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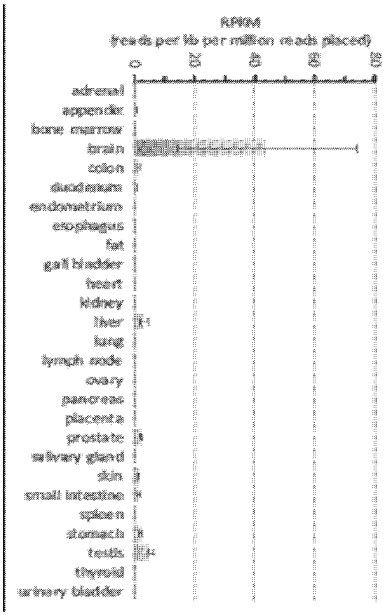

Figure 1

(57) Abstract: Disclosed are methods and compositions for treating cognitive decline in subjects in need. More specifically, disclosed are methods of administrating exogenous adropin to subjects suffering from, or at risk of, cognitive decline. Also disclosed are subjects who would benefit from such treatment and pharmaceutical acceptable compositions comprising adropin, adropin$^{34-76}$, and derivatives or variations thereof.

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[Continued on next page]

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METHODS AND COMPOSITIONS FOR TREATING DECREASED COGNITIVE ABILITY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application 62682717, filed June 8, 2018, hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT CLAUSE

[0002] The work disclosed herein was supported by award no. R21NS108138. The U.S. Government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The invention relates to methods and compositions for treating reduced cognitive ability. More specifically, the invention relates to using the polypeptide adropin to treat subjects with decreased cognitive ability, including decreased cognitive ability due to age related dementia.

BACKGROUND

[0004] 'Dementia' as a general term describes memory loss and other cognitive deficits severe enough to make normal daily life difficult or impossible. Neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease and the degenerative dementias (Lewy body, Frontotemporal) represent a pressing health problem for industrialized nations. 'Mild Cognitive Impairment' describes an intermediate stage between cognitive decline that is expected with aging, and the more serious declines observed in dementia. The incidence of these conditions is predicted to increase owing to an aging populace. By 2030, 1 in 5 Americans will be aged 65 years or older. Metabolic disease may also be a predisposing risk factor for neurodegenerative conditions. Type 2 diabetes is a predisposing risk factor for cognitive decline and dementia. Cardiovascular disease, obesity and insulin resistance are also associated with increased risk for neurocognitive decline. The number of people considered obese or overweight now outnumbers those considered to have a healthy

body mass index by 2 to 1. The conditions are thus being established for a dramatic rise in the incidence of debilitating neurodegenerative disorders. An increased occurrence of dementias in an aging and increasingly obese population thus represents a clear challenge to the US economy and the health care system. In the absence of new clinical interventions to treat cognitive decline, more than 12 million Americans may suffer from neurodegenerative diseases within 30 years. The search for treatments that delay or prevent cognitive decline is thus an imperative.

[0005] Unfortunately, few options are currently available for treating dementias. Health and longevity are maintained, at least in part, by secreted peptides signaling metabolic condition at a cellular and organismal level. The secreted peptide adropin was identified by one of the inventors as such a signal. The involvement of adropin in metabolic control, was documented, showing that the putative secreted domain (adropin$^{34-76}$) rapidly alters activity of intracellular signaling molecules (e.g., SIRT1, Pgc1α) regulating glucose and fatty acid metabolism. While adropin is most abundant in the central nervous system, its functions as a neuropeptide remain unknown. The Inventors have made the surprising discovery that the decline in cognitive performance associated with aged subjects may be slowed, delayed, or prevented by sustained adropin activity in the brain.

SUMMARY OF THE INVENTION

[0006] A method of treating cognitive decline in a subject in need, the method comprising administering an effective amount of Adropin, Adropin$^{34-76}$, or variants or derivatives thereof, to the subject parenterally.

[0007] A method of treating cognitive decline, whereby the effective amount of Adropin, Adropin$^{34-76}$, is 1000 nmol/kg/day to about 1 nmol/kg/day, preferably about 450 nmol/kg or most preferably about 90 nmol/kg/day.

[0008] A method of treating cognitive decline, in a subject wherein the subject is a human subject.

[0009] A method of treating cognitive decline, in a subject at risk, including subjects diagnosed with mild cognitive impairment, dementia, hypercholesterolemia, type 2 diabetes, a metabolic dysregulation disorder or advanced age.

[0010] A method of treating cognitive decline, wherein the effective amount is administered one or more times a day, over a period of 2 weeks or over the remaining life of the subject.

[0011] A method of treating cognitive decline, wherein administering an effective amount consists of administrating an oligonucleotide enabled to express an effective amount of Adropin or Adropin$^{34-76}$.

[0012] A method of treating cognitive decline, wherein administering an effective amount includes intraperitoneal, subcutaneous, intramuscular, or intravenous injection.

[0013] A composition for treating age related dementia, comprising Adropin, Adropin$^{34-76}$, or variants or derivatives thereof, prepared in a pharmaceutically acceptable aqueous solution.

[0014] A composition for treating age related dementia, comprising Adropin, Adropin$^{34-76}$, or variants or derivatives thereof, prepared in a pharmaceutically acceptable aqueous solution further including but not limited to one or more pharmaceutically acceptable salts, proteins, polypeptides, and preservatives glycerol, liquid polyethylene glycol, and pharmaceutically acceptable oils,

[0015] A composition for treating age related dementia, comprising Adropin, Adropin$^{34-76}$, or variants or derivatives thereof, prepared in a dehydrated or concentrated form, for later use after hydration.

[0016] A composition for treating age related dementia, comprising Adropin, Adropin$^{34-76}$, prepared in pharmaceutically acceptable aqueous solution, whereby the composition is not found in nature.

REFERENCE TO COLOR FIGURES

[0017] The application file contains at least one figure executed in color. Copies of this patent application publication with color photographs will be provided by the Office upon request and payment of the necessary fee.

DESCRIPTION OF THE FIGURES

[0018] **Figure 1** Illustrates the relative expression of the ENHO transcript in human tissues (adapted from Mol Cell Proteomics. 2014 Feb13(2):397-406).

[0019] **Figure 2** Illustrates the modification of the mouse *Enho* gene with the internal ribosomal entry site (IRES) and causes-recombination (Cre) coding sequence inserted into the 3'-UTR in exon 2. B6.*Enho-Cre* mice were mated with B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J (Ai9) strain that have a loxP-flanked STOP cassette that prevents transcription of a CAG-promoter-driven variant of the red fluorescent protein (tdTomato). In cells expressing the Enho transcript, expression of Cre results in removal of the STOP cassette by site-specific recombination.

[0020] **Figure 3** Illustrates (A) Analysis of adropin protein in tissues by western blot. Tissues were homogenized in RIPA buffer; 50 µg of total protein was used in the blot. (B) Schematic of the B6.*Enho-Cre* mouse. An IRES ("internal ribosomal entry site")-Cre inserted in the 3' UTR of the Enho gene of C57BL/6J (B6) mice drives Cre-mediated recombination of genomic DNA flanked by LoxP sites. B6.*Enho-Cre* mice were crossed with B6.Cg- Gt(ROSA)26 Sor$^{tm9(CAG\text{-}tdTomato)Hze}$/J (tdTomato) mice. Enho(+ve) cells are identified by activation of tdTomato expression, resulting from Cre-mediated removal of a transcriptional block (TB) 5' of the tdTomato open reading frame; the tdTomato can be visualized directly when exposed to yellow-green light or by immunohistochemistry (IHC).

[0021] **Figure 4** representative photomicrographs showing tdTomato expression in the hippocampus (A-D). Enho-Cre mice crossed with Ai9 reporter strain allow visualization of adropin expressing cells in the hippocampus. (A-C) Codetection of GFAP (A) and tdTomato (B). The merged image (C) suggests that hippocampal astrocytes are devoid of adropin; (D-F) Codetection of NeuN with tdTomato.

[0022] **Figure 5** Illustrates prevention of a decline in adropin protein in the nervous system with aging improves cognition in old B6 mice. (A) Western blot showing adropin protein in 4 and 18 month old AdrTG and age-matched wild type (WT) controls (n=3/group); HSP90 is presented as a loading control. (B-C) Learning and memory of male 18 mo. old AdrTG is significantly improved relative to aged matched WT controls (NOR: novel object recognition test; Acq: memory acquisition in aversive T-maze test; Ret: memory retention in aversive T-maze test) (AdrTG, n=11-14; WT, n=11-14). Significantly different from WT; ** $p<0.01$; ***, $p<0.001$ (Student's t-test).

[0023] **Figure 6** illustrates reduced expression of inflammatory cytokines in cortical (A) and hippocampal (B) tissue samples from 18 mo. old AdrTG relative to age-matched controls. *, $p<0.05$; **, $p<0.01$.

[0024] **Figure 7** illustrates Western blots indicating increased 4-HNE with aging in male B6 mice (A), that suggest lower 4-HNE in 18-month old adropin transgenic mice (AdrTG) compared to age-matched control mice (B). In panel B, the western blot and quantitated data are shown.

[0025] **Figure 8** Body composition (A-B) and glucose tolerance (C-D) are normal in 18 mo. old AdrTG mice compared to age-matched controls.

[0026] **Figure 9** illustrates reduced JNK phosphorylation (Thr183/Tyr185) in cortical extracts from 18-month old AdrTG mice compared to age-matched controls. Western blot showing phosphorylated JNK (pJNK), total JNK and Actin immunoreactivity (A). Quantitation of pJNK, total JNK (adjusted for actin) and the pJNK/JNK ratio (B).

[0027] **Figure 10** illustrates body weight in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin$^{34-76}$.

[0028] **Figure 11** illustrates Novel Object Recognition in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin$^{34-76}$.

[0029] **Figure 12** illustrates T-maze Acquisition and T-maze Retention in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin$^{34-76}$.

[0030] **Figure 13** illustrates body weight in C57BL/6J (B6), LdLr-/-, and ADRTG/LdLr-/-, male and female mice.

[0031] **Figure 14** illustrates body composition in C57BL/6J (B6), LdLr-/-, and ADRTG/LdLr-/-, male and female mice.

[0032] **Figure 15** illustrates Discrimination index, the percent of total time spent exploring with the novel object , in the Novel Object Recognition test. Controls (C57BL/6J (B6)), LdLr-/-, and ADRTG/LdLr-/-, transgenic mice.

DETAILED DESCRIPTION OF THE INVENTION

[0033] Neurodegenerative diseases are a pressing health problem for industrialized nations for which few treatments are currently available. Health and longevity are maintained, at least in part, by secreted peptides signaling metabolic condition at an organismal and cellular level. One of the Inventors identified the secreted peptide adropin as such a signal in 2008. Full length adropin protein (76 aa) is encoded by the Energy Homeostasis Associated (ENHO) gene which is located on human chromosome 9p13.3. Adropin is a secretory signal peptide. Early analyses of adropin function suggested links with insulin resistance and the control of carbohydrate and lipid metabolism in the periphery. Recent studies suggest a protective role in cerebral ischemia. (Yang et al. (2018) *Aging and Disease.*;9 (2): 322-330). The Inventors have reported associations between plasma adropin concentrations and relative intake of fats and carbohydrates, and further demonstrated a metabolic response to exogenously administered adropin$^{34-76}$, when they delivered the putative secretory domain (adropin$^{34-76}$) to mice by intraperitoneal injection. (Stevens, et al., (2016) *Obesity* 24, 1731-1740; St-Onge, et al., (2014) *Obesity* 22,1056---1063). Initial examination of ENHO expression using northern blot suggested abundant expression in mouse brain and liver (Kumar, et al., (2008) *Cell metabolism*, 8, 468-481), whereas recent studies using RNA-seq in humans suggest adropin expression is most abundant in brain (Fig.1). An independent lab reported adropin protein in mice is most abundant in the CNS. (Wong, et al., (2014) *The Journal of biological chemistry* 289, 25976-25986).The Inventors' analysis using a monoclonal antibody confirms abundant adropin immunoreactivity in the CNS relative to peripheral tissues of male C57BL/6J (B6) mice

Fig. 3A). Adropin may function primarily as a neuropeptide, with circulating adropin originating predominately from the CNS.

[0034] Analysis of *Enho* expression in the mouse brain using in situ hybridization in one of the inventor's laboratories, initially indicated a very broad distribution but were unable to define the cell-types and areas of expression (Kumar, et al., (2008) *Cell metabolism*, 8, 468---481). The Inventors were able to show, using a Cre-inducible reporter mouse, that adropin is expressed in hippocampal neurons. To identify cell-types expressing adropin, the "ENHO-Cre" strain was developed (Fig. 2). Mating ENHO-Cre mice with the tdTomato reporter strain (Madisen, et al, (2010) *Nature Neuroscience* 13, 133-140) allows for visualizing cell-types expressing adropin, mediated by Cre- removal of a LoxP-flanked transcription block (TB) (Fig. 2B, C). These studies indicate hippocampal pyramidal neurons express adropin (Fig. 4), suggesting a potential role in cognition.

[0035] Analysis of mRNA expression indicates that adropin is most abundant in the CNS (Kumar et al., (2008) *Cell metabolism* 8(6):468-81; Wong et al., (2014) *The Journal of biological chemistry* 289(37):25976--86). Immunohistochemistry also indicates high expression of adropin in the CNS (Fig 3A).

[0036] The inventors have engineered transgenic mice that over express adropin ("AdrTG") in the nervous system and peripheral tissues. They found that continuous elevated adropin in the CNS protects cognitive function during aging. In addition, reduced JNK phosphorylation and expression of cytokines indicates reduced neuroinflammatory processes. Inflammation is also increasingly considered an important predisposing risk factor for age-related neurodegeneration (Irwin & Vitiello (2019) *Lancet Neurol.* 18(3):296-306; Madhavan et a., Nat Rev Cardiol. 15(12):744-756). The transgenic mice demonstrate a delay or prevention in the decline of learning and memory associated with aging. The inventors demonstrated improved cognition in older subjects with continuous elevated adropin compared to age-matched controls, through the use of cognitive assessments: novel object recognition; spatial memory acquisition; and retention, using an aversive T-maze. When the inventors compared C57BL/6J adropin transgenic mice (AdrTG) with littermate controls, they found that

while cognitive performance is normal in young (less than 12 month) AdrTG, mice, old (18 month) AdrTG mice exhibit significantly better memory acquisition and retention compared to age-matched controls. In addition, the inventors have also shown that adropin levels may be increased systemically by endogenous administration. When adropin was administered via intraperitoneal injection, the subjects with diet-induced obesity experienced metabolic changes suggesting improvements in glucose control and reduced risk for type 2 diabetes. (Kumar *et al.* 2008; Gao *et al.* 2014, 2015)

[0037] The Inventors disclose a method of treating cognitive declines in aged subjects by increasing levels of adropin in the subject's brain by way of exogenous administration.

[0038] In one embodiment, subjects experiencing or at risk of age-related dementia are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin^{34--76}, or variants or derivatives thereof.

[0039] In another embodiment, is an injectable preparation of adropin, adropin^{34--76}, or variants or derivatives thereof, and a pharmaceutical acceptable preparation. A pharmaceutical acceptable preparation may also include a preservative, a composition to block non-specific binding of adropin, by way of example a protein such as gelatin or albumin, or a compound, such as a surfactant, by way of example, Tween 20 preferably at 0.1 to 1 percent. The solution may also include compounds to prevent degradation or aggregation of the peptide while staying within physiological acceptable parameters for injection. By way example, the pH may vary from 5.0 to 6.0, 6.0 to 7.0, 7.0, to 8.0, or 9.0 to 10.0. One or more salts may also be included. By way of non-limiting example, a phycological acceptable concentration of salt may be a concentration, plus or minus up to 5, 10, or 15 percent that normally found in the subject. Non-naturally occurring salts may also be included, by way of example, Tris, HCL. Formulations of adropin may be concentrated to some degree or lyophilized for storage and later hydrated before use.

[0040] The aqueous solution may further contain various salts or buffers that are well known in the art. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable

dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are, Ringer's solution, or isotonic sodium chloride solution.

[0041] Adropin Sequences

[0042] Human adropin Amino Acid Sequence (SEQ ID NO.:1):

```
          10         20         30         40         50
MGAAISQGAL IAIVCNGLVG FLLLLLWVIL CWACHSRSAD VDSLSESSPN
          60         70
SSPGPCPEKA PPPQKPSHEG SYLLQP
```

[0043] Adropin$^{34-76}$, peptide used in the examples for inducing metabolic and cognitive activity (SEQ ID NO.:2):

```
34                                                    76
CHSRSAD VDSLSESSPN SSPGPCPEKA PPPQKPSHEG SYLLQP
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[0044] The sequences disclosed herein represent non-limiting examples. It is anticipated that variations of Adropin and Adropin^{34--76}, may also be effective, particularly those with conservative amino acid substitutions. In addition, variants, analogs and derivatives of Adropin polypeptide sequences are anticipated including but not limited to the following list of possible modifications. Chemical design strategies to avoid aggregation, including corruption of hydrophobic patches by means of amino acid substitutions or *N*-methylation of amino acids. Physicochemical properties of adropin may be improved by the introduction of stabilizing α-helixes, introduction of salt bridge formations or lactam bridges. The half-life may be extended through identifying cleavage sites and substitution of amino acids, stabilizing the secondary structure of the molecule, peptide acylation, insertion of albumin-binding elements into the peptides backbone, conjunction to antibody fragments, or polyethylene-glycol (PEG)-ylation.

[0045] Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences. In sequence comparisons, the two sequences being compared are aligned using the Clustal method (Higgins et al, Cabios 8:189-191, 1992) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, Wis.). In this method, multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment =10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise alignment=5. The residue weight table used for the alignment program is PAM250 (Dayhoff et al., in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NBRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

[0046] Variations in the amino acid sequence will likely be comprised of conservative amino acid substitutions, or substitutions of amino acids outside of function regions. It is anticipated that polypeptides with 85 percent or more, 90 percent or more, 95 percent or more or 98 percent of more identity to Adropin and Adropin^{34--76}, may be effective at treating cognitive decline. It is also anticipated that the variants described herein include variants not found in nature.

Subjects

[0047] It is envisioned that subjects selected for treatment would include human subjects, particularly human subjects diagnosed with dementia, or suffering from cognitive decline or at risk for cognitive decline. Human subjects at risk for cognitive decline, would include, by way of example, the elderly, subjects at risk for, or who have

experienced: stroke, Parkinson's Disease, people identified as being "at risk" for early-onset Alzheimer's disease including those with a family history or with risk genes: APOE-e4, amyloid precursor protein, presenilin-1, presenilin-2), traumatic brain injury, chronic poorly-controlled hypertension, type 2 diabetes, sleep apnea, disruption of normal sleeping patterns, elevated biomarkers of systemic and/or cerebral inflammation, hypercholesterolemia, and tests for biomarkers in the blood plasma that are currently under development. Surgical interventions have also been indicated to induce inflammation in the nervous system and dementia. The inventors anticipate administration of endogenous adropin or adropin$^{34-76}$ may be used as an effective post-operative prophylactic to reduce risk for dementia in elderly patients.

[0048] Other subjects who may benefit from Adropin treatment include, by way of non-limiting example, laboratory test animals, livestock, show animals and house hold pets.

Treatment

[0049] Adropin, Adropin$^{34-76}$, or variants. analogs, or derivates thereof, may be administered to a subject parenterally. By way of non-limiting example, by subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Solutions or suspensions of the peptide may be prepared in pharmaceutically acceptable salts as an aqueous solution. Peptide solutions may also be prepared in glycerol, liquid polyethylene glycols, or mixtures thereof of pharmaceutically acceptable oils. A preservative may be added to the peptide preparation to prevent microbial growth. Preparations may also contain a pharmaceutically acceptable protein, such as gelatin or albumin, to inhibit nonspecific binding of the protein to storage vehicles. By way of example, studies in mice using adropin$^{34-76}$ to improve cognition have used 0.1% bovine serum albumin for this purpose.

[0050] Using mice, the Inventors have shown that administration of adropin$^{34-76}$, in doses as low as 90 nmol/kg/day, delivered as two 45 nmol/kg injections, can be effective in producing changes in metabolism. Dose as high as 900 nmol/kg/d are well

tolerated in mice. While the doses required for a desired clinical effect in humans is expected to vary between patients, the 90 nmol/kg dose administered by subcutaneous, intramuscular, or intravenous injection, either once or twice a day, may represent a preferred treatment protocol. It is anticipated that the treatments would be administered daily, although long-acting formulations involving weekly injections are envisaged. The optimum dose and frequency for a particular subject may be determined by the patient's treating physician, based on the patient's cognitive response. It is anticipated that adropin therapy may or may not improve a subject's cognitive function relative to current baseline, however adropin therapy may result in significant delays in the advancement of the disease. Improvement may manifest as a significant delay or a reduced rate of subsequent cognitive decline.

[0051] In one non-limiting embodiment, is a human subject, exhibiting evidence of age-related cognitive decline or dementia, treated with an injection of about 450 nmol/kg of adropin$^{34-76}$, a dose known to affect metabolic control in male B6 mice. Symptoms of dementia can vary greatly between human subjects. At least two of the following core mental functions must be significantly impaired for a diagnosis of dementia to be considered: memory, communication and language, ability to focus and pay attention, reasoning and judgement, visual perception. Medical doctors will diagnose Alzheimer's and other types of dementia based on a careful examination of medical history; physical examination; laboratory tests; characteristic changes in thinking; and assessment of day-to-day function and behavior associated with each type and can determine that a person has dementia with a high level of certainty. It is more difficult to determine the exact type of dementia because symptoms and changes in brain morphology of the different types of dementias can overlap. In some cases, a doctor may diagnose "dementia" and not specify a type. The term dementia, as used herein, is meant to include all forms of dementia. Non-limiting symptoms of dementia included loss of: memory, communication and language skills, an ability to focus and pay attention, reasoning and judgment, as well as visual perception. It is anticipated that treatment would be initiated soon after the diagnosis of dementia. The treatment will be repeated daily for the remaining life of the subject. Similar subjects may receive sham treatment. After an appropriate period of time for the particular subject, both groups will

be subjected to cognitive testing. It is anticipated that subjects receiving adropin or continuous adropin administration, will perform better on cognitive testing compared to subjects receiving sham treatment.

[0052] Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

EXAMPLES

Materials and Methods

[0053] Using a Cre-inducible reporter model developed by the Inventors (Fig. 3), adropin expression was observed in hippocampal neurons (Fig. 4) typically related to learning and memory (Eichenbaum et al., (2017) Curr Opin Behav Sci. 2017;17, 65--70).

[0054] The AdrTG model used for these experiments was developed by the Inventors using a human β–actin promotor to drive overexpression of the adropin open reading frame in a synthetic gene in B6 mice (Kumar, et al., (2008) Cell metabolism, 8, 468---481; Gao, et al., (2014) Diabetes 63, 3242---3252). Increased expression of adropin is evident in all tissues examined, including the nervous system (*Id.*) and data not shown, particularly in tissues where expression of the endogenous gene is low (for e.g., skeletal and cardiac muscle, adipose tissue). An analysis of adropin expression in the hippocampus of male mice housed at SLU suggests a 40% increase in relative expression (WT, 1.00±0.02, n=6;; AdrTG, 1.42±0.07, n=7;; $p<0.01$ by Student's t--test).

Transgenic Mouse Model

[0055] AdrTG were generated and maintained as previously described (Kumar et al., (2008) Cell metabolism.;8(6):468-481; Ghoshal et al., (2018) Molecular

metabolism 8:51-64; Gao S, et al., (2014) Diabetes. 2014;63(10):3242-3252) For aging studies, male mice were maintained in group housing (3-4/cage) on standard rodent chow. Fasting glucose and glucose clearance were assessed in mice fasted for 6h. Glucose tolerance tests were performed using 1 mg/kg dextrose administered intraperitoneally (ip.), as previously described. (Kumar, et al., (2008) Cell metabolism, 8, 468---481; Gao, et al., (2014) Diabetes 63, 3242-3252)

[0056] For visualization of cells expressing adropin, an IRES-Cre was inserted into the 3'untranslated region (UTR) of the adropin open reading frame in exon 2 (Fig. 1). The targeting vector was constructed using recombineering system. Isogenic DNA containing the Enho locus was retrieved from genomic colony RP23-100C7 of C57Bl/6 BAC genomic library via gap repair. An IRES-Cre-Frt-neo-Frt was inserted into 3' 53 bp downstream of the translational stop codon in exon 2. For gene targeting, 50µg of linearized targeting vector consisting of 3.5kb 5'arm and 7.2 kb 3' arm was electroporated into Bruce4 B6 embryonic stem (ES) cells. Correct homologous recombination in targeted clones was confirmed with Fidelity PCR at the 5' and 3' ends. The fragments produced from Fidelity PCR with these primers were sequenced to further verify the correctness of recombination. Correctly targeted ES cells were injected into Albino B6 blastocysts; germline transmitting chimeric mice were obtained and mated with Albino B6 mice to generate heterozygous carriers of the EnhoIRES-Cre-Frt-neo-Frt on the B6 background. The Frt-neo-Frt sequence was removed using B6;SJL-Tg(ACTFLPe)9205Dym/J transgenic mice purchased from the Jackson laboratory. EnhoIRES-Cre mice were then crossed onto the B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J strain in which a loxP-flanked stop cassette prevents transcription of a red fluorescent protein variant (tdTomato) driven by a CAG promotor (Madisen et al., (2010) Nature neuroscience.;13(1):133-140).

[0057] Gene expression was assessed using qRT-PCR as previously described. Hippocampal and cortical tissue samples were dissected from fresh brains and snap frozen brains on dry-ice cold isopentane. Total RNA was extracted using Trizol (Invitrogen, Life Technologies) and treated with a DNAfree kit (Ambion, Life Technologies). 800 ng of total RNA were used for cDNA synthesis (Superscript III

Reverse transcription kit, Invitrogen). Quantitative PCR was performed in 96-well plates using Taqman gene expression and QuantStudio 7 Detection Systems (Applied Biosystems, Life Technologies); 36B4 was used as the reference gene.

Assessment of Cognitive Function.

[0058] Memory retention and acquisition were assessed using an aversive T-maze and novel objective recognition, as previously described (Farr et al., (2016) J Alzheimers Dis.; 54(4):1339-1348). Briefly, the T-maze consists of a black plastic alley with a start box at one end and two goal boxes at the other. An electrifiable floor of stainless steel rods run throughout the maze to deliver a foot-shock using a scrambled grid floor shocker (Model E13-08, Coulbourn Instruments, Whitehall, PA). Mice are not permitted to explore the maze prior to training. A block of training trials begins when mice are placed in the start box. The guillotine door is raised and a cue buzzer sounded simultaneously (doorbell type, at 55dB); 5s later a mild aversive foot-shock is applied with an intensity of 0.35mA. The arm of the maze entered on the first trial is designated “incorrect” and the mild foot-shock continued until the mouse enters the other goal box, which in all subsequent trials is designated “correct” for each mouse. Mice are trained until they made one active avoidance, with an inter-trial interval of 30–35s. The number of trials to make one active avoidance is the measure of acquisition. Retention is tested 7d later, with training continued until the criterion of making five active avoidances in six consecutive trials is achieved. The number of trials needed to reach this criterion is the measure of retention(Butler et al., (2012) The Journal of clinical endocrinology and metabolism.;97(10):3783-3791; Wong et al., (2014) The Journal of biological chemistry.; 289(37):25976-25986).

[0059] For the novel object recognition task, mice are habituated to an empty apparatus, a 58×66×11-cm white plastic box, for 5min a day for 3 consecutive days prior to entry of objects. On the first day of training, mice are placed in the testing apparatus with two identical objects (A and B). 24h later the animal is reintroduced to the arena but this time one of the original objects is removed and a new object (C). Total time spent exploring each of the two objects is recorded. Anxiety will be assessed using open field and elevated plus maze tests. The experimental apparatus consists of

a central platform, two open arms and two equal-sized closed arms opposite to each other. The test consisted of placing a mouse in the central platform facing an enclosed arm and allowing it to freely explore the maze for 5 min. The number of entries into the open and closed arms and the time spent in open arms was measure by an observer blind to treatment

[0060] Statistical Analysis. Data were compiled in Microsoft Excel. Statistical analysis requiring analysis of covariance used univariate analysis in SPSS Statistics Version 23 (IBM). Student's t-test was used for comparisons of 2 groups.

EXAMPLE 1

[0061] The Inventors assessed cognitive performance using a strain of B6 mice over expressing the adropin open reading frame under the control of a human bβ-–actin promotor (AdrTG) (Kumar, et al., (2008) Cell metabolism, 8, 468---481; Gao, et al., (2014) Diabetes 63, 3242---3252) . Analysis of male adropin transgenic mice (AdrTG), mice aged <1yr, suggested normal performance in tests of declarative memory, and of spatial learning and memory (data not shown). However, at 18 months of age, clear differences in performance were noted (Fig. 3), indicating a neuroprotective role from age related cognitive decline. Alternatively, there may be a "ceiling effect" in younger mice, with improved cognitive performance only observed when the ability for memory consolidation declines in the control group. Performance in tests of declarative memory, and of spatial learning and memory, were not significantly different in AdrTG and littermate controls aged <12 months (data not shown). However, at 18 months AdrTG exhibited significantly improved cognitive performance (Fig. 5). Adropin signaling in the CNS may thus prevent and/or delay cognitive decline observed with aging of male B6 mice. The transgenic animals created by the inventors demonstrate that if adropin levels in the brain are maintained, cognitive decline may be slowed, delayed or prevented.

EXAMPLE 2

[0062] Continuous exposure to adropin reduces stress in transgenic mice. Adropin transgenic mice show reduced stress indicators compared to wide type. Reduced inflammation (Figure 6) and reduced oxidative stress (Figure 7) are seen in AdrTG transgenic mice. Reduced expression of inflammatory cytokines in cortical (Figure 6A) and hippocampal (Figure 6B) are seen in tissue samples from 18 mo. old AdrTG relative to age-matched controls. *, $p<0.05$; **, $p<0.0$. In addition, increased 4-HNE is seen with aging in male B6 mice (Figure 7A), but less so in adropin transgenic mice (AdrTG) (Figure 7B) suggesting a relative reduction in oxidative stress in the AdrTG transgenic mice. Body composition (Figure 8A-B) and glucose tolerance (Figure 8C-D) are normal in 18 mo. old AdrTG mice compared to age-matched controls. Reduced SAPK/JNK activity in the nervous system of 18-month old adropin transgenic mice is consistent with reduced neuroinflammation (Figure 9). The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses including UV and gamma radiation, ceramides, inflammatory cytokines, and in some instances, growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-MEKK4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4/7. Upon activation, MKKs phosphorylate and activate the SAPK/JNK kinase (Ichijo, (1999) Oncogene 18, 6087-93.). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (Kyriakis and Avruch, (2001) Physiol Rev 81, 807-69.). Both Rac1 and cdc42 mediate the stimulation of MEKKs and MLKs (*Id.*). Alternatively, MKK4/7 can be activated in a GTPase-independent mechanism via stimulation of a germinal center kinase (GCK) family member (Kyriakis (1999) J Biol Chem 274, 5259-62.). There are three SAPK/JNK genes each of which undergoes alternative splicing, resulting in numerous isoforms (Kyriakis and Avruch, (2001) Physiol Rev 81, 807-69.). SAPK/JNK, when active as a dimer, can translocate to the nucleus and regulate transcription through its effects on c-Jun, ATF-2, and other transcription factors (Kyriakis and Avruch, (2001) Physiol Rev 81, 807-69; Leppä and Bohmann (1999) Oncogene 18, 6158-62.).

[0063] Total JNK was measured by Western Blot using Recombinant Anti-JNK2 antibody [EP1595Y] (ab76125) from Abcam. Phosphorylation of JNK (an indicator

of activation) was measured using Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251 (Cell Signaling Technology). The Phospho-SAPK/JNK (Thr183/Tyr185) Antibody detects endogenous levels of p46 and p54 SAPK/JNK dually phosphorylated at threonine 183 (Thr183) and tyrosine 185 (Tyr185). Actin was detected using Cell Signaling 3700s (monoclonal antibody), and is used to estimate loading.

EXAMPLE 3

[0064] To examine the effects of endogenous adropin on a subject's cognitive ability, 34 male C57BL/6J (B6) mice, aged 18 months, were treated with exogenous adropin$^{34-76}$. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME). After acclimation for 1 month, mice were separated into two weight matched groups of 15 mice: Group 1 adropin$^{34-76}$ treatment group and Group 2 saline vehicle control.

[0065] The mice were administered a single intraperitoneal injection (volume 100 µl) each morning (0900h) for 4 weeks. Group 1 was administered adropin$^{34-76}$ at a dose of 90 nmol/kg/day. Group 2 was administered saline. The peptide was purchased from Neoscientific Inc. Behavioral testing began after 2 weeks of injections. Injections continued though testing until 4 weeks.

[0066] There was no significant effect of adropin$^{34-76}$ treatment on body weight (mean ± SD for weight gain in grams for group 1, 0.7 ± 3.1; group 2, 0.6 ± 2.3, p=0.95 by Student's t-test). One mouse in group 1 died on 08/23/19. (Figure 10)

EXAMPLE 4

[0067] Adropin treatment improved cognitive performance as measured by the Novel Object Recognition test (Figure 11, Table 1). A non-amnesic subject will spend more time exploring the novel object than the familiar one. When adropin treated and vehicle treated mice were subjected to the Novel Object Recognition test, adropin treated mice spent more time exploring the novel object (Figure 11, Table 1)

[0068] Table 1 Novel Object Recognition test 24 Hour delay % Novel Object

	Vehicle	Adropin

65.00	40.74
43.48	64.58
46.88	62.50
40.00	58.82
31.48	55.56
44.44	77.50
0.00	66.67
42.50	79.10
60.00	51.06
40.00	100.00
52.94	51.61
33.33	46.43
52.63	42.86
71.79	53.06
33.96	37.50

[0069] Statistical analysis of Table 1.

Statistical analysis	Data 1
Column B	Adropin
vs.	vs.
Column A	Vehicle
Unpaired t test	
P value	0.0183

P value summary	*
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.505 df=28
How big is the difference?	
Mean ± SEM of column A	43.90 ± 4.350, n=15
Mean ± SEM of column B	58.88 ± 4.106, n=15
Difference between means	14.99 ± 5.982
95% confidence interval	2.733 to 27.24
R squared	0.1831
F test to compare variances	
F,DFn, Dfd	1.122, 14, 14
P value	0.8320
P value summary	ns
Significantly different? (P < 0.05)	No

Example 5

[0070] Adropin treatment also improved performance during the Acquisition T-maze test. Mice prepared with adropin or placebo in Example 3 were subjected to the Acquisition T-maze test. Adropin treated mice performed better (reduced time in maze) then placebo treated mice (Figure 12, Table 2).

[0071] Table 2. T- maze acquisition

Vehicle	Adropin
8.	7.
13.	6.

10.	7.
7.	8.
8.	8.
12.	7.
9.	8.
10.	9.
14.	6.
12.	9.
13.	7.
13.	6.
10.	8.
11.	7.
12.	

[0072] Statistical analysis of Table 2

Statistical analysis	T-maze Act
Column B	Adropin
vs.	vs.
Column A	Vehicle
Unpaired t test	
P value	< 0.0001
P value summary	****

Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=5.464 df=27
How big is the difference?	
Mean ± SEM of column A	10.80 ± 0.5538, n=15
Mean ± SEM of column B	7.357 ± 0.2695, n=14
Difference between means	-3.443 ± 0.6301
95% confidence interval	-4.736 to -2.150
R squared	0.5251
F test to compare variances	
F, DFn, Dfd	4.525, 14, 13
P value	0.0099
P value summary	**
Significantly different? (P < 0.05)	Yes

[0073] Adropin treated mice also showed superior performance in the T maize retention. (Figure 12, Table 3) Mice were retested in the maze after 7 days and Adropin treated mice retained learned memories and complete the maze in lets time.

[0074] Table 3. T-maze Retention

Vehicle	Adropin
9.	6.
9.	
10.	7.

11.	6.
7.	6.
9.	7.
9.	7.
11.	8.
9.	6.
9.	6.
9.	9.
9.	7.
10.	7.
7.	7.
10.	7.

[0075] Statically analysis of Table 3

Statically analysis	T-maze Ret
Column B	Adropin
vs.	vs.
Column A	Vehicle
Unpaired t test	
P value	< 0.0001
P value summary	****
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed

t, df	t=6.178 df=27
How big is the difference?	
Mean ± SEM of column A	9.200 ± 0.2960, n=15
Mean ± SEM of column B	6.857 ± 0.2310, n=14
Difference between means	-2.343 ± 0.3792
95% confidence interval	-3.121 to -1.565
R squared	0.5857
F test to compare variances	
F,DFn, Dfd	1.759, 14, 13
P value	0.3169
P value summary	ns
Significantly different? (P < 0.05)	No

Example 6

[0076] Epidemiological studies suggest that the development of hypercholesterolemia in the mid- (as opposed to late-) stages of life associates with increased risk for Alzheimer's disease. Type 2 diabetes is also a major risk factor for dementia and is commonly associated with dyslipidemia. The inventors set out to demonstrate that adropin would be protective under such conditions and to this end, crossbreed transgenic mice to produce mice with severe metabolic dysregulation, which were also continuously exposed to elevated levels of adropin. Low-density lipoprotein receptor deficient (Ldlr-/-) mice were used as a model of hypercholesterolemia (see Ishibashi, et. al. J. Clin. Invest., 92 (1993), pp. 883-893). Adropin transgenic mice (AdrTG) were crossed onto the Ldlr-/- background (see Ghoshal et. al., Mol Metab. 2018 Feb;8:51-64.). It was found that the adropin transgene did not prevent hypercholesterolemia due to loss of LDLR.

[0077] To further enhance the effects of metabolic dysregulation in their transgenic mouse model, the inventors placed these mice on a high fat/high sugar diet (HDF) (Research Diets 12451, 45% kcal/fat, 35% kcal/sucrose, 20% kcal/protein) for 3 months. At the end of this time, spatial learning and memory testing was compared between AdrTG;Ldlr-/- mice and Ldlr-/- mice. A group of 10 age-matched male C57BL/6J mice maintained on stand low-fat rodent chow were included as controls.

[0078] Ldlr-/- mice (11 females, 7 males), AdrTG;Ldlr-/- mice (6 females, 10 males) and age-matched C57BL/6J mice (n=12) were placed on a high fat diet for 3 months. Note all transgenic mice are on the same C57BL/6J background. Body composition was determined at baseline and after 3 months, after which cognitive function was assessed by Novel Object Recognition and Aversive T-maze testing. All mice gained weight during the study irrespective of sex (Figure 13). Mice fed HFD gained more weight than B6 mice fed chow (13 grams vs. 3 grams). Body composition indicated increases in fat mass (FM) relative to total body weight in animals fed the HFD compared to the control group fed chow. (Figure 14)

[0079] Fed blood glucose levels in all animals were high (>350 mg/dL), indicating dysregulation of glucose metabolism. (Table 4)

[0080] Table 4. Blood glucose levels in transgenic mice on HFC diets

Dependent Variable: Blood Glucose				
group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Female, Ldlr-/-	400	24	353	448
Male, Ldlr-/-	443	29	384	501
Female, AdrTG;Ldlr-/-	374	29	315	432
Male, AdrTG;Ldlr-/-	462	25	411	513
B6 chow	356	21	313	399

[0081] Performance in the novel objective recognition (NOR) test was significantly improved in AdrTG;Ldlr-/- mice relative to Ldlr-/- mice, suggesting improved learning and memory. AdrTG;Ldlr-/- mice spent significantly more time exploring the novel object compared to Ldlr-/- mice. Values are adjusted for sex (Figure 15).

[0082] The inventors demonstrated that the adropin transgene improves cognitive function in a mouse model of type 2 diabetes and hypercholesterolemia. Older patients with type 2 diabetes who are at increased risk for dementia, and who may exhibit symptoms of mild cognitive impairment, could therefore benefit from treatment with adropin$^{34-76}$ administered by daily injection or any of the treatment methods discussed herein.

[0083] All publications and patents cited in this specification are hereby incorporated by reference in their entirety. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

CLAIMS

What is claimed is:

1. A method of treating cognitive decline, in a subject in need, the method comprising administering an effective amount of (SEQ ID NO.:1), (SEQ ID NO.:2) or variants or derivatives thereof, to the subject parenterally.
2. The method of claim 1, wherein (SEQ ID NO.:1), (SEQ ID NO.:2) or variants or derivatives thereof, consists of (SEQ ID NO.:2) and (SEQ ID NO.:1).
3. The method of claim 1, wherein the effective amount is 1000 nmol/kg/day to about 1 nmol/kg/day.
4. The method of claim 1 wherein, an effective amount is about 450 nmol/kg/day.
5. The method of claim 1, wherein the effective amount is about 90 nmol/kg/day.
6. The method of claim 1, wherein the subject is a human subject.
7. The method of claim 1 wherein, the subject in need has been diagnosed with dementia.
8. The method of claim 1, wherein a subject in need, comprises a subject at risk, the subject at risk selected from the group consisting of subjects with: mild cognitive impairment, hypercholesterolemia, type 2 diabetes, or a metabolic dysregulation disorder which results in elevated serum levels of cholesterol and/or triglyceride).
9. The method of claim 1 wherein the subject is a post-surgery subject with symptoms of neuroinflammation.
10. The method of claim 1, wherein the effective amount is administered daily.
11. The method of claim 1 wherein the effective amount is administered one or more times a day, over a period of 2 or more weeks.

12. The method of claim 1 wherein administering an effective amount consists of administering an oligonucleotide enabled to be express SEQ ID NO.:1 or SEQ ID NO.:2.

13. The method of claim 1 wherein, parenterally administration is selected from the group consisting of intraperitoneal, subcutaneous, intramuscular, and intravenous injection.

14. The method of claim 1 further comprising a response from the subject wherein the subject's cognitive decline is arrested, reduced or slowed.

15. A composition for treating age related dementia, comprising (SEQ ID NO.:1), (SEQ ID NO.:2), variants or derivatives thereof, prepared in pharmaceutically acceptable aqueous solution.

16. The composition of claim 15, wherein, ((SEQ ID NO.:1), (SEQ ID NO.:2), variants or derivatives thereof, is selected form the group consisting of (SEQ ID NO.:1) and (SEQ ID NO.:2).

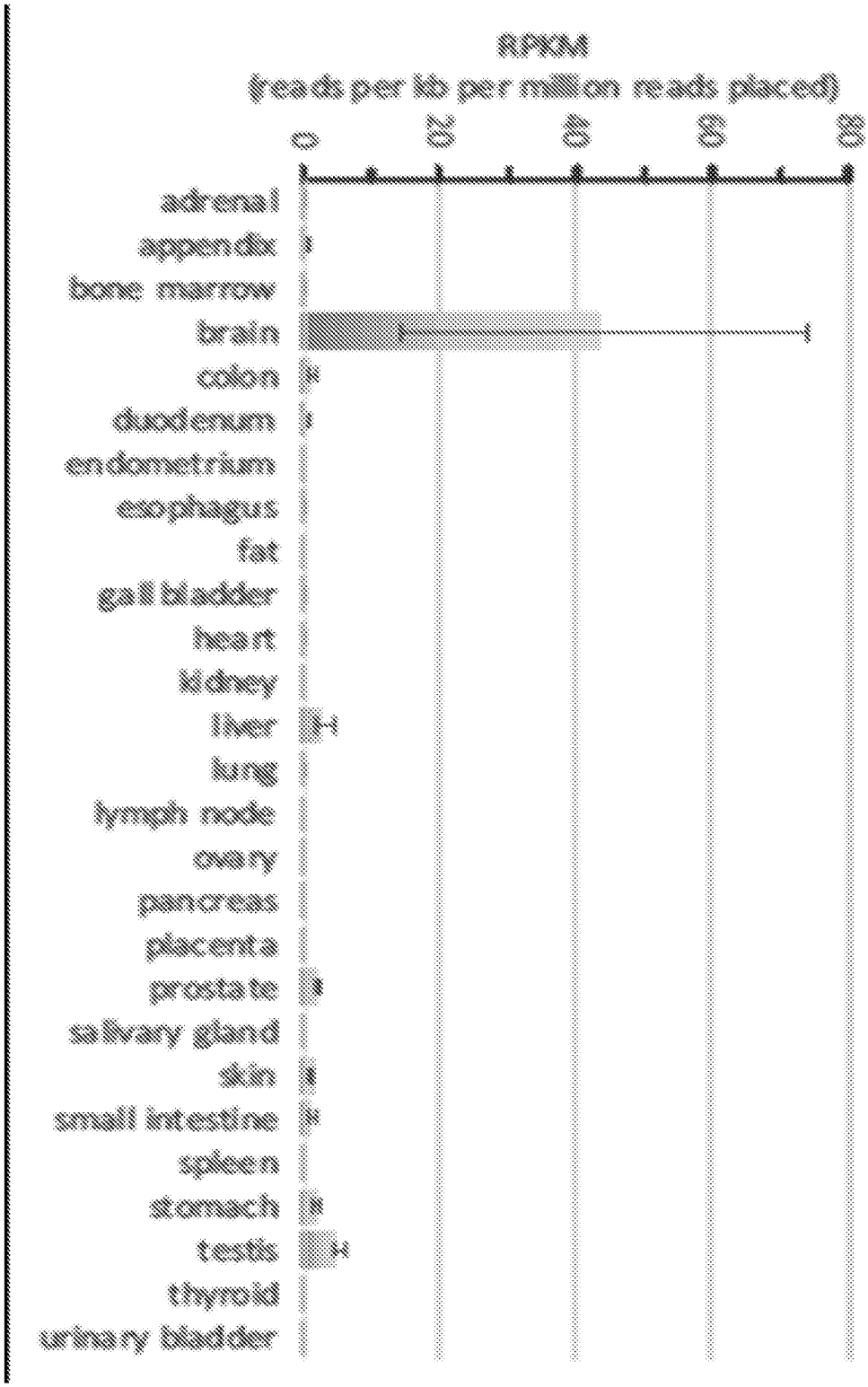
17. The composition of claim 16, further comprising one or more pharmaceutically acceptable compounds selected from the group consisting of, salts, proteins, surfactants, polypeptides, and preservatives.

18. The composition of claim 16, further comprising one or more compounds selected from the group consisting of: glycerol, liquid polyethylene glycol, and pharmaceutically acceptable oils.

19. The composition of claim 16, whereby the composition is not found in nature.

20. A composition for treating age related dementia, comprising a polypeptide with 90 percent or more identity to (SEQ ID NO.:1) or (SEQ ID NO.:2), wherein the composition is effective at treating the subject's cognitive decline.

21. The composition of claim 20, wherein the polypeptide with 90 percent or more identity to (SEQ ID NO:1) or (SEQ ID NO:2), is not found in nature.


RPKM
(reads per kb per million reads placed)
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2
4
6
8
adrenal
appendix
bone marrow
brain
colon
duodenum
endometrium
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gall bladder
heart
kidney
liver
lung
lymph node
ovary
pancreas
placenta
prostate
salivary gland
skin
small intestine
spleen
stomach
testis
thyroid
urinary bladder

Figure 1

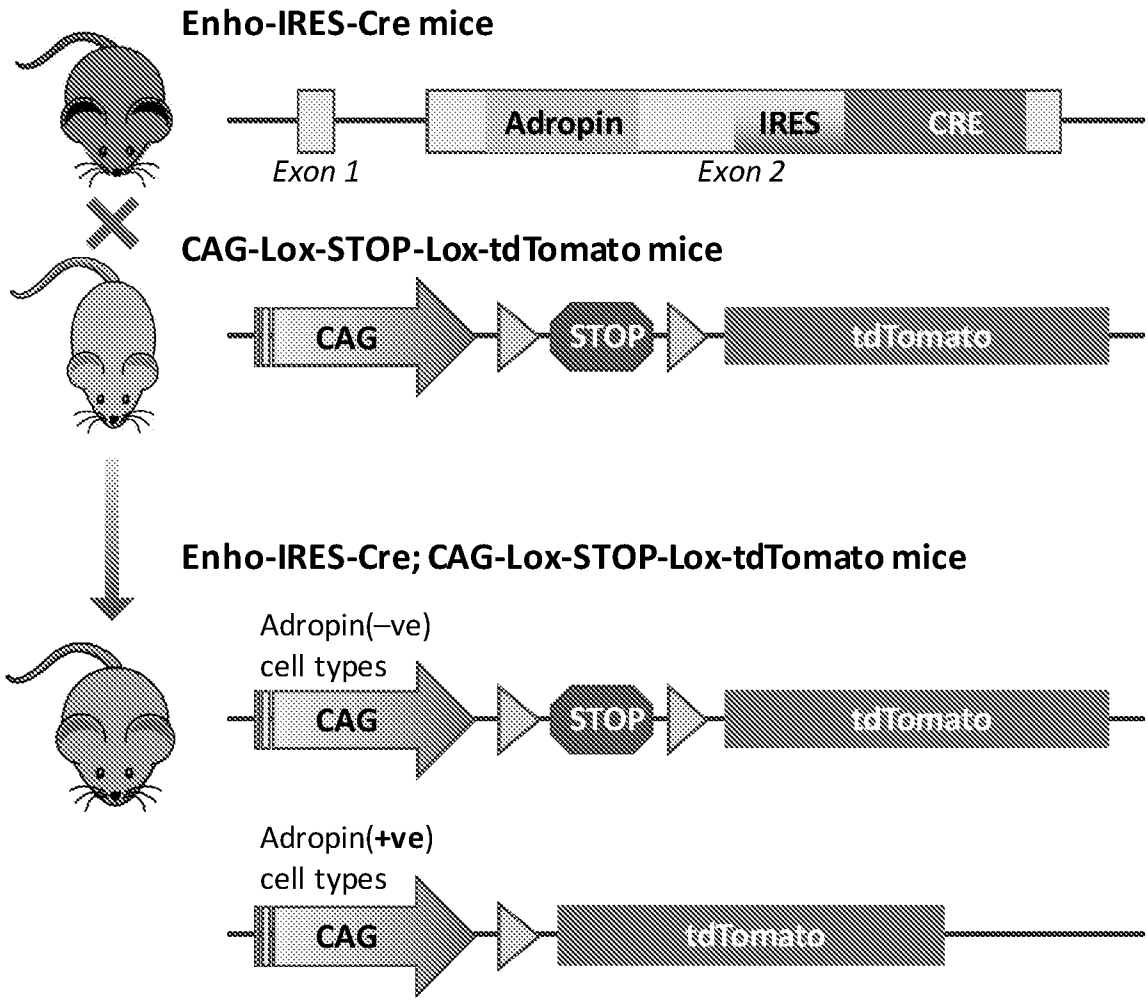

Enho-IRES-Cre mice
Adropin
IRES
CRE
Exon 1
Exon 2
CAG-Lox-STOP-Lox-tdTomato mice
CAG
STOP
tdTomato
Enho-IRES-Cre; CAG-Lox-STOP-Lox-tdTomato mice
Adropin(–ve)
cell types
CAG
STOP
tdTomato
Adropin(+ve)
cell types
CAG
tdTomato

Figure 2

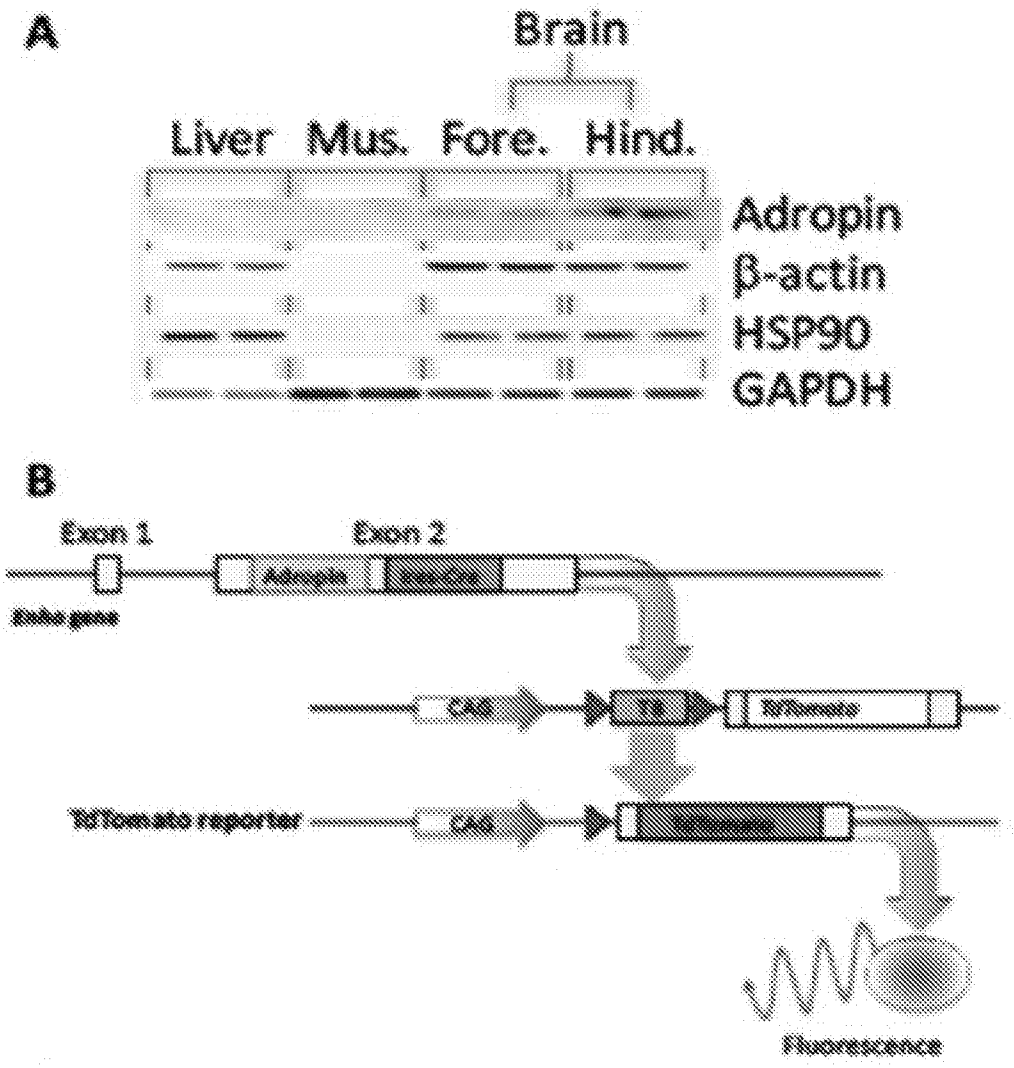

A
Brain
Liver
Mus.
Fore.
Hind.
Adropin
β-actin
HSP90
GAPDH
B
Exon 1
Exon 2
CAG
TdTomato reporter
CAG
Fluorescence

Figure 3

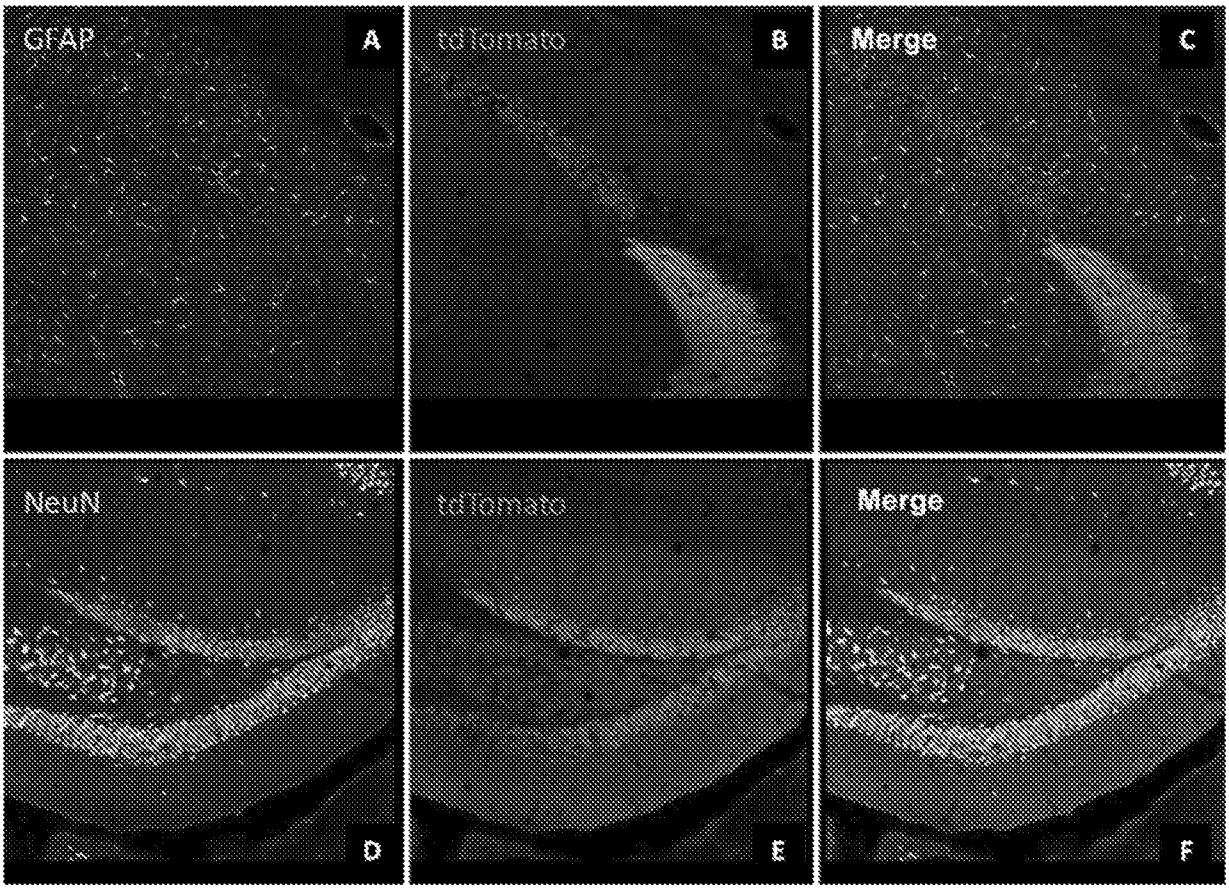


Figure 4

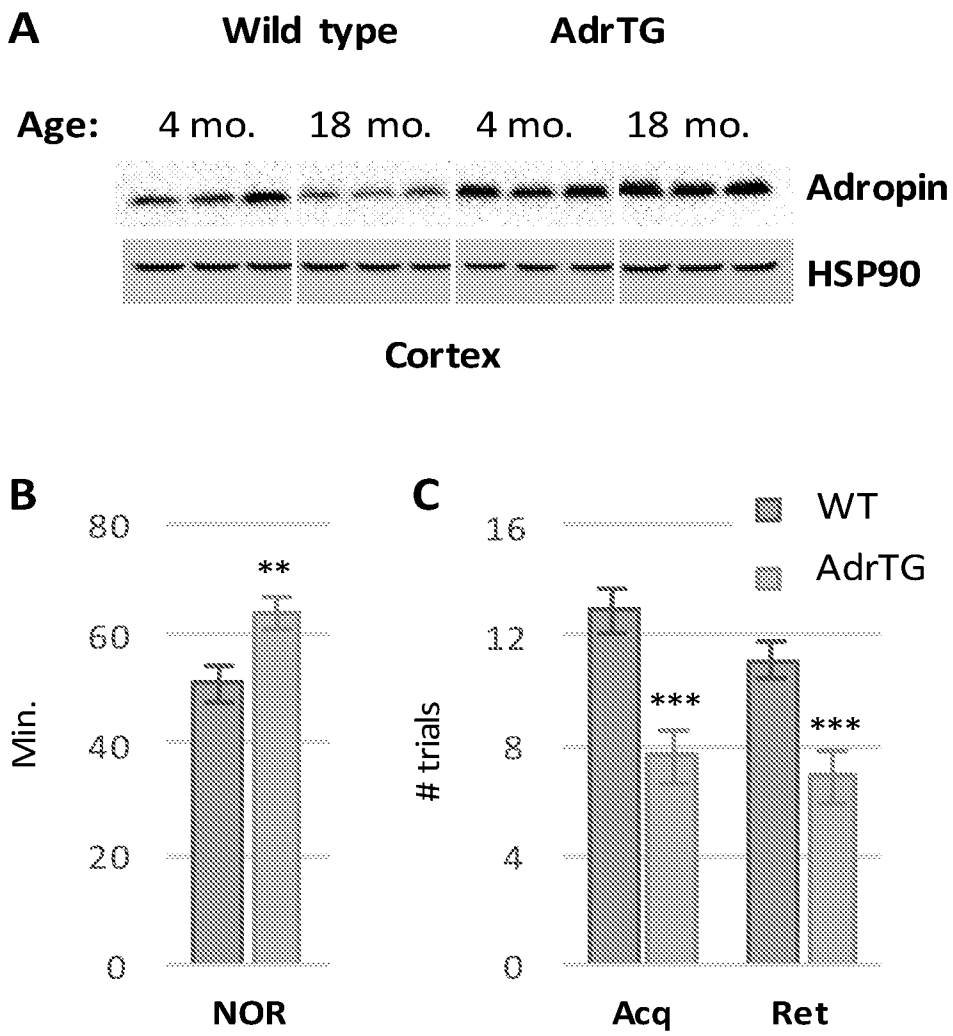
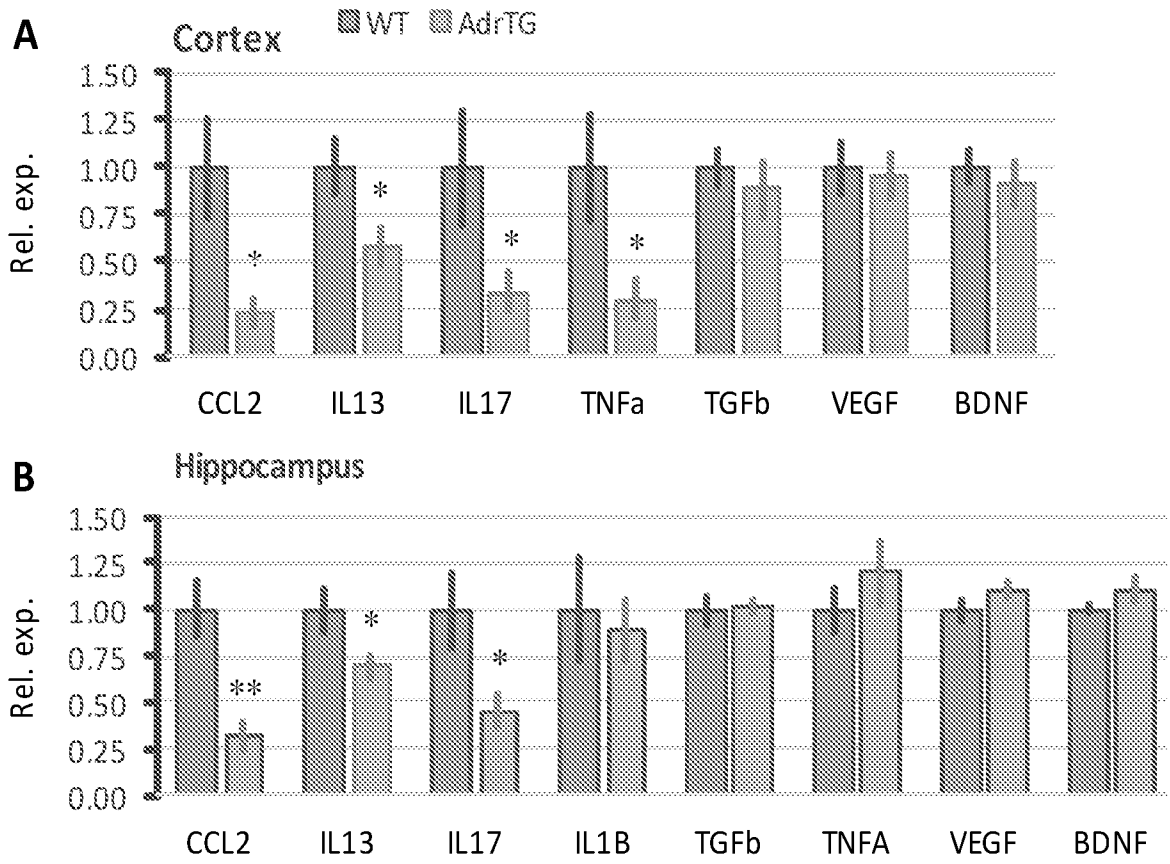

A
Wild type
AdrTG
Age:
4 mo.
18 mo.
4 mo.
18 mo.
Adropin
HSP90
Cortex
B
Min.
80
60
40
20
0
**
NOR
C
trials
16
12
8
4
0
WT
AdrTG
***
***
Acq
Ret

Figure 5


A
Cortex
WT
AdrTG
Rel. exp.
1.50
1.25
1.00
0.75
0.50
0.25
0.00
CCL2
IL13
IL17
TNFa
TGFb
VEGF
BDNF
B
Hippocampus
Rel. exp.
1.50
1.25
1.00
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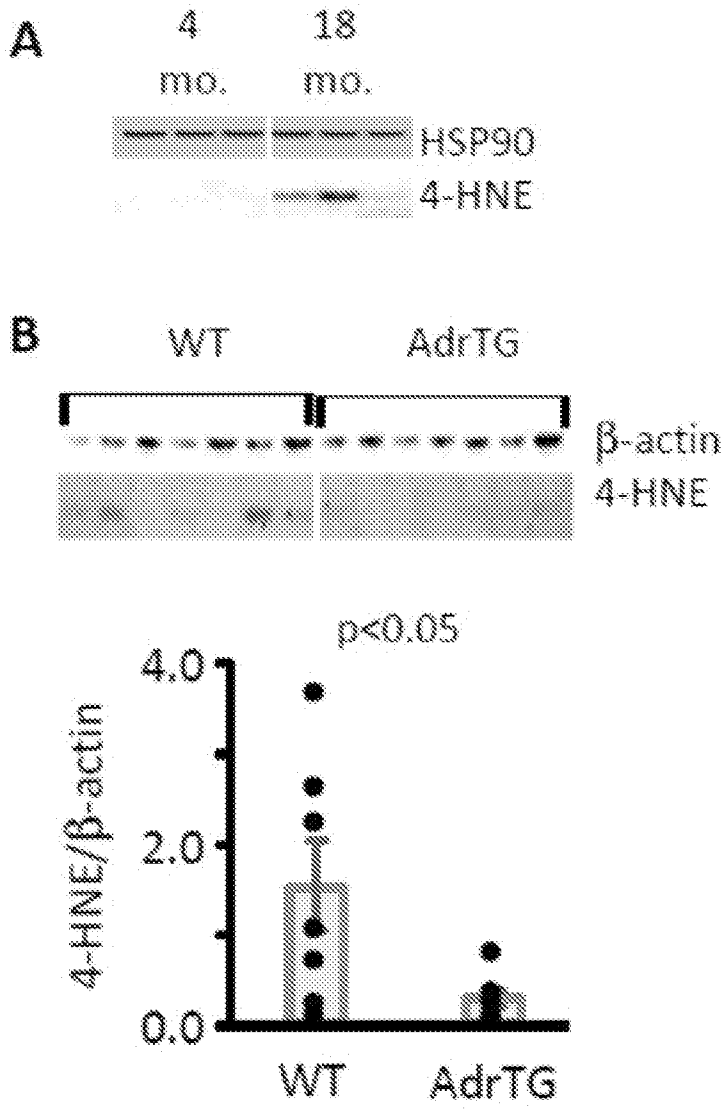
IL1B
TGFb
TNFA
VEGF
BDNF

Figure 6


A
4
mo.
18
mo.
HSP90
4-HNE
B
WT
AdrTG
β-actin
4-HNE
p<0.05
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2.0
0.0
4-HNE/β-actin
WT
AdrTG

Figure 7

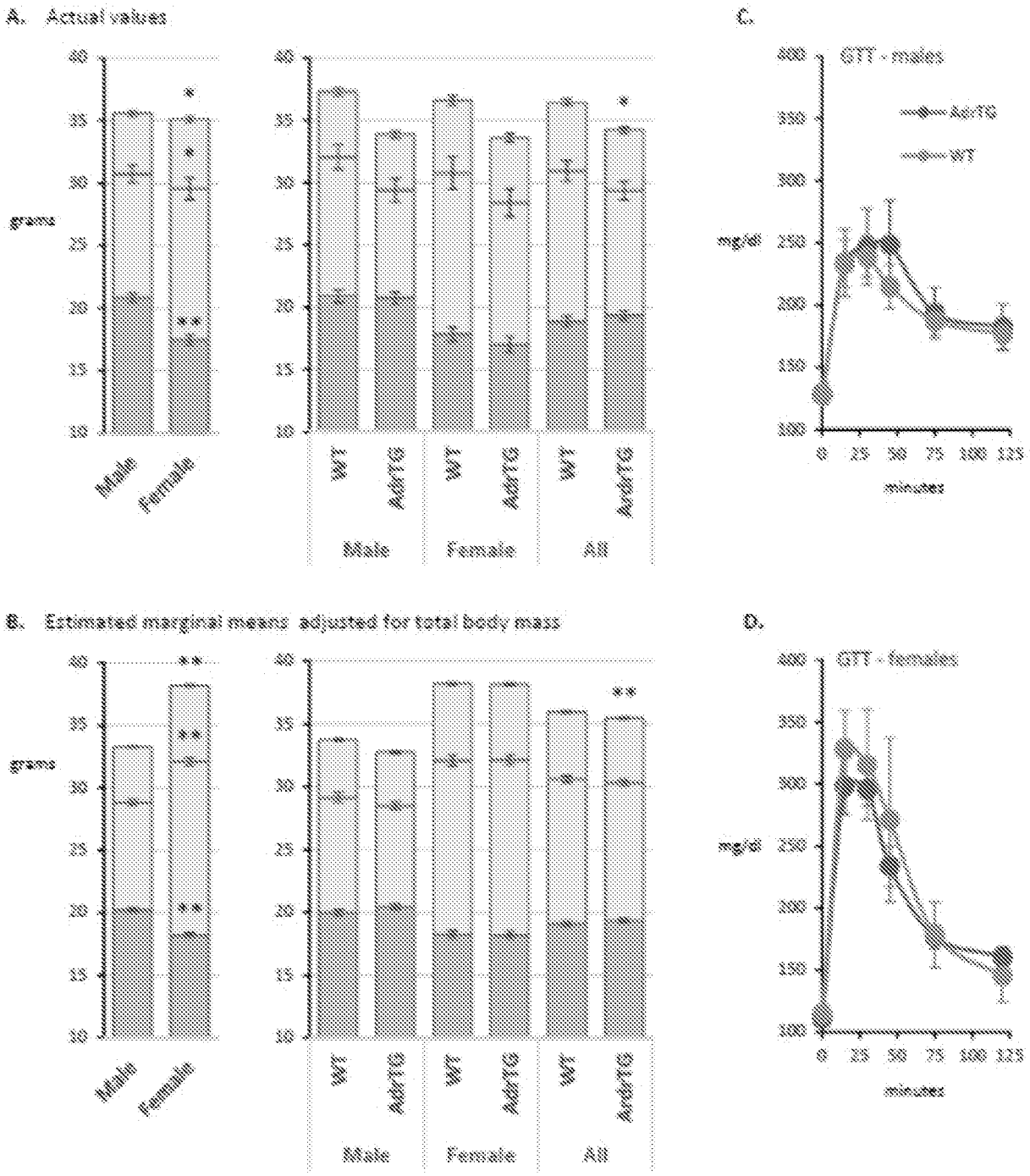

A. Actual values
grams
Male
Female
WT
AdrTG
Male
Female
All
C.
GTT - males
AdrTG
WT
mg/dl
minutes
B. Estimated marginal means adjusted for total body mass
D.
GTT - females

Figure 8

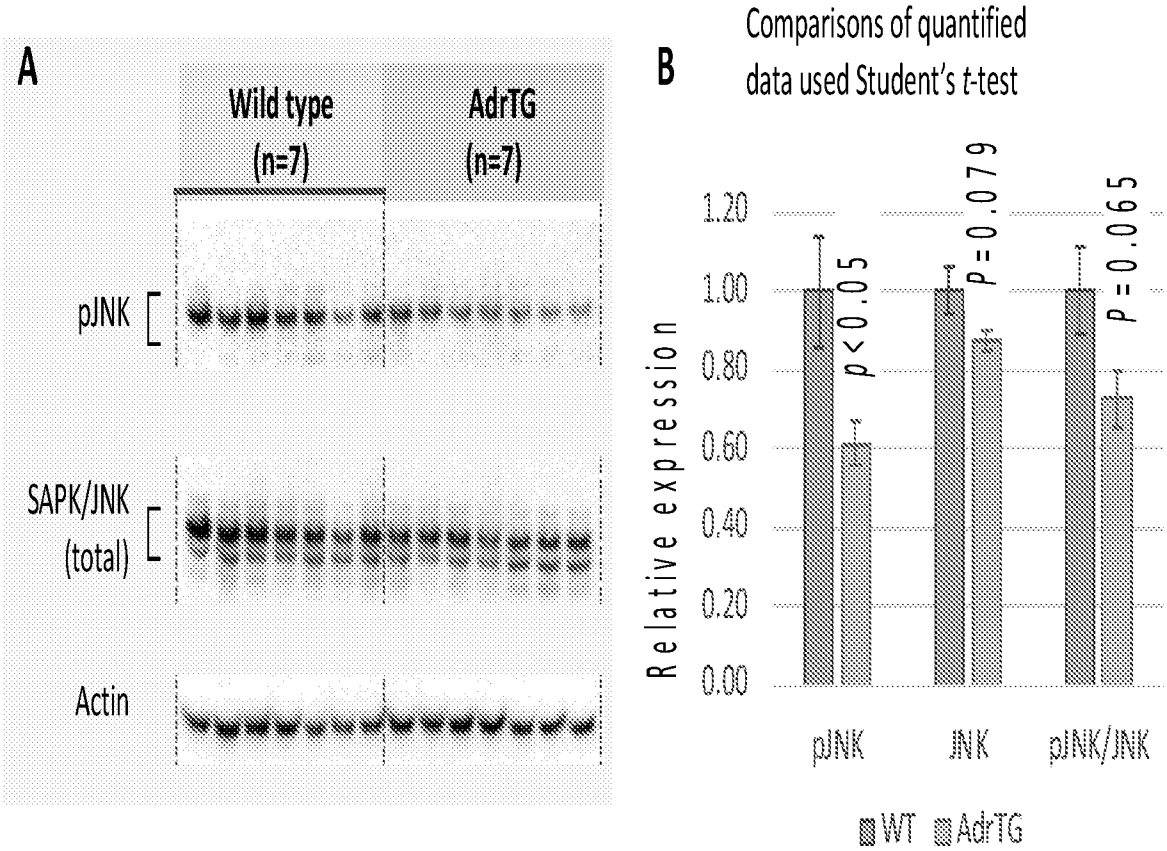
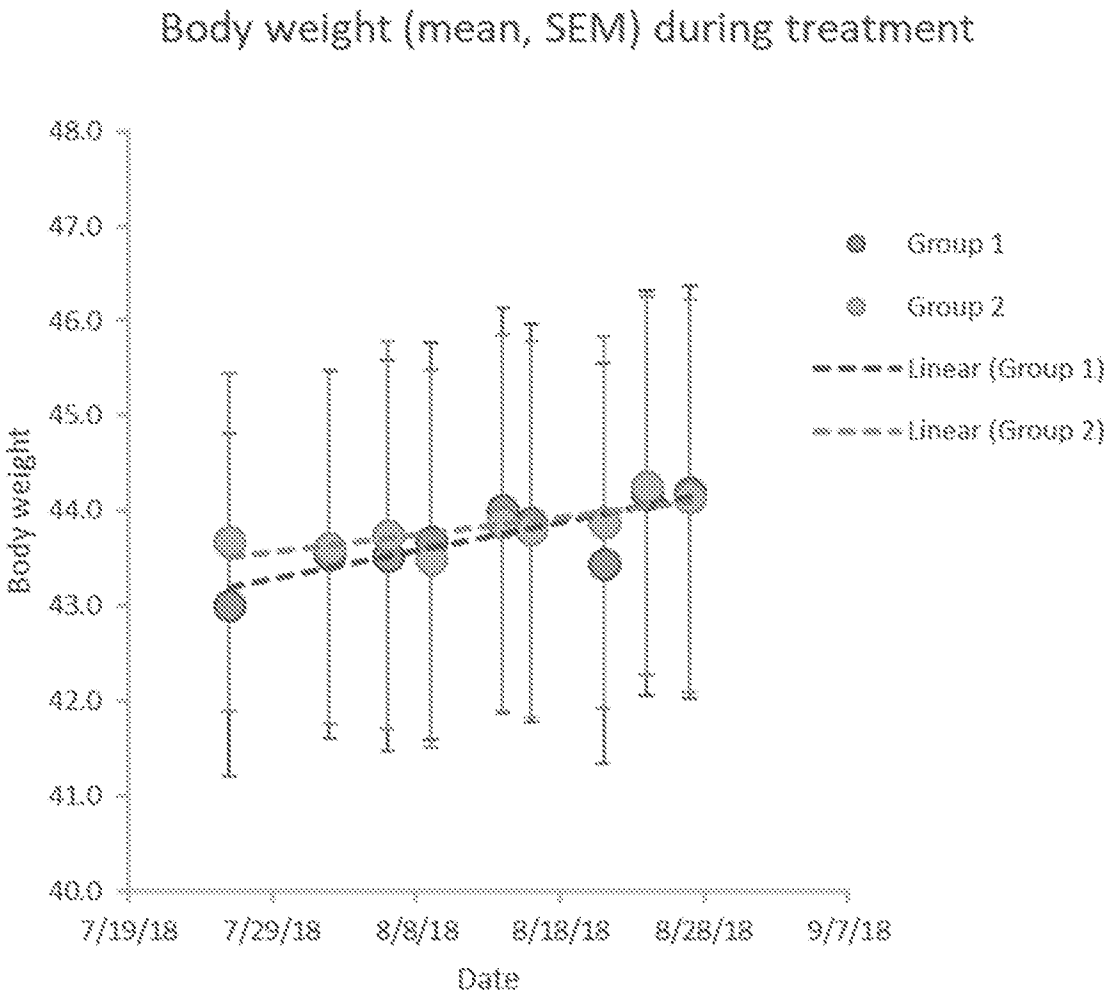

A
Wild type
(n=7)
AdrTG
(n=7)
pJNK
SAPK/JNK
(total)
Actin
B
Comparisons of quantified
data used Student's t-test
Relative expression
1.20
1.00
0.80
0.60
0.40
0.20
0.00
p < 0.05
p = 0.079
p = 0.065
pJNK
JNK
pJNK/JNK
WT
AdrTG

Figure 9


Body weight (mean, SEM) during treatment
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47.0
46.0
45.0
44.0
43.0
42.0
41.0
40.0
Body weight
7/19/18
7/29/18
8/8/18
8/18/18
8/28/18
9/7/18
Date
Group 1
Group 2
Linear (Group 1)
Linear (Group 2)

Figure 10

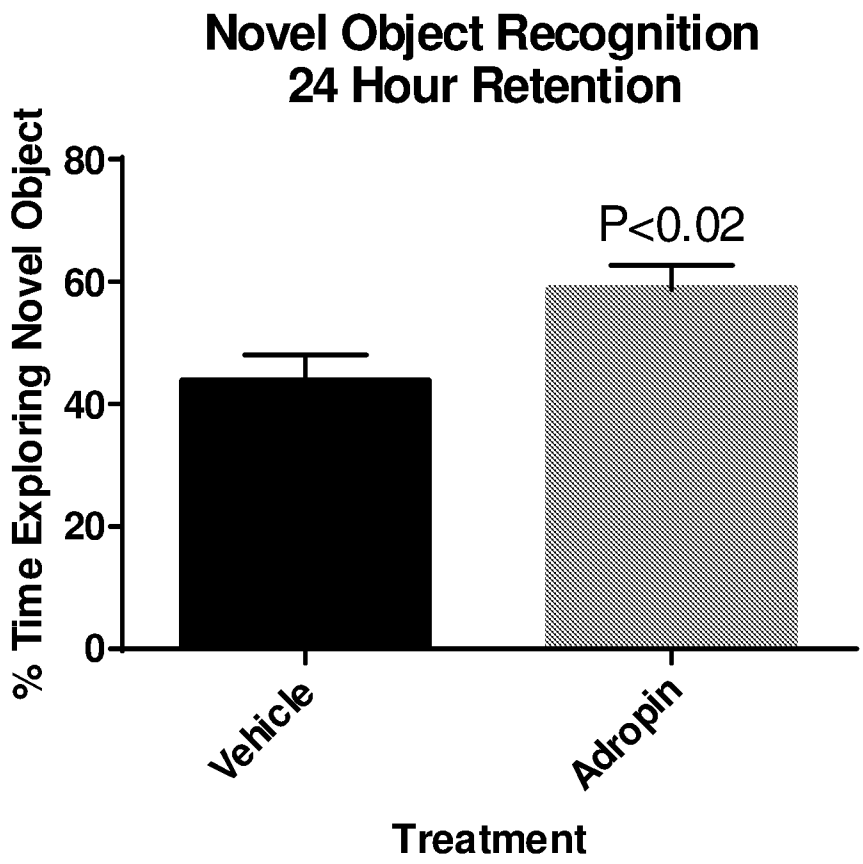
Novel Object Recognition
24 Hour Retention
% Time Exploring Novel Object
80
60
40
20
0
P<0.02
Vehicle
Adropin
Treatment

Figure 11

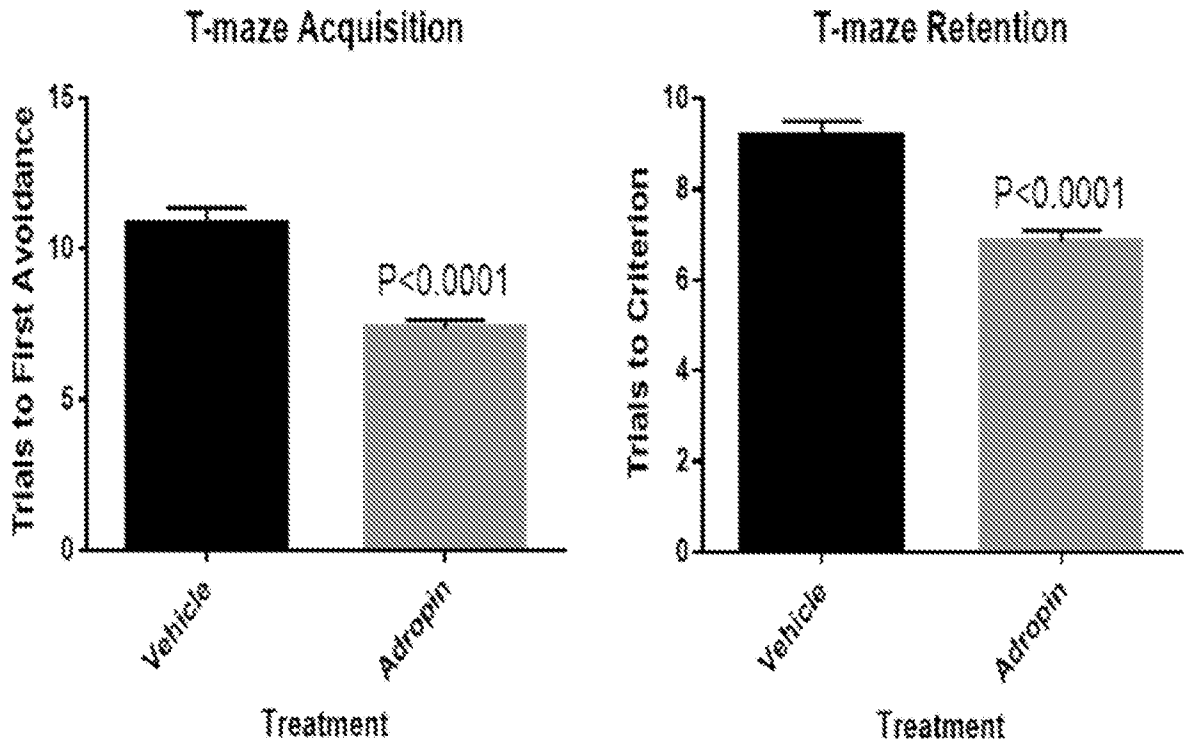
T-maze Acquisition
Trials to First Avoidance
15
10
5
0
P<0.0001
Vehicle
Atropin
Treatment
T-maze Retention
Trials to Criterion
10
8
6
4
2
0
P<0.0001
Vehicle
Atropin
Treatment

Figure 12

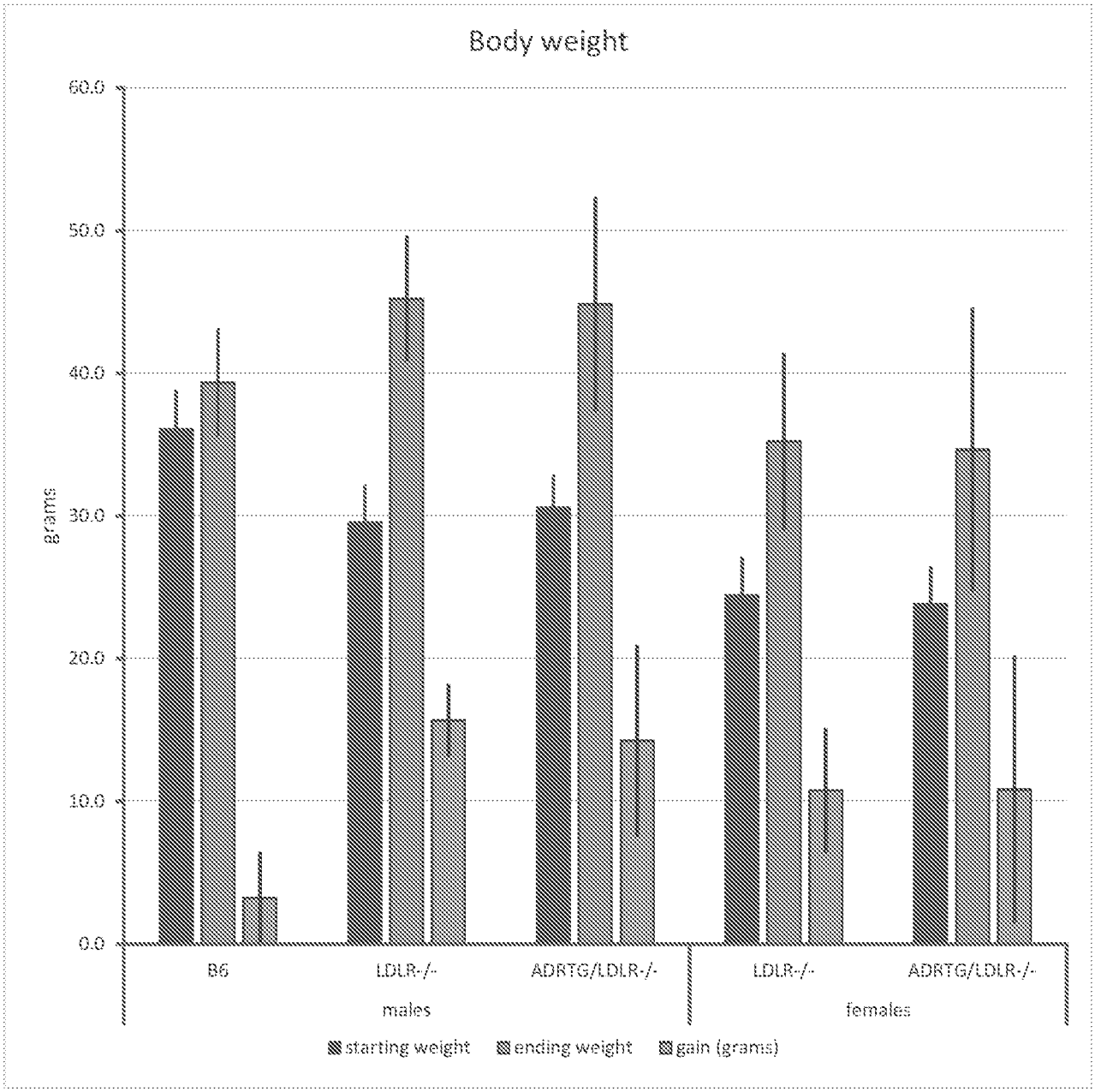
Body weight
60.0
50.0
40.0
30.0
20.0
10.0
0.0
grams
B6
LDLR-/-
ADRTG/LDLR-/-
LDLR-/-
ADRTG/LDLR-/-
males
females
starting weight
ending weight
gain (grams)

Figure 13

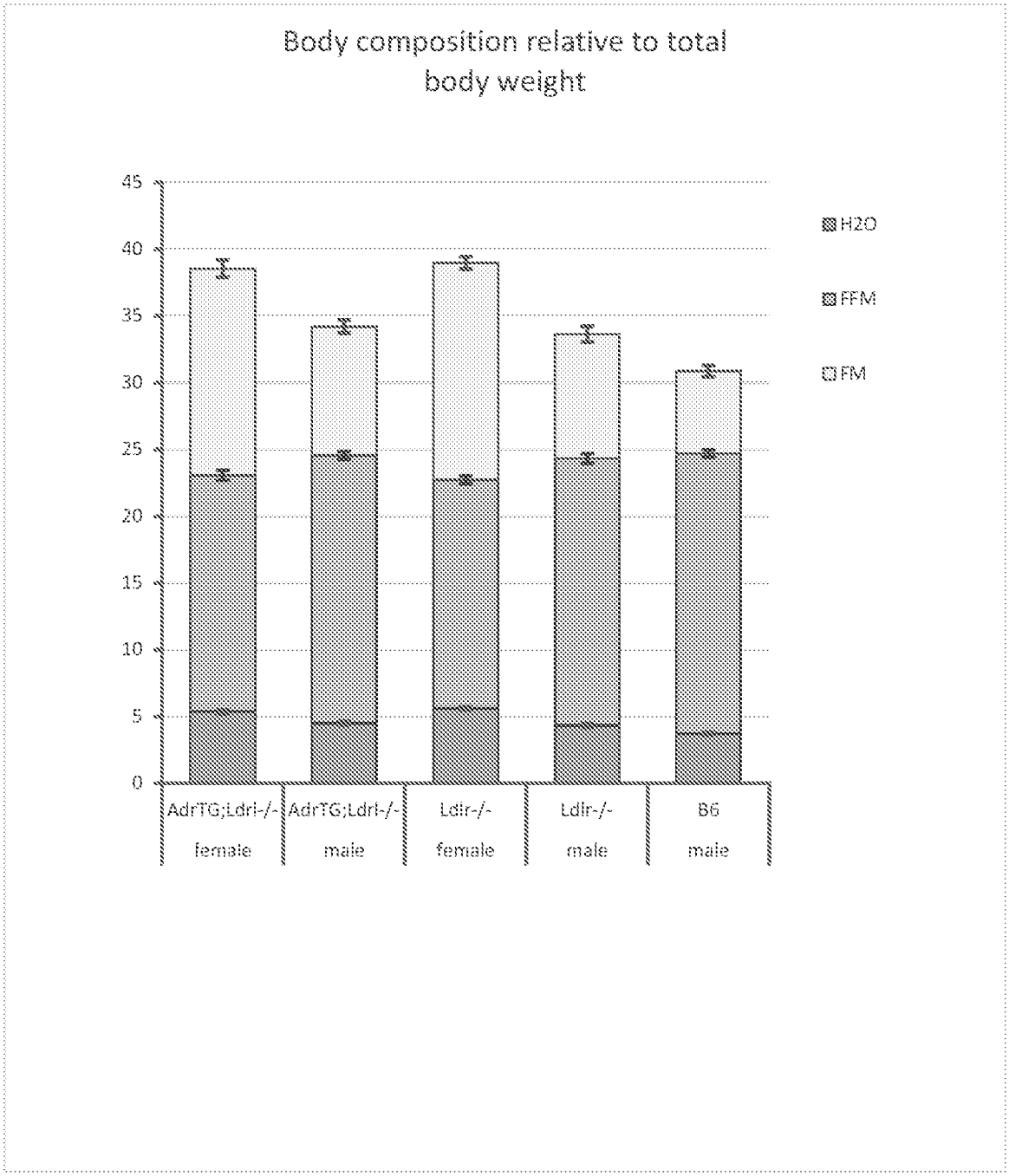
Body composition relative to total body weight
45
40
35
30
25
20
15
10
5
0
H2O
FFM
FM
AdrTG;Ldrl-/- female
AdrTG;Ldrl-/- male
Ldlr-/- female
Ldlr-/- male
B6 male

Figure 14

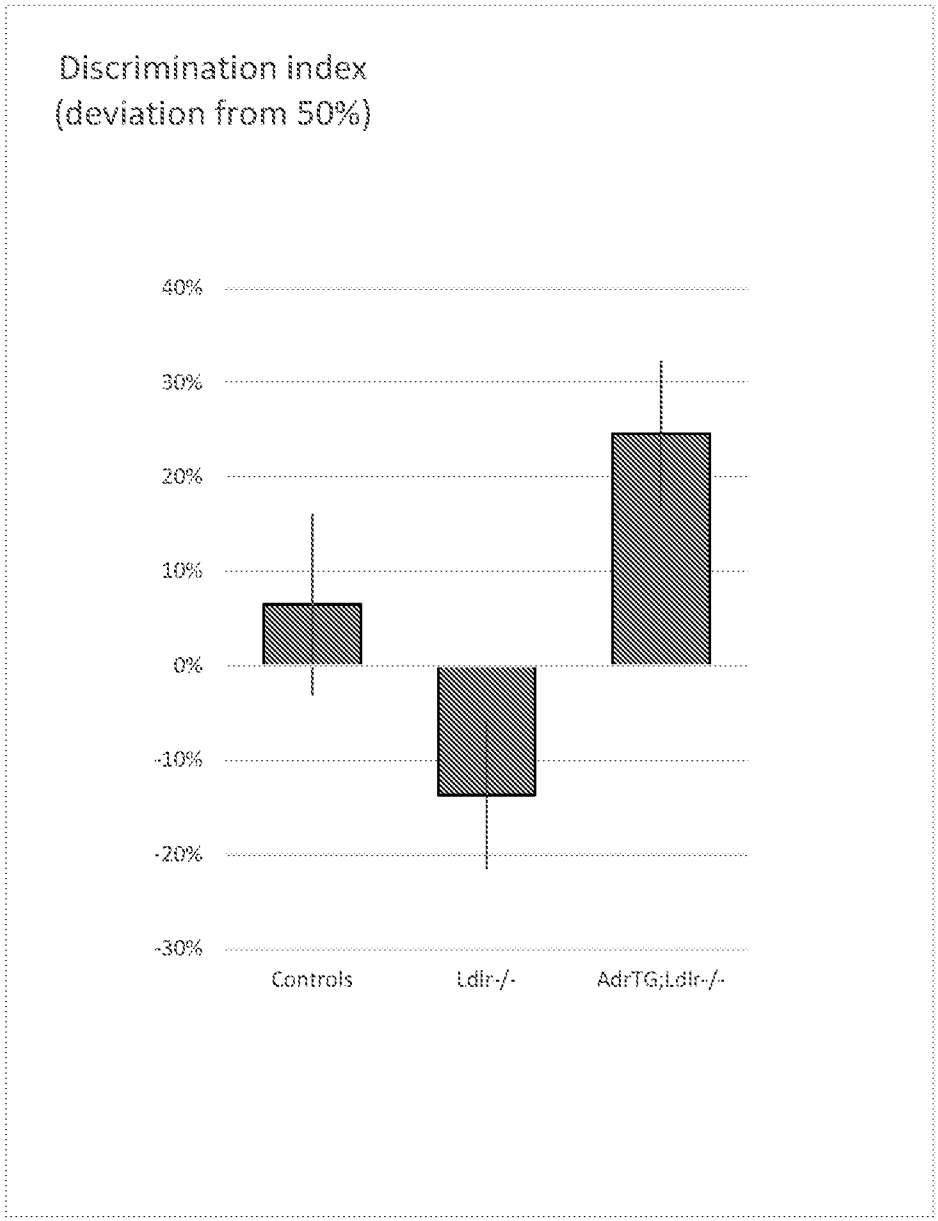
Discrimination index
(deviation from 50%)
40%
30%
20%
10%
0%
-10%
-20%
-30%
Controls
Ldlr-/-
AdrTG;Ldlr-/-

Figure 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/35428

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/35428

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.:
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:

-***-Please see supplemental page-***-

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.:
1-21; SEQ ID NO: 1

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/US19/35428

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/7088, 38/16, 38/22; A61P 3/04, 9/02, 9/12, 25/28; C07K 14/435, 14/575 (2019.01)

CPC - A61K 31/7088, 38/16, 38/22; A61P 3/04, 9/02, 9/12, 25/28; C07K 14/435, 14/575, 14/705; C12N 5/06, 15/09, 15/63; C12P 21/02

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 2003/0096951 A1 (JACOBS, K et al.) 22 May 2003; paragraphs [1767]-[1768], [2864], [3390], [3409], [3431], [3433], [3437]-[3441], [3450]	1-9,12-21 ---------- 10-11
D, Y	(KUMAR, KG et al.) Identification of Adropin as a Secreted Factor Linking Dietary Macronutrient Intake with Energy Homeostasis and Lipid Metabolism. Cell Metabolism. December 2008, Vol. 8, No. 6; pages 468-481; abstract; page 7, 3rd paragraph; DOI: 10.1016/j.cmet.2008.10.011	10-11
A	US 2010/0306866 A1 (BUTLER, AA et al.) 2 December 2010; entire document	1-21
D, A	(YANG, C et al.) Age-Dependent Decrease in Adropin is Associated with Reduced Levels of Endothelial Nitric Oxide Synthase and Increased Oxidative Stress in the Rat Brain. Aging and Disease. April 2018, Vol. 9, No. 2; pages 322-330; DOI: 10.14336/AD.2017.0523	1-21
A	(GHOSHAL, S et al.) Adropin: An endocrine link between the biological clock and cholesterol homeostasis. Molecular Metabolism. February 2019, Epub 30 December 2017, Vol. 8; pages 51-64; DOI: 10.1016/j.molmet.2017.12.002	1-21

☐ 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
7 October 2019 (07.10.2019)	05NOV 2019
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 Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/35428

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

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 examined, the appropriate additional examination fees must be paid.

Group I+, Claims 1-21 and SEQ ID NO: 1 (Human Adropin Amino Acid Sequence) are directed toward methods and compositions for treating cognitive decline in subjects in need.

The methods and compositions will be searched to the extent that they encompass SEQ ID NO: 1 (first exemplary Human Adropin Amino Acid Sequence). Applicant is invited to elect additional Human Adropin Amino Acid Sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and available as an option within at least one searchable claim, to be searched. Additional Human Adropin Amino Acid Sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-21 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (Human Adropin Amino Acid Sequence). Applicants must specify the claims that encompass any additionally elected Human Adropin Amino Acid Sequence(s). 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/examined. An exemplary election would be SEQ ID NO: 2 (Human Adropin Amino Acid Sequence).

No technical features are shared between the Human Adropin Amino Acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method of treating cognitive decline, in a subject in need, the method comprising administering an effective amount of a polypeptide or variants or derivatives thereof, to the subject parenterally; a composition for treating age related dementia, comprising a polypeptide or variants or derivatives thereof, prepared in pharmaceutically acceptable aqueous solution; and a composition for treating age related dementia, comprising a polypeptide with 90 percent or more identity to a sequence, wherein the composition is effective at treating the subject's cognitive decline.

However, these shared technical features are previously disclosed by US 9.458,209 B2 (NOVARTIS AG) (hereinafter 'Novartis').

Novartis discloses a method of treating cognitive decline (method of treating age-related dementia (cognitive decline); column 10, lines 56-61), in a subject in need (column 15, lines 50-53), the method comprising administering an effective amount of a polypeptide (the method comprising administering an effective amount of a polypeptide; abstract; column 16, lines 48-61) or variants or derivatives thereof, to the subject parenterally (column 27, lines 32-35); a composition for treating age related dementia (composition for treating age-related dementia (cognitive decline); column 10, lines 56-61; column 26, lines 60-64), comprising a polypeptide or variants or derivatives thereof (abstract), prepared in pharmaceutically acceptable aqueous solution (prepared in pharmaceutically acceptable aqueous solution; column 26, lines 60-64); and a composition for treating age related dementia (composition for treating age-related dementia (cognitive decline); column 10, lines 56-61; column 26, lines 60-64), comprising a polypeptide with 90 percent or more identity to a sequence (comprising a polypeptide with 90 percent or more identity to a sequence; column 25, lines 18-23), wherein the composition is effective at treating the subject's cognitive decline (wherein the composition is effective at treating the subject's cognitive decline; column 10, lines 56-61).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Novartis reference, unity of invention is lacking.