGCATGA AGAACAAGATCCTGATCTTCGGGCTGTTTGAGGAGACGGCCCTGGCTGCCTTCCTGTCC TACTGCCCCGGCATGGACGTGGCCCTGCGCATGTACCCTCTCAAGCCCAGCTGGTGGTT CTGTGCCTTCCCCTACAGTTTCCTCATCTTCGTCTACGACGAAATCCGCAAACTCATCC TGCGCAGGAACCCAGGGGGTTGGGTGGAGAAGGAAA (SEQ ID NO:21)

[00151] In other embodiments, the recombinant AAV vector comprises AAV1. In one embodiment, the recombinant AAV vector comprises a nucleotide sequence encoding ATP1A3, the vector comprising AAV1-pBK828-1-ATP1A3-cherry. An AAV1-pBK828-1-ATP1A3-cherry vector can comprise an ITR, a human Syn promoter, an *ATP1A3* open reading frame (ORF), linkers, a Myc tag, a FLAG tag, a P2A sequence, a mCherry open reading frame, a Sv40 PolyA tail, and a flanking ITR. In some embodiments, the AAV1-pBK828-1-ATP1A3-cherry vector can comprise the following elements operably linked in order of 5' to 3': an AAV-ITR, a human Syn promoter, an *ATP1A3* transgene, a linker sequence, a Myc tag, a linker sequence, a FLAG tag, a linker sequence, a mCherry tag, a Sv40 PolyA sequence, and an AAV-ITR.

[00152] In yet another embodiment, the recombinant AAV vector comprises AAV1-hSyn-mCherry-pBK292-1, which does not contain a transgene can be used as a control vector to the AAV1-pBK828-1-ATP1A3-cherry vector.

[00153] As used herein, the term "open reading frame (ORF)" refers to the parts of a reading frame that has the ability to be translated. An ORF can be a continuous chain of codons that begins with a start codon (e.g., ATG) and ends at a stop codon (e.g., TAA, TAG, TGA). A reading frame is a sequence of nucleotides that are read as codons specifying amino acids.

[00154] In some embodiments, the AAV vector is pseudotyped, which refers to the practice of creating hybrids of certain AAV strains to be able to refine the interaction with desired target cells. The hybrid AAV can be created by taking a capsid from one strain and the genome from another strain. For example, AAV2/5, a hybrid with the genome of AAV2 and the capsid of AAV5, can be used to achieve more accuracy and range in brain cells than AAV2 would be able to achieve unhybridized. Production of pseudotyped rAAV is disclosed in, for example, WO01/83692.

[00155] Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic *et al.*, Molecular Therapy, 22(11): 1900-1909 (2014). It is understood that the nucleotide sequences of the genomes of various AAV serotypes are known in the art.

[00156] Examples of recombinant AAV that can be constructed to comprise the nucleic acid molecules of the disclosure are set out in International Patent Application No. PCT/US2012/047999 (WO 2013/016352) incorporated by reference herein in its entirety.

[00157] Any suitable method known in the art can be used to produce AAV vectors. In one particular method, AAV stocks can be produced by co-transfection of a rep/cap vector plasmid encoding AAV packaging functions and the vector plasmid containing the recombinant AAV genome into human cells infected with the helper adenovirus. General principles of recombinant AAV production are reviewed in, for example, Carter, 1992, Current Opinions in Biotechnology, 1533-539; and Muzyczka, (1992) *Curr. Topics in Microbial. and Immunol.*, 158:97-129). Various approaches are described in Ratschin *et al.*, *Mol. Cell. Biol.* 4:2072 (1984); Hermonat *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6466 (1984); Tratschin *et al.*, Mol. Cell. Biol. 5:3251 (1985); McLaughlin *et al.*, J. Virol., 62:1963 (1988); and Lebkowski *et al.*, 1988 Mol. Cell. Biol., 7:349 (1988). Samulski *et al.* (1989, J. Virol., 63:3822-3828); U.S. Pat. No. 5,173,414; U.S. Pat. No. 5,658,776; WO 95/13392; WO 96/17947; WO 97/09441; WO 97/08298; WO 97/21825; WO 97/06243; WO 99/11764; Perrin *et al.* (1995) Vaccine 13:1244-1250; Paul *et al.* (1993) Human Gene Therapy 4:609-615; Clark *et al.* (1996) Gene Therapy 3:1124-1132; U.S. Pat. Nos. 5,786,211; 5,871,982; and 6,258,595. The foregoing documents are hereby incorporated by reference in their entirety herein, with particular emphasis on those sections of the documents relating to recombinant AAV production.

[00158] The recombinant viral vectors (e.g., rAAV) may be purified by methods standard in the art such as by column chromatography or cesium chloride gradients. Methods for purifying recombinant viral vectors from helper virus are known in the art.

[00159] The nucleic acid encoding ATP1A3 can be provided to the cell using any method known in the art. For example, the template can be supplied by a non-viral (e.g., plasmid) or viral vector.

[00160] The AAV rep and/or cap genes can alternatively be provided by a packaging cell that stably expresses the genes. A method of generating a packaging cell is to create a cell line that stably expresses all the necessary components for viral (e.g., AAV) particle production. For example, in one embodiment, a plasmid (or multiple plasmids) comprising a viral rAAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the rAAV genome, and a selectable marker, such as a neomycin resistance gene, are integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing (Samulski *et al.*, 1982, Proc. Natl. Acad. S6. USA, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin *et al.*, 1983, Gene, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, J. Biol. Chem., 259:4661-4666). The packaging cell line is then infected with a helper virus such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of rAAV. Other examples of suitable methods employ adenovirus or baculovirus rather than plasmids to introduce rAAV genomes and/or rep and cap genes into packaging cells.

[00161] In one embodiment, packaging cells can be stably transformed cancer cells such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another embodiment, packaging cells are cells that are not transformed cancer cells, such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[00162] In still further embodiments, the delivery vector is a hybrid Ad-AAV delivery vector. Briefly, the hybrid Ad-AAV vector comprises an adenovirus vector genome comprising adenovirus (i) 5′ and 3′ cis-elements for viral replication and encapsidation and, further, (ii) a recombinant AAV vector genome comprising the AAV 5′ and 3′ inverted terminal repeats (ITRs), an AAV packaging sequence, and a heterologous sequence(s) flanked by the AAV ITRs, where the recombinant AAV vector genome is flanked by the adenovirus 5′ and 3′ cis-elements. The adenovirus vector genome can further be deleted, as described above.

[00163] Another vector for use in the present disclosure comprises Herpes Simplex Virus (HSV). HSV can be modified for the delivery of transgenes to cells by producing a vector

that exhibits only the latent function for long-term gene maintenance. HSV vectors are useful for nucleic acid delivery because they allow for a large DNA insert of up to or greater than 20 kilobases; they can be produced with extremely high titers; and they have been shown to express transgenes for a long period of time in the central nervous system as long as the lytic cycle does not occur.

[00164] Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway et al. (1999) *Gene Therapy* 6:986 and WO 00/17377.

[00165] In other embodiments of the present disclosure, the delivery vector of interest is a retrovirus. Retroviruses normally bind to a species specific cell surface receptor, e.g., CD4 (for HIV); CAT (for MLV-E; ecotropic Murine leukemic virus E); RAM1/GLVR2 (for murine leukemic virus-A; MLV-A); GLVR1 (for Gibbon Ape leukemia virus (GALV) and Feline leukemia virus B (FeLV-B)). The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes. A replication-defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques.

[00166] Yet another suitable vector is a lentiviral vector. Lentiviruses are a subtype of retroviruses but they have the unique ability to infect non-dividing cells, and therefore can have a ride range of potential applications.

[00167] Yet another suitable vector is a poxvirus vector. These viruses contain more than 100 proteins. Extracellular forms of the virus have two membranes while intracellular particles only have an inner membrane. The outer surface of the virus is made up of lipids and proteins that surround the biconcave core. Poxviruses are very complex antigenically, inducing both specific and cross-reacting antibodies after infection. Poxvirus can infect a wide range of cells. Poxvirus gene expression is well studied due to the interest in using vaccinia virus as a vector for expression of transgenes.

[00168] In another representative embodiment, the nucleic acid sequence encoding ATP1A3 is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus comprising the nucleic acid sequence encoding ATP1A3 can be stably integrated into the chromosome of the cell.

[00169] To enhance virus titers, helper virus functions *(e.g.,* adenovirus or herpesvirus) that promote a productive AAV infection can be provided to the cell. Helper virus sequences necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, e.g., as a non-infectious adenovirus miniplasmid that carries all of the helper genes that promote efficient AAV production.

[00170] Further, the helper virus functions may be provided by a packaging cell with the helper sequences embedded in the chromosome or maintained as a stable extrachromosomal element. Generally, the helper virus sequences cannot be packaged into AAV virions, e.g., are not flanked by TRs.

[00171] In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed. Many non-viral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In particular embodiments, non-viral delivery systems rely on endocytic pathways for the uptake of the nucleic acid molecule by the targeted cell. Exemplary nucleic acid delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

[00172] In particular embodiments, plasmid vectors are used in the practice of the present disclosure. Naked plasmids can be introduced into cells by injection into the tissue. Expression can extend over many months. Cationic lipids can aid in introduction of DNA into some cells in culture. Injection of cationic lipid plasmid DNA complexes into the circulation of mice can result in expression of the DNA in organs (e.g., the lung). One advantage of plasmid DNA is that it can be introduced into non-replicating cells.

[00173] In a representative embodiment, a nucleic acid molecule (e.g., a plasmid) can be entrapped in a lipid particle bearing positive changes on its surface and, optionally, tagged with antibodies against cell surface antigens of the target tissue.

[00174] Liposomes that consist of amphiphilic cationic molecules are useful non-viral vectors for nucleic acid delivery *in vitro* and *in vivo*. The positively charged liposomes are believed to complex with negatively charged nucleic acids via electrostatic interactions to form lipid:nucleic acid complexes. The lipid:nucleic acid complexes have several advantages as gene transfer vectors. Unlike viral vectors, the lipid:nucleic acid complexes can be used to transfer expression cassettes of essentially unlimited size. Since the complexes lack proteins,

they can evoke fewer immunogenic and inflammatory responses. Moreover, they cannot replicate or recombine to form an infectious agent and have low integration frequency.

[00175] Amphiphilic cationic lipid:nucleic acid complexes can be used for *in vivo* transfection both in animals and in humans and can be prepared to have a long shelf-life.

[00176] In addition, vectors according to the present disclosure can be used in diagnostic and screening methods, whereby a nucleic acid encoding ATP1A3 is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model screening methods, whereby a nucleic acid of interest is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

[00177] The vectors of the present invention can also be used for various non-therapeutic purposes, including but not limited to use in protocols to assess gene targeting, clearance, transcription, translation, etc., as would be apparent to one skilled in the art. The vectors can also be used for the purpose of evaluating safety (spread, toxicity, immunogenicity, etc.). Such data, for example, are considered by the United States Food and Drug Administration as part of the regulatory approval process prior to evaluation of clinical efficacy.

[00178] **Gene Therapy Methods, Pharmaceutical Formulations, and Modes of Administration**

[00179] Another aspect of the present disclosure provides a composition, pharmaceutical formulation comprising, consisting, or consisting essentially of vector comprising the an *ATPase* transgene (e.g., *ATP1A3*) and/or nucleic acid expression cassettes as described herein.

[00180] In some embodiments, compositions of the present disclosure comprise, consist of, or consist essentially of a recombinant viral vector (e.g., rAAV) and/or a pharmaceutically acceptable carrier and/or excipient, and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and optionally can be in solid or liquid particulate form.

[00181] By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject along with the isolated nucleic acid or vector without causing any undesirable biological effects such as toxicity. Thus, such a pharmaceutical composition can be used, for example, in transfection of a cell *ex vivo* or in administering an isolated nucleic acid or vector directly to a subject.

[00182] The compositions may also comprise other ingredients such as diluents and adjuvants. Acceptable carriers, diluents and adjuvants are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and may include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter ions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

[00183] The pharmaceutical carriers, diluents or excipients suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00184] In some embodiments, sterile injectable solutions are prepared by incorporating the recombinant viral vector (e.g., rAAV) in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-

drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[00185] For purposes of intramuscular injection, solutions in an adjuvant such as sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of recombinant viral vector (e.g., rAAV) as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxpropylcellulose. A dispersion of recombinant viral vector (e.g., rAAV) can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

[00186] Pharmaceutical compositions can be prepared as injectable formulations or as topical formulations to be delivered to the subject by transdermal transport. Numerous formulations for both intramuscular injection and transdermal transport have been previously developed and can be used in the practice of the invention. The recombinant viral vector can be used with any pharmaceutically acceptable carrier and/or excipient for ease of administration and handling.

[00187] Titers of recombinant viral vectors (e.g., rAAV) to be administered according to the methods of the present disclosure will vary depending, for example, on the particular recombinant viral vector, the mode of administration, the treatment goal, the individual, and the cell type(s) being targeted, and may be determined by methods standard in the art.

[00188] In the case of a viral vector, virus particles can be contacted with the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of virus to administer can vary, depending upon the target cell type and the particular virus vector, and can be determined by those of skill in the art. Typically, at least about 10^3 virus particles, at least about 10^5 particles, at least about 10^7 particles, at least about 10^9 particles, at least about 10^{11} particles, or at least about 10^{12} particles are administered to the cell. In exemplary embodiments, about 10^7 to about 10^{15} particles, about 10^7 to about 10^{13} particles, about 10^8 to about 10^{12} particles, about 10^{10} to about 10^{15} particles, about 10^{11} to about 10^{15} particles, about 10^{12} to about 10^{14} particles, or about 10^{12} to about 10^{13} particles are administered Dosages may also be expressed in units of viral genomes (vg).

[00189] The cell to be administered the vectors of the disclosure can be of any type, including but not limited to neuronal cells (including cells of the peripheral and central nervous systems), retinal cells, epithelial cells (including dermal, gut, respiratory, bladder, pulmonary, peritoneal and breast tissue epithelium), muscle (including cardiac, smooth muscle, including pulmonary smooth muscle cells, skeletal muscle, and diaphragm muscle), pancreatic cells (including islet cells), kidney cells, hepatic cells (including parenchyma), cells of the intestine, fibroblasts (e.g., skin fibroblasts such as human skin fibroblasts), fibroblast-derived cells, endothelial cells, intestinal cells, germ cells, lung cells (including bronchial cells and alveolar cells), prostate cells, stem cells, progenitor cells, dendritic cells, and the like. Moreover, the cells can be from any species of origin, as indicated above

[00190] Methods of transducing a target cell with a vector according to the present disclosure are contemplated by the present disclosure. The term "transduction" is used herein to refer to the administration/delivery of an *ATPase* transgene to a recipient cell either *in vivo* or *in vitro*, via a replication-deficient recombinant viral vector (e.g., rAAV) of the present disclosure thereby resulting in expression of an ATPase by the recipient cell. Thus, the present disclosure provides methods of administering an effective dose (or doses, administered essentially simultaneously or doses given at intervals) of a recombinant viral vector (e.g., rAAV) that encodes ATP1A3 to a subject in need thereof.

[00191] The *in vivo* transduction methods comprise the step of administering an effective dose, or effective multiple doses, of a nucleic acid expression cassette or composition comprising a recombinant viral vector of the present disclosure to an animal (including a human being) in need thereof. If the dose is administered prior to development of a disorder/disease, the administration is prophylactic. If the dose is administered after the development of a disorder/disease, the administration is therapeutic. In embodiments of the present disclosure, an effective dose is a dose that alleviates (eliminates or reduces) at least one symptom associated with the disorder/disease state being treated, that slows or prevents progression to a disorder/disease state, that slows or prevents progression of a disorder/disease state, that diminishes the extent of disease, that results in remission (partial or total) of disease, and/or that prolongs survival. An example of a disease contemplated for prevention or treatment with methods of the present disclosure is AHC.

[00192] Transduction with a recombinant viral vector (e.g., rAAV) may also be carried out *in vitro*. In one embodiment, desired target cells are removed from the subject, transduced with recombinant viral vector (e.g., rAAV) and reintroduced into the subject. Alternatively,

syngeneic or xenogeneic target cells can be used where those cells will not generate an inappropriate immune response in the subject.

[00193] Suitable methods for the transduction of a recombinant viral vector (e.g., rAAV) or the reintroduction of transduced cells into a subject are known in the art. In one embodiment, cells can be transduced *in vitro* by combining the recombinant viral vector (e.g., rAAV) with target cells, *e.g.*, in appropriate media, and screening for those cells harboring the DNA of interest using conventional techniques such as Southern blots and/or PCR, or by using selectable markers. A recombinant viral vector (e.g., rAAV) or transduced cells can then be formulated into pharmaceutical compositions, and the composition introduced into the subject by various techniques, such as by intramuscular, intravenous, subcutaneous and intraperitoneal injection, by injection into smooth and cardiac muscle, using *e.g.*, a catheter, intrathecal, intracisteranl, intraventricular or intraparenchymal into the brain.

[00194] Transduction of cells with recombinant viral vector (e.g., rAAV) of the present disclosure can result in the in sustained expression of *ATP1A3* or another *ATPase* gene. The present disclosure thus provides methods of administering/delivering a recombinant viral vector (e.g., rAAV) that expresses, for example, *ATP1A3* to a subject (e.g., a human patient). These methods include transducing tissues (including, but not limited to, tissues such as nervous system and muscle, organs such as brain, heart, liver, and glands such as salivary glands) with one or more recombinant viral vector (e.g., rAAV) of the present disclosure. Transduction may be carried out with gene cassettes comprising tissue specific control elements as described herein.

[00195] In some embodiments, gene editing is accomplished by transducing cells with a nuclease, such as zinc finger nuclease(s) or CRISPR/Cas9, plus normal ATPase sequences flanking a mutation in ATPase as a donor template. In such embodiments, the transduction with the nuclease cleaves the ATPase gene near a pathogenic variant/mutation followed by homology directed repair to correct the variant/mutation causing symptoms for an individual patient thereby treating and/or preventing ATPase -related diseases.

[00196] The isolated nucleic acids, vectors, and compositions of the present disclosure may further be used in various methods.

[00197] Another aspect of the present disclosure provides a method of treating or preventing an ATPase-mediated disease in a subject, the method comprising, consisting of, or consisting essentially of administering to the subject a therapeutically effective amount of the nucleic acid expression cassette, vector, composition, or pharmaceutical compositions

comprising a nucleic acid encoding an ATPase (e.g., ATP1A3) as described in the present disclosure such that the ATPase-mediated disease in the subject is prevented.

[00198] As used herein, the term "subject" and "patient" are used interchangeably and refer to both human and nonhuman animals. The term "nonhuman animals" of the disclosure includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dog, cat, horse, cow, chickens, amphibians, reptiles, and the like. The subject can be a human patient that is at risk for, or suffering from, an ATP1A3-mediated disease. The subject can also be a human patient that is at risk for, or suffering from, a disease caused by a mutation in the *ATP1A3* gene. The human patient can be of any age (e.g., an infant, child, or adult).

[00199] As used herein, "treatment" or "treating" refers to the clinical intervention made in response to a disease, disorder or physiological condition manifested by a patient or to which a patient may be susceptible. The aim of treatment includes the alleviation or prevention of symptoms, slowing or stopping the progression or worsening of a disease, disorder, or condition and/or the remission of the disease, disorder or condition.

[00200] An "effective amount" or "therapeutically effective amount" as used herein means an amount which provides a therapeutic or prophylactic benefit. Effective amounts of the nucleic acid molecules and/or compositions and/or pharmaceutical compositions can be determined by a physician with consideration of individual differences in age, weight, and condition of the patient (subject).

[00201] An effective amount of a therapeutic agent is one that will decrease or ameliorate the symptoms normally by at least 10%, more normally by at least 20%, most normally by at least 30%, typically by at least 40%, more typically by at least 50%, most typically by at least 60%, often by at least 70%, more often by at least 80%, and most often by at least 90%, conventionally by at least 95%, more conventionally by at least 99%, and most conventionally by at least 99.9%.

[00202] The term "disease" as used herein includes, but is not limited to, any abnormal condition and/or disorder of a structure or a function that affects a part of an organism. It may be caused by an external factor, such as an infectious disease, or by internal dysfunctions, such as cancer, cancer metastasis, and the like.

[00203] The term "administration" or "administering" as it applies to a human, primate, mammal, mammalian subject, animal, veterinary subject, placebo subject, research subject, experimental subject, cell, tissue, organ, or biological fluid, refers without limitation to contact of an exogenous ligand, reagent, placebo, small molecule, pharmaceutical agent,

therapeutic agent, diagnostic agent, or composition to the subject, cell, tissue, organ, or biological fluid, and the like. "Administration" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, placebo, and experimental methods. Treatment of a cell encompasses exposure of the cell to a reagent (e.g., a nucleic acid molecule), as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administering" also encompasses in vitro and ex vivo treatments, e.g., of a cell, by a reagent, diagnostic, binding composition, or by another cell.

[00204] Administration of an effective dose of the isolated nucleic acids, vectors, and compositions may be by routes standard in the art including, but not limited to, intrathecal, intra-cisterna magna, intracerebroventircular, intrahippocampal, intramuscular, parenteral, intravenous, oral, buccal, nasal, pulmonary, intracranial-intra-parenchymal, intraosseous, or intraocular. Intrahippocampal administration can comprise injecting the isolated nucleic acid, vector, or composition into the substance of the hippocampal brain (intra-parenchymal). Intracerebroventricular can comprise injecting the isolated nucleic acid, vector, or composition into the ventricle, which is filled with cerebrospinal fluid and is next to the hippocampus but not part of it.

[00205] Route(s) of administration and serotype(s) of viral (e.g., AAV) components of the recombinant viral vector (e.g., rAAV, and in particular, the AAV ITRs and capsid protein) of the present disclosure may be chosen and/or matched by those skilled in the art taking into account the disease state being treated and the target cells/tissue(s) that are to express the ATP1A3.

[00206] The present disclosure further provides for local administration and systemic administration of an effective dose of rAAV and compositions of the present disclosure including combination therapy as provided herein. For example, systemic administration is administration into the circulatory system so that the entire body is affected. Systemic administration includes enteral administration such as absorption through the gastrointestinal tract and parenteral administration through injection, infusion or implantation.

[00207] In particular, actual administration of a vector (e.g., rAAV) of the present disclosure can be accomplished by using any physical method that will transport the vector into the target tissue of the subject.

[00208] The virus vectors and virus capsids can be administered to tissues of the CNS (e.g., brain, eye) and may advantageously result in broader distribution of the virus vector or capsid than would be observed in the absence of the present disclosure.

[00209] Administration according to the present disclosure includes, but is not limited to, injection into the ventricles, cisterna magna, spinal theca, muscle, the bloodstream and/or directly into the brain.

[00210] In some embodiments, the nucleic acid molecules, vectors, and/or compositions of the disclosure can be administered to the CNS (e.g., to the brain or to the eye). The virus vector and/or capsid may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes cortex, basal ganglia, hippocampus and portaamygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The nucleic acid molecules, vectors, and/or compositions of the disclosure can also be administered to different regions of the eye such as the retina, cornea and/or optic nerve.

[00211] The nucleic acid molecules, vectors, and/or compositions can be delivered into the cerebrospinal fluid (e.g, by lumbar puncture) for more disperse administration of the delivery vector.

[00212] The nucleic acid molecules, vectors, and/or compositions can be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intracerebroventricular, intra-cisterna magnal, intraparenchymal, intracranial, intrathecal, intra-ocular, intracerebral, intraventricular, intravenous (e.g., in the presence of a sugar such as mannitol), intranasal, intra-aural, intra-ocular (e.g., intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (e.g., sub-Tenon's region) delivery as well as intramuscular delivery with retrograde delivery to motor neurons.

[00213] In other embodiments, the nucleic acid molecules, vectors, and/or compositions can be administered in a liquid formulation by direct injection (e.g., stereotactic injection) to the desired region or compartment in the CNS. In other embodiments, the virus vector can be provided by topical application to the desired region or by intra-nasal administration of aerosol formulation. Administration to the eye, may be by topical application of liquid droplets. As a further alternative, the virus vector and/or capsid may be administered as a solid, slow-release formulation.

[00214] In other embodiments, more than one route of administration can be utilized (e.g., ICV and ICM administration).

[00215] For example, resuspending the recombinant viral vector (e.g., rAAV) in phosphate buffered saline (PBS) can be sufficient to provide a vehicle useful for muscle tissue expression, and there are no known restrictions on the carriers or other components that can

be co-administered with the recombinant viral vector (e.g., rAAV, although compositions that degrade DNA should be avoided in the normal manner with rAAV). In cases where the recombinant viral vector comprises rAAV, the capsid proteins of a rAAV may be modified so that the rAAV is targeted to a particular target tissue of interest such as muscle.

[00216] Dosages will depend upon the mode of administration, the severity of the disease or condition to be treated, the individual subject's condition, the particular vector, and the gene to be delivered, and can be determined in a routine manner. In some embodiments, the isolated nucleic acid molecule or vector is administered to the subject in a therapeutically effective amount, as that term is defined above.

[00217] The dose of vector (e.g., rAAV) to be administered in methods disclosed herein will vary depending, for example, on the particular recombinant viral vector, the mode of administration, the treatment goal, the individual, and the cell type(s) being targeted, and may be determined by methods standard in the art. Titers of each recombinant viral vector (e.g., rAAV) administered may range from about $1x10^6$, about $1x10^7$, about $1x10^8$, about $1x10^9$, about $1x10^{10}$, about $1x10^{11}$, about $1x10^{12}$, about $1x10^{13}$, about $1x10^{14}$, or to about $1x10^{15}$ or more per ml. Dosages may also be expressed in units of viral genomes (vg) (*i.e.*, $1x10^7$ vg, $1x10^8$ vg, $1x10^9$ vg, $1x10^{10}$ vg, $1x10^{11}$ vg, $1x10^{12}$ vg, $1x10^{13}$ vg, $1x10^{14}$ vg, $1x10^{15}$ respectively). Dosages may also be expressed in units of viral genomes (vg) per kilogram (kg) of bodyweight (*i.e.*, $1x10^{10}$ vg/kg, $1x10^{11}$ vg/kg, $1x10^{12}$ vg/kg, $1x10^{13}$ vg/kg, $1x10^{14}$ vg/kg, $1x10^{15}$ vg/kg respectively). Methods for titering viral vectors such as AAV are described in Clark *et al.*, *Hum. Gene Ther.*, 10:1031-1039 (1999).

[00218] In some embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., daily, weekly, monthly, or yearly.

[00219] Delivery to a target tissue can also be achieved by delivering a depot comprising the virus vector and/or capsid. In representative embodiments, a depot comprising the vector and/or capsid is implanted into skeletal, cardiac and/or diaphragm muscle tissue or the tissue can be contacted with a film or other matrix comprising the virus vector and/or capsid.

[00220] Combination therapies are also contemplated by the present disclosure. Combination as used herein includes both simultaneous treatment and sequential treatments. Combinations of methods of the present disclosure with standard medical treatments are specifically contemplated, as are combinations with alternative vectors mentioned above, novel vectors that are engineered and generated to enhance the effect of therapy and novel therapies.

[00221] **Kits**

[00222] Other aspects of the present disclosure provides a kit for the prevention and/or treatment of a cancer in subject, the kit comprising, consisting of, or consisting essentially of a composition as provided herein and instructions for use.

[00223] Yet another aspect of the present disclosure provides all that is disclosed and illustrated herein.

[00224] The following Examples are provided by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Mouse Model that Reproduces the Manifestations of AHC Provides Evidence for Translation of Gene Therapy into Human Application

[00225] There are currently six mouse models of *ATP1A3* mutations. (Clapcote *et al.* (2006) *Proc Natl Acad Sci USA,* 106(33): 14085-14090; Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468; Masoud *et al.* (2017) *Curr Treat Options Neurol.* 19(2):8; Heinzen *et al.* (2012) *Nat Genet.* 44(9):1030-1034; Helseth *et. al.* (2018) *Neurobiol Dis.* 119:100-112; Holm *et al.* (2016) *Biochim Biophys Acta.* 1857(11):1807-1828; Ikeda *et al.* (2017) *Brain Res.* 1666: 27–37). The heterozygous Mashlool and the Matoub mouse models are the only models that reproduce all the manifestations of AHC (Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Helseth *et. al.* (2018) *Neurobiol Dis.* 119:100-112). Each of these knock-in mice, as reported, carries one of the two most common mutations on a C57BL/6J background. Mashlool ($Mashl^{+/-}$) carries the D801N mutation, which is the most common mutation (40% of AHC patients) that causes AHC of average severity in humans. The Matoub mouse ($Matb^{+/}$) carries the E815K mutation, which is the second most common mutation (26% of AHC patients) and causes the most severe phenotype of AHC in humans. The Mashlool and the Matoub mice have spontaneous as well as stress (cold-water exposure) induced hemiplegias, dystonias and epileptic seizures. Both also, similar to human phenotype, have reduction in induced hemiplegia in response to flunarizine but no other benefits from this medication (Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468; Masoud *et al.* (2017) *Curr Treat Options Neurol.* 19(2):8; Mikati *et al.* (2000) *Pediatr Neurol.* 23(2):134-141; Helseth *et*

al. (2018) *Neurobiol Dis.* 119:100-112). The response to flunarizine is another unique validation of these two models that has not been demonstrated in any other model.

[00226] For the Examples described herein, the D801N (*Mashl*$^{+/-}$) mutant was chosen to study because it is the most common mutation in humans over the E815K (*Matb*$^{+/}$) mutant that has prohibitive mortality limiting the ability to maintain a thriving colony and to carry out long term experimentation. In addition to paroxysmal spells, prepubescent and adult D801N mice also manifest behavioral abnormalities that closely parallel AHC morbidity in humans. These include increased mortality as well as balance, impulsivity, memory, and gait, abnormalities. (Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468). D801N mice also have predisposition to spreading depolarization, increased neuronal excitability and impaired firing of GABAergic fast spiking inhibitory interneurons. (Hunanyan *et al.* (2015) *Epilepsia* 56(1):82-93; Hunanyan *et al.* (2018) *Epilepsia* 59(7): 1455-1468). Thus, the D801N (*Mashl*$^{+/-}$) model can be used to help develop novel therapies that can be translated to clinical applications to treat AHC and other *ATP1A3* related diseases mentioned above. In most of the experiments described herein, the P10 age group was selected to study as it corresponds to infancy in humans the age at which AHC symptoms start. The behavioral testing was performed at around P40, which corresponds to adolescence in humans, the age at which the AHC manifestations have been established for at least 10 years.

Example 2: Intracerebroventricular (ICV) Injection of AAV9 Active Vector Results in Robust Increases in ATPase Activity in Brain Regions Contiguous to the Injection site

[00227] To determine whether ICV injection of AAV9 active vector can result in increases in ouabain sensitive ATPase activity in brain regions we performed ATPase enzyme activity assays as described previously (Clapcote *et al.* (2006) *Proc Natl Acad Sci USA,* 106(33): 14085-14090; Ye *et al.* (2017) *EMBO J.*, 36(16): 2419-2434).

[00228] Briefly, WT mice were injected intrahippocampally with $4x10^{10}$ vg (1µl in each side) of AAV9-Syn-ATP1A3-p2a-Cherry-SV40polyA (**FIG. 1**) or with a similar dose of control vector (vector lacking the ATP1A3 transgene). A 22% increase in total ATPase activity in hippocampus in mice injected with AAV9-Syn-ATP1A3-p2a-Cherry-SV40polyA (n=2) was observed as compared those injected with the control vector (n=2), and even higher (375%) in the ouabain sensitive fraction (**FIG. 4**). There was no increase in the ouabain insensitive fraction indicating that the increase in total activity was due to increases in the oubain sensitive fraction as calculated from **FIG. 4** (AAV-ATP1A3-ouabain-sensitive-

fraction = 0.93-0.55 = 0.38; AAV-cherry-ouabain-sensitive-fraction = 0.74-0.66 = 0.08; ratio = 0.38/0.08 = 4.75 fold or 375% increase). AAV injected 1 μL/per hemisphere, intra-parenchymal, into each dorsal hippocampus ($4x10^{13}$ vg/mL, total 2 μL per mouse).

[00229] P40 wild-type mice injected unilaterally, at P10, ICV with active vector (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA, V=5 μl, $4x10^{13}$ vg/ml, n=3/group) showed, as compared to control vector (vector lacking the ATP1A3 transgene), that ouabain-sensitive ATPase activity was 102% higher in the ipsilateral hippocampus (**FIG. 5,** p=0.046 one tailed Student's t-test). In other ipsilateral areas the changes did not achieve statistically significant differences (number of mice was only 3 per group) but areas closer to the hippocampus showed increased ouabain-sensitive ATPase activity as compared to control vector. These were 37% higher in the cerebral cortex, 26% higher in the basal ganglia/thalamus, 16% higher in brainstem and none in the cerebellum (all comparisons $p>0.05$) after the above unilateral ICV injection.

Example 3: Transduction after unilateral intrahippocampal and intracerebroventricular injections assessed by mCherry expression

[00230] Intrahippocampal injections of 4×10^{10} vg (V=5 μL of $4x10^{13}$ vg/mL of AAV9-Syn-ATP1A3-p2a-mCherry-SV40polyA (AAV9-ATP1A3) in $Mashl^{+/-}$ and WT-littermates injected at ages P0-P10 and in adult mice were performed in 24 mice. These were sacrificed one month later.

[00231] The results demonstrated excellent transduction efficacy of active viral vector in all ages as detected by the mCherry signal and as illustrated in the hippocampus of an adult WT mouse (**FIGS. 6A-6B**). Consistently, the percent of neurons expressing mCherry in CA1 was >90%, and in CA3 ~70%. Expression was robust in both the pyramidal cells and in interneurons.

[00232] In addition, unilateral intracerebroventricular injection (ICV) of AAV9-ATP1A3 ($4x10^{13}$ vg/mL; V=5 μl) in ~P10 mice showed at P40 excellent expression of mCherry seen in the cytoplasm in various brain regions including hippocampus, basal ganglia, thalamus, cortex and even in the, relatively distant, cerebellum (**FIGS. 7A-7B**).

Example 4: Restoring motor performance on balance beam test in mutant mice

[00233] To determine whether unilateral intrahippocampal AAV9-Syn-ATP1A3-p2a-Cherry-SV40polyA could restore motor performance in mutant mice, a balance beam test was performed. Adult mutants $Mashl^{+/-}$ mice (2-3 month old mice) were treated with

intrahippocampal AAV9-Syn-ATP1A3-p2a-Cherry-SV40polyA (20 10^{10} vg in 5µl of $4x10^{13}$, n=6 mice) and tested 21 days post-injection. $Mashl^{+/-}$ mice treated with AAV-Syn-ATP1A3-p2a-Cherry-SV40polyA (n=3) had better performance (i.e., they crossed the beam faster) than $Mashl^{+/-}$ mice treated with control vector AAV-cherry (n=3 per group, p=0.053, Student's t-test one tailed) (**FIG. 8**). Injection of AAVs was performed on 2-3 month old mice.

Example 5: ICV and Intra-Cisterna Magna (ICM) Injections of AAV9 Active Vector Result in Robust FLAG Reporter Gene Expression in Brain Regions Contiguous to the Injection Sites

[00234] To determine the extent of reporter gene expression after ICV and ICM injections we performed the following. 1) Unilateral ICV injection of $20x10^{10}$ vg at P10 with sacrifice of animals at P40 (**FIGS. 9A-9D**). 2) ICM injection of $15x10^{10}$ vg at P10 with sacrifice at P40 (**FIG. 9F**). Incremental dose study of combined ICM and bilateral ICV injections using three doses of the same AAV active vector injected at P10 and sacrificed at P40 (AAV9-hSyn-ATP1A3-FLAG-p2a-Cherry-SV40polyA, 4 WT mice/dose, **FIGS. 10A-10H**). The three doses studied were $3x10^{10}$ vg in each site (cisterna magna and the two lateral ventricles, total $9x10^{10}$ vg/mouse), $6x10^{10}$ vg in each site (total $18x10^{10}$ vg/mouse) and $9x10^{10}$ vg in each site (total $27x10^{10}$ vg/mouse).

[00235] Robust transgene expression was observed with the higher doses (**FIGS. 9A-9D, 9F** and **FIGS 10A-10H**) but not with the lower two doses of the incremental dose study. Two reporter genes, FLAG after the 3' terminal of *ATP1A3* DNA followed by mCherry with a cleavage site in between them, were used to assess transgene expression. Specifically, the hippocampus showed FLAG expression nearly 100% of cells in the CA3 and CA1 hippocampal regions on the side of the ICV injections whether after a unilateral or bilateral ICV injections (**FIGS. 9A-9D** and **FIGS. 10A-10B)**. After unilateral ICV injections, there was minimal expression on the contralateral side. With combined ICM and bilateral ICV injections cortex and cerebellum showed expression in about 10% of total cells with much higher percentages in areas close to the injection sites: almost all the cells in those regions showed robust expression (**FIGS. 10A-10E)** while more distant areas showed very low if any expression. Deep brain structures including basal ganglia, thalamus, and brainstem also showed hardly any expression (**FIGS. 10F-10H**).

[00236] In these experiments, mCherry was detected in the cytoplasm since it is cleaved from the ATP1A3-FLAG, whereas ATP1A3-FLAG fusion protein was detected in the cell membrane the functional locale of the pump (**FIGS. 9A-9F** and **FIGS. 10A-10H**). This

indicates that the ATP1A3 transgene is not only expressed but also that the ATP1A3 protein is transported to its functional locale, the cell membrane. In the active vector, since FLAG coding DNA sequence is at the 3' end of the *ATP1A3* coding sequence, it is expected to be transcribed only after *ATP1A3* coding sequence is transcribed. Thus, the demonstration of a positive signal for FLAG indicates the expression and transcription of exogenous *ATP1A3* through the active vector.

[00237] Whether administration of the entire high dose unilaterally ICV would increase transduction beyond the immediately contiguous areas using two doses one almost triple the other was studied. The two doses were: $7.5x10^{10}$ vg unilaterally ICV and $20x10^{10}$ vg unilaterally ICV. With both, nearly 100% of hippocampal neurons expressed FLAG on the side ipsilateral to the injection site with about 10% in cortex and essentially no expression in other areas. These data indicated that dosing regimen providing the highest level of transgene expression without using needlessly additional vector was about $7.5x10^{10}$ vg per injection site and that ICM and bilateral ICV injections are needed. This justified the use of the combination ICM and bilateral ICV dose of $7.5x10^{10}$ in each site for a total of $22.5x10^{10}$ vg/animal in studies looking at survival and behavior.

Example 6: Combined ICV and ICM Active Vector Injections Result in Improvement in Mashl$^{+/-}$ Phenotype

[00238] The effects of active vector and control vector administered via ICM and bilateral ICV ($22.5x10^{10}$ vg/animal, $7.5x10^{10}$ in each site) injections at P10 were compared at P40. The studied groups were wild type untreated (WT Naïve, n=10), wild type control (WT-CTL, n=10), wild type treatment (WT-Tr, n=4), mutant control (Het-CTL, n=10) and mutant treatment (Het-Tr, n=9) groups.

[00239] It was found that Mashl$^{+/-}$ mice treated with the active vector experienced significantly decreased occurrence of hemiplegia spells (**FIG. 11,** p=0.0128, Het control n=4, Het treatment n=6**)** and a trend for shorter duration of dystonia spells (**FIG. 12)** induced by the cold-water immersion test (p= 0.00359 for comparison of all groups using one way ANOVA and 0.062 for comparison of Het-treatment and Het-control groups using one tailed Student's t-test, Het control n=4, Het treatment n=6). None of the mice of the three WT groups had dystonia so these groups are not shown in **FIG. 12**. Additionally, none of the mice receiving active vector had cold-water induced epileptic seizures while 25% of those receiving control vector had seizure (**FIG. 13** Het control n=4, Het treatment n=6**)**. The latter

comparison did not achieve statistical significance ($p>0.05$), but the first two did as illustrated by the p values above.

[00240] Significant improvements on the 8 mm balance beam test were also observed. In particular, mutant Mashl$^{+/-}$ mice receiving active vector had significantly shorter times in crossing the beam (**FIG. 14**, $p \leq 0.001$for comparisons among the groups using ANOVA, $p=0.0105$ two tailed Student's t-test for comparison between the Het-Tr and Het-CTRL groups, WT naïve n=6, WT-CTRL n=10, WT-Tr n=4, Het-CTRL n=6, Het-Tr n=9).

Example 7: Combined ICV and ICM Active Vector Injections Result in Long Term Improved Survival

[00241] When the above groups of mice were followed into adulthood through P90, the group treated with the active vector showed much better and statistically significant ($p=0.009$) survival as compared to the mutant group injected with the control vector (HET-CTL) (**FIG. 15**). When animals were sacrificed after P90 Western blot showed expression of the FLAG antibody in both hippocampus and cerebellum in mice injected with active vector as illustrated by sample Western blots (**FIG. 16**) and by comparative densitometry (**FIG. 17,** $p=0.032$). The Western blots shown in **FIG. 17** illustrates that there is continued expression of the FLAG transgene into adulthood at P90 in both cerebellum and hippocampus. The FLAG band intensity was much stronger in hippocampal lysate than in the cerebellar lysates (**FIG. 16**). The graph shown in **FIG. 17** (WT-CTRL n=2, WT-Tr n=2, Het-CTRL n=2, Het-Tr n=3, $p=0.032$ for comparison of control and the treatment groups using one way ANOVA with post-hoc Student-Newman-Keuls method) illustrates the continued expression of the FLAG transgene into adulthood at P90 in the WT and Het treatment groups.

Example 8: AAV-Mediated Gene Therapy of Alternating Hemiplegia of Childhood (AHC) in Humans

[00242] Examples 1-8 demonstrate efficacy of AAV-mediated gene therapy in Mashl$^{+/-}$ mice, which contains the D801N mutation, the most commonly found mutation in human AHC patients. These results indicate that AAV-mediated gene therapy with the ATP1A3 transgene is a therapeutic approach to treating humans with ATP1A3-mediated diseases, including AHC.

[00243] To demonstrate efficacy and safety of this AAV-mediated gene therapy approach in humans, the following steps will be taken.

[00244] 1. Optimize the vector, dose and delivery route.

[00245] 2. Further optimize dosing regimens and initiate toxicity studies.

[00246] 3. Initiate human study plans. Duke is the leading center in the International AHC consortium with over 100 patients in the AHC program database.

[00247] One skilled in the art will readily appreciate that the present disclosure is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present disclosure described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the present disclosure. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the present disclosure as defined by the scope of the claims.

[00248] No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference, unless explicitly indicated otherwise.

[00249] The present disclosure shall control in the event there are any disparities between any definitions and/or description found in the cited references.

Claims:

1. A nucleic acid expression cassette comprising a nucleic acid sequence encoding an ATPase.

2. The nucleic acid expression cassette of claim 1, wherein the ATPase is selected from the group consisting of ATP1A1, ATP1A2, ATP1A3, ATP2C1, ATP6A1, ATP6V1B1, ATP6V0A4, ATP7A, ATP7B, or ATP11C.

3. The nucleic acid expression cassette of claim 1, wherein the nucleic acid sequence comprises the sequence set forth in SEQ ID NO:01, or a fragment, isoform, or homologue thereof.

4. The nucleic acid expression cassette of claim 2, wherein the nucleic acid sequence comprises a sequence having at least 80% identity to the sequence set forth in SEQ ID NO:01.

5. The nucleic acid expression cassette of claim 1, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO:02, or a fragment, isoform, or homologue thereof.

6. The nucleic acid expression cassette of claim 4, wherein the nucleic acid sequence comprises a sequence having at least 80% identity to sequence set forth in SEQ ID NO:02.

7. The nucleic acid expression cassette of claim 1, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO:07, or a fragment, isoform, or homologue thereof.

8. The nucleic acid expression cassette of claim 4, wherein the nucleic acid sequence comprises a sequence having at least 80% identity to the sequence set forth in SEQ ID NO:07.

9. The nucleic acid expression cassette of any of claims 1-7, wherein the expression cassette further comprises a nucleotide sequence encoding ATP1A3 that is codon-optimized to reduce CpG methylation sites and for mammalian expression.

10. The nucleic acid expression cassette of any of claims 1-8, wherein the nucleic acid sequence encoding ATP1A3 is operably linked to a promoter and a polyadenylation sequence.

11. The nucleic acid expression cassette of claim 9, wherein the promoter comprises a neuron-specific promoter.

12. The nucleic acid expression cassette of claim 10, wherein the neuron-specific promoter is selected from the group consisting of synapsin 1, calcium/calmodulin-dependent protein kinase II, tubulin alpha 1, neuron-specific enolase, and platelet-derived growth factor beta chain promoters.

13. The nucleic acid expression cassette of claim 11, in which the promoter is a human synapsin promoter.

14. The nucleic acid expression cassette of claim 12, wherein the human synapsin promoter comprises the proximal region of the synapson 1 promoter (-422 to -22).

15. The nucleic acid expression cassette of claim 13, wherein the human synapsin promoter comprises the nucleic acid sequence set forth in SEQ ID NO:05.

16. The nucleic acid expression cassette of claim 12, wherein the human synapsin promoter comprises the nucleic acid sequence set forth in SEQ ID NO:04 or SEQ ID NO:09.

17. The nucleic acid expression cassette of claim 9, wherein the promoter is selected from a group of constitutively active promoters.

18. The nucleic acid expression cassette of claim 16, wherein the constitutively active promoter is selected from the group consisting of human β-actin, human elongation factor-1α, chicken β-actin combined with cytomegalovirus early enhancer, cytomegalovirus (CMV), simian virus 40, and herpes simplex virus thymidine kinase.

19. The nucleic acid expression cassette of any of claims 1-17, wherein the nucleic acid expression cassette further comprises a transcriptional termination signal selected from the

group consisting of bovine growth hormone polyadenylation signal (BGHpA), Simian virus 40 polyadenylation signal (SV40pA), and a synthetic polyadenylation signal.

20. A vector comprising a nucleic acid sequence encoding an ATPase.

21. The vector of claim 19, wherein the vector is a viral vector or non-viral vector.

22. The vector of claim 20, wherein the vector is a recombinant viral vector.

23. The vector of claim 20, wherein the viral vector is selected from the group consisting of an adenovirus vector, an adeno-associated virus vector, a herpes simplex virus vector, a retrovirus vector, a lentivirus vector, and alphavirus vector, a flavivirus vector, a rhabdovirus vector, a measles virus vector, a Newcastle disease viral vector, a poxvirus vector, or a picornavirus vector.

24. The vector of claim 21, wherein the adenovirus vector is an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVrh74, AAV8, AAV9, AAV10, AAV11, AAV12 and AAV13.

25. The vector of claim 23, wherein the adenovirus vector is an AAV serotype selected from the group consisting of AAV1, AAV8, or AAV9.

26. The vector of claim 23, wherein the adenovirus vector comprises an AAV9 vector.

27. The vector of claim 23, wherein the adenovirus vector comprises an AAV8 vector.

28. The vector of claim 23, wherein the adenovirus vector comprises an AAV1 vector.

29. The vector of claim 19, wherein the vector comprises the AAV9-hSyn-ATP1A3-p2A-mCherry (pBK828) vector.

30. The vector of claim 28, wherein the vector comprises the nucleic acid sequence set forth in SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21.

31. The vector of claim 28, wherein the vector comprises a nucleic acid sequence nucleic acid having at least 80% identity to the sequence set forth in set forth in SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21.

32. The vector of claim 20, wherein the non-viral vector is selected from the group consisting of a polymer based vector, a peptide based vector, a lipid nanoparticle, a solid lipid nanoparticle, or a cationic lipid based vector.

33. The vector of claim 19, wherein the nucleic acid sequence is contained in a nucleic acid expression cassette.

34. The vector of claim 19, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO:1, or a fragment, isoform, or homologue thereof.

35. The vector of claim 19, wherein the nucleic acid sequence comprising a sequence having at least 80% identity to SEQ ID NO:01.

36. The vector according to claim 19, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO:02 or SEQ ID NO:07, or a fragment, isoform, or homologue thereof.

37. The vector according to claim 19, wherein the nucleic acid sequence comprising a sequence having at least 80% identity to SEQ ID NO:02 or SEQ ID NO:07.

38. The vector according to claim 19, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO:06, or a fragment, isoform, or homologue thereof.

39. The vector according to claim 19, wherein the nucleic acid sequence comprises a sequence having at least 80% identity to the sequence set forth in SEQ ID NO:06.

40. The vector as in any of claims 19-38, the vector further comprising one or more of the following elements: (a) an inverted terminal repeat sequence (ITR); (b) a promoter; (c) transcription terminator; and (d) a flanking inverted terminal repeat sequence (ITR).

41. The vector of claim 40, wherein the promoter comprises a neuron-specific promoter.

42. The vector of claim 40, wherein the neuron-specific promoter is selected from the group consisting of synapsin 1, calcium/calmodulin-dependent protein kinase II, tubulin alpha 1, neuron-specific enolase, and platelet-derived growth factor beta chain promoters.

43. The vector of claim 40, wherein the promoter is derived from the human synapsin promoter.

44. The vector of claim 42, wherein the human synapsin promoter comprises the proximal region of the synapson 1 promoter (-422 to -22).

45. The vector of claim 42, wherein the human synapsin promoter comprises the nucleic acid sequence set forth in SEQ ID NO:04.

46. The vector of claim 42, wherein the human synapsin promoter comprises the nucleic acid sequence set forth in SEQ ID NO:05 or SEQ ID NO:09.

47. The vector of claim 40, wherein the promoter is a constitutively active promoter.

48. The vector of claim 46, wherein the constitutively active promoter is selected from the group consisting of human β-actin, human elongation factor-1α, chicken β-actin combined with cytomegalovirus early enhancer, cytomegalovirus (CMV), simian virus 40, and herpes simplex virus thymidine kinase.

49. The vector of 39, wherein the transcription terminator is selected from the group consisting of bovine growth hormone polyadenylation signal (BGHpA), Simian virus 40 polyadenylation signal (SV40pA), and a synthetic polyadenylation signal.

50. A composition comprising the vector of any of claims 20-48.

51. A pharmaceutical composition comprising a vector of any of clams 20-48 and a pharmaceutically acceptable carrier and/or excipient.

52. A method of preventing an ATPase-mediated disease in a subject, the method

comprising administering to the subject a therapeutically effective amount of the vector of any of claims 20-48 or the composition of claim 50 or the pharmaceutical composition of clam 51 such that the ATPase -mediated disease in the subject is prevented.

53. A method of treating an ATPase-mediated disease in a subject, the method comprising administering to the subject a therapeutically effective amount of the vector of any of claims 20-48 or the composition of claim 50 or the pharmaceutical composition of clam 51 such that the ATPase-mediated disease in the subject is treated.

54. The method of any of claims 52 or 53, wherein the vector or composition thereof is administered by a route selected from the group consisting of intramuscular injection, systemically, parenterally by injection, infusion or implantation, intracerebraoventricularly, intra-cisterna magna, intrahippocampal, and intrathecally.

55. The method of any of claims 52 or 53, wherein the vector or composition thereof is administered by intracerebroventricular and intra-cisterna magna injections.

56. The method of any of claims 52 or 53, wherein the subject is a human.

57. The method of any of claims 52 or 53, wherein the subject has an ATP1A3 protein mutation selected from the group consisting of a E815K mutation, a D801N mutation, an I180N mutation, a R756C mutation, or a V589F mutation.

58. The method of any of claims 52 or 53, wherein the ATPase-mediated disease is selected from the group consisting of rapid-onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (AHC), epileptic encephalopathy (EE), cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss (CAPOS) syndrome, fever induced paroxysmal weakness and encephalopathy (FIPWE), recurrent episodes of cerebellar ataxia (RECA), early-onset schizophrenia Dystonia Dysmorphism of the face Encephalopathy MRI abnormalities without hemiplegia (D-DEMO) syndrome and Childhood Rapid Onset ataxia (CROA).

59. The method of any of claims 52 or 53, wherein the ATPase-mediated disease is AHC.

60. A use of the vector of any of claims 20-48 or the composition of claim 50 or the pharmaceutical composition of clam 51 for the preparation of a medicament for the treatment or prevention of AHC.

61. A kit for the treatment and/or prevention of an ATPase-mediated disease in a subject, the kit comprising a composition of any of claims 50-51 and instructions for use.

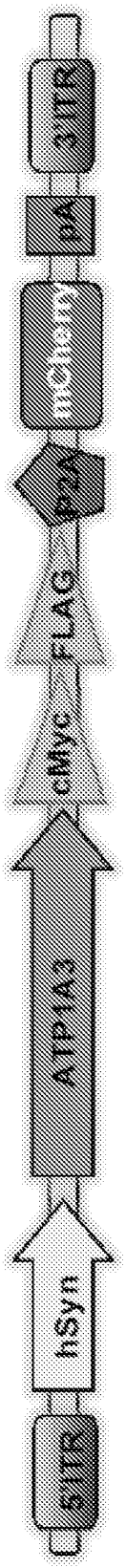

FIG. 1

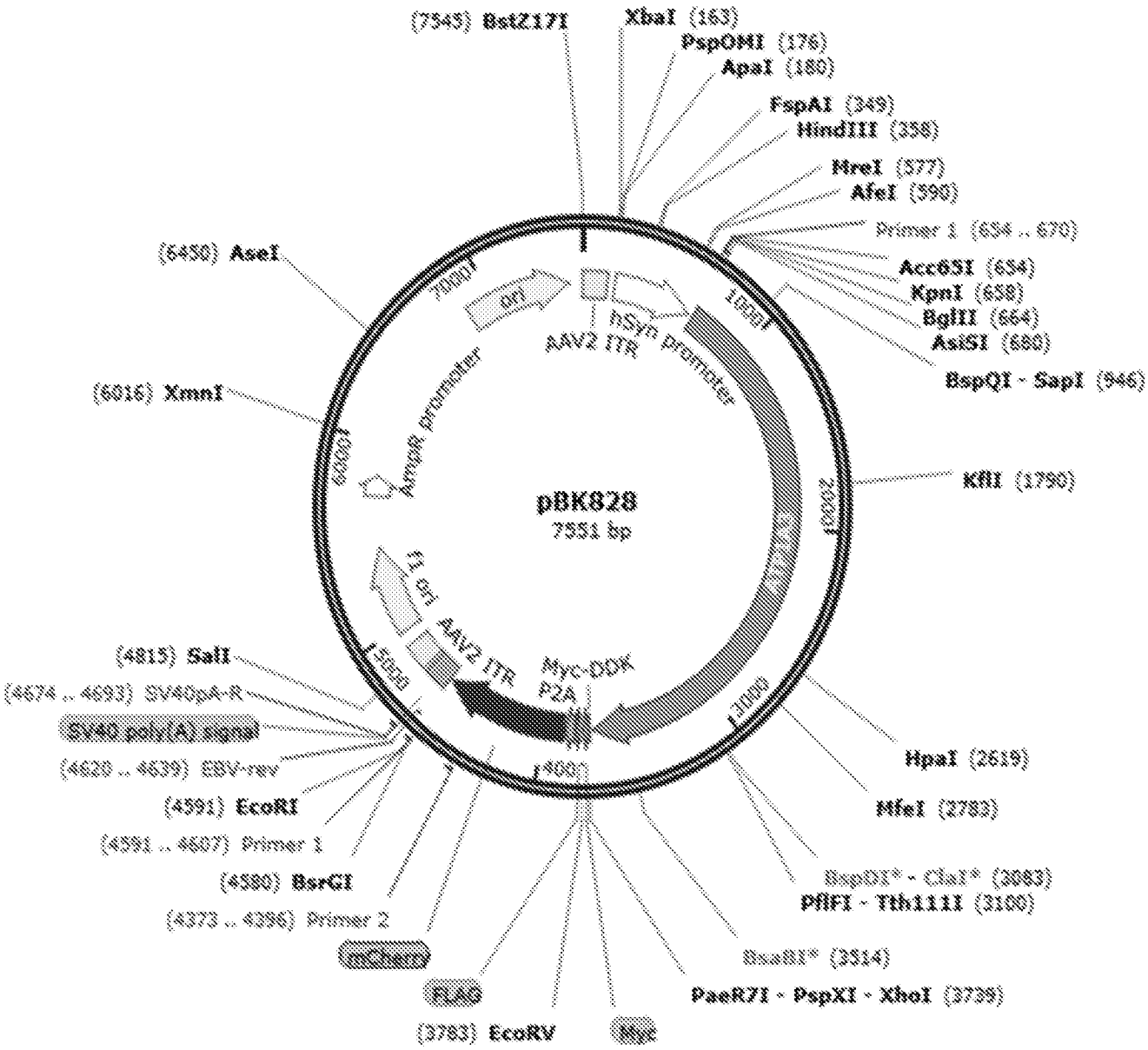
(7545) BstZ17I
XbaI (163)
PspOMI (176)
ApaI (180)
FspAI (349)
HindIII (358)
NreI (577)
AfeI (590)
Primer 1 (634 .. 670)
Acc65I (654)
KpnI (658)
BglII (664)
AsiSI (680)
BspQI - SapI (946)
KflI (1790)
HpaI (2619)
MfeI (2783)
BspQI* - ClaI* (3083)
PflFI - Tth111I (3100)
BsaBI* (3514)
PaeR7I - PspXI - XhoI (3739)
Myc
(3783) EcoRV
FLAG
mCherry
(4373 .. 4396) Primer 2
(4580) BsrGI
(4591 .. 4607) Primer 1
(4591) EcoRI
(4620 .. 4639) EBV-rev
SV40 poly(A) signal
(4674 .. 4693) SV40pA-R
(4815) SalI
(6458) AseI
(6016) XmnI
pBK828
7551 bp
ori
AAV2 ITR
hSyn promoter
Ampicillin promoter
Myc-DDK
P2A
AAV2 ITR
1000
2000
3000
4000
5000
6000
7000

FIG. 2

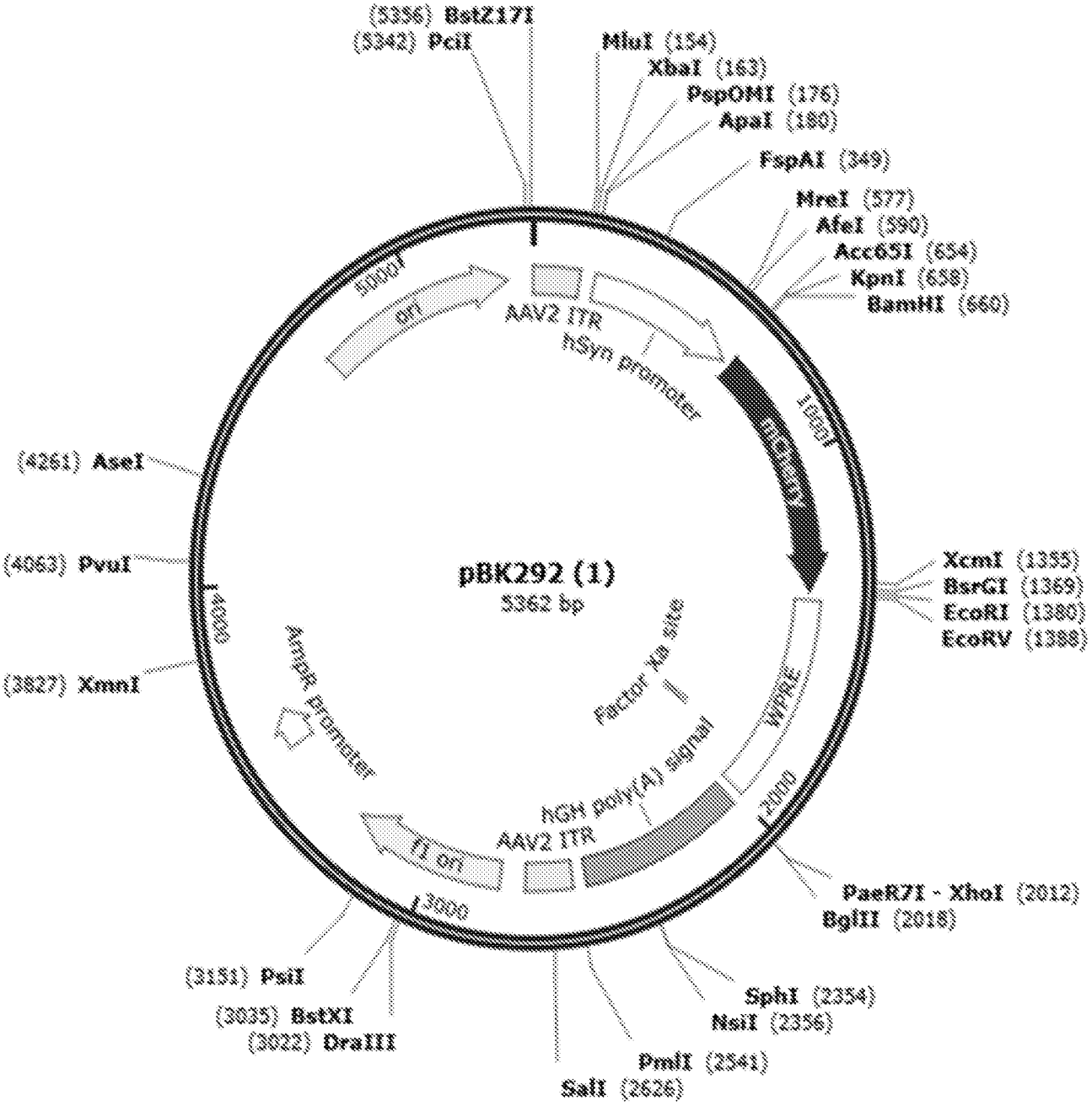
(5356) BstZ17I
(5342) PciI
MluI (154)
XbaI (163)
PspOMI (176)
ApaI (180)
FspAI (349)
MreI (577)
AfeI (590)
Acc65I (654)
KpnI (658)
BamHI (660)
5000
ori
AAV2 ITR
hSyn promoter
1000
(4261) AseI
(4063) PvuI
4000
(3827) XmnI
pBK292 (1)
5362 bp
Factor Xa site
WPRE
AmpR promoter
NGH poly(A) signal
2000
f1 ori
AAV2 ITR
3000
XcmI (1355)
BsrGI (1369)
EcoRI (1380)
EcoRV (1388)
PaeR7I - XhoI (2012)
BglII (2018)
(3151) PsiI
(3035) BstXI
(3022) DraIII
SphI (2354)
NsiI (2356)
PmlI (2541)
SalI (2626)

FIG. 3

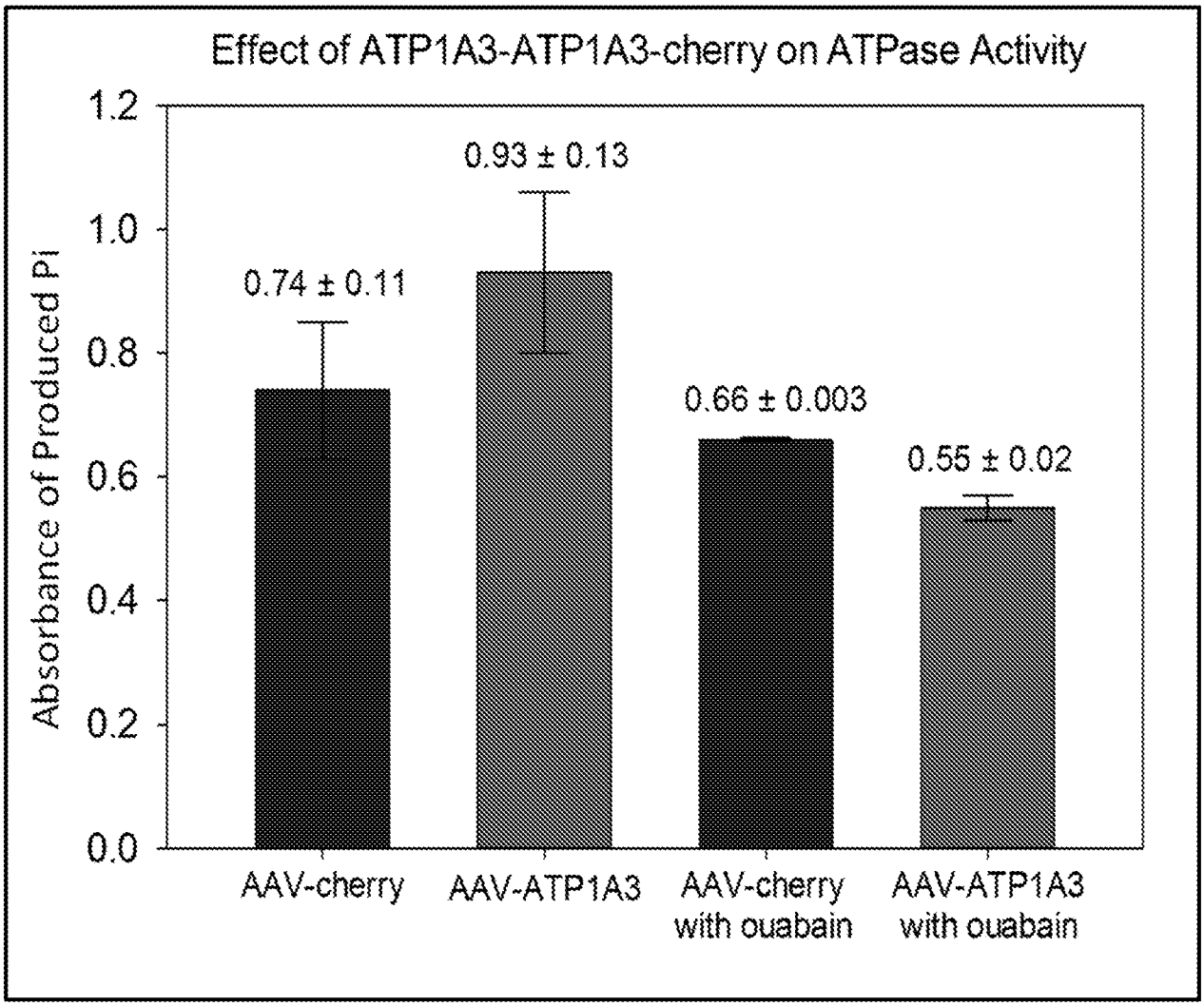
Effect of ATP1A3-ATP1A3-cherry on ATPase Activity
Absorbance of Produced Pi
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.74 ± 0.11
0.93 ± 0.13
0.66 ± 0.003
0.55 ± 0.02
AAV-cherry
AAV-ATP1A3
AAV-cherry with ouabain
AAV-ATP1A3 with ouabain

FIG. 4

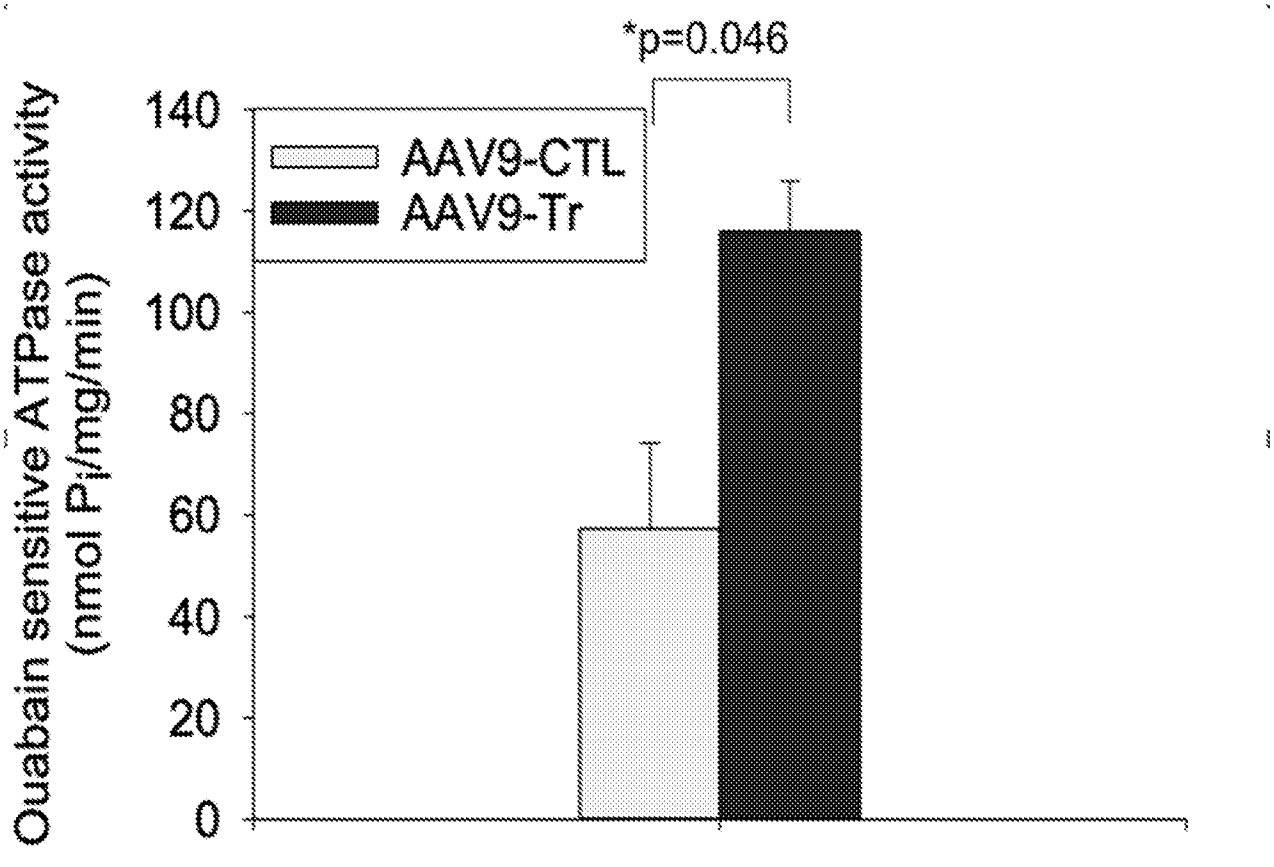
*p=0.046
AAV9-CTL
AAV9-Tr
Ouabain sensitive ATPase activity
(nmol Pi/mg/min)
140
120
100
80
60
40
20
0

FIG. 5

FIG. 6A

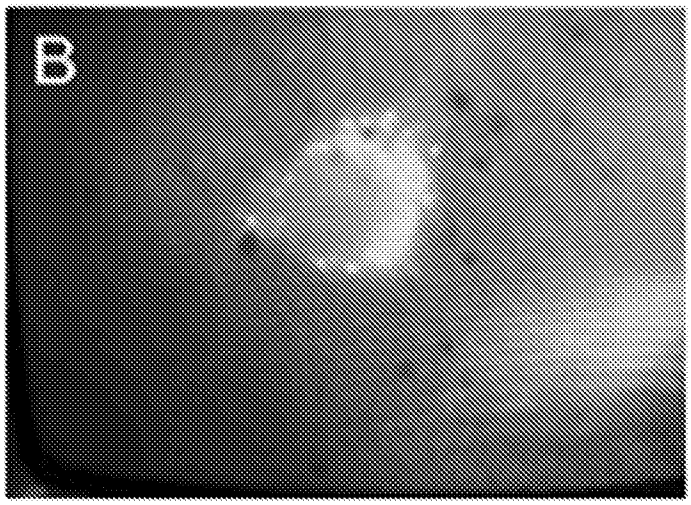

FIG. 6B

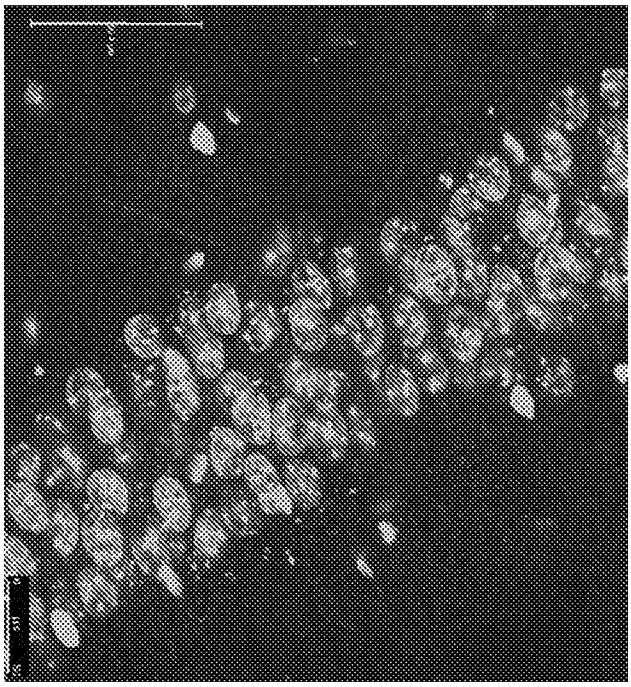

FIG. 7A

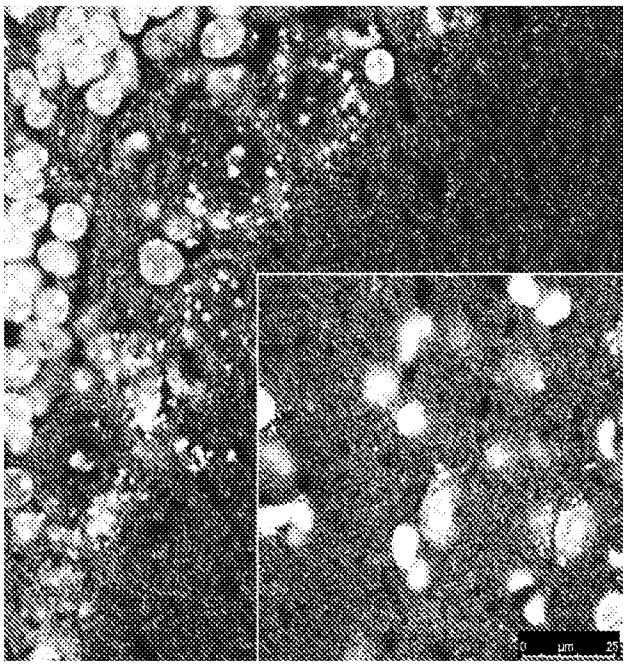

FIG. 7B

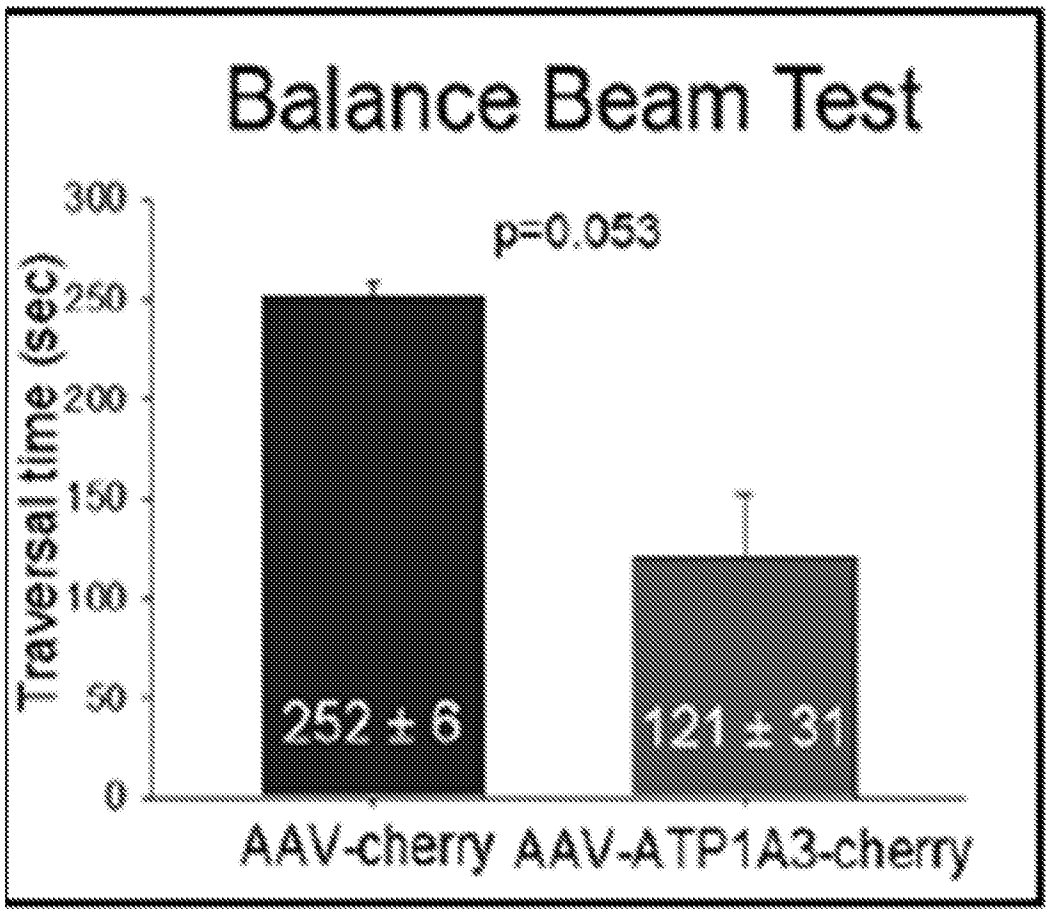
Balance Beam Test
p=0.053
Traversal time (sec)
300
250
200
150
100
50
0
252 ± 6
121 ± 31
AAV-cherry
AAV-ATP1A3-cherry

FIG. 8

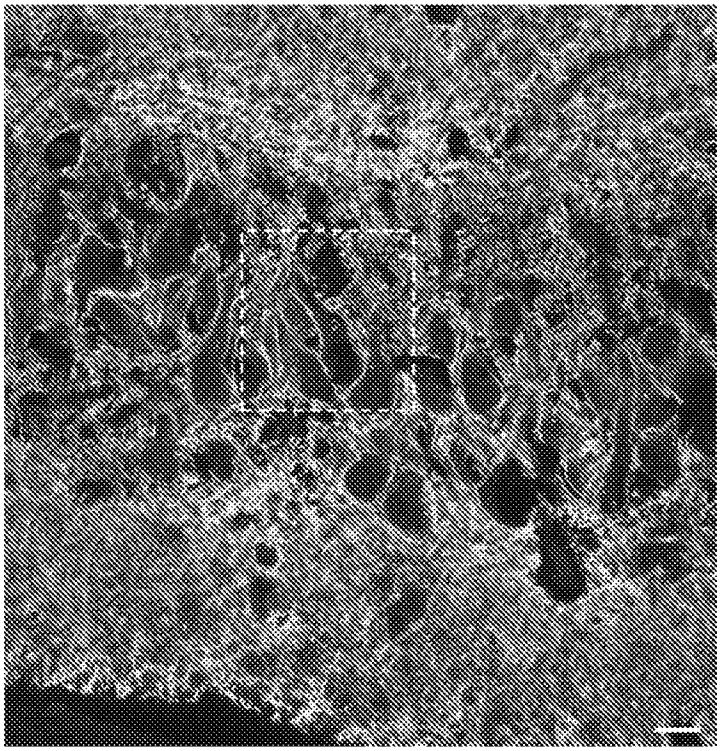

FIG. 9A

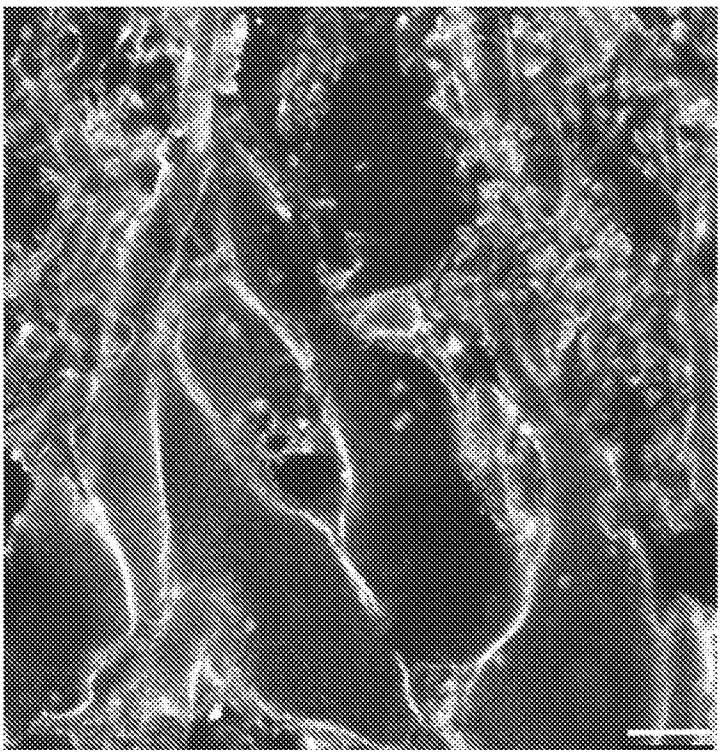

FIG. 9B

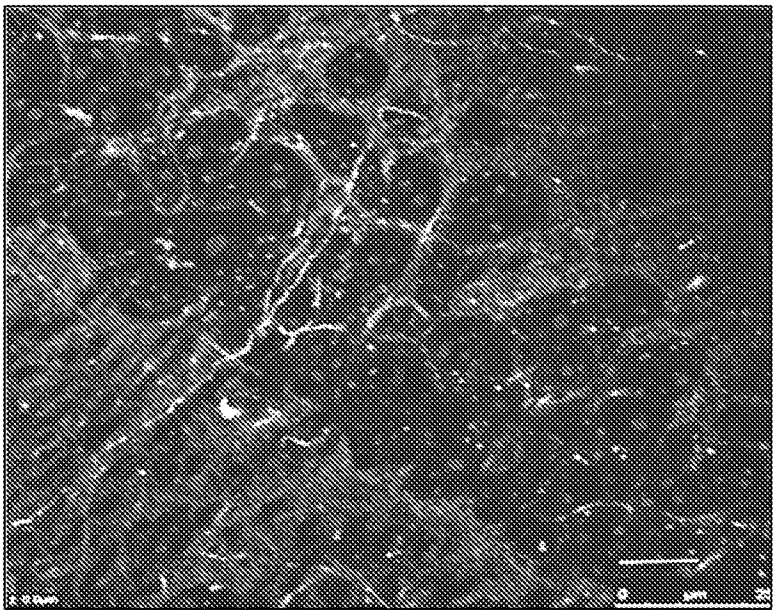

FIG. 9C

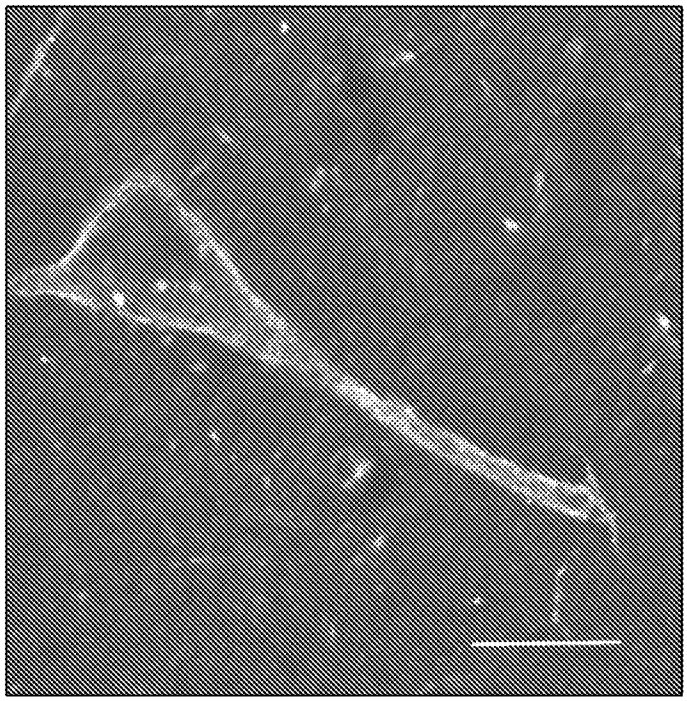

FIG. 9D

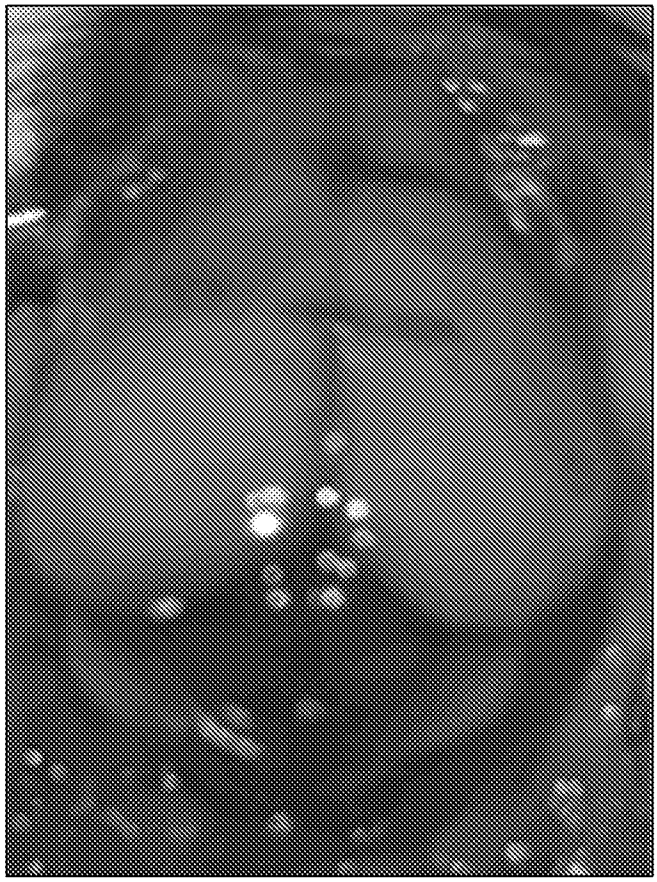

FIG. 9E

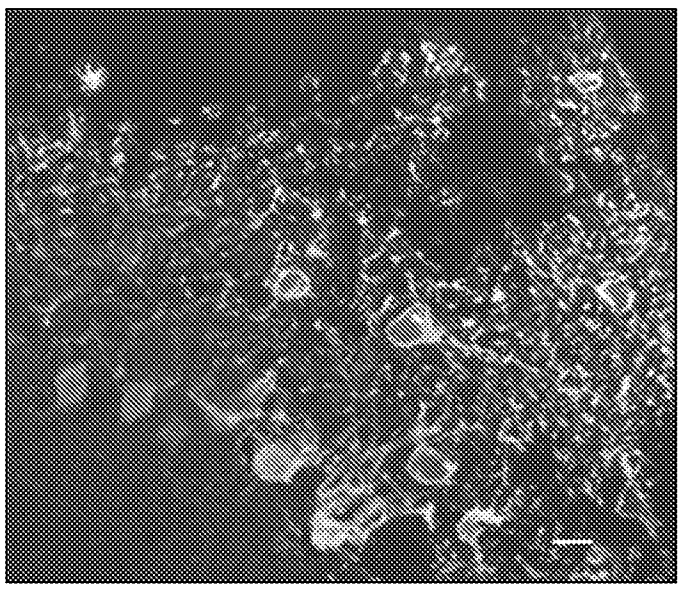

FIG. 9F

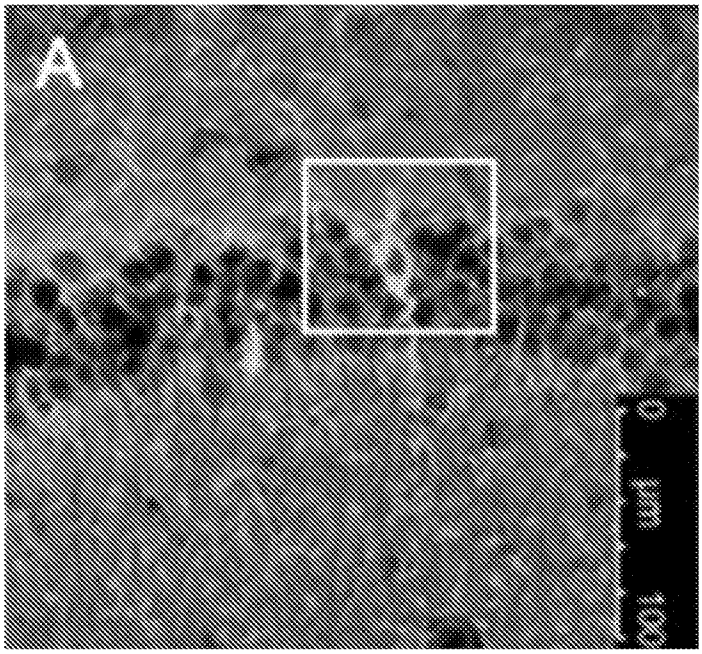

FIG. 10A

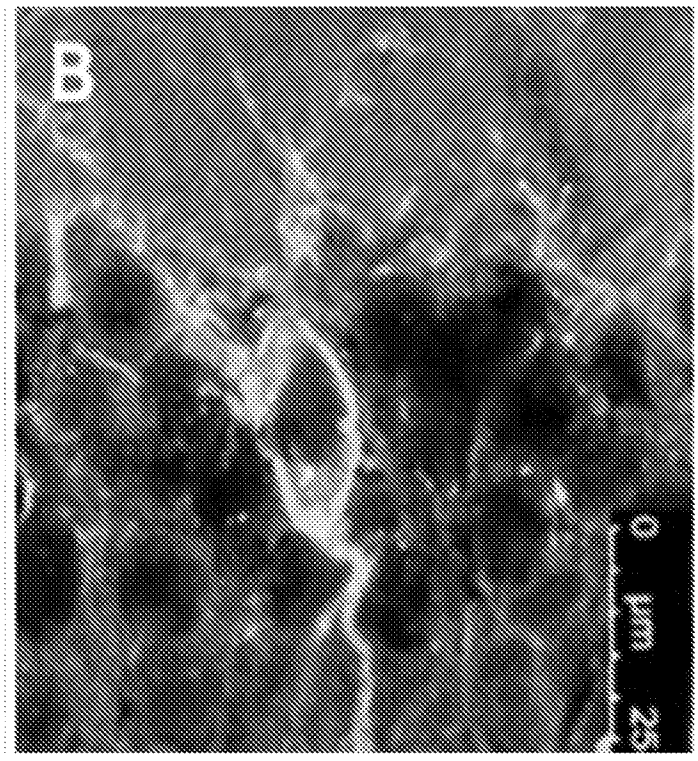

FIG. 10B

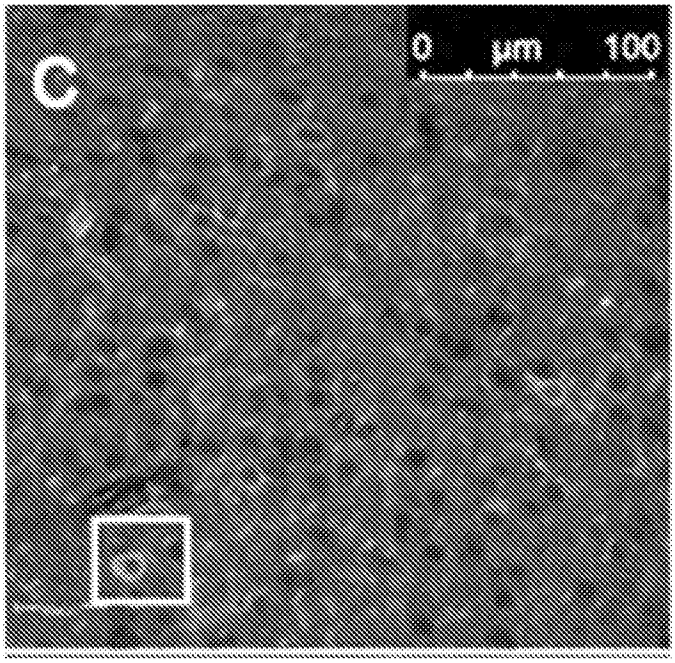

FIG. 10C

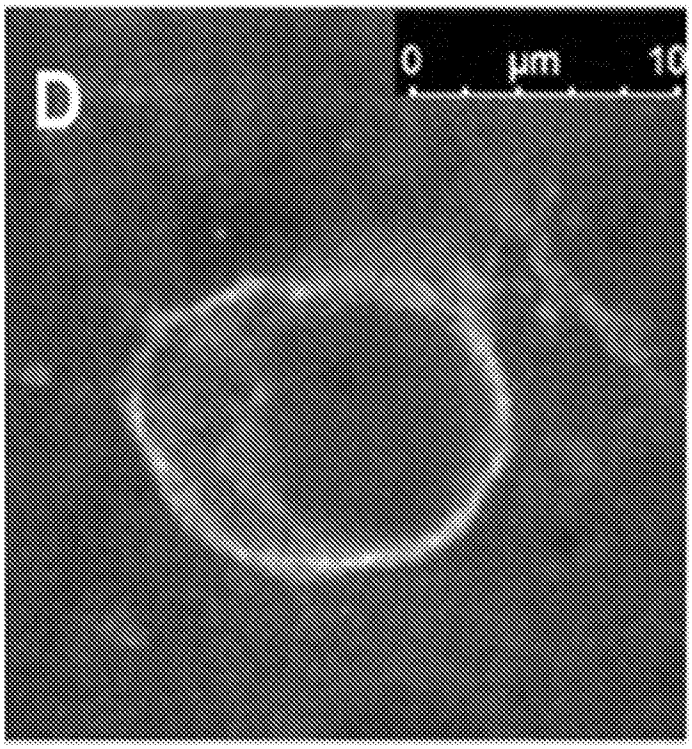

FIG. 10D

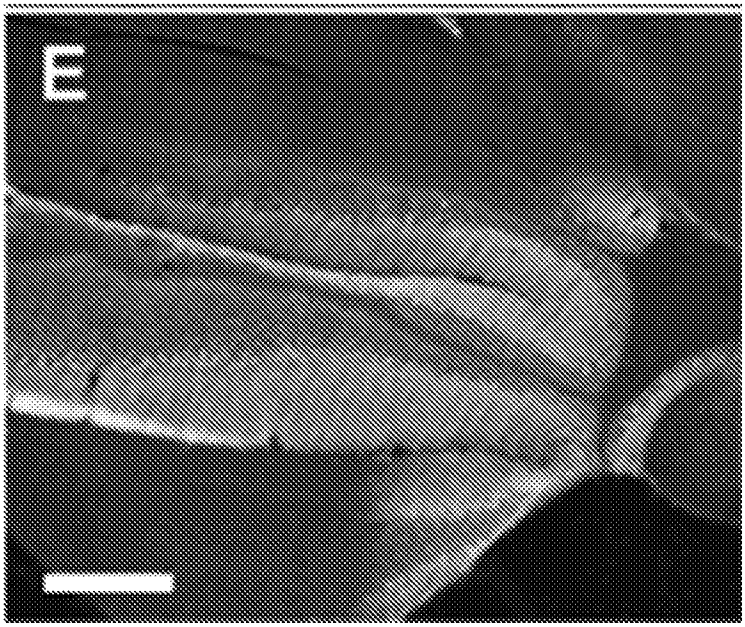

FIG. 10E

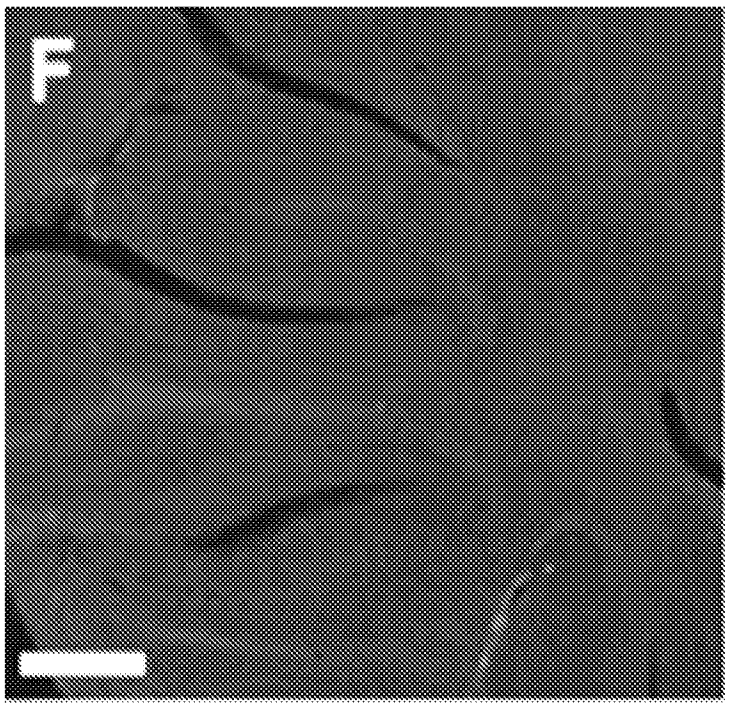

FIG. 10F

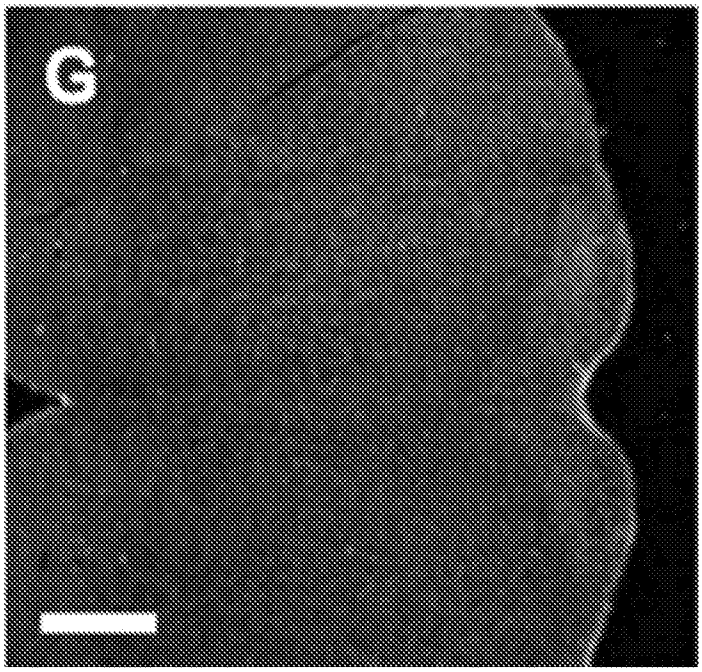

FIG. 10G

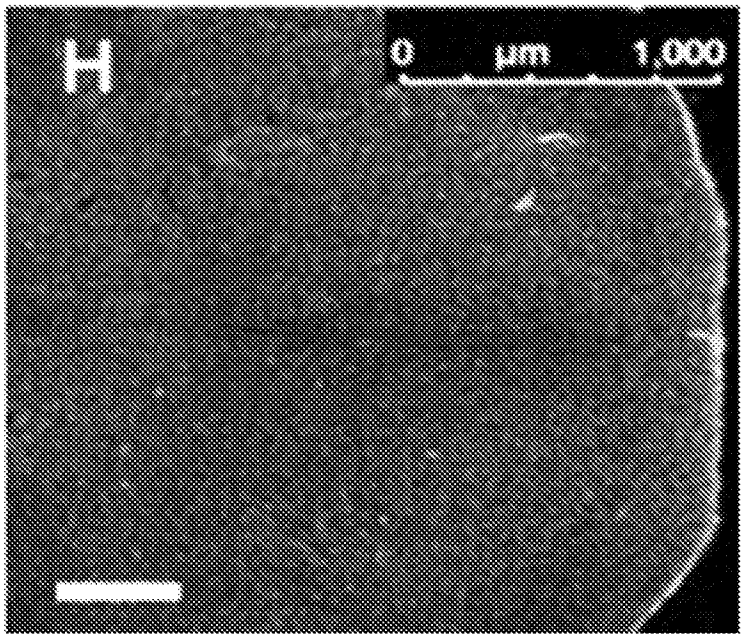

FIG. 10H

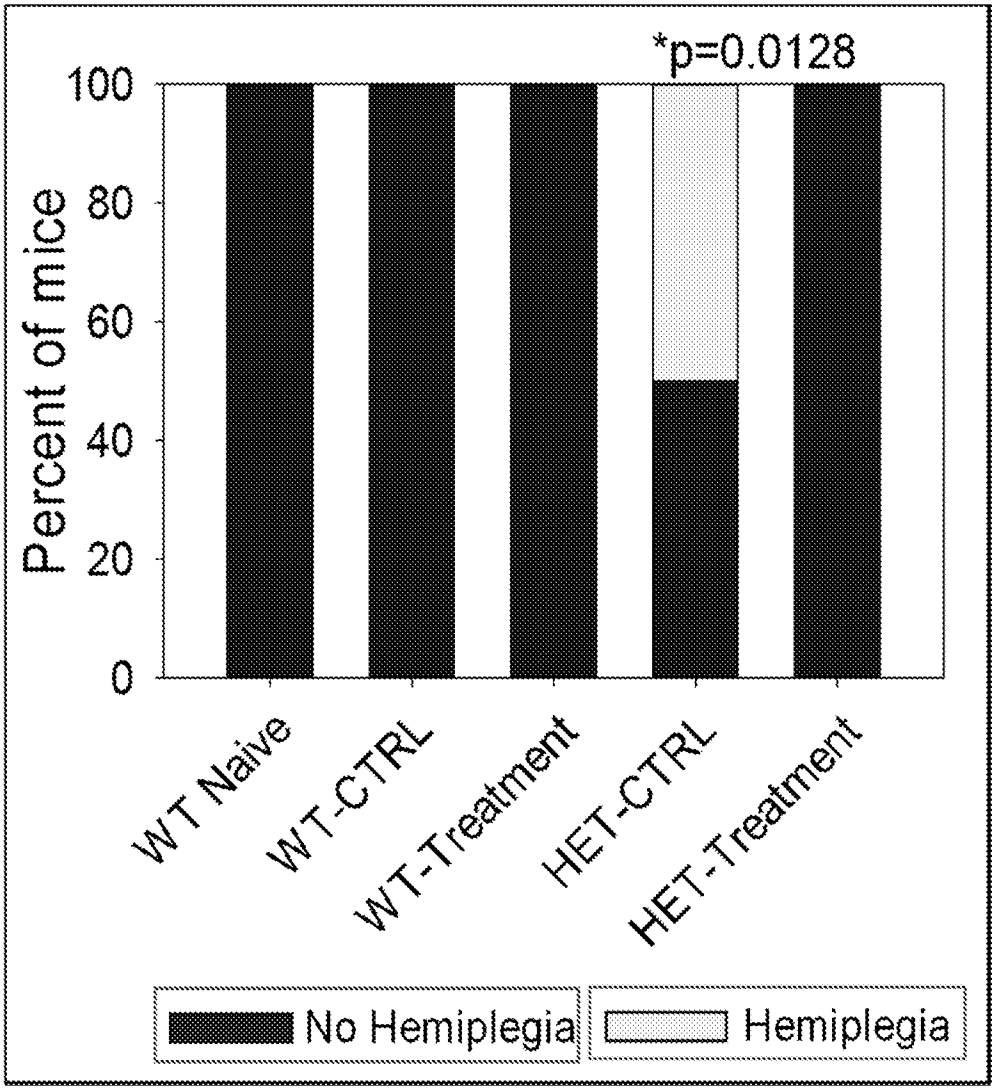
*p=0.0128
Percent of mice
100
80
60
40
20
0
WT Naive
WT-CTRL
WT-Treatment
HET-CTRL
HET-Treatment
No Hemiplegia
Hemiplegia

FIG. 11

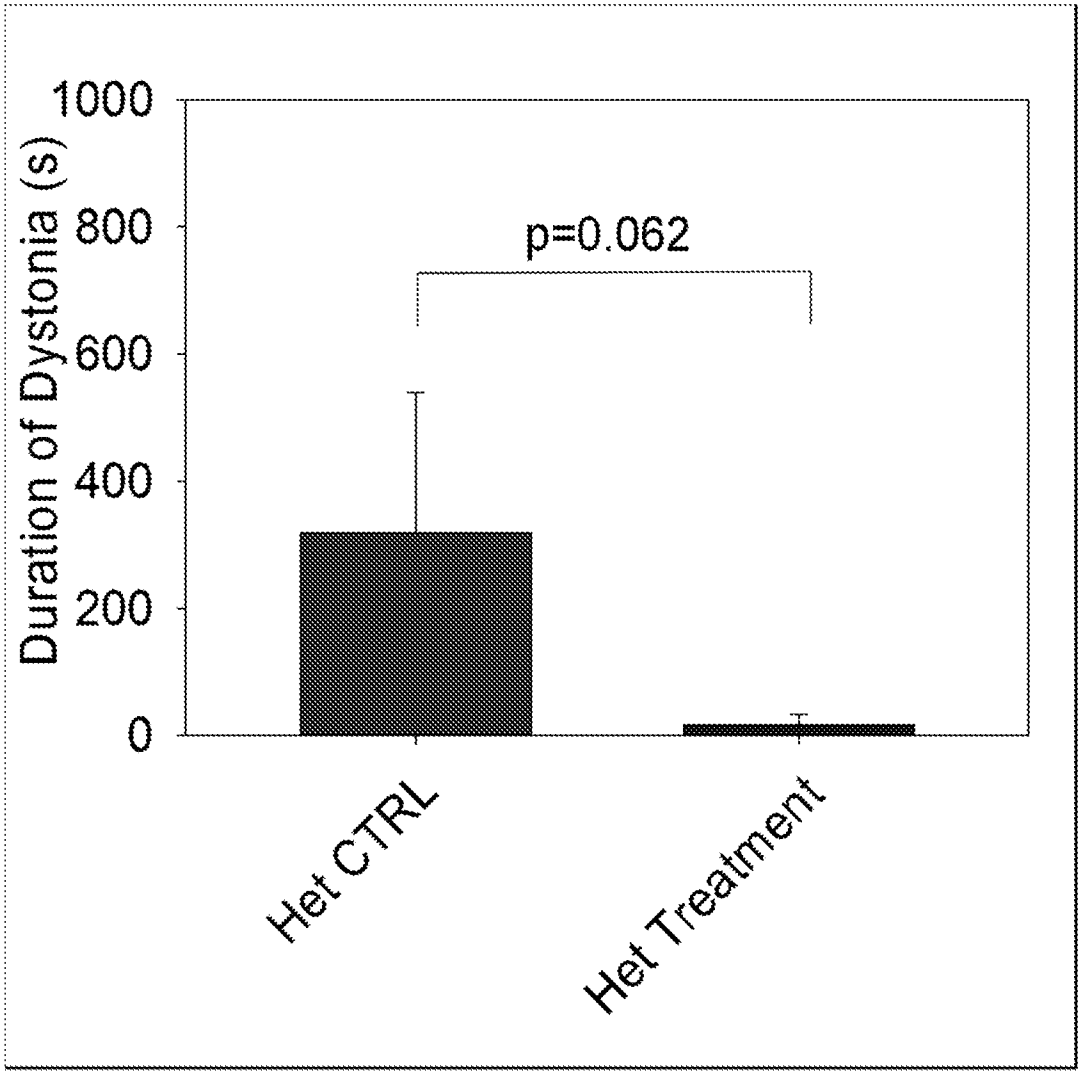
Duration of Dystonia (s)
1000
800
600
400
200
0
p=0.062
Het CTRL
Het Treatment

FIG. 12

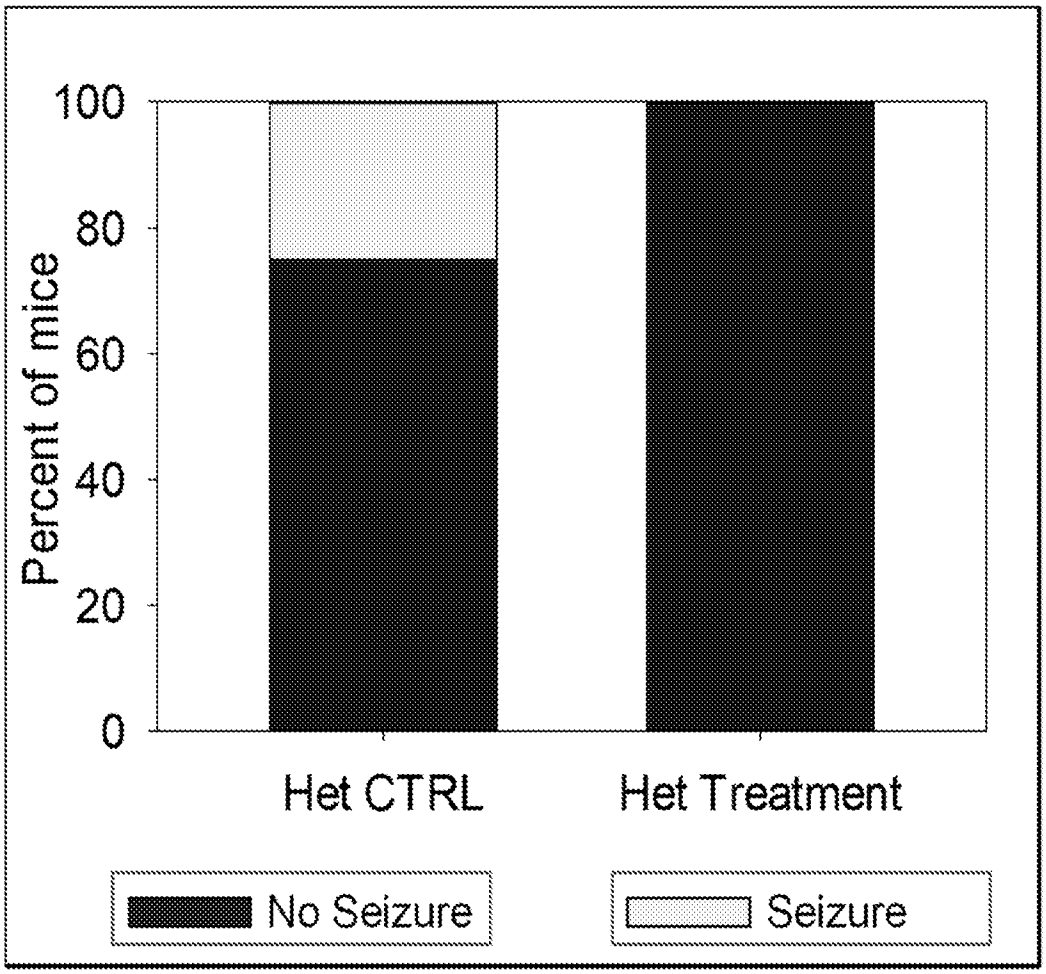
100
80
60
40
20
0
Percent of mice
Het CTRL
Het Treatment
No Seizure
Seizure

FIG. 13

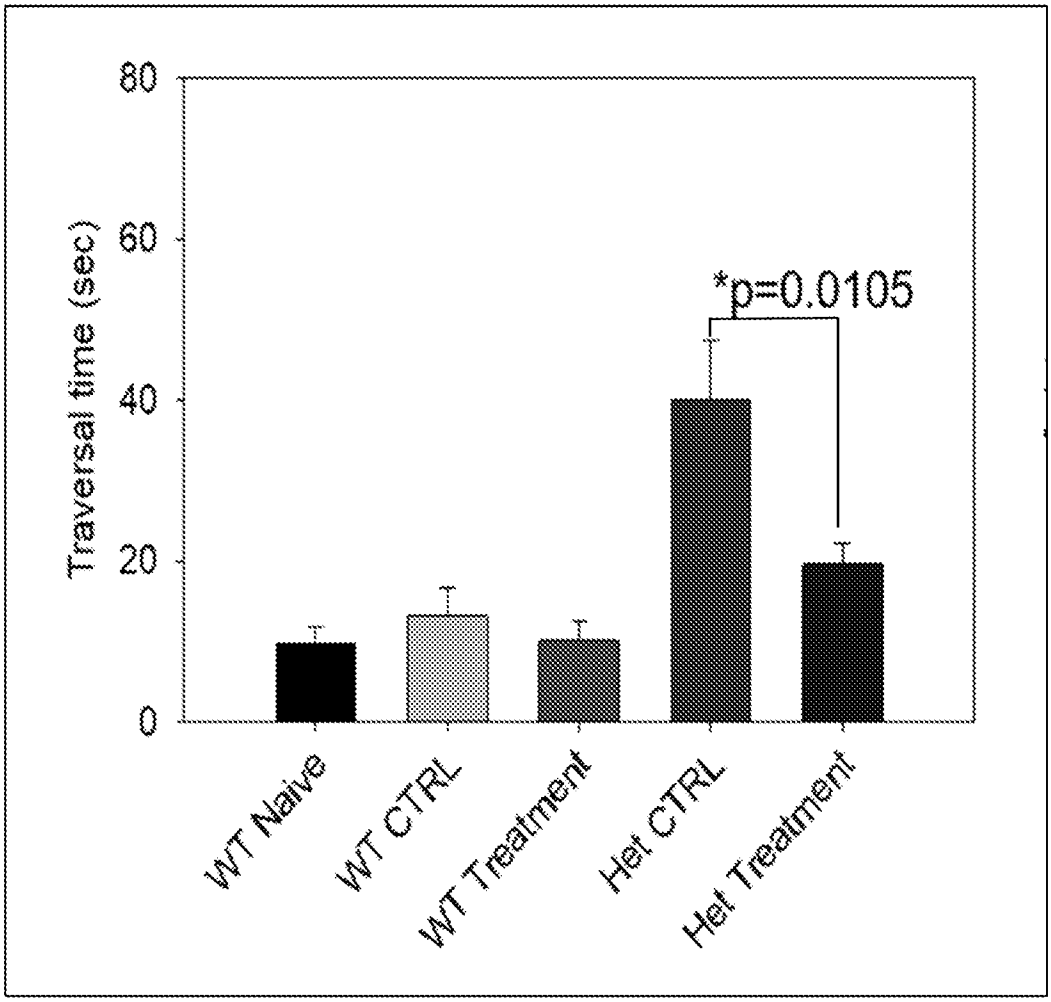
Traversal time (sec)
80
60
40
20
0
*p=0.0105
WT Naive
WT CTRL
WT Treatment
Het CTRL
Het Treatment

FIG. 14

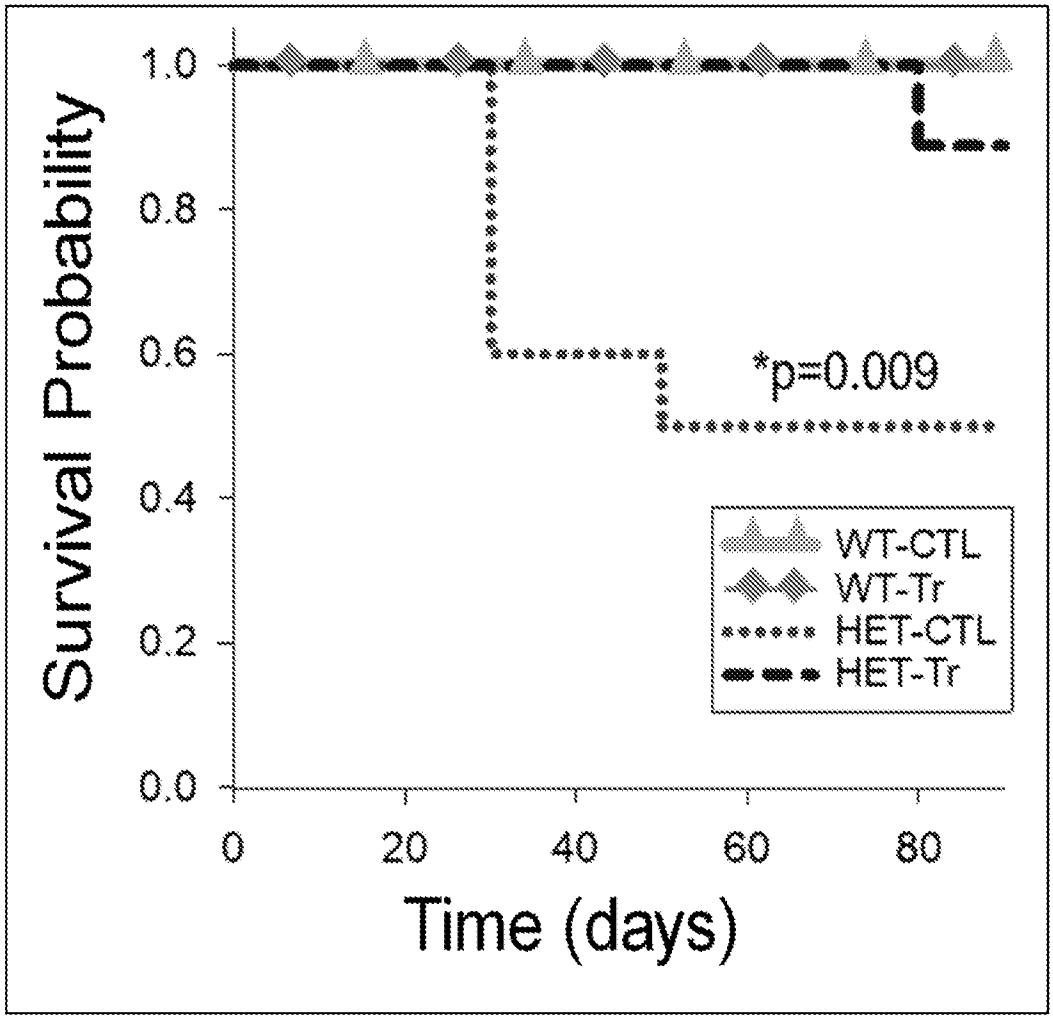
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Time (days)
*p=0.009
WT-CTL
WT-Tr
HET-CTL
HET-Tr

FIG. 15

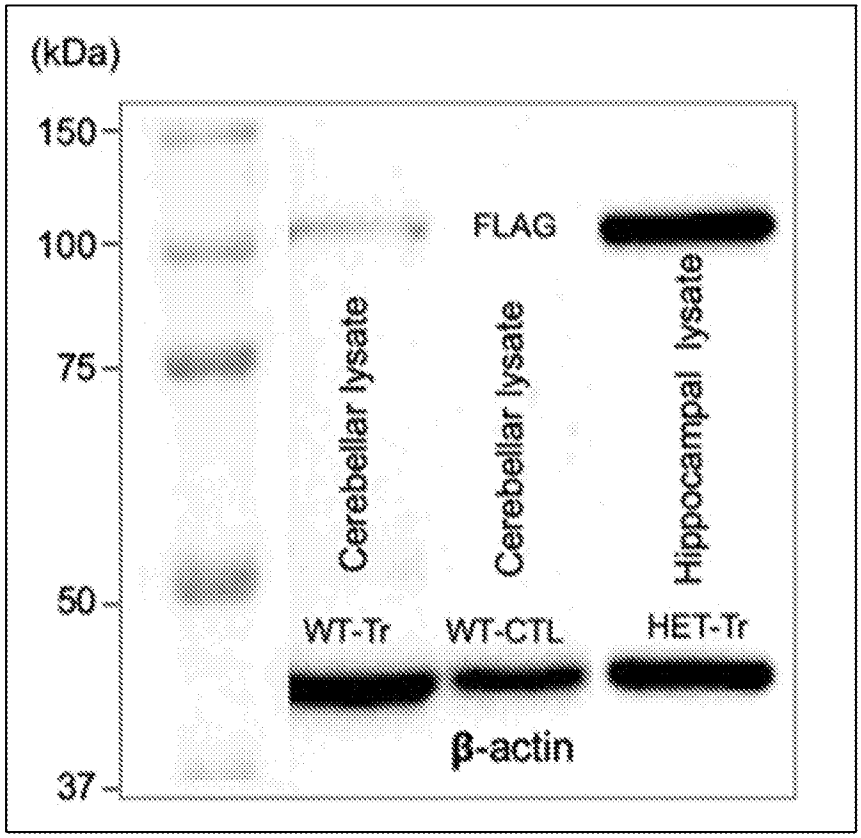
(kDa)
150
100
75
50
37
FLAG
Cerebellar lysate
Cerebellar lysate
Hippocampal lysate
WT-Tr
WT-CTL
HET-Tr
β-actin

FIG. 16

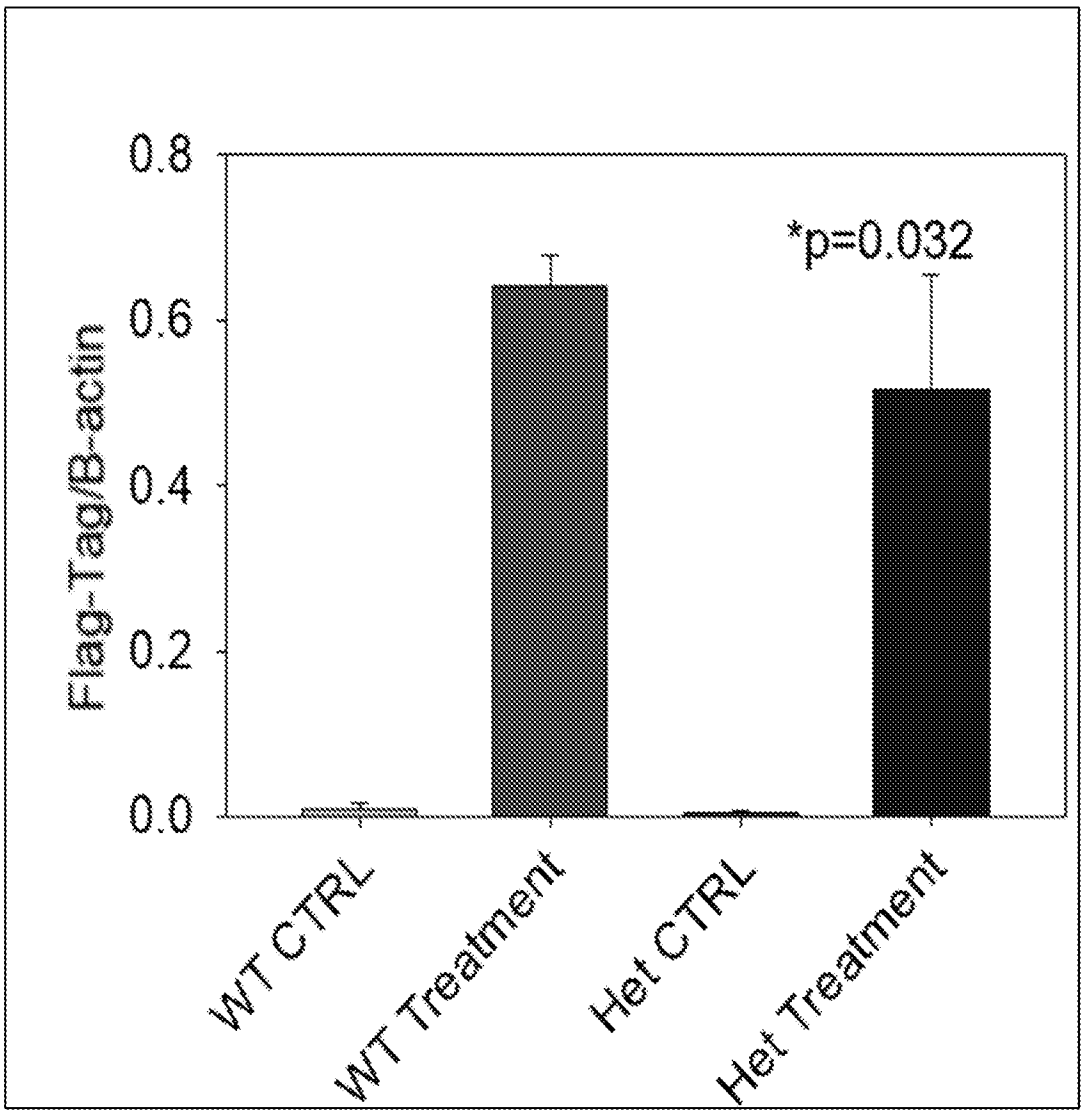
0.8
0.6
0.4
0.2
0.0
Flag-Tag/B-actin
*p=0.032
WT CTRL
WT Treatment
Het CTRL
Het Treatment

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/32978

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86, C12N 15/79, C12N 15/63 (2020.01)

CPC - C12N 2015/8518, C12N 15/8645, C12N 15/8676, C12N 15/8695

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)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---------- Y	US 2003/0225017 A1 (MURDIN et al.) 4 December 2003 (04.12.2003); abstract; para [0025], [0063], [0064], [0070], [0078]	1, 20-23, 33, 40/(20-23, 33) ---------- 2, 9, 24-25, 28, 40/(24-25, 28)
Y	WO 2012/178173 A1 (CENTROSE, LLC) 27 December 2012 (27.12.2012); pg 7, para 1	2, 9/2
Y	WO 2014/007858 A1 (UNIVERSITY OF IOWA RESEARCH FOUNDATION) 9 January 2014 (09.01.2014); pg 10, ln 22-30; pg 10, ln 31-34	24-25, 28, 40/(24-25, 28)
Y	US 2010/0095387 A1 (SMITH et al.) 15 April 2010 (15.04.2010); para [0296]	9/1, 9/2
Y	US 2014/0057969 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 27 February 2014 (27.02.2014); para [0240]	9/1, 9/2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" 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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" 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
"X" 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
"Y" 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

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2020	15 OCT 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 20/32978

Continuation of Box No. III Observations where unity of invention is lacking:

Group I+: Claims 1-9 and 20-49, directed to an isolated nucleic acid expression cassette and a vector comprising the cassette, comprising a nucleic acid sequence encoding a ATPase, a vector for delivery of the cassette, the vector having one or more sequences (e.g. inverted terminal repeat sequence (ITR), a promoter). The ATPase expression cassette and vector will be searched to the extent that the ATPase is ATP1A1, the vector is an adeno-associated virus vector (AAV) serotype AAV1, and comprises an ITR. It is believed that claims 1-2, 9, 20-25, 28, 33, and 40(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass an ATPase expression cassette. Additional ATPase expression cassettes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected ATPase expression cassettes. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a vector comprising an ATPase expression cassette, the ATPase comprising ATP1A2, the vector is AAV serotype AAV2, and the vector further comprises the human synapsin promoter which comprises the nucleic acid sequence set forth in SEQ ID NO:04 (claims 1-2, 7-9, 20-24, 33 and (40-45)(in part)).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the nucelkic acid features including a specific ATPase gene, a vector type (viral or non-viral), and further features of the vector comprising viral regulatory/replication sequences, a specific promoter, not required by any of the other inventions.

Common Technical Features

The feature shared by the inventions listed as Group I+ is a nucleic acid cassette comprising an ATPase, one or more elements, such as a promoter, for expression of the gene, and a vector for delivery of the cassette.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2003/0225017 A1 to Murdin et al. (hereinafter "Murdin").

Murdin teaches a nucleic acid expression cassette comprising a nucleic acid sequence encoding an ATPase (Abstract "The present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of Chlamydia, specifically C. pneumoniae, employing a vector containing a nucleotide sequence encoding a membrane ATPase of a strain of Chlamydia pneumoniae and a promoters to etfect expression of the membrane ATPase in the host. Modifications are possible within the scope of this invention."; para [0064] "Accordingly, a second aspect of the inventions encompasses (i) an expression cassette containing a DNA molecule of the invention placed under the control of the elements required for expression, in particular under the control of an appropriate promoter"), and further teaches a vector comprising a nucleic acid sequence encoding an ATPase (Abstract "The present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of Chlamydia, specifically C. pneumoniae, employing a vector containing a nucleotide sequence encoding a membrane ATPase of a strain of Chlamydia pneumoniae and a promoters to etfect expression of the membrane ATPase in the host. Modifications are possible within the scope of this invention.").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Group I+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

Item 4, continued: claims 10-19 and 50-61 are not not drafted in accordance with the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

Note: Claims 11 and 17 are objected to for lack of antecedent basis. As drafted, claims 11 and 17 depend from claim 9, which fails to recite a promoter. For the purposes of this application, claim claims 11 and 17 are construed as though depending from claim 10 "wherein the nucleic acid sequence encoding ATP1A3 is operably linked to a promoter".

Note: Claims 21, 29, 33-39, and 40-49 (claim 19 only) are objected to for lack of antecedent basis, a vector. As drafted, claims 21, 29, 33-39, and 40-49 (claim 19 only) depend from claim 19, which fails to recite a vector. For the purposes of this application, claims 21, 29, 33-39, and 40-49 (claim 19 only) are construed as though depending from claim 20, "A vector comprising a nucleic acid sequence encoding an ATPase".

Note: Claim 49 is objected to for lack of antecedent basis. As drafted, claim 49 depends from claim 39, which fails to recite a transcription terminator. For the purposes of this application, claim 49 is construed as though depending from claim 40.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/32978

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims 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. ☒ Claims Nos.: 10-19 and 50-61
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

*****Continued in Supplemental Box*****

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 claims 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 claims Nos.:
1-2, 9, 20-25, 28, 33, and 40(in part)

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.