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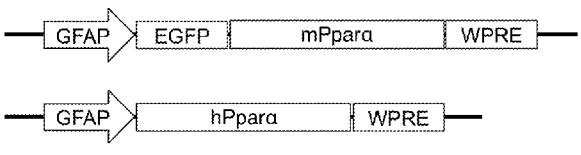
(54) Title: COMPOSITIONS AND METHODS OF TREATING, PREVENTING, OR DELAYING THE PROGRESSION OF NEURODEGENERATIVE DISEASE

FIG. 1



(57) Abstract: Methods for manipulating metabolism in astrocytes to improve astrocytic and neuronal function, as well as individual pathology hallmarks and symptoms during the development of neurodegeneration by increasing PPARa expression and activity specifically in astrocytes within the central nervous system to treat a variety of neurodegenerative conditions including but not limited to conditions involving neuronal lipid dysregulation, oxidative damage, accumulation of β-amyloid or other protein aggregates, dementia, and motor dysfunction.

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COMPOSITIONS AND METHODS OF TREATING, PREVENTING, OR DELAYING THE PROGRESSION OF NEURODEGENERATIVE DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 63/578,608 filed August 24, 2023, the specification of which is incorporated herein in their entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. AG068175 awarded by National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention is in the field of molecular biology, more particularly, the present invention relates to methods and compositions for manipulating metabolism in astrocytes to improve astrocytic and neuronal function, as well as individual pathology hallmarks and symptoms during the development of neurodegeneration. In particular, it relates to increasing PPARα expression and activity, specifically in astrocytes within the central nervous system, to treat a variety of neurodegenerative conditions, and specifically those which cause neuronal lipid dysregulation, synaptic loss, oxidative damages, accumulation of β-amyloid or other protein aggregates, dementia, and motor dysfunction.

BACKGROUND OF THE INVENTION

[0004] Neurodegenerative diseases, including but not limited to Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and prion diseases, are increasingly being recognized to share common cellular and molecular mechanisms related to abnormal lipid metabolism. Fatty acids are the essential component of most lipid species. The brain critically depends on astrocytes to eliminate fatty acids (FAs) and maintain lipid balance. Recently, It was revealed that impaired FA removal by astrocytic mitochondria induces lipid accumulation, followed by neurodegeneration that recapitulates key features of Alzheimer's disease (AD), including synaptic loss, neuroinflammation, demyelination, and cognitive impairment. Further, it was discovered that impaired FA degradation is an early event in the brain of a mouse model of AD. Findings suggest that increasing the activity of FA degradation in astrocytes, but not in neurons, may have better therapeutic benefits. Peroxisome proliferator-activated receptor α (PPARα) is a metabolic regulator of lipid metabolism, particularly in the degradation of excessive lipids in the form of free fatty acids. While the agonists of PPARα have shown protective effects in some animal experiments or pre-clinical studies of AD, the expression and function of PPARα in modulating metabolism and its potential adverse effects in treating neurodegenerative diseases,

including AD, remain unclear and contradictory. Therefore, there is a pressing need for novel therapeutics and effective gene therapies to modulate PPARα expression precisely and delay or reverse the development of neurodegeneration by restoring astrocyte metabolic function. Thus, it is necessary to elucidate the cell type-specific role of PPARα in the brain and in the development of neurodegenerative diseases, as well as to develop precise therapeutic strategies that target PPARα and its downstream pathways with minimal adverse effects. These efforts could ultimately lead to efficacious treatments for patients with neurodegenerative diseases for which disease-modifying treatments are lacking.

BRIEF SUMMARY OF THE INVENTION

[0005] It is an objective of the present invention to provide methods that allow for the treatment of neurodegenerative diseases by specifically upregulating the expression of PPARα in astrocytes, as specified in the independent claims. Embodiments of the invention are given in the dependent claims. Embodiments of the present invention can be freely combined with each other if they are not mutually exclusive.

[0006] In some embodiments, the present invention features a method of treating a neurodegenerative disease in a subject in need thereof. The method may comprise administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0007] In other embodiments, the present invention features a method of delaying the onset of or preventing a neurodegenerative disease in a subject in need thereof. The method may comprise administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene to a subject at risk of having a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0008] Non-limiting examples of neurodegenerative disease may include but are not limited to Alzheimer's disease (AD), Parkinson's disease (PD) and other forms of Parkinsonism, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases.

[0009] One of the unique and inventive technical features of the present invention is the use of a viral vector, e.g., AAV-Astrocyte-PPARα, that increases the expression of PPARα in astrocytes. Without wishing to limit the invention to any theory or mechanism, it is believed that the technical feature of the present invention advantageously provides for targeted treatment of neurodegenerative disorders, e.g., AD. None of the presently known prior references or works have the unique inventive technical feature of the present invention.

[0010] Moreover, the prior references teach away from the present invention. For example, current PPARα agonists lack cell selectivity, affecting all cells in the brain—including neurons, astrocytes, and other cell types—as well as other organs throughout the body. Specifically, targeting neurons may suppress pyruvate metabolism, which is critical for ATP production in the brain, and there are concerns about the potential carcinogenic effects of PPARα agonists on the liver.

[0011] Furthermore, the inventive technical features of the present invention contributed to a surprising result. For example, selectively upregulating PPARα protein expression in astrocytes can limit the progression of neurodegenerative diseases such as Alzheimer's disease (AD) while minimizing adverse effects on other cell types within the central nervous system and other organs.

[0012] In some embodiments, the present invention provides a method of using a viral vector, e.g., an adeno-associated viral (AAV) comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, e.g., AAV-Astrocyte-PPARα, for treating or preventing neurodegenerative diseases. For example, provided herein are methods for treating or preventing AD in a subject. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of viral vectors that upregulates the expression of PPARα specifically inside astrocytes in the subject. More specifically, the methods disclosed herein are useful in modulating the expression of PPARα protein in astrocytes for the treatment or delay the development, onset, and symptoms related to neurodegenerative diseases. For example, the disclosed AAV-Astrocyte-PPARα is useful in the treatment of AD. In contrast to the

prior art administration of PPARα agonists nonselective to treat AD, demonstrated herein is the novel and unexpected finding that

[0013] In one embodiment, the neurodegenerative disease is associated with protein aggregation, such as β-amyloid plaques. In another embodiment, neurodegenerative disease is associated with neuroinflammation, oxidative stress, and lipid dysregulation. In certain embodiments, the neurodegenerative disease is selected from the group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), frontotemporal dementia, Lewy Body dementia, stroke, depression, vascular dementia, and prion diseases.

[0014] Another aspect of the invention provides a method for improving neuronal health and brain function. In one embodiment, the AAV-Astrocyte-PPARα decreases the level of reactive oxygen species (ROS), diminishing the production of PLIN2 in brain tissues, and restoring the morphology and phenotype of astrocytes to non-disease conditions. In another embodiment, the AAV-Astrocyte-PPARα increases synaptic, improves the short-term and long-term plasticity, rescues the nest building and the novel object recognition behavior compared to those in AD subjects.

[0015] The subject may be a human subject, for example, a human subject exhibiting symptoms of AD and dementia. The composition may be administered by intravenous injection with appropriate AAV vectors. Alternatively, the composition is administered by an intranasal delivery, direct injection into the brain (including through a small hole in the skull or using a catheter), implantable devices, Nanoparticle-based delivery (transport drugs across the blood-brain barrier).

[0016] Any feature or combination of features described herein are included within the scope of the present invention provided that the features included in any such combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art. Additional advantages and aspects of the present invention are apparent in the following detailed description and claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0017] The features and advantages of the present invention will become apparent from a consideration of the following detailed description presented in connection with the accompanying drawings in which:

[0018] FIG. 1 shows the map of the design of the AAV5-GFAP-PPARα construct in mouse (top) and human (bottom) isoforms.

[0019] FIG. 2 shows the expression of PPARα in astrocytes in the 5xFAD mouse brain which are injected with AAV5-GFAP-EGFP-*mPpara*-WPRE. EGFP expression (co-expressed with PPARα), GFAP expression, DAPI levels, and a merged image are shown. 88.5% of the GFAP positive cells are also expressing EGFP (co-expressed with PPARα). Scale bar, 50 μm.

[0020] FIGs. 3A, 3B,and 3C show AAV5-GFAP-EGFP-PPARα treatment improves fEPSP and long-term potentiation (LTP) in 5xFAD mice. FIG. 3A shows a brightfield photomicrograph of a hippocampal slice on a MED64 electrode array. FIG. 3B shows a time course for Theta-burst induced LTP. FIG. 3C shows the mean LTP 35-40 min after Theta-burst. Closed circle, wildtype (WT); triangle, AAV5-GFAP-EGFP treated 5xFAD (5xFAD); square, AAV5-GFAP-EGFP-PPARα treated 5xFAD mice (5xFAD-PPARα). n = 3 mice for each group. ** $p < 0.01$, *** $p < 0.001$.

[0021] FIGs. 4A and 4B show AAV5-GFAP-EGFP-PPARα treatment rescues the short-term plasticity (STP) in 5xFAD mice. FIG. 4A shows the mean STP 15-20 min after Theta-burst, *** $p < 0.001$. FIG. 4B shows paired-pulse ratio (PPR) measured at different inter-stimulus intervals (30, 40, and 50 ms). Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. n = 3 mice for each group. **WT vs. 5xFAD, ##5xFAD vs. 5xFAD-PPARα. * $p < 0.05$; ** or ## $p < 0.01$.

[0022] FIGs. 5A and 5B show AAV5-GFAP-EGFP-PPARα treatment alleviates behavioral deficits in 5xFAD mice. FIG. 5A shows results from nest building tests, including Nest scores and unshredded nestle weight. FIG. 5B. shows the discrimination index by novel object recognition (NOR) test. Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. n = 4-6 mice for each group. * $p < 0.05$.

[0023] FIG. 6 shows immunostaining results of perilipin-2 (PLIN2) from the hippocampal region of 5xFAD mice injected with AAV5-GFAP-EGFP-PPARα. Left: PLIN2 and DAPI single-channel images and merged images are shown. Right: quantification of the PLIN2 expression. Scale bar, 50 μm. Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. n = 4 mice for each group. * $p < 0.05$, ** $p < 0.01$.

[0024] FIG. 7 shows immunostaining results of 4-Hydroxynonenal (4-HNE; a marker for lipid peroxidation and oxidative stress) from the hippocampal region of 5xFAD mice injected with AAV5-GFAP-EGFP-PPARα. Left: 4-HNE and DAPI single-channel images and merged images. Right: quantification of the 4-HNE levels. Scale bar, 50 μm. Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. n = 4 mice for each group. * $p < 0.05$, ** $p < 0.01$.

[0025] FIG. 8 shows immunostaining results of amyloid antibody 6E10 from the hippocampal region of 5xFAD mice injected with AAV5-GFAP-EGFP-PPARα. Left: 6E10 and DAPI single-channel images and merged images. Right: quantification of the amyloid levels. Scale

bar, 50 μm. Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. n = 4 mice for each group. ** $p < 0.01$, *** $p < 0.001$.

[0026] FIG. 9 shows immunostaining results of GFAP from the hippocampal region of 5xFAD mice injected with AAV5-GFAP-EGFP-PPARα. Left: GFAP and DAPI single-channel images and merged images. Right: quantification of the length of astrocyte processes including primary branches and secondary branches. Each dot indicates the average branch length of one individual cell from 4 mice per group. Scale bar, 50 μm. Closed circle, WT; triangle, 5xFAD; square, 5xFAD-PPARα. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

DETAILED DESCRIPTION OF THE INVENTION

[0027] Disclosed are the various compounds, solvents, solutions, carriers, and/or components to be used to prepare the compositions to be used within the methods disclosed herein. Also disclosed are the various steps, elements, amounts, routes of administration, symptoms, and/or treatments that are used or observed when performing the disclosed methods, as well as the methods themselves. These and other materials, steps, and/or elements are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed, while specific reference of each various individual and collective combination and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein.

[0028] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which a disclosed invention belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "comprising" means that other elements can also be present in addition to the defined elements presented. The use of "comprising" indicates inclusion rather than limitation. Stated another way, the term "comprising" means "including principally, but not necessary solely". Furthermore, variation of the word "comprising", such as "comprise" and "comprises", have correspondingly the same meanings. In one respect, the technology described herein related to the herein described compositions, methods, and respective component(s) thereof, as essential to the invention, yet open to the inclusion of unspecified elements, essential or not ("comprising").

[0029] Suitable methods and materials for the practice and/or testing of embodiments of the disclosure are described below. Such methods and materials are illustrative only and are not intended to be limiting. Other methods and materials similar or equivalent to those described herein can be used. For example, conventional methods well known in the art to which the disclosure pertains are described in various general and more specific references, including, for

example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Sambrook et al., Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Press, 2001; Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates, 1992 (and Supplements to 2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, 4th ed., Wiley & Sons, 1999; Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1990; and Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999, Gene Expression Technology (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, Calif.), "Guide to Protein Purification" in Methods in Enzymology (M. P. Deutshcer, ed., (1990) Academic Press, Inc.); PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, Calif.), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R. I. Freshney. 1987. Liss, Inc. New York, N.Y.), Gene Transfer and Expression Protocols, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, Tex.), the disclosures of which are incorporated in their entirety herein by reference.

[0030] As used herein, the terms “subject” and “patient” are used interchangeably. As used herein, a subject can be a mammal such as a non-primate (e.g., cows, pigs, horses, cats, dogs, rats, etc.) or a primate (e.g., monkey and human). In specific embodiments, the subject is a human. In one embodiment, the subject is a mammal (e.g., a human) having a disease, disorder, or condition described herein. In another embodiment, the subject is a mammal (e.g., a human) at risk of developing a disease, disorder, or condition described herein. In certain instances, the term patient refers to a human.

[0031] As used herein, the terms "treat," “treating,” or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, with the objective of preventing, reducing, slowing down (lessen), inhibiting, or eliminating an undesired physiological change, symptom, disease, or disorder. For example, the disease may be a neurodegenerative disease. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented or onset delayed. Optionally, the subject or patient may be identified (e.g., diagnosed)

as one suffering from the disease or condition prior to administration of the compositions of the invention. Subjects at risk for the disease can be identified by, for example, any or a combination of appropriate diagnostic or prognostic assays known in the art.

[0032] As used herein, “clinical improvement” may refer to a noticeable reduction in the symptoms of a disorder, or cessation thereof.

[0033] The terms “manage,” “managing,” and “management” refer to preventing or slowing the progression, spread, or worsening of a disease or disorder, or of one or more symptoms thereof. In certain cases, the beneficial effects that a subject derives from a prophylactic or therapeutic agent do not result in a cure of the disease or disorder.

[0034] The terms “administering” and “administration” refer to methods of providing a pharmaceutical preparation, composition, or formulation to a subject. The compositions described herein can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Such methods are well known to those skilled in the art and include, but are not limited to, administering the compositions orally, intranasally, parenterally (e.g., intravenously and subcutaneously), by intramuscular injection, by intraperitoneal injection, intrathecally, transdermally, extracorporeally, topically or the like.

[0035] A “therapeutically effective amount” refers to an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side effects. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, single-dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days, weekly, twice weekly, etc. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

[0036] The exact amount of the compositions required will vary from subject to subject,

depending on the species, age, weight, and general condition of the subject, the severity of the disorder being treated, the particular composition used, its mode of administration, and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

[0037] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety for all purposes. In case of conflict, the present specification, including explanations of terms, will control.

[0038] Although methods and materials similar or equivalent to those described herein can be used to practice or test the disclosed technology, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting.

[0039] Referring now to FIGs. 1-9, the present invention features methods and compositions for manipulating metabolism in astrocytes to improve astrocytic and neuronal function, as well as individual pathology hallmarks and symptoms during the development of neurodegeneration. In particular, it relates to increasing PPARα expression and activity, specifically in astrocytes within the central nervous system, to treat a variety of neurodegenerative conditions, specifically those that cause neuronal lipid dysregulation, oxidative damages, accumulation of β-amyloid or other protein aggregates, dementia, and motor dysfunction.

[0040] The present invention features methods of treating a neurodegenerative disease in a subject in need thereof. In some embodiments, the method comprises administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0041] The present invention may also feature methods of delaying the onset of or preventing a neurodegenerative disease in a subject in need thereof. In some embodiments, the method comprises administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion PPARA gene to a subject at risk of having a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an

astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene

[0042] In some embodiments, the present invention features a method of preventing or treating a neurodegenerative disease in a subject in need thereof, the method comprising: (a) identifying the subject presenting with the neurodegenerative disease; and (b) administering a viral vector with an astrocyte-specific promoter that carries and upregulates expression of PPARA gene to promote lipid metabolism. In some embodiments, identifying a subject presenting a neurodegenerative disease involves clinically diagnosing the subject with at least one of the neurodegenerative diseases mentioned herein.

[0043] Examples of neurodegenerative diseases that may be treated or prevented using the methods and viral vectors described herein include, but are not limited to, the following: Alzheimer's disease (AD), Parkinson's disease (PD) and other forms of Parkinsonism, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases. Non-limiting examples of prion disease may include but are not limited to Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease.

[0044] In some embodiments, the viral vector is an adeno-associated viral (AAV) vector. In other embodiments, the viral vector is a lentiviral vector. Non-limiting examples of AAV vectors include but are not limited to AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes. Other efficient gene delivery and selective transduction systems may also be employed in the methods described herein.

[0045] In some embodiments, the astrocyte-specific promoter is a Glial Fibrillary Acidic Protein (GFAP) promoter. In some embodiments, the astrocyte-specific promoter is a human GFAP promoter, e.g., a 2.2 kb human GFAP promoter (e.g., gfa2). In other embodiments, the astrocyte-specific promoter is monkey GFAP promoter (e.g., GfaABC1D, 0.7kb). In some embodiments, the astrocyte-specific promoter is a gfa2 (2.2 kb), gfa2(B)3 (2.6kb), or gfa2(ABD)3. In accordance with the viral vectors described herein, other astrocyte-specific promoters may be utilized. Additionally, truncated or variant forms of these promoters may be employed, including, but not limited to, human or rat ALDH1L1 (0.9-2.1 kb), mouse Slc1a3 (Glast) (0.64 kb), and human GJB3 (Cx30) (0.5 kb).

[0046] In some embodiments, the viral vector may be administered intravenously, intranasally, intrathecally, intracisternally, intracerebroventricularly, or a combination thereof. In some

embodiments, the viral vector may be administered via intravenous injection, subcutaneous injection, intramuscular injection, or a combination thereof. In other embodiments, the viral vector may be administered via intranasal delivery, intrathecal delivery, or a combination thereof. In other embodiments, the viral vector may be administered directly into the brain, such as through a small hole in the skull, using a catheter, or via intraparenchymal or intracerebral injection. In further embodiments, the viral vector may be administered via a nanoparticle delivery system. Regardless of the administration method, the viral vectors described herein are capable of crossing the blood-brain barrier.

[0047] In certain embodiments, the viral vector is administered intravenously. Thus, without wishing to limit the present invention to any theory or mechanism, it is believed that for intravenous (IV) administration targeting brain delivery, the viral vector must exhibit enhanced blood-brain barrier penetrability. In some embodiments, this requirement may be achieved through the use of specific AAV serotypes, such as AAV-PHP.eB, AAV-PHP.B, AAVrh10, and AAV9, or through other modifications of existing AAV serotypes.

[0048] Thus, in some embodiments, the present invention may feature methods of treating a neurodegenerative disease in a subject in need thereof. In some embodiments, the method comprises administering an AAV vector comprising an astrocyte-specific promoter (e.g., a GFAP promoter) operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease. In other embodiments, the method comprises intravenously administering an AAV vector comprising an astrocyte-specific promoter (e.g., a GFAP promoter) operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease.

[0049] In other embodiments, the present invention may feature methods of delaying the onset of a neurodegenerative disease in a subject in need thereof. In some embodiments, the method comprises administering an AAV vector comprising an astrocyte-specific promoter (e.g., a GFAP promoter) operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease. In other embodiments, the method comprises intravenously administering an AAV vector comprising an astrocyte-specific promoter (e.g., a GFAP promoter) operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease.

[0050] In some embodiments, a therapeutically effective dose of the viral vector is administered to treat (or prevent) the neurodegenerative disease. The precise amount required will vary depending on the species, age, weight, and general condition of the subject, as well as the severity of the disorder being treated, the specific viral vector used, and its mode of

administration. Therefore, it is not feasible to specify an exact dosage for every situation. However, one of ordinary skill in the art can determine the appropriate amount through routine experimentation, given the teachings herein.

[0051] In some embodiments, the present invention features a method of delaying the onset of, treating, or preventing a neurodegenerative disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a viral vector comprising a nucleic acid molecule encoding for one or more homologous genes encoding PPARα (e.g., a functional isoform or functional fragment) which upregulate the expression and/or activity of PPARα selectively inside of astrocytes in the central nervous system. In some embodiments, the viral vector further comprises an astrocytes-specific promoter operatively linked to the PPARα nucleic acid molecule. As used herein, “homologous genes” may refer to genes that share a common evolutionary origin with the human PPARA gene but from a different species (e.g., mouse Ppara gene).

[0052] In some embodiments, the nucleic acid molecule encoding PPARα for upregulating the expression of PPARα and its functional fragments or isoforms. As used herein, a 'functional fragment' or 'functional isoform' refers to a nucleic acid that encodes a polypeptide capable of functioning, such as a transcription factor, in a subject, similar to the full-length nucleic acid encoding PPARα.

[0053] In some embodiments, the astrocyte-specific promoter is selected from the nucleic acid molecule consisting of different forms of GFAP promoter (human gfa2, 2.2kb; GfaABC1D, 0.7kb; gfa2(B)3, 2.6kb; gfa28, 0.45kb) or other astrocyte-specific promoters and their truncates or variants (human / rat ALDH1L1, 0.9-2.1kb; mouse Slc1a3(Glast), 0.64 kb; human GJB3 (Cx30), 0.5kb).

[0054] In some embodiments, the viral vector is an adeno-associated viral (AAV) vector. In other embodiments, the viral vector is a lentiviral vector. Non-limiting examples of AAV vectors include but are not limited to AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes.

[0055] In certain embodiments, methods described herein, e.g., administering a therapeutically effective dose of a viral vector, e.g., AAV-Astrocyte-PPARα, reduces the levels of reactive oxygen species (ROS) and oxidative stress in brain tissues affected by neurodegenerative diseases such as Alzheimer's disease (AD). Oxidative stress, primarily caused by the generation of ROS, is a hallmark of AD, leading to damage to proteins, lipids, and DNA, as well as inflammation and cell death.

[0056] In some embodiments, the methods described herein, such as administering a therapeutically effective dose of a viral vector like AAV-Astrocyte-PPARα, reduce the accumulation of neutral lipid species in brain tissues affected by neurodegenerative diseases such as Alzheimer's disease (AD). The accumulation of neutral lipids in the form of lipid droplets is a hallmark of AD brains and is associated with disease progression.

[0057] In certain embodiments, the methods described herein, such as administering a therapeutically effective dose of a viral vector like AAV-Astrocyte-PPARα, increases synaptic function, including improvements in both short-term and long-term plasticity in the subject. Additionally, in some embodiments, the methods described herein restore cognitive function, including cognitive and spatial memory, in the subject through the therapeutically effective dose of AAV-Astrocyte-PPARα.

[0058] In some embodiments, methods described herein alleviates Alzheimer's disease-associated astrocyte reactivity and morphological features in the subject.

[0059] In some embodiments, the upregulation of PPARα can be effectuated by administering a small molecule that selectively targets astrocytes and promotes PPARα expression and/or activity. Such small molecules can be identified by routine drug screening protocols. For example, to screen for a PPARα agonist, a cell line such as HepG2 can be engineered to express a reporter gene, like luciferase, under the control of a PPARα-responsive promoter. After treating the cells with potential agonist compounds, the activity of the reporter gene (e.g., luciferase luminescence) is measured. In some embodiments, an increase in reporter activity compared to a vehicle-treated control group indicates activation of PPARα by the tested compound, suggesting it may be a potential agonist. The screening methods described herein are well-established for identifying PPARα agonists, and variations of these methods may be utilized in accordance with the present invention. In certain embodiments, the cell lines used for the aforementioned screening methods may include HepG2, HEK293, HepaRG, or C2C12. Non-limiting examples of reporter genes or reporters may include luciferase, green fluorescent protein (GFP), or β-Galactosidase (LacZ).

[0060] The present invention may also feature a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene for use in a method of treating a neurodegenerative disease in a subject diagnosed with a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises

an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0061] In other embodiments, the present invention may feature a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene for use in a method of preventing a neurodegenerative disease in a subject at risk of having a neurodegenerative disease. In some embodiments, the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0062] The present invention may further feature the use of a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene in the manufacture of a medicament for the treatment of a neurodegenerative disease. In some embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a full-length PPARA gene. In other embodiments, the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment (e.g., a functional fragment) of a PPARA gene.

[0063] **EXAMPLES**

[0064] The following are non-limiting examples of the present invention. It is to be understood that said examples are not intended to limit the present invention in any way. Equivalents or substitutes are within the scope of the present invention.

[0065] **Example 1- Therapeutic AAV vector upregulating astrocytic PPARα expression rescued hippocampal synaptic function in an amyloidosis mouse model of Alzheimer's disease.**

[0066] *Alzheimer's disease mouse model:* 5xFAD hemizygous (B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax, Strain #034848-JAX) and its wildtype littermates were produced by crossing with C57BL/6J females.

[0067] *Virus production*: The pAAV-GFAP-EGFP-T2A-*mPpara*[NM_011144.6]-WPRE was packaged into AAV5 serotype viral vector (FIG. 1 upper construct). The AAV5-GFAP-EGFP-T2A-*mPpara*[NM_011144.6]-WPRE (1.5×10^{13} GC/mL), and AAV5-GFAP-EGFP-WPRE (1.3×10^{13} GC/mL) were used for injection.

[0068] *Stereotactic virus injection:* 5xFAD hemizygous mice and their wildtype littermates were produced by crossing with C57BL/6J females. 5xFAD mice were injected with AAV particles into bilateral hippocampi at 2 months of age. Briefly, mice were anesthetized with Ketamine (100

mg/kg) and Xylazine (10 mg/kg), and their heads placed in a stereotactic apparatus (KOPF Instruments, USA). The skull was exposed, and a small craniotomy was performed. To cover the hippocampal region, mice were bilaterally microinjected using the following coordinates: anteroposterior (AP), -3.6 mm from bregma, mediolateral (ML), ± 2.3 mm, dorsoventral (DV), 2.7 mm. All microinjections were carried out using a 5 µL syringe (Hamilton Company, USA) and a glass pipette (WPI, USA). The injection volume and flow rate (100 nL/min) were controlled by an injection pump (WPI). Each mouse received 500 nL of virus on each site individually. Following each injection, the needle was left in place for 10 additional minutes to allow for diffusion of the viral vector away from the needle track and was then slowly withdrawn. The incision was closed using Vetbond tissue adhesive. For postoperative care, mice were subcutaneously injected with Ethiqa XR (3.25 mg/kg).

[0069] *Immunostaining*: Mice brains were perfused and sectioned 5 months after treatment. GFAP antibody was used to identify the distribution and morphological changes of astrocytes. DAPI was used to mark the nuclei.

[0070] *Multi-Electrode Array (MEA) recording*: Five months after virus injection mice were anesthetized. Acute hippocampal slices were prepared by a vibratome (Leica VT1200, Leica Microsystems Inc., USA) and positioned over a multi-microelectrode array on a MED64 probe (FIG. 3A) as previously described. Three slices per animal (three animals for each group) were used. Evoked extracellular field recordings were acquired with Med64 Mobius software (Alpha Med Scientific Inc., Japan). Schaffer collateral/commissural pathways were stimulated with biphasic current pulses (200µs). The Input-Output (I/O) curves were obtained by applying stimuli with increasing amplitudes from 10 to 100 µA. Then stimulation intensity was applied to elicite 30% of the maximum response to evoke fEPSPs. Paired pulse facilitation was measured with two stimulations applied at a different inter-pulse varying from 10 to 50 ms. The percentage of facilitation was determined by calculating the paired-pulse ratio (PPR), i.e., dividing the fEPSP slope of the second response by the fEPSP slope of the first response. Then long-term potentiation (LTP) was induced with a theta burst stimulation (TBS) protocol comprising 10 trains (200 ms duration) of 4 pulses at 5kHz with 2 s intervals between trains. Before TBS, a stable baseline was established for at least 15 min. The magnitude of LTP was quantified as the percentage change in the fEPSP initial slope (10-40%), measured during the 40-60 min interval after the TBS. Data were analyzed with Mobius software (FIG. 3B).

Statistics: All data were presented as the mean±SEM. Statistical analyses were performed with GraphPad Prism9 (GraphPad Software). Electrophysiology results were compared with two-way ANOVA, followed by Tukey's test for IO curves, and PPRs. LTP analysis was performed by Kruskal-Wallis one-way ANOVA for ranks followed by Dunn's multiple comparisons test. In other

experiments, among groups differences were determined with one-way ANOVA, followed by Tukey's multiple comparisons test. *P*-values < 0.05 were considered statistically significant.

[0071] The AAV5-GFAP-EGFP-T2A-*mPpara*[NM_011144.6]-WPRE transduction allows the specific expression of PPARα in astrocytes (FIG. 2). 5xFAD mice were injected with either AAV5-GFAP-EGFP-T2A-*mPpara*[NM_011144.6]-WPRE virus (5xFAD-PPARα) or vehicle control viruses without the *mPpara* sequence (5xFAD-EGFP). Wildtype mice were also injected with control viruses (WT-EGFP).

[0072] Results from the multielectrode array (MED64) showed that impaired long-term synaptic plasticity and short-term synaptic plasticity in 5xFAD mice are rescued in 5xFAD-PPARα mice (FIG. 3D and FIG. 4A-4B). Field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating Schaffer collateral fibers and recorded in the CA1 stratum radiatum in slices obtained from 7 months old WT-EGFP, 5xFAD-EGFP, and 5xFAD-PPARα mice.

[0073] Time course for Theta-burst induced LTP showed that the level of potentiation is dramatically reduced in slices from 7 months old 5xFAD-EGFP mice compared to slices from WT-EGFP mice, but partly rescued in 5XFAD-PPARα mice. Kruskal-Wallis one-way ANOVA by ranks followed by Dunn's multiple comparison test ($F = 41.16$) (n = 9 slices from 3 animals per group, $^{***}p < 0.001$, FIG. 3B). Mean LTP was measured from 35 min to 40 min after Theta-burst. The slices from both 5xFAD-EGFP and 5xFAD-PPARα mice exhibited a reduction in LTP than those from WT-EGFP mice. The 5xFAD-PPARα group showed a significantly improved LTP response compared to the slices from the 5xFAD-EGFP group (FIG. 3C). Thus, the upregulation of PPARα in astrocytes rescued the LTP decline in 7 months old 5xFAD mice, which contributes to the improvement in long-term plasticity.

[0074] Mean short-term plasticity (STP), measured during 15 min to 20 min post Theta-burst, indicated a significant deficit in 5XFAD-EGFP mice, which was rescued in 5XFAD-PPARα mice (FIG. 4A). Also, the paired-pulse ratio (PPR) measured at different inter-stimulus intervals (30, 40, and 50 ms) were notably attenuated in slices from 5XFAD-EGFP mice from 30 and 40 ms intervals stimulation, but not 50 ms stimulation relative to WT-EGFP controls (FIG. 4B). The PPR differences between 5xFAD-PPARα and WT-EGFP mice were diminished when the intervals reached more than 30 ms. These suggested that the enhancing PPARα expression in astrocytes improved the impairment of short-term potentiation, as well as PPR in 5xFAD mice, which are important parameters of short-term plasticity.

[0075] These data suggest that the enhancing PPARα expression in astrocytes of the hippocampal region improves the long-term and short-term plasticity in Alzheimer's disease mouse models.

[0076] **Example 2- Therapeutic AAV vector upregulating astrocytic PPARα expression alleviated the behavioral impairments in an amyloidosis mouse model of Alzheimer's disease.**

[0077] *Cognitive function*: Mice were assessed for novel object recognition (NOR) and novel object placement, tasks that measure recognition memory, presumed to critically involve perirhinal cortex and dorsal hippocampus. Nest-building indexes, which have been identified as highly correlated with motor and hippocampal impairments, were assessed.

[0078] Nest building behavior was significantly impaired in 5xFAD-EGFP mice compared to WT-EGFP mice, indicative of motor and/or hippocampal impairments (FIG. 5A). Also, NOR test (24 hours interval) indicated a decrease in long-term memory in 5xFAD-EGFP mice (FIG. 5B). Further, a complete reversal of impairment in nest building and a trend toward improved novel object recognition were observed in 5xFAD-PPARα mice compared to 5xFAD-EGFP mice (FIG. 5A-5B).

[0079] **Example 3- Enhancing PPARα expression in hippocampal astrocytes reduced lipid accumulation and lipid peroxidation in an amyloidosis mouse model of Alzheimer's disease.**

[0080] *Immunostaining*: The procedure was performed as described in Example 1. PLIN2 antibody was used to indicate the expression and distribution of PLIN2, a lipid droplet marker protein. The PLIN2 expression level has been found highly correlated with triacylglycerol content and lipid droplet load. 4-HNE antibody was applied to determine the levels of 4-HNE, which is a product of lipid peroxidation and is widely accepted as a stable marker for oxidative stress.

[0081] PPARα overexpression in hippocampal astrocytes substantially reduced LD marker PLIN2 (-60%) (FIG. 6) and lipid peroxidation marker 4-HNE (-73%) in the hippocampus (FIG. 7). Thus, by enhancing PPARα expression in hippocampal astrocytes, a remarkable reduction in lipid droplet load and lipid peroxidation was observed in the 5xFAD mouse brains.

[0082] **Example 4- Enhancing PPARα expression in hippocampal astrocytes reduced amyloid deposition in an amyloidosis mouse model of Alzheimer's disease.**

[0083] *Immunostaining:* The procedure was performed as described in Example 1. Amyloid antibody (6E10) was used to indicate the amount and distribution of amyloid deposition.

[0084] The upregulation of PPARα in hippocampal astrocytes led to a 77% decline in amyloid deposition in the 5xFAD mouse brain (Figure 8). Selective enhancement of PPARα expression in hippocampal astrocytes effectively reduced amyloid deposition, supporting its potency in mitigating amyloid proteinopathy, a key pathological hallmark of Alzheimer's disease.

[0085] Together, these data strongly support that astrocytic PPARα, potentially via increasing FA metabolism and lipid turnover, alleviates AD pathologies and cognitive impairment.

[0086] **Example 5- Enhancing PPARα expression in hippocampal astrocytes led to prolongation of both primary and secondary branch length of astrocytes in an amyloidosis mouse model of Alzheimer's disease.**

[0087] *Immunostaining*: The process is described as Example 1. GFAP antibody was used to indicate the activation, distribution and morphological changes of astrocytes, and the images were analyzed by ImageJ.

[0088] Upregulation of PPARα expression in hippocampal astrocytes led to a remarkable rescue of both primary (WT-EGFP: 22.3 ± 0.707 nm, 5xFAD-EGFP: 17.9 ± 0.925 nm, 5xFAD-PPARα: 24.9 ± 1.540 nm) and secondary branch lengths (WT-EGFP: 10.10 ± 0.763 nm, 5xFAD-EGFP: 6.95 ± 0.534 nm, 5xFAD-PPARα: 11.20 ± 1.040 nm) in 5xFAD mouse brains. Prominent astrocyte reactivity was also observed in 5xFAD-EGFP mouse brains, which is a common neuropathological finding in Alzheimer's disease, but not in 5xFAD-PPARα mouse brains.

[0089] This notable effect suggests the potential of PPARα modulation as a promising therapeutic approach to counteract the adverse effects of Alzheimer's disease-associated hippocampal astrocytes reactivity.

[0090] Although there has been shown and described the preferred embodiment of the present invention, it will be readily apparent to those skilled in the art that modifications may be made thereto which do not exceed the scope of the appended claims. Therefore, the scope of the invention is only to be limited by the following claims. In some embodiments, the figures presented in this patent application are drawn to scale, including the angles, ratios of dimensions, etc. In some embodiments, the figures are representative only and the claims are not limited by the dimensions of the figures. In some embodiments, descriptions of the inventions described herein using the phrase "comprising" includes embodiments that could be described as "consisting essentially of" or "consisting of", and as such the written description requirement for claiming one or more embodiments of the present invention using the phrase "consisting essentially of" or "consisting of" is met.

WHAT IS CLAIMED IS:

1. A method of treating a neurodegenerative disease in a subject in need thereof, the method comprising administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene to a subject diagnosed with a neurodegenerative disease, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.
2. A method of delaying the onset of or preventing a neurodegenerative disease in a subject in need thereof, the method comprising administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion PPARA gene to a subject at risk of having a neurodegenerative disease, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.
3. The method of claim 1 or claim 2, wherein the neurodegenerative disease is selected from a group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases.
4. The method of any one of claims 1–3, wherein the viral vector is an adeno-associated viral (AAV) vector or a lentiviral vector.
5. The method of claim 4, wherein the lentiviral vector is selected from a group consisting of AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes.
6. The method of any one of claims 1–5, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a full length PPARA gene.
7. The method of any one of claims 1–5, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment of a PPARA gene.
8. The method of any one of claims 1–7, wherein the astrocyte-specific promoter is a Glial Fibrillary Acidic Protein (GFAP) promoter.
9. The method of any one of claims 1–7, wherein the viral vector is administered intravenously, intranasally, intrathecally, intracisternally, intracerebroventricularly, or a combination thereof.
10. The method of any one of claims 1–7, wherein the viral vector is administered via direct injection into the brain.
11. The method of any one of claims 1–10, wherein the viral vector is administered via a nanoparticle delivery system.
12. The method of any one of claims 1–11, wherein the viral vector is transported across the blood-brain barrier.

13. The method of any one of claims 1–12, wherein the viral vector decreases the level of reactive oxygen species (ROS) and oxidative stresses in brain tissues affected by the neurodegenerative diseases.
14. The method of any one of claims 1-13, wherein the viral vector diminishes the accumulation of neutral lipid species in brain tissues affected by the neurodegenerative disease.
15. The method of any one of claims 1-14, wherein the viral vector increases synaptic function, wherein synaptic function includes short-term and long-term plasticity.
16. The method of any one of claims 1–15, wherein the viral vector rescues cognitive function; wherein cognitive function includes cognitive memory and spatial memory.

17. A viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene for use in a method of treating a neurodegenerative disease in a subject diagnosed with a neurodegenerative disease, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.
18. A viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene for use in a method of preventing a neurodegenerative disease in a subject diagnosed with a neurodegenerative disease, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.
19. The viral vector of claim 17 or claim 18, wherein the neurodegenerative disease is selected from a group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy Body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases.
20. The viral vector of any one of claims 17–19, wherein the viral vector is an adeno-associated viral (AAV) vector or a lentiviral vector.
21. The method of claim 4, wherein the lentiviral vector is selected from a group consisting of AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes.
22. The viral vector of any one of claims 17–21, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a full length PPARA gene.
23. The viral vector of any one of claims 17–21, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment of a PPARA gene.
24. The viral vector of any one of claims 17–23, wherein the astrocyte-specific promoter is a Glial Fibrillary Acidic Protein (GFAP) promoter.

25. The viral vector of any one of claims 17–24, wherein the viral vector is administered intravenously, intranasally, intrathecally, intracisternally, intracerebroventricularly, or a combination thereof.
26. The viral vector of any one of claims 17–24, wherein the viral vector is administered via direct injection into the brain.
27. The viral vector of any one of claims 17–24, wherein the viral vector is administered via a nanoparticle delivery system.
28. The viral vector of any one of claims 17–27, wherein the viral vector is transported across the blood-brain barrier.
29. The viral vector of any one of claims 17–28, wherein the viral vector decreases the level of reactive oxygen species (ROS) and oxidative stresses in brain tissues affected by the neurodegenerative diseases.
30. The viral vector of any one of claims 17-29, wherein the viral vector diminishes the accumulation of neutral lipid species in brain tissues affected by the neurodegenerative disease.
31. The viral vector of any one of claims 17-30, wherein the viral vector increases synaptic function, wherein synaptic function includes short-term and long-term plasticity.
32. The viral vector of any one of claims 17–31, wherein the viral vector rescues cognitive function; wherein cognitive function includes cognitive memory and spatial memory.

33. Use of a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene in the manufacture of a medicament for the treatment of a neurodegenerative disease.
34. The viral vector of claim 33, wherein the neurodegenerative disease is selected from a group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy Body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases.
35. The viral vector of claim 33 or claim 34, wherein the viral vector is an adeno-associated viral (AAV) vector or a lentiviral vector.
36. The method of claim 35, wherein the lentiviral vector is selected from a group consisting of AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes.
37. The viral vector of any one of claims 33–36, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a full length PPARA gene.
38. The viral vector of any one of claims 33–36, wherein the viral vector comprises an astrocyte-specific promoter operatively linked to a fragment of a PPARA gene.

39. The viral vector of any one of claims 33–38, wherein the astrocyte-specific promoter is a Glial Fibrillary Acidic Protein (GFAP) promoter.
40. The viral vector of any one of claims 33–39, wherein the viral vector is administered intravenously, intranasally, intrathecally, intracisternally, intracerebroventricularly, or a combination thereof.
41. The viral vector of any one of claims 33–40, wherein the viral vector is administered via direct injection into the brain.
42. The viral vector of any one of claims 33–40, wherein the viral vector is administered via a nanoparticle delivery system.
43. The viral vector of any one of claims 33–40, wherein the viral vector is transported across the blood-brain barrier.
44. The viral vector of any one of claims 33–43, wherein the viral vector decreases the level of reactive oxygen species (ROS) and oxidative stresses in brain tissues affected by the neurodegenerative diseases.
45. The viral vector of any one of claims 33-44, wherein the viral vector diminishes the accumulation of neutral lipid species in brain tissues affected by the neurodegenerative disease.
46. The viral vector of any one of claims 33-45, wherein the viral vector increases synaptic function, wherein synaptic function includes short-term and long-term plasticity.
47. The viral vector of any one of claims 33–46, wherein the viral vector rescues cognitive function; wherein cognitive function includes cognitive memory and spatial memory.

48. A method of screening for a PPARα agonist, the method comprising:
    a) obtaining a cell line comprising a PPARα-responsive promoter operatively linked to a reporter gene; and
    b) contacting the cell line with a potential PPARα agonist compound;
    wherein a potential PPARα agonist compound is confirmed as a PPARα agonist when expression of the reporter gene increases compared to a vehicle-treated control.
49. The method of claim 48, wherein the cell line is a HepG2.
50. The method of claim 48, wherein the reporter gene is a luciferase.

51. A method of delaying the onset of, treating, or preventing a neurodegenerative disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a viral vector comprising a nucleic acid molecule encoding for one or

more homologous genes encoding PPARα which upregulate the expression and/or activity of PPARα selectively inside of astrocytes in the central nervous system.

52. The method of claim 51, wherein the viral vector further comprises an astrocytes specific promoter operatively linked to the PPARα nucleic acid molecule.
53. The method of claim 52, wherein the astrocyte-specific promoter is a Glial Fibrillary Acidic Protein (GFAP) promoter.
54. The method of any one of claim 51-53, wherein nucleic acid molecule encoding for one or more homologous genes upregulates expression of PPARα and its functional fragments or isoforms.
55. The method of any one of claim 51-54, wherein the viral vector is an adeno-associated viral (AAV) vector or a lentiviral vector.
56. The method of claim 55, wherein the lentiviral vector is selected from a group consisting of AAV2, AAV5, AAV6, AAV9, AAV-PHP.eB, AAV-PHP.B, or AAVrh10 serotypes.
57. The method of any on of claim 51-56, wherein the neurodegenerative disease is selected from a group consisting of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), frontotemporal dementia, Lewy Body dementia, Gaucher's disease, progressive supranuclear palsy, stroke, depression, vascular dementia, and prion diseases.
58. The method of any one of claim 51-57, wherein the viral vector is administered intravenously, intranasally, intrathecally, intracisternally, intracerebroventricularly, or a combination thereof.
59. The method of any one of claim 51-57, wherein the viral vector is administered via direct injection into the brain.
60. The method of any one of claim 51-57, wherein the viral vector is administered via a nanoparticle delivery system.
61. The method of any one of claim 51-57, wherein the viral vector is transported across the blood-brain barrier.
62. The method of any one of claims 51–61, wherein the viral vector is transported across the blood-brain barrier.
63. The method of any one of claims 51-62, wherein the viral vector decreases the level of reactive oxygen species (ROS) and oxidative stresses in brain tissues affected by the neurodegenerative diseases.
64. The method of any one of claims 51-63, wherein the viral vector diminishes the accumulation of neutral lipid species in brain tissues affected by the neurodegenerative disease.

65. The method of any one of claims 51-64, wherein the viral vector increases synaptic function, wherein synaptic function includes short-term and long-term plasticity in the subject.
66. The method of any one of claims 51-65, wherein the viral vector rescues cognitive function; wherein cognitive function includes cognitive memory and spatial memory.

FIG. 1

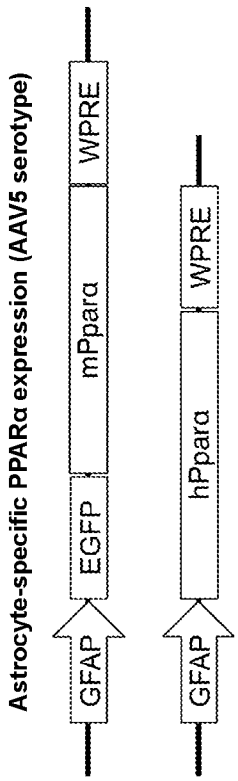

Astrocyte-specific PPARα expression (AAV5 serotype)
GFAP
EGFP
mPparα
WPRE
GFAP
hPparα
WPRE

FIG. 2

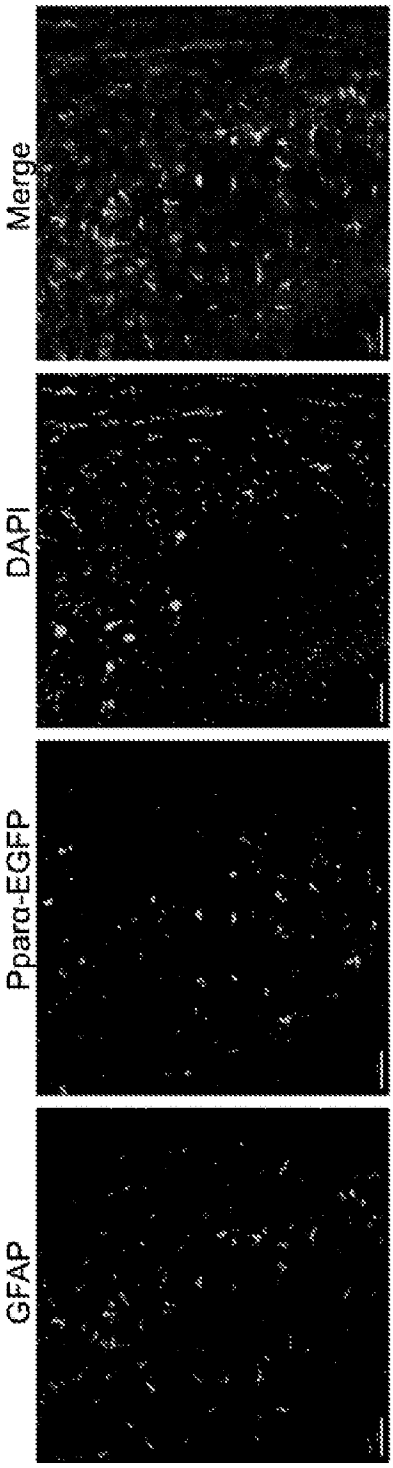

GFAP
Pparα-EGFP
DAPI
Merge

FIG. 3C

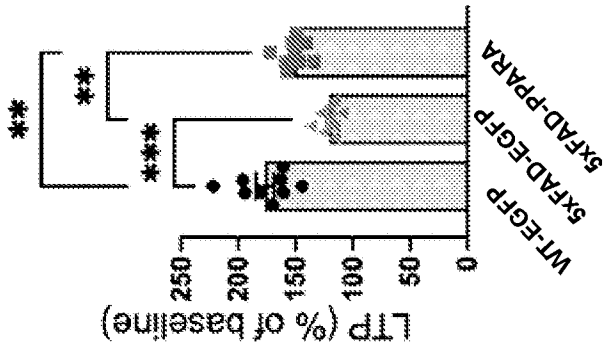


FIG. 3B

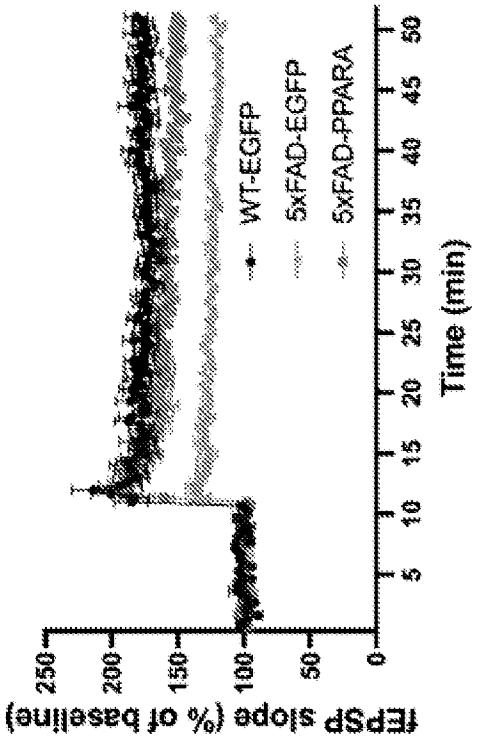


FIG. 3A

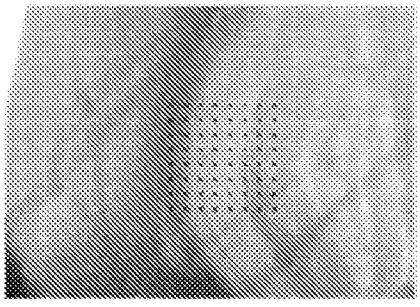


FIG. 4A

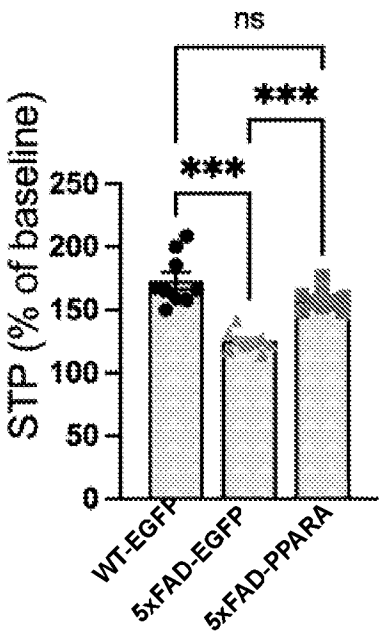


FIG. 4B

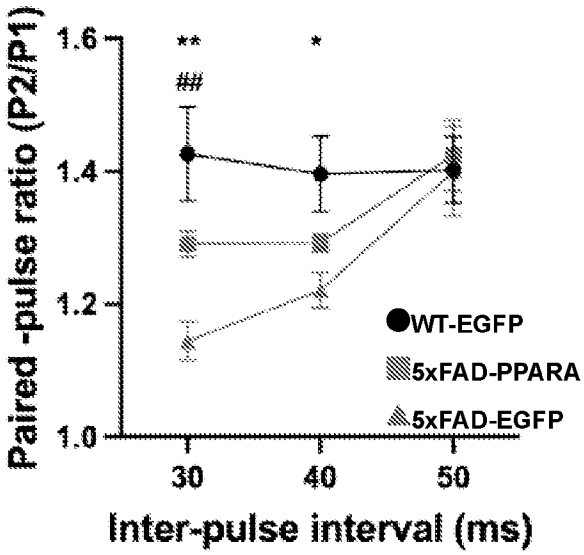


FIG. 5A

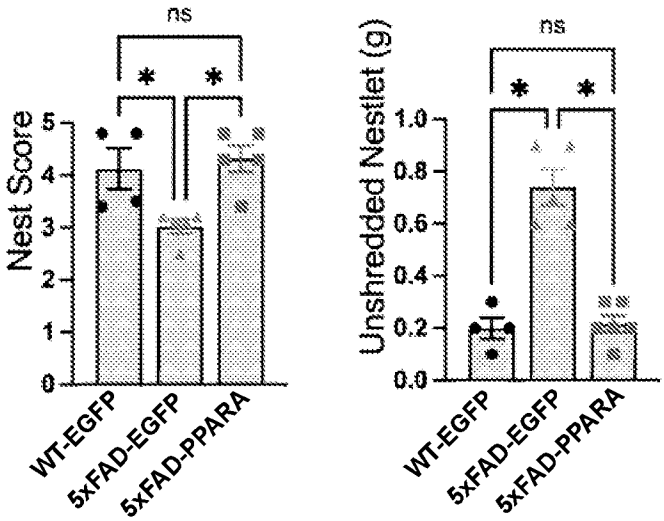


FIG. 5B

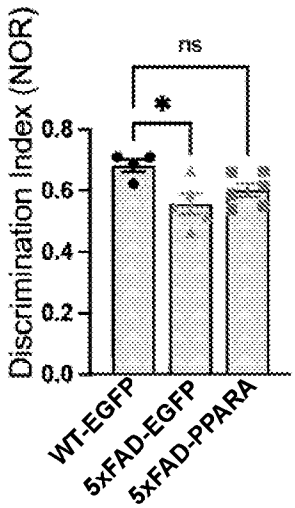
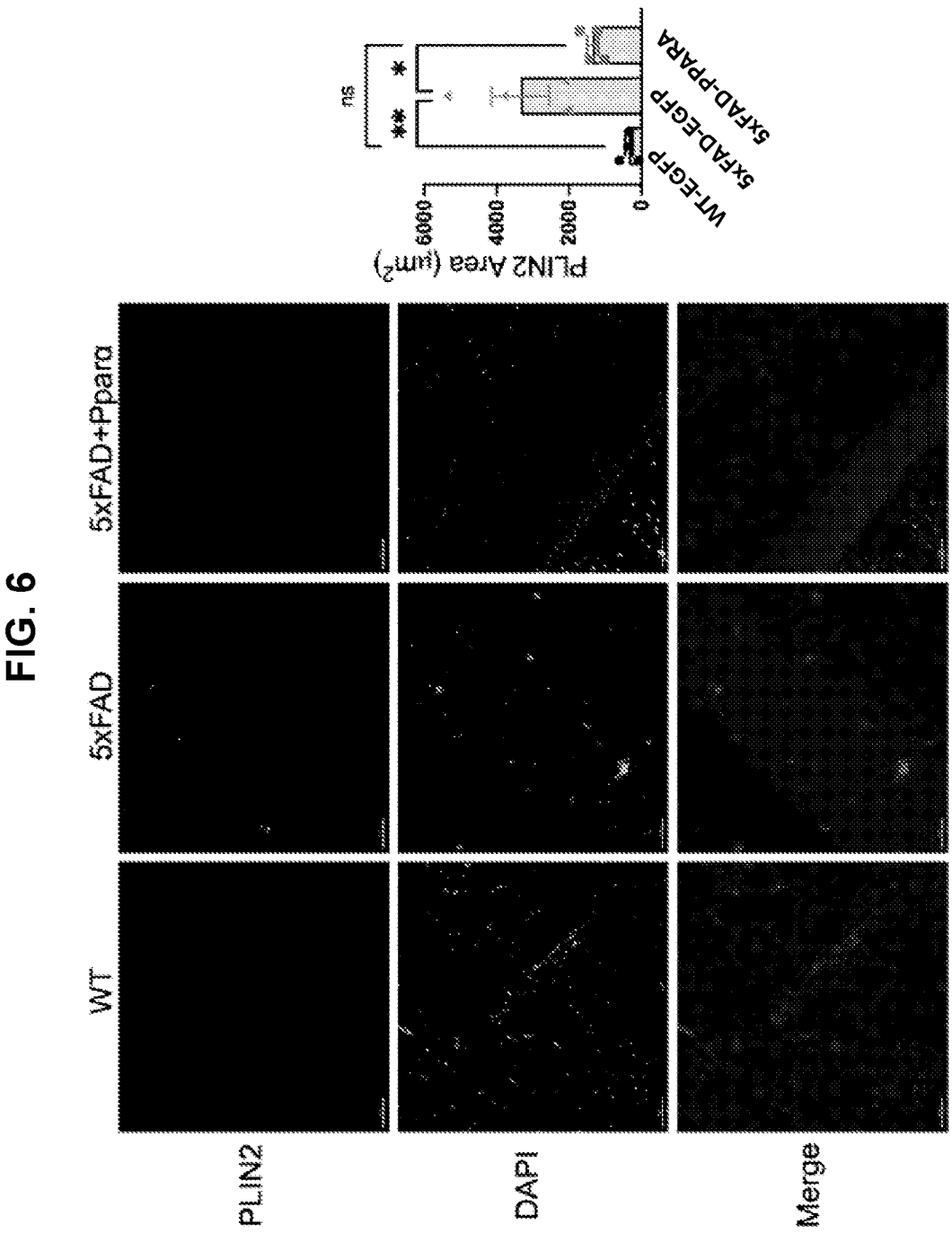


FIG. 6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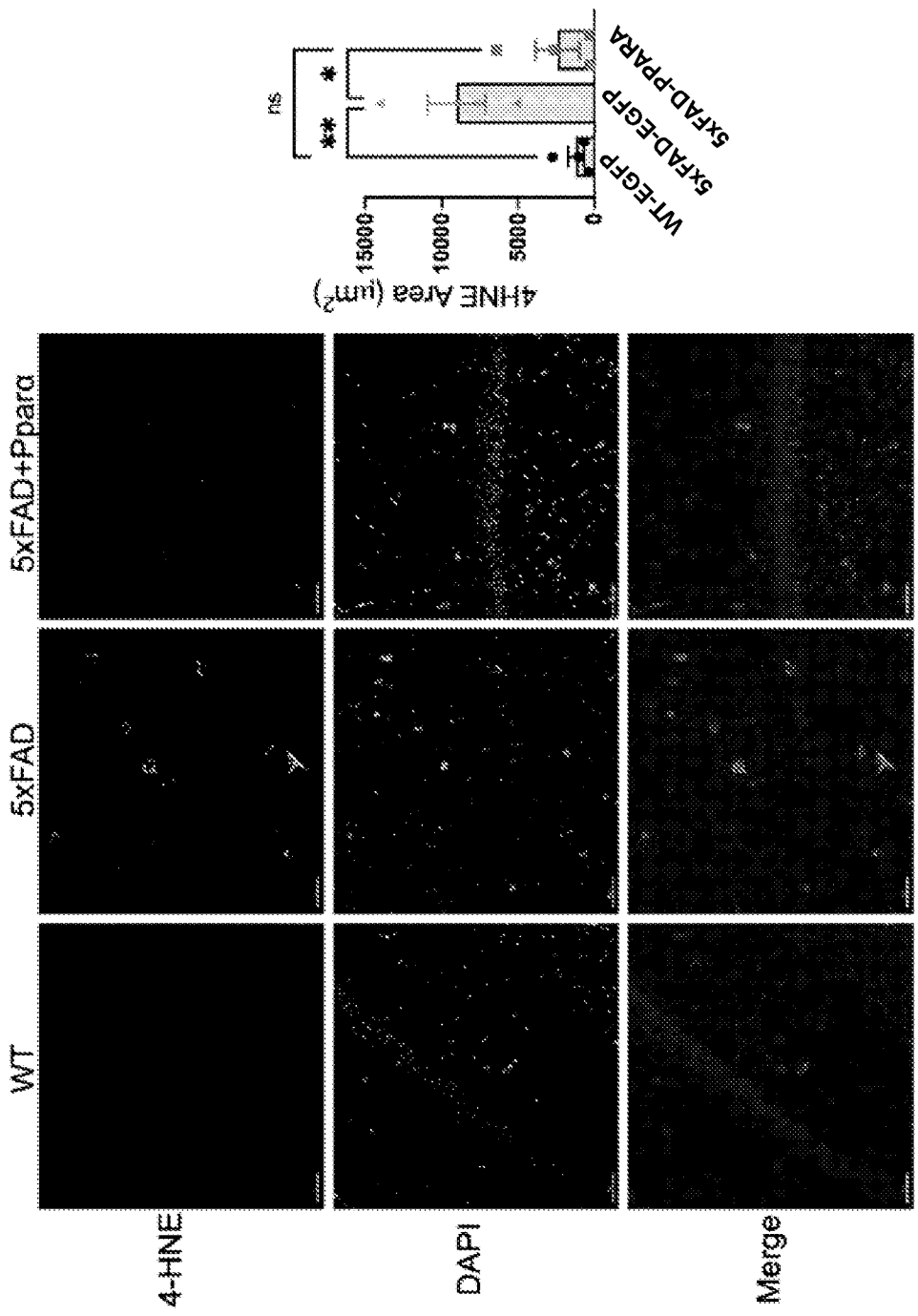
5xFAD
5xFAD+Pparα
PLIN2
DAPI
Merge
PLIN2 Area (μm²)
6000
4000
2000
0
WT-EGFP
5xFAD-EGFP
5xFAD-PPARA
ns
*
**

FIG. 7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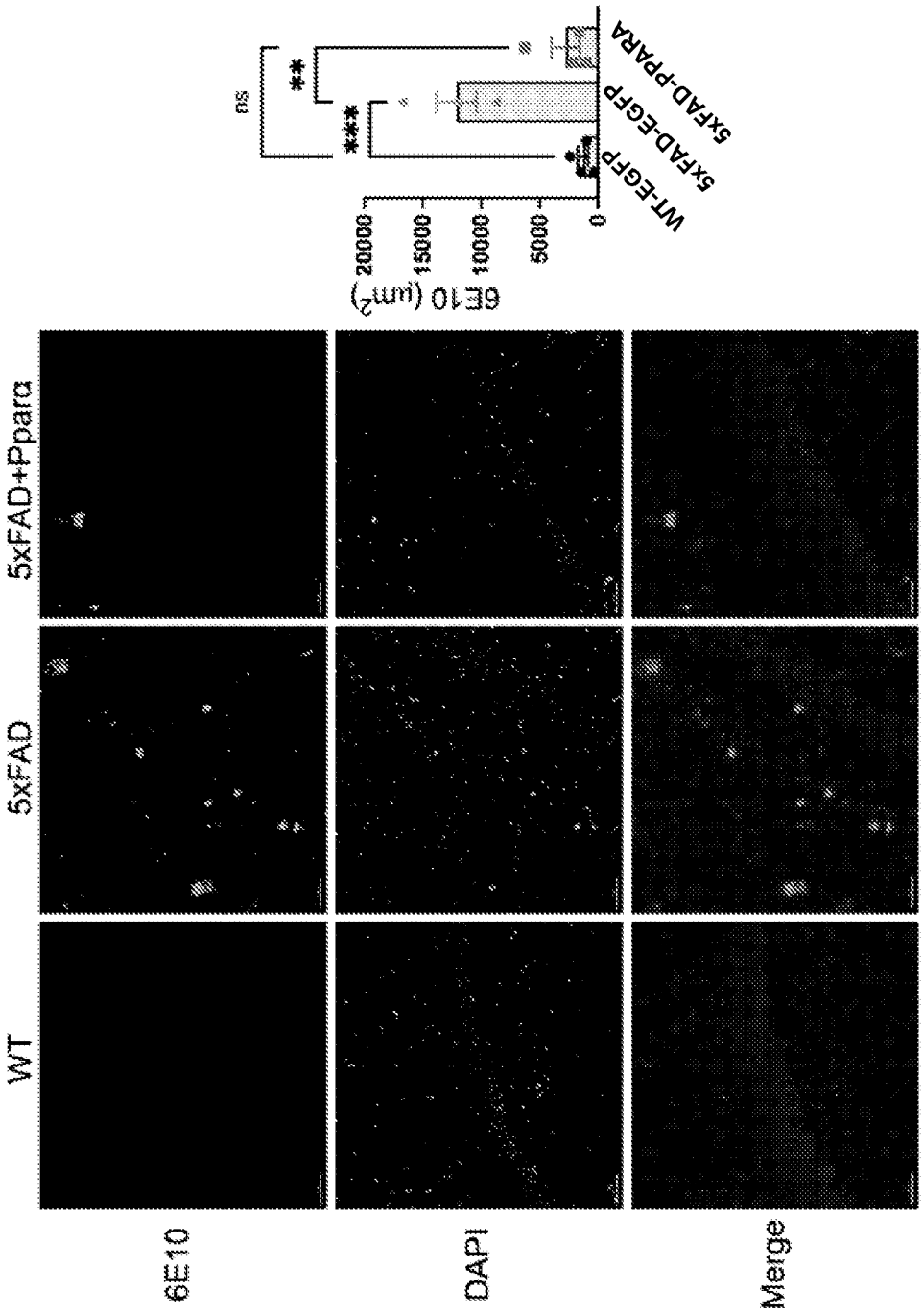
5xFAD+Pparα
4-HNE
DAPI
Merge
4HNE Area (μm²)
15000
10000
5000
0
WT-EGFP
5xFAD-EGFP
5xFAD-PPARA
ns
**
*

FIG. 8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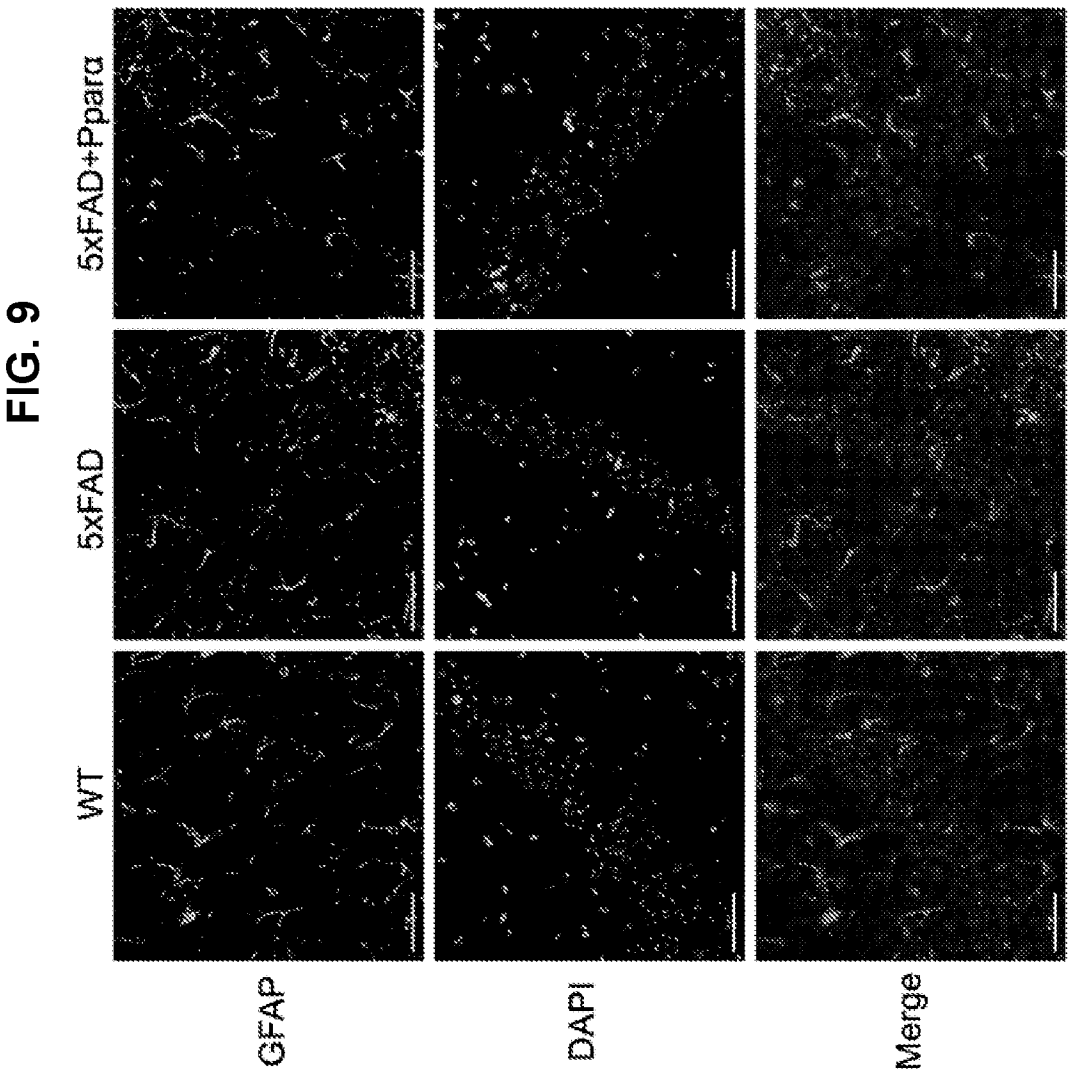
5xFAD+Pparα
6E10
DAPI
Merge
6E10 (μm²)
20000
15000
10000
5000
0
WT-EGFP
5xFAD-EGFP
5xFAD-PPARA
ns
**
***
**

FIG. 9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5xFAD
5xFAD+Ppara
GFAP
DAPI
Merge
2nd branch length (μm)
Primary branch length (μm)
WT
5xFAD
5xFAD-PP
ns
*
**
***

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/042331

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 48/00*** (2023.01); ***A61K 38/17*** (2023.01); ***A61P 25/28*** (2023.01); ***C12N 15/86*** (2023.01); ***C12N 15/864*** (2023.01)

CPC: ***A61K 48/0058; A61K 38/1783; A61P 25/28; C12N 15/86; C12N 15/8645***

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	WO 2023/039476 A1 (THE BROAD INSTITUTE et al.) 16 March 2023 (16.03.2023) abstract; para [0040]-[0041], [0046], [0121], [0159], [0161], [0237], [0249], [0349], [0354], [0478], [0480]	1-3
A	US 2008/0286260 A1 (GOLZ et al.) 20 November 2008 (20.11.2008) entire document	1-3

☐ 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
04 December 2024 (04.12.2024)	**12 December 2024 (12.12.2024)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**KARI RODRIQUEZ**
Facsimile No. **571-273-8300**	Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/042331

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

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.: **1-3**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/042331

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.

Group I: Claims 1-3, directed to methods of treating, delaying the onset of, or preventing a neurodegenerative disease in a subject comprising administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.

Group II: Claims 17-19, directed to a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.

Group III: Claims 33-36, directed to use of a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene in the manufacture of a medicament for the treatment of a neurodegenerative disease.

Group IV: Claims 48-50, directed to a method of screening for a PPARa agonist.

Group V: Claims 51-54, directed to a method of delaying the onset of, treating, or preventing a neurodegenerative disease in a subject comprising administering a viral vector comprising a nucleic acid molecule encoding for one or more homologous genes encoding PPARa which upregulate the expression and/or activity of PPARa selectively inside of astrocytes in the central nervous system.

The inventions listed as Groups I-V 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

Group I requires administering a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain, not required by Groups II-V.

Group II requires a composition of matter comprising or consisting of a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain, not required by Groups I or III-V.

Group III requires use of a viral vector in the manufacture of a medicament for the treatment of a neurodegenerative disease, not required by Groups I-II or IV-V.

Group IV requires a method of screening for a PPARa agonist comprising obtaining a cell line comprising a PPARa-responsive promoter operatively linked to a reporter gene; and contacting the cell line with a potential PPARa agonist compound; wherein a potential PPARa agonist compound is confirmed as a PPARa agonist when expression of the reporter gene increases compared to a vehicle-treated control, not required by Groups I-III or V.

Group V requires administering to the subject a therapeutically effective amount of a viral vector comprising a nucleic acid molecule encoding for one or more homologous genes encoding PPARa which upregulate the expression and/or activity of PPARa selectively inside of astrocytes in the central nervous system, not required by Groups I-IV.

Common Technical Features

The feature shared by Groups I-V is: a viral vector comprising a nucleotide encoding PPARA or a homolog thereof.

The feature shared by Groups I-II is: a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.

The feature shared by Groups I-III is: a viral vector comprising an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene.

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

The feature shared by Groups I and V is: a method of treating, delaying the onset of, or preventing a neurodegenerative disease in a subject in need thereof comprising administering a viral vector comprising a portion of a PPARA gene or homolog thereof to a subject diagnosed with a neurodegenerative disease, wherein administering upregulates the expression of PPARA.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2008/0286260 A1 to Golz et al. (hereinafter 'Golz') in view of WO 2023/039476 A1 to the Broad Institute et al. (hereinafter 'Broad').

Golz discloses a method of treating, delaying the onset of, or preventing a neurodegenerative disease in a subject in need thereof comprising administering a viral vector comprising a portion of a PPARA gene or homolog thereof to a subject diagnosed with a neurodegenerative disease, wherein administering upregulates the expression of PPARA (para [0206] - "The regulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of PPARA. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein... In one embodiment, the agent stimulates one or more of the biological activities of PPARA. Examples of such stimulatory agents include the active PPARA and nucleic acid molecules encoding a portion of PPARA... These regulatory methods can be performed in vitro... or, alternatively, in vivo (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by unwanted expression or activity of PPARA... In another embodiment, the method involves administering a regulator of PPARA as therapy to compensate for reduced or undesirably low expression or activity of PPARA or a protein in the PPARA signaling pathway."; para [0210] - "The nucleic acid molecules... of the invention can be incorporated into pharmaceutical compositions suitable for administration. "; para [0104] - "A number of viral-based expression systems can be used to express PPARA in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding PPARA can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence."; para [0213]-[0214] - "In another embodiment of the invention, the polynucleotides encoding PPARA, or any fragment or complement thereof, may be used for therapeutic purposes... Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population."; para [0205] - "The present invention provides for both prophylactic and therapeutic methods for cardiovascular diseases, gastroenterological diseases, cancer, inflammation, hematological diseases, respiratory diseases, neurological diseases and urological diseases."; para [0168]-[0169] - "CNS disorders include disorders of the central nervous system as well as disorders of the peripheral nervous system. CNS disorders include... Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy...within the meaning of the definition are also considered to be CNS disorders.").

Golz does not disclose an astrocyte-specific promoter operatively linked to at least a portion of a PPARA gene, wherein the viral vector upregulates the expression of PPARA to promote lipid metabolism in the brain.

Broad discloses a method of treating, delaying the onset of, or preventing a neurodegenerative disease in a subject in need thereof comprising administering a viral vector (para [0161] - "provided herein are methods of treating a subject in need thereof by delivering an engineered AAV particle, engineered AAV capsid, engineered AAV capsid vector or system thereof, an engineered cell, and/or formulation thereof to the subject."; para [0039]-[0040] - "In certain example embodiments, the AAV viral particle is an engineered AAV1, AAV2, AAV3... viral particle. In certain example embodiments, the optional cargo is capable of treating or preventing a CNS, a muscle disease or disorder, or both."; para [0121] - "In certain example embodiments, the central nervous system disease or disorder is Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glutl Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angleman Syndrome, or a combination thereof."; para [0123] - " In certain example embodiments, the expanded repeat disease is Huntington's disease, a Myotonic Dystrophy, or Facioscapulohumeral muscular dystrophy (FSHD).") comprising a portion of a PPARA gene or homolog thereof (para [0478] - "In some embodiments, a therapeutic or preventive, such as the engineered AAV capsids and systems thereof as described elsewhere herein, can be delivered to a subject in need thereof or a cell thereof to treat a brain, neuron, neurological, and/or central nervous system disease or disorder

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

(CNS). In some embodiments the brain, neuron, neurological, and/or CNS disease or disorder can be caused, directly or indirectly, by one or mutations in one or more of the following genes as compared to normal or non-pathological variant of the same... in the case of diseases or disorders associated with or involving aberrant, pathologic, and/or abnormal PPAR (e.g. PPAR alpha, PPAR beta, PPAR delta, and/or PPAR gamma) regulation or signaling in the brain, neurons, and/or CNS and/or diseases or disorders thereof:... PPARA"; para [0249] - "In embodiments, the anti-fibrotic protein is a peroxisome proliferator-activated receptor (PPAR) or may include one or more PPARs. In some embodiments, the protein is PPARa, PPAR y is a dual PPARa/y") to a subject diagnosed with a neurodegenerative disease (para [0161]; para [0039]-[0040]; para [0121]; para [0123]; para [0119] - "described in certain example embodiments herein are methods of treating a muscle disease, disorder, or a symptom thereof, or both a muscle and a central nervous system disease, disorder, or a symptom thereof comprising: administering, to the subject in need thereof, a composition as in any one of the above paragraphs and elsewhere herein a vector system as in any one of the above paragraphs and elsewhere herein"), and where an astrocyte-specific promoter may be used (para [0351] - "To express a polynucleotide, the vector can include one or more transcriptional and/or translational initiation regulatory sequences, e.g., promoters, that direct the transcription of the gene and/or translation of the encoded protein in a cell."; para [0354] - "Suitable neuronal tissue/cell specific promoters include, but are not limited to, GFAP promoter (astrocytes)").

Since Golz discloses treating a neurodegenerative disease with a viral vector encoding PPARA, and Broad discloses treating a neurodegenerative disease with a viral vector encoding PPARA and where an astrocyte-specific promoter may be used, it would have been obvious to one of ordinary skill in the art to modify the method, as disclosed by Golz, to include or instead use an astrocyte-specific promoter, since this would simply allow expression of PPARA in a specific cell type associated with a tissue known to be associated with PPARA expression, thereby providing any improved ability to correct aberrant expression or activity of PPARA, such as by promoting/restoring lipid metabolism in the brain, and any associated improved patient clinical outcome, and therefore any improved value of the therapeutic method of Golz.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I-V therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

RE: Item 4

Claims 4-16, 20-32, 37-47, 55-66 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Note, Claim 36 is objected to for lack of antecedent basis. As drafted, claim 36 depends from claim 35 and recites "the method of claim 35", though claim 35 is directed to a viral vector. For the purposes of this application, the phrase has been interpreted as reciting "the viral vector of claim 35".