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(54) Title: DIABETES AND ALZHEIMER'S DISEASE RAT MODEL AND USES THEREOF

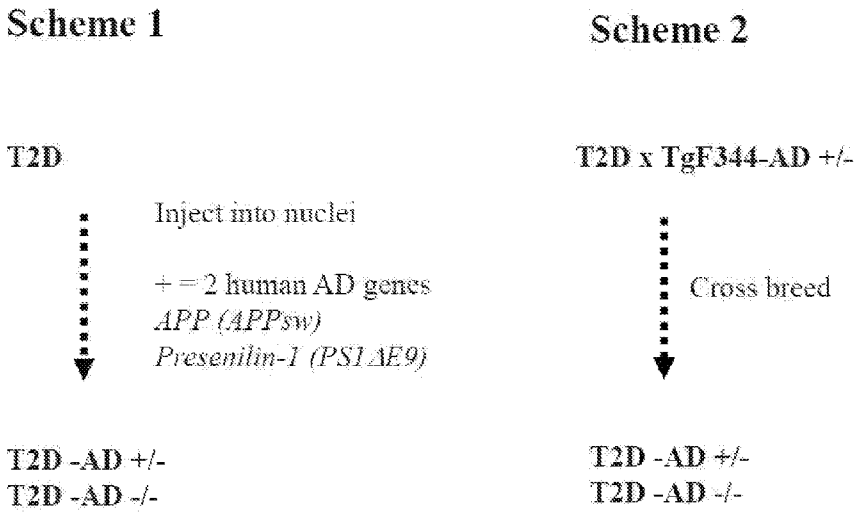

FIG. 1

(57) Abstract: Disclosed herein is a type-2 diabetes-Alzheimer's disease (T2D-AD) animal model having insulin resistance, a defect in pancreatic beta-cell function but normal leptin signaling, transgenic hemizygous human amyloid precursor protein (APP) expression, and transgenic human presenilin-1 (PS1) expression. Histology analysis of the amyloid burden showed that the T2D-AD$^{+/-}$ rat had amyloid plaque at 6 months of age. In a reciprocal interaction, AD appeared to increase the risk of T2D.

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DIABETES AND ALZHEIMER'S DISEASE RAT MODEL AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 63/506,239, filed June 5, 2023, which is hereby incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with Government Support under Grant Nos. DK092993 and DK135074 awarded by the National Institutes of Health. The Government has certain rights in the invention.

SEQUENCE LISTING

[0003] This application contains a sequence listing filed in ST.26 format entitled "320317-2010 Sequence Listing" created on May 30, 2024, having 9,868 bytes. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND OF THE INVENTION

[0004] The most commonly used rodent models of Type 2 diabetes mellitus (T2DM)include the Zucker diabetic fatty (ZDF) rat, the Otsuka Long Evans Tokushima fatty (OLETF) rat, and the db/db mouse, all of which exhibit obesity-associated insulin resistance and impaired β-cell function, resulting in diabetes. While these animal models have contributed substantially to understanding the pathophysiology and treatment of T2DM and its complications, the basic mechanisms underlying the pathogenesis of diabetes in these models do not correspond with what occurs in most human patients with T2DM.

[0005] Research shows that people with prediabetes or type 2 diabetes have a higher risk of getting Alzheimer's disease and other types of dementia later in life. These effects on the brain are so strong that some scientists feel that Alzheimer's related to insulin resistance should be called "type 3 diabetes." However, the mechanism for this is poorly understood, and there is no animal model that adequately models this interaction.

SUMMARY OF THE INVENTION

[0006] Disclosed herein is a type-2 diabetes-Alzheimer's disease (T2D-AD) animal model having insulin resistance, a defect in pancreatic beta-cell function but normal leptin signaling, transgenic hemizygous human amyloid precursor protein (APP) expression, and transgenic human presenilin-1 (PS1) expression.

[0007] In some embodiments, the animal model is produced by a process comprising cross-breeding a first diabetic rodent having insulin resistance and a defect in pancreatic beta-

cell function but normal leptin signaling with a second rodent containing hemizygous human amyloid precursor protein (APP) and presenilin-1 (PS1) alleles. In some embodiments, the first rodent is produced by a process comprising cross-breeding a polygenic obesity rodent model with a rodent having a defect in pancreatic beta-cell function but normal leptin signaling. In some embodiments, the first rodent is produced by a process comprising cross-breeding the polygenic obesity rodent model with a with Zucker diabetic fatty (ZDF)-lean rat.

[0008] For example, in some embodiments, the animal model is derived from a cross breeding of the UCD_T2DM Sprague Dawley rat and a Fischer TgF344AD$^{+/-}$ rat containing 2 hemizygous human AD transgenes (*APP* and *PS1*). For the diabetes trait, the UCD_T2DM rat showed a similar characteristic of T2D observed in humans: rising glucose level, rising and then falling insulin level, and obesity (Cummings et al, AJPhysiol, 295, R1782, 2008). For the AD trait, the TgF344AD$^{+/-}$ rat containing the AD genes displayed the hallmark of AD: amyloid burden, tau hyperphosphorylation, and poor cognitive performance (Cohen et al, J Neurosci, 33, 6245, 2013). DNA analysis identified animals with the APP and PS1 genes, and serum glucose analysis further categorized animals with hyperglycemia (>350 mg/dl). By filial generation 9, the breeding had selected colony founders that exhibited both the AD and T2D phenotype.

[0009] The rats had a hemizygous insertion of the AD genes and received the designation T2D-AD$^{+/-}$. The control litter mate received the designation T2D-AD$^{-/-}$. Gene insertion analysis determined that in both T2D-AD$^{+/-}$ and the TgF344AD$^{+/-}$ models, the AD genes intercalated into chromosome 4. Histology analysis of the amyloid burden showed that the T2D-AD$^{+/-}$ rat had amyloid plaque at 6 mos. of age. In contrast, the TgF344AD$^{+/-}$ showed no amyloid until 16 mos. of age. Cognitive measurement as assessed with the Barnes Maze Test showed T2D$^{+/-}$ performed poorly in comparison to the T2D$^{-/-}$. In a reciprocal interaction, AD appeared to increase the risk of T2D. The time to diabetes as measured by a diabetes incidence plot decreased by a month in T2D-AD$^{+/-}$ animals. The glucose and insulin profile confirmed the early onset of T2D in the T2D-AD$^{+/-}$ rats.

[0010] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF FIGURES

[0011] FIG. 1 shows two schemes for producing animal models having type 2 diabetes and Alzheimer's disease.

[0012] FIG. 2 shows colony founders with APP and PS1 transgenes and resulting glucose.

[0013] FIGs. 3A to 3C show amyloid burden in brain of T2D-/- rats at 6 months (FIG. 3A), T2D+/- rats at 6 months (FIG. 3B), and T2D+/- rats at 9 months (FIG. 3C). Propidium Iodide (PI) in red. F1-Fluoro-2,5-bis[(E)-3-carboxy-4-hydroxystyryl]benzene solution (FSB)in blue. Rats never spontaneously develop AD. T2D-AD$^{+/-}$ shows amyloid burden at 6 mos. Tg344AD$^{+/-}$ shows amyloid burden at 16 months.

[0014] FIGs. 4A to 4C show cognitive performance of T2D-AD$^{+/-}$ and T2D-AD$^{-/-}$ rats. FIG. 4A shows escape latency as a function of time for T2D-AD$^{+/-}$ (square) and T2D-AD$^{-/-}$ (circle) rats. FIGs. 4B and 4C show % use as a function of time for T2D-AD$^{-/-}$ (FIG. 4B) and T2D-AD$^{+/-}$ (FIG. 4C) rats.

[0015] FIG. 5 shows diabetic incidence (%) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats. Diabetes incidence is glucose > 350 mg/dL.

[0016] FIG. 6 shows non-fasting glucose levels (mg/dL) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats.

[0017] FIG. 7 shows insulin levels (ng/mL) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats.

[0018] FIGs. 8A to 8C show Metabolite dynamics of Pyr, Lac and Bic signals in representative control (FIG. 8A), T2D-AD$^{-/-}$ (FIG. 8B), and T2D-AD$^{+/-}$ (FIG. 8C) animals after an injection of hyperpolarized ~100mM [1-^{13}C]Pyr solution.

[0019] FIG. 9 is a comparison of spectra from three representative animals highlights the increase in lactate and reduction of bicarbonate production in a T2D-AD$^{-/-}$ animal compared to a control, which is further amplified in a T2D-AD$^{+/-}$ animal.

[0020] FIG. 10 shows Bic/Lac expression in control, T2D-AD$^{-/-}$, and T2D-AD$^{+/-}$, Tg344-AD$^{-/-}$, and Tg344-AD$^{+/-}$ rats.

[0021] FIG. 11 shows astrocyte neuron lactate shuttle (ANLS).

[0022] FIG. 12 shows an agarose DNA gel electrophoresis following polymerase chain reaction of PS1, Prp, and APP genes. The far-left lane contains the DNA ladder. All rats (TgF344-AD -/-, TgF344-AD +/-, T2D-AD -/-, T2D-AD +/-, and T2D) contain a band at 799 bp, indicating the presence of the genomic Prp product. Only the TgF344-AD +/- and T2D-AD +/- rats contain the bands at 1,300 and 400 bp, indicating the presence of the PS1 and APP genes, respectively. The TgF344-AD -/- and T2D-AD -/- do not display the bands at 1,300 and 400 bp.

[0023] FIG. 13 shows a comparison of amyloid plaques in brain regions of TgF344-AD and T2D-AD rats. 9-month-old TgF344-AD -/- and T2D-AD -/- rat brains display no amyloid plaques (panels A, B). Nine-month-old TgF344-AD +/- and T2D-AD +/- rat brains display amyloid plaques in hippocampus (red), cortex (green), and thalamus (panels C, D).

[0024] FIG. 14 shows quantitative analysis (ImageJ (NIH)) amyloid plaque distribution. TgF344AD +/- rat: hippocampus (2.92%±0.34%), cortex (1.11%±0.26%), and thalamus (0.12%±0.04%); T2D-AD +/- rats: hippocampus (5.39%±0.18%), cortex (2.65%±0.13%), and thalamus (0.32%±0.14%).

[0025] FIG. 15 shows non-fasting blood glucose concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 8 and for T2D-AD +/- rats, n = 10. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in non-fasting blood glucose concentrations at and after diabetes onset ($F_{7,3} = 15.44$, $p = 1.56\times10^{-5}$). A two-tail t-test indicates a significant difference between non-fasting blood glucose concentrations in T2D-AD -/- and T2D-AD +/- rats at onset (*), at 1 month (**) and 2 months after onset (***). Data points are mean ± SEM.

[0026] FIG. 16 shows fasting blood glucose concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 7, and for T2D-AD +/- rats, n = 10. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in fasting blood glucose concentrations after diabetes onset ($F_{6,3} = 5.20$, $p = 0.009$). A two-tail t-test indicates a significant difference between fasting blood glucose concentrations in T2D-AD -/- and T2D-AD +/- rats 1 month after onset (*) and 2 months after onset (**). Data points are mean ± SEM.

[0027] Fig. 17 shows fasting insulin concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 7, and for T2D-AD +/- rats, n = 8. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in fasting insulin concentrations before and after diabetes onset ($F_{6,3} = 6.82$, $p = 2.89\times10^{-3}$). A two-tail t-test indicates a significant difference between fasting insulin concentrations in T2D-AD -/- and T2D-AD +/- rats at onset (*), at 1 month after onset (**), and at 2 months after onset (***). Data points are mean ± SEM.

[0028] FIG. 18 shows Barnes maze escape latencies. Escape latencies for the T2D-AD -/- and T2D-AD +/- rats for days 2-6 (n = 18 animals per genotype). T2D-AD +/- rats show no change, while the T2D-AD -/- rats decrease their escape latencies over time. T-test indicates a significant difference between T2D-AD -/- and T2D-AD +/- latencies on day 5 (*, $t(110) = -3.656$, $p = 3.950\times10^{-4}$) and day 6 (**, $t(144) = -3.097$, $p = 2.345\times10^{-3}$). Data points are shown as mean ± standard error of the mean (SEM).

[0029] FIG. 19 shows Barnes maze escape latencies for TgF344-AD rats for days 2-6. TgF344-AD -/- (n = 10). TgF344-AD +/- (n = 7). TgF344-AD -/- latencies for days 2-6 TgF344-AD +/- rats show no change, while the TgF344-AD -/- rats decrease their escape latencies over time. T-test indicates a significant difference on day 5 (*, $t(41) = -1.795$, $p = 0.040$) and day 6

(**, t(61) = -2.028, p =0.023). Data points are shown as mean ± standard error of the mean (SEM).

[0030] FIGs. 20A and 20B show Barnes maze search strategies for T2D-AD -/- (FIG. 20A) T2D-AD +/- (FIG. 20B) rats. T2D-AD -/- rats used the direct search strategy much more than T2D-AD +/- rats. Direct strategy reflects cognitive skills. Chi-square analysis collapsing data across all trials revealed a significant difference in the distribution of search patterns between T2D-AD -/- and T2D-AD +/- rats ($p = 2.46\times10^{-4}$).

[0031] FIGs. 21A and 21B show Barnes maze search strategies for T2D-AD -/- (FIG. 20A) T2D-AD +/- (FIG. 20B) rats. TgF344AD -/- rats used the direct search strategy much more than TgF344AD +/- rats. Direct strategy reflects cognitive skills. Chi-square analysis collapsing data across all trials revealed a significant difference in the distribution of search patterns between Tg344-AD -/- and Tg344-AD +/- rats (p = 0.0172).

[0032] FIG. 22 shows Bic/lac ratio before and after dichloroacetate (DCA). Dichloroacetate activates pyruvate dehydrogenase. Control rats (n=3) displayed a bic/lac ratio of 0.164±0.012. Data not available for post DCA. T2D-AD -/- (n=8) displayed a bic/lac ratio of 0.075±0.005. After DCA (n=2) bic/lac ratios increased to 0.207±0.014. T2D-AD +/- rats displayed (n=7) a bic/lac ratio of 0.042±0.004, almost 50% lower than the T2D-AD -/- rats. Post-DCA (n=2) the bic/lac ratio increased to 0.263±0.068. The Tg344-AD -/- rats (n=1) displayed a bic/lac ratio of 0.275. Post DCA bic/lac ratio jumps to 0.653. Tg344-AD +/- rat (n=1) displayed a bic/lac ratio of 0.071. Post-DCA (n=1) rat showed a bic/lac ratio of 0.361.

DETAILED DESCRIPTION

[0033] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[0034] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

[0036] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

[0037] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

[0038] Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of chemistry, biology, and the like, which are within the skill of the art.

[0039] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to perform the methods and use the probes disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C, and pressure is at or near atmospheric. Standard temperature and pressure are defined as 20 °C and 1 atmosphere.

[0040] Before the embodiments of the present disclosure are described in detail, it is to be understood that, unless otherwise indicated, the present disclosure is not limited to particular materials, reagents, reaction materials, manufacturing processes, or the like, as such can vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It is also possible in the present disclosure that steps can be executed in different sequence where this is logically possible.

Definitions

[0041] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0042] As used herein, the term "APP" or "Amyloid Precursor Protein" denotes an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation, neural plasticity and iron export. APP is best known as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a 37 to 49 amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients.

[0043] As used herein, the term "PS1" or "Presenilin 1" denotes a protein encoded by the PSEN1 gene. Presenilin 1 is one of the four core proteins in presenilin complex, which mediate the regulated proteolytic events of several proteins in the cell, including gamma secretase. Gamma-secretase is considered to play a strong role in generation of beta amyloid, accumulation of which is related to the onset of Alzheimer's disease, from the beta-amyloid precursor protein.

[0044] A "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in the production of that RNA, polypeptide, protein, or enzyme, i.e., the nucleotide sequence encodes an amino acid sequence for that polypeptide, protein or enzyme. A coding sequence for a protein may include a start codon (usually ATG) and a stop codon.

[0045] "Complementary," as used herein, refers to the subunit sequence complementarity between two nucleic acids, e.g., two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs).

[0046] A "deletion mutation" means a type of mutation that involves the loss of genetic material, which may be from a single base to an entire piece of chromosome. Deletion of one or more nucleotides in the DNA could alter the reading frame of the gene; hence, it could result in a synthesis of a nonfunctional protein due to the incorrect sequence of amino acids during translation.

[0047] The terms “express” and “expression” mean allowing or causing the information in a gene or DNA sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an “expression product” such as a protein. The expression product itself, e.g., the resulting protein, may also be said to be “expressed”. An expression product can be characterized as intracellular, extracellular or secreted. The term “intracellular” means something that is inside a cell. The term “extracellular” means something that is outside a cell. A substance is “secreted” by a cell if it appears in significant measure outside the cell, from somewhere on or inside the cell.

[0048] The term “gene”, also called a “structural gene” means a DNA sequence that codes for or corresponds to a particular sequence of amino acids which comprise all or part of one or more proteins or enzymes, and may or may not include introns and regulatory DNA sequences, such as promoter sequences, 5′-untranslated region, or 3′-untranslated region which affect for example the conditions under which the gene is expressed. Some genes, which are not structural genes, may be transcribed from DNA to RNA, but are not translated into an amino acid sequence. Other genes may function as regulators of structural genes or as regulators of DNA transcription.

[0049] By “genetically modified” is meant a gene that is altered from its native state (e.g., by insertion mutation, deletion mutation, nucleic acid sequence mutation, or other mutation), or that a gene product is altered from its natural state (e.g., by delivery of a transgene that works in trans on a gene's encoded mRNA or protein, such as delivery of inhibitory RNA or delivery of a dominant negative transgene).

[0050] By “exon” is meant a region of a gene which includes sequences which are used to encode the amino acid sequence of the gene product.

[0051] The term “heterologous” refers to a combination of elements not naturally occurring. For example, heterologous DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell. A heterologous expression regulatory element is such an element operatively associated with a different gene than the one it is operatively associated with in nature.

[0052] As used herein, the term “homology” refers to the subunit sequence identity or similarity between two polymeric molecules e.g., between two nucleic acid molecules, e.g., between two DNA molecules, or two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, e.g., if a position in each of two polypeptide molecules is occupied by phenylalanine, then they are identical at that position. The

homology between two sequences, most clearly defined as the % identity, is a direct function of the number of identical positions, e.g., if half (e.g., 5 positions in a polymer 10 subunits in length) of the positions in two polypeptide sequences are identical then the two sequences are 50% identical; if 70% of the positions, e.g., 7 out of 10, are matched or homologous, the two sequences share 70% identity. By way of example, the polypeptide sequences ACDEFG and ACDHIK share 50% identity and the nucleotide sequences CAATCG and CAAGAC share 50% identity. It should be understood that a sequence identity of at least 90%, such as 90% identity, 91% identity, 92% identity, 93% identity, 94% identity, 95% identity, 96% identity, 97% identity, 98% identity, or 99% identity applies to all sequences disclosed in the present application.

[0053] "Homologous recombination" is the physical exchange of DNA expedited by the breakage and reunion of two non-sister chromatids. In order to undergo recombination the DNA duplexes must have complimentarily. The molecular mechanism is as follows: DNA duplexes pair, homologous strands are nicked, and broken strands exchange DNA between duplexes. The region at the site of recombination is called the hybrid DNA or heteroduplex DNA. Second nicks are made in the other strand, and the second strand crosses over between duplexes. After this second crossover event the reciprocal recombinant or splice recombinant is created. The duplex of one DNA parent is covalently linked to the duplex of another DNA parent. Homologous recombination creates a stretch of heteroduplex DNA.

[0054] A "hypomorphic mutation" is a change to the genetic material (usually DNA or RNA), which can be caused by any form of genetic mutation, and causes an decrease in normal gene function without causing a complete absence of normal gene function.

[0055] The term "inbred animal" is used herein to refer to an animal that has been interbred with other similar animals of the same species in order to preserve and fix certain characteristics, or to prevent other characteristics from being introduced into the breeding population.

[0056] The term "insertional mutation" is used herein to refer the translocation of nucleic acid from one location to another location which is in the genome of an animal so that it is integrated into the genome, thereby creating a mutation in the genome. Insertional mutations can also include knocking out or knocking in of endogenous or exogenous DNA via gene trap or cassette insertion. Exogenous DNA can access the cell via electroporation or chemical transformation. If the exogenous DNA has homology with chromosomal DNA it will align itself with endogenous DNA. The exogenous DNA is then inserted or disrupts the endogenous DNA via two adjacent crossing over events, known as homologous recombination. A targeting vector can use homologous recombination for insertional mutagenesis. Insertional mutagenesis of

endogenous or exogenous DNA can also be carried out via DNA transposon. The DNA transposon is a mobile element that can insert itself along with additional exogenous DNA into the genome. Insertional mutagenesis of endogenous or exogenous DNA can be carried out by retroviruses. Retroviruses have a RNA viral genome that is converted into DNA by reverse transcriptase in the cytoplasm of the infected cell. Linear retroviral DNA is transported into the nucleus, and become integrated by an enzyme called integrase. Insertional mutagenesis of endogenous or exogenous DNA can also be done by retrotransposons in which an RNA intermediate is translated into DNA by reverse transcriptase, and then inserted into the genome.

[0057] The term "gene knockdown" refers to techniques by which the expression of one or more genes is reduced, either through genetic modification (a change in the DNA of one of the organism's chromosomes) or by treatment with a reagent such as a short DNA or RNA oligonucleotide with a sequence complementary to either an mRNA transcript or a gene. If genetic modification of DNA is done, the result is a "knockdown organism" or "knockdowns".

[0058] [0043] A "mutation" is a detectable change in the genetic material in the animal, which is transmitted to the animal's progeny. A mutation is usually a change in one or more deoxyribonucleotides, the modification being obtained by, for example, adding, deleting, inverting, or substituting nucleotides. Exemplary mutations include but are not limited to a deletion mutation, an insertion mutation, a non-sense mutation or a missense mutation. Thus, the terms "mutation" or "mutated" as used herein are intended to denote an alteration in the "normal" or "wild-type" nucleotide sequence of any nucleotide sequence or region of the allele. As used herein, the terms "normal" and "wild-type" are intended to be synonymous, and to denote any nucleotide sequence typically found in nature. The terms "mutated" and "normal" are thus defined relative to one another; where a cell has two chromosomal alleles of a gene that differ in nucleotide sequence, at least one of these alleles is a "mutant" allele as that term is used herein. Based on these definitions, an "endogenous SCID gene" is the "wild-type" gene that exists normally in a cell, and a "mutated SCID gene" defines a gene that differs in nucleotide sequence from the wild-type gene.

[0059] By "knock-out" is meant an alteration in the nucleic acid sequence that reduces the biological activity of the polypeptide normally encoded therefrom by at least 80% compared to the unaltered gene. The alteration may be an insertion, deletion, frameshift mutation, or missense mutation. Preferably, the alteration is an insertion or deletion, or is a frameshift mutation that creates a stop codon.

[0060] The term "outbred animal" is used herein to refer to an animal that breeds with any other animal of the same species without regard to the preservation of certain characteristics.

[0061] As used herein, the term "phenotype" means any property of a cell or organism. A phenotype can simply be a change in expression of an mRNA or protein. Examples of phenotypes also include, but are in no way limited to, cellular, biochemical, histological, behavioral, or whole organismal properties that can be detected by the artisan. Phenotypes include, but are not limited to, cellular transformation, cell migration, cell morphology, cell activation, resistance or sensitivity to drugs or chemicals, resistance or sensitivity to pathogenic protein localization within the cell (e.g., translocation of a protein from the cytoplasm to the nucleus), resistance or sensitivity to ionizing radiation, profile of secreted or cell surface proteins, (e.g., bacterial or viral) infection, post-translational modifications, protein localization within the cell (e.g., translocation of a protein from the cytoplasm to the nucleus), profile of secreted or cell surface proteins, cell proliferation, signal transduction, metabolic defects or enhancements, transcriptional activity, recombination intermediate joining, DNA damage response, cell or organ transcript profiles (e.g., as detected using gene chips), apoptosis resistance or sensitivity, animal behavior, organ histology, blood chemistry, biochemical activities, gross morphological properties, life span, tumor susceptibility, weight, height/length, immune function, organ function, any disease state, and other properties known in the art. In certain situations and therefore in certain embodiments of the invention, the effects of mutation of one or more genes in a cell or organism can be determined by observing a change in one or more given phenotypes (e.g., in one or more given structural or functional features such as one or more of the phenotypes indicated above) of the mutated cell or organism compared to the same structural or functional feature(s) in a corresponding wild-type or (non-mutated) cell or organism (e.g., a cell or organism in which the gene(s) have not been mutated).

[0062] By "transgenic" is meant any animal which includes a nucleic acid sequence which is inserted by artifice into a cell and becomes a part of the genome of the animal that develops from that cell. Such a transgene may be partly or entirely heterologous to the transgenic animal. Although transgenic mice represent another embodiment of the invention, other transgenic mammals including, without limitation, transgenic rodents (for example, hamsters, guinea pigs, rabbits, and rats), and transgenic pigs, cattle, sheep, and goats are included in the definition.

Animal Model

[0063] Disclosed herein is a type-2 diabetes-Alzheimer's disease (T2D-AD) animal model having insulin resistance, a defect in pancreatic beta-cell function but normal leptin signaling, transgenic hemizygous human amyloid precursor protein (APP) expression, and transgenic human presenilin-1 (PS1) expression. In some embodiments, the animal model is derived from a cross breeding of the UCD_T2DM Sprague Dawley rat and a Fischer TgF344AD$^{+/-}$ rat containing 2 hemizygous human AD transgenes (*APP* and *PS1*).

UCD_T2DM Rats

[0064] Obesity and insulin resistance in most animal models of T2DM result from monogenic mutations that are rare in human and animal populations and present multiple problems in terms of applying these models to clinical T2DM. Because the development of T2DM in humans requires both peripheral insulin resistance and inadequate β-cell compensation, the UCD_T2DM rat model was developed, demonstrating polygenic adult-onset obesity and diabetes development in both sexes with preserved leptin signaling and fertility.

[0065] The UCD_T2DM rat model was created by crossing obese Sprague-Dawley rats having insulin resistance from polygenic adult-onset obesity with a Zucker diabetic fatty (ZDF)-lean rats that have a defect in pancreatic beta-cell function but normal leptin signaling. This is described in Cummings, et al. Am J Physiol Regul Integr Comp Physiol. 2008 295(6):R1782-93, which is incorporated by reference in its entirety for the teaching of this rat model.

[0066] It is understood that in some embodiments, any polygenic obesity model having insulin resistance can be crossed with ZDF-lean rats to obtain T2DM rats. Polygenic obesity models better reflect the human obese phenotype. Obesity in these models isn't caused by one mutation, but rather from errors at multiple sites within the genome. Such animals are fed a high-fat diet (as high as 60% fat (kcal)), which causes diet-induced obesity (DIO). According to the literature, standard Sprague-Dawley® and Long-Evans rats are the two most common outbred animal stock used for DIO studies.

[0067] The Zucker diabetic fatty (ZDF) has a missense mutation in the gene coding the leptin receptor (fa/fa). ZDF-lean rats have fa/+ alleles. Both of these rat models are commercially available from Charles River Laboratories (Wilmington, MA).

[0068] These two lines of non-diabetic rats were crossed, each of which has only one of the primary defects associated with T2DM. Selective breeding was then used to enrich for diabetes in subsequent generations. One founder line consisted of OSD rats that exhibit polygenic, adult-onset obesity and insulin resistance on a standard laboratory rat chow diet but do not develop diabetes due to robust β-cell compensation. The second founder line, ZDF-lean rats, did not possess the leptin receptor mutation but had a defect in pancreatic β-cell insulin

production that results in diabetes only in the setting of insulin resistance. This breeding strategy successfully produced diabetes in both male and female animals with animals exhibiting adult-onset obesity, insulin resistance, impaired glucose tolerance, and eventual β-cell decompensation. This model provided several advantages over currently available models including obesity of polygenic rather than monogenic origin, a later age of onset, preserved fertility, and development of diabetes in both sexes.

[0069] It is also understood that one of ordinary skill in the art could cross the OSD and ZDF-lean rats with selective breading for diabetes with a similar expectation of success.

TgF344-AD Rat

[0070] The TgF344-AD rat is an Alzheimer's rat model overexpressing human amyloid precursor protein (*APP*) and presenilin-1 (*PS1*) alleles.

[0071] The cDNA sequence for *APP* is disclosed in Genbank under access number Gene ID: 351 and has the nucleic acid sequence:

ATGCTGCCCGGACTGGCTCTGCTGCTGCTGGCCGCTTGGACCGCCAGAGCCCTGGAAGT
GCCCACCGATGGCAATGCTGGCCTGCTGGCCGAGCCCCAGATCGCCATGTTCTGCGGCA
GACTGAACATGCACATGAACGTGCAGAACGGCAAGTGGGACAGCGACCCCAGCGGCACC
AAGACCTGCATCGACACCAAAGAGGGCATCCTGCAGTATTGCCAGGAAGTGTACCCCGAG
CTGCAGATCACCAACGTGGTGGAAGCCAACCAGCCCGTGACCATCCAGAACTGGTGCAAG
CGGGGCAGAAAGCAGTGCAAGACCCACCCCCACTTCGTGATCCCTTACCGGTGCCTGGTC
GGAGAGTTCGTGTCCGACGCCCTGCTGGTGCCCGACAAGTGCAAGTTCCTGCATCAGGAA
CGGATGGACGTCTGCGAGACACATCTGCACTGGCACACCGTGGCCAAAGAGACATGCAG
CGAGAAGTCCACCAACCTGCACGACTACGGCATGCTGCTGCCCTGCGGCATCGACAAGTT
CCGGGGCGTGGAATTCGTGTGCTGCCCCCTGGCCGAGGAATCCGACAACGTGGACAGCG
CCGACGCCGAAGAGGACGACAGCGACGTGTGGTGGGGCGGAGCCGACACCGATTACGC
CGACGGCAGCGAGGACAAGGTCGTGGAAGTGGCTGAAGAGGAAGAGGTGGCCGAGGTC
GAAGAAGAGGAAGCCGACGACGACGAGGATGACGAGGACGGCGACGAAGTGGAAGAAGA
AGCCGAGGAACCCTACGAGGAAGCCACCGAGCGGACCACCTCTATCGCCACCACCACCA
CAACCACTACCGAGAGCGTGGAAGAGGTGGTGCGCGAAGTGTGCAGCGAGCAGGCCGAG
ACAGGCCCCTGCCGGGCCATGATCAGCCGGTGGTACTTCGACGTGACCGAGGGCAAGTG
CGCCCCCTTCTTCTATGGCGGCTGCGGCGGCAACCGGAACAACTTCGACACCGAGGAATA
CTGCATGGCCGTGTGCGGCAGCGCCATCCCTACCACAGCCGCCAGCACCCCCGACGCCG
TGGACAAGTACCTGGAAACCCCTGGCGACGAGAACGAGCACGCCCACTTCCAGAAGGCC
AAAGAGCGGCTGGAAGCCAAGCACCGCGAGCGGATGAGCCAGGTGATGAGAGAGTGGGA
AGAGGCCGAGAGACAGGCCAAGAACCTGCCCAAGGCCGACAAGAAAGCCGTGATCCAGC

ACTTCCAGGAAAAGGTCGAAAGCCTGGAACAGGAAGCCGCCAACGAGCGGCAGCAGCTG
GTGGAAACCCACATGGCCAGAGTGGAAGCCATGCTGAACGACCGGCGGAGACTGGCCCT
GGAAAACTACATCACCGCCCTGCAGGCCGTGCCCCCCAGACCCAGACACGTGTTCAACAT
GCTGAAGAAATACGTGCGGGCCGAGCAGAAGGACCGGCAGCACACCCTGAAGCACTTCG
AGCACGTGCGGATGGTGGACCCCAAGAAGGCCGCCCAGATCCGCTCTCAGGTCATGACC
CACCTGAGAGTGATCTACGAGAGAATGAACCAGAGCCTGAGCCTGCTGTACAATGTGCCC
GCCGTGGCCGAAGAAATCCAGGACGAGGTGGACGAGCTGCTGCAGAAAGAGCAGAACTA
CAGCGACGACGTGCTGGCCAACATGATCAGCGAGCCCCGGATCAGCTACGGCAACGACG
CCCTGATGCCCAGCCTGACCGAGACAAAGACCACCGTGGAACTGCTGCCCGTGAACGGC
GAGTTCAGCCTGGACGACCTGCAGCCCTGGCACAGCTTTGGCGCTGATAGCGTGCCCGC
CAACACCGAGAACGAGGTGGAACCCGTGGACGCCAGACCTGCCGCCGACAGAGGCCTGA
CCACAAGACCTGGCAGCGGCCTGACCAACATCAAGACCGAAGAGATCAGCGAAGTGAACC
TGGACGCCGAGTTCCGGCACGACAGCGGCTACGAGGTGCACCACCAGAAACTGGTGTTC
TTCGCCGAGGACGTGGGCAGCAACAAGGGCGCCATCATCGGCCTGATGGTCGGAGGCGT
GGTGATCGCCACCGTGATCATCATCACCCTGGTGATGCTGAAAAAGAAGCAGTACACCAG
CATCCACCACGGCGTGGTCGAAGTGGACGCCGCTGTGACCCCCGAGGAACGGCACCTGA
GCAAGATGCAGCAGAACGGCTACGAGAACCCCACCTACAAGTTCTTCGAGCAGATGCAGA
ACTGA (SEQ ID NO: 1).

[0072] The protein sequence of the APP protein has the following protein sequence:
MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTKTC
IDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVGEFVSDA
LLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFRGVEFVCCPL
AEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEEEADDDEDDED
GDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPCRAMISRWYFDVTEG
KCAPFFYGGCGGNRNNFDTEEYCMAVCGSAIPTTAASTPDAVDKYLETPGDENEHAHFQKAK
ERLEAKHRERMSQVMREWEEAERQAKNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETH
MARVEAMLNDRRRLALENYITALQAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVD
PKKAAQIRSQVMTHLRVIYERMNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISE
PRISYGNDALMPSLTETKTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAA
DRGLTTRPGSGLTNIKTEEISEVNLDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV
VIATVIIITLVMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN (SEQ
ID NO: 2).

[0073] In some embodiments, the APP has the Swedish and London mutations (APPsl) and has the following protein sequence:

MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTKTC
IDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVGEFVSDA
LLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFRGVEFVCCPL
AEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEEEADDDEDDED
GDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPCRAMISRWYFDVTEG
KCAPFFYGGCGGNRNNFDTEEYCMAVCGSAIPTTAASTPDAVDKYLETPGDENEHAHFQKAK
ERLEAKHRERMSQVMREWEEAERQAKNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETH
MARVEAMLNDRRRLALENYITALQAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVD
PKKAAQIRSQVMTHLRVIYERMNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISE
PRISYGNDALMPSLTETKTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAA
DRGLTTRPGSGLTNIKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV
VIATVIVITLVMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN (SEQ
ID NO: 3).

[0074] The cDNA sequence for PS1 is disclosed in Genbank under access number Gene ID: 5663 and code for the following protein sequence:
MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQ
VVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATIKSVSFYTRKDGQLIYTPFTEDTETVG
QRALHSILNAAIMISVIVVLTILLVVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYI
TVALLIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLILAVISVYDLVAVLC
PKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAEGDPEAQRRVSKNSKYNAESTERESQ
DTVAENDDGGFSEEWEAQRDSHLGPHRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIF
YSVLVGKASATASGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATDYLVQPF
MDQLAFHQFYI (SEQ ID NO: 4).

[0075] In some embodiments, the PS1 protein may be modified (PS1 M146L) and may have the protein sequence sequence:
MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQ
VVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATIKSVSFYTRKDGQLIYTPFTEDTETVG
QRALHSILNAAIMISVIVVMTILLVVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYI
TVALLIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLILAVISVYDLVAVLC
PKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAEGDPEAQRRVSKNSKYNAESTERESQ
DTVAENDDGGFSEEWEAQRDSHLGPHRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIF
YSVLVGKASATASGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATDYLVQPF
MDQLAFHQFYI (SEQ ID NO: 5).

[0076] It is also understood that one of ordinary skill in the art could cross genetically engineer rats to overexpress APP and PS1 along with selective breading for Alzheimer's disease symptoms with a similar expectation of success.

Methods of Making Mutant Animals

[0077] The animal models disclosed herein can be produced by methods that involve a combination of genetic introduction methods, genetic modification or mutagenesis mechanisms, and vector delivery methods. For all genetic modification or mutagenesis mechanisms one or more introduction and delivery method may be employed.

Genetic Introduction Methods

[0078] In some embodiments, a gene is inserted or mutated directly in the germ cells of an adult animal. This method usually involves the creation of a transgenic founder animal by pronuclear injection. Rat oocytes can be microinjected with DNA into the male pronucleus before nuclear fusion. The microinjected DNA creates a transgenic founder rat. In this method, a female rat is mated and the fertilized eggs are flushed from their oviducts. After entry of the sperm into the egg, the male and female pronuclei are separate entities until nuclear fusion occurs. The male pronucleus is larger and can be identified via dissecting microscope. The egg can be held in place by micromanipulation using a holding pipette. The male pronucleus is then microinjected with DNA that can be genetically modified. The microinjected eggs are then implanted into a surrogate pseudopregnant female which was mated with a vasectomized male for uterus preparation. The foster mother gives birth to transgenic founder animals. If the transgenic DNA encodes the appropriate components of a mutagenesis system, such as transposase and a DNA transposon, then mutagenesis will occur directly in the germ cells of founder animals and some offspring will contain new mutations. Chemical mutagenesis can also be used to cause direct germ line mutations.

[0079] In some embodiments, a gene is inserted or mutated in the early embryo of a developing animal. The mutant embryonic cells develop to constitute the germ cells of the organism, thereby creating a stable and heritable mutation. Several forms of mutageneis mechanisms can be introduced this way including, but not limited to, zinc finger nucleases and delivery of gene traps by a retrovirus.

[0080] In some embodiments, a gene is inserted or mutated is mutated in a pluripotent cell. These pluripotent cells can proliferate in cell culture and be genetically modified without affecting their ability to differentiate into other cell types including germ line cells. Genetically modified pluripotent cells from a donor can be microinjected into a recipient blastocyst, or in the case of spermatogonial stem cells can be injected into the rete testis of a recipient animal.

Recipient genetically modified blastocysts are implanted into pseudopregnant surrogate females. The progeny which have a genetic modification to the germ line can then be established, and lines homozygous for the genetic modification can be produced by interbreeding.

[0081] In some embodiments, a gene is inserted or mutated in a somatic cell and then used to create a genetically modified animal by somatic cell nuclear transfer. Somatic cell nuclear transfer uses embryonic, fetal, or adult donor cells which are isolated, cultured, and/or modified to establish a cell line. Individual donor cells are fused to an enucleated oocyte. The fused cells are cultured to blastocyst stage, and then transplanted into the uterus of a pseudopregnant female. Alternatively the nucleus of the donor cell can be injected directly into the enucleated oocyte. See U.S. Appl. Publ. No. 20070209083.

Genetic Modification Methods

Mobile DNA Technology

[0082] DNA transposons are discrete mobile DNA segments that are common constituents of plasmid, virus, and bacterial chromosomes. These elements are detected by their ability to transpose self-encoded phenotypic traits from one replicon to another, or to transpose into a known gene and inactivate it. Transposons, or transposable elements, include a piece of nucleic acid bounded by repeat sequences. Active transposons encode enzymes (transposases) that facilitate the insertion of the nucleic acid into DNA sequences.

[0083] The lifecycle and insertional mutagenesis of DNA transposon Sleeping Beauty (SB) is depicted in FIG. 1. In its lifecycle, the SB encodes a transposase protein. That transposase recognizes the inverted terminal repeats (ITRs) that flank the SB transposon. The transposase then excises SB and reintegrates it into another region of the genome. Mutagenesis via Sleeping Beauty is depicted. The mechanism is similar to the life cycle, but transposase is not encoded by the transposon, but instead is encoded elsewhere in the genome

[0084] The Sleeping Beauty (SB) mutagenesis breeding and screening scheme is depicted in FIG. 2. One rat referred to as the “driver” rat contains the (SB) transposase within its genome. A second rat, the “donor” rat contains the transposon which has the transposase-recognizable inverted terminal repeats (ITRs). The two rats are bred to create the “seed” rat which has an active transposon containing transposase and ITRs. The transposon recognizes the ITRs, excises the transposon, and inserts it elsewhere in the rat's genome. This insertion event often disrupts coding, regulatory, and other functional regions in the genome to create knockout rat models. The “seed” rat is bred with wild type rats which beget heterozygous G1 mutants. If the transposon has inserted into the genome, the event will be recorded via size

comparison of DNA by Southern blot analysis. The exact location of the transposon insertion is determined by PCR-based amplification methods combined with sequencing of the DNA flanking the new insertion.

[0085] The sequences for the DNA transposons Sleeping Beauty (SB) piggyBac (PB) functional domains are shown in FIG. 3. The SB and PB transposase sequences encode the protein that recognizes the ITRs and carries out the excision and re-integration. The 3′ and 5′ ITRs are the flanking sequences which the respective transposases recognizes in order to carry out excision and reintegration elsewhere in the genome.

[0086] The DNA transposon Sleeping Beauty (SB) was used by the inventors to create a knockout rat in the Ada gene. The mechanism is depicted in FIG. 4, and is the same as that described above. The transposase is encoded, and the protein recognizes the ITRs of the transposon. The transposon is then excised and reinserted into the seventh intron of the rat Ada gene which resides on chromosome 3, location 3q42.

[0087] In some embodiments, insertion or mutation can involve the transposon piggyBac, and sequence configurations outside of piggyBac, for use as a mobile genetic element as described in U.S. Pat. No. 6,962,810. The Lepidopteran transposon piggyBac is capable of moving within the genomes of a wide variety of species, and is gaining prominence as a useful gene transduction vector. The transposon structure includes a complex repeat configuration consisting of an internal repeat (IR), a spacer, and a terminal repeat (TR) at both ends, and a single open reading frame encoding a transposase.

[0088] The Lepidopteran transposable element piggyBac transposes via a unique cut-and-paste mechanism, inserting exclusively at 5′ TTAA 3′ target sites that are duplicated upon insertion, and excising precisely, leaving no footprint (Elick et al., 1996b; Fraser et al., 1996; Wang and Fraser 1993).

[0089] In some embodiment, the insertion or mutation involves the Sleeping Beauty transposon system for genome manipulation as described, for example, in U.S. Pat. No. 7,148,203. In one embodiment, the system utilizes synthetic, salmonid-type Tc1-like transposases with recognition sites that facilitate transposition. The transposase binds to two binding-sites within the inverted repeats of salmonid elements, and appears to be substrate-specific, which could prevent cross-mobilization between closely related subfamilies of fish elements.

[0090] In some embodiment, the insertion or mutation involves a transposon gene transfer system to introduce DNA into the DNA of a cell comprising: a nucleic acid fragment comprising a nucleic acid sequence positioned between at least two inverted repeats wherein

the inverted repeats can bind to a SB protein and wherein the nucleic acid fragment is capable of integrating into DNA of a cell; and a transposase or nucleic acid encoding a transposase. In one embodiment, the transposase is provided to the cell as a protein and in another the transposase is provided to the cell as nucleic acid. In one embodiment the nucleic acid is RNA and in another the nucleic acid is DNA. In yet another embodiment, the nucleic acid encoding the transposase is integrated into the genome of the cell. The nucleic acid fragment can be part of a plasmid or a recombinant viral vector. Preferably, the nucleic acid sequence comprises at least a portion of an open reading frame and also preferably, the nucleic acid sequence comprises at least a regulatory region of a gene. In one embodiment the regulatory region is a transcriptional regulatory region and the regulatory region is selected from the group consisting of a promoter, an enhancer, a silencer, a locus-control region, and a border element. In another embodiment, the nucleic acid sequence comprises a promoter operably linked to at least a portion of an open reading frame.

[0091] If the transgene flanked by the terminal repeats, the terminal repeats can be derived from one or more known transposons. Examples of transposons include, but are not limited to the following: Sleeping Beauty (Izsvak Z, Ivics Z. and Plasterk R H. (2000) Sleeping Beauty, a wide host-range transposon vector for genetic transformation in vertebrates. J. Mol. Biol. 302:93-102), mosl (Bessereau J L, et al. (2001) Mobilization of a *Drosophila* transposon in the *Caenorhabditis elegans* germ line. Nature. 413(6851):70-4; Zhang L, et al. (2001) DNA-binding activity and subunit interaction of the mariner transposase. Nucleic Acids Res. 29(17):3566-75, piggyBac (Tamura T. et al. Germ line transformation of the silkworm *Bombyx mori* L. using a piggyBac transposon-derived vector. Nat Biotechnol. 2000 January; 18(1):81-4), Himar1 (Lampe D J, et al. (1998) Factors affecting transposition of the Himar1 mariner transposon in vitro. Genetics. 149(11):179-87), Hermes, Tol2 element, Pokey, Tn5 (Bhasin A, et al. (2000) Characterization of a Tn5 pre-cleavage synaptic complex. J Mol Biol 302:49-63), Tn7 (Kuduvalli P N, Rao J E, Craig N L. (2001) Target DNA structure plays a critical role in Tn7 transposition. EMBO J 20:924-932), Tn916 (Marra D, Scott J R. (1999) Regulation of excision of the conjugative transposon Tn916. Mol Microbiol 2:609-621), Tc1/mariner (Izsvak Z, Ivics Z4 Hackett P B. (1995) Characterization of a Tc1-like transposable element in zebrafish (*Danio rerio*). Mol. Gen. Genet. 247:312-322), Minos and S elements (Franz G and Savakis C. (1991) Minos, a new transposable element from *Drosophila hydei*, is a member of the Tc1-like family of transposons. Nucl. Acids Res. 19:6646; Merriman P J, Grimes C D, Ambroziak J, Hackett D A, Skinner P, and Simmons M J. (1995) S elements: a family of Tel-like transposons in the genome of *Drosophila melanogaster*. Genetics 141:1425-1438), Quetzal elements (Ke Z,

Grossman G L, Comel A J, Collins F H. (1996) Quetzal: a transposon of the Tc1 family in the mosquito *Anopheles albimanus*. Genetica 98:141-147); Txr elements (Lam W L, Seo P, Robison K, Virk S, and Gilbert W. (1996) Discovery of amphibian Tel-like transposon families. J Mol Biol 257:359-366), Tc1-like transposon subfamilies (Ivics Z, Izsvak Z, Minter A, Hackett P B. (1996) Identification of functional domains and evolution of Tel-like transposable elements. Proc. Natl. Acad Sci USA 93: 5008-5013), Tc3 (Tu Z. Shao H. (2002) Intra- and inter-specific diversity of Tc-3 like transposons in nematodes and insects and implications for their evolution and transposition. Gene 282:133-142), ICESt1 (Burrus Vet al. (2002) The ICESt1 element of *Streptococcus thermophilus* belongs to a large family of integrative and conjugative elements that exchange modules and change their specificity of integration. Plasmid. 48(2): 77-97), maT, and P-element (Rubin G M and Spradling A C. (1983) Vectors for P element-mediated gene transfer in *Drosophila*. Nucleic Acids Res. 11:6341-6351). These references are incorporated herein by reference in their entirety for their teaching of the sequences and uses of transposons and transposon ITRs.

[0092] Translocation of Sleeping Beauty (SB) transposon requires specific binding of SB transposase to inverted terminal repeats (ITRs) of about 230 bp at each end of the transposon, which is followed by a cut-and-paste transfer of the transposon into a target DNA sequence. The ITRs contain two imperfect direct repeats (DRs) of about 32 bp. The outer DRs are at the extreme ends of the transposon whereas the inner DRs are located inside the transposon, 165-166 bp from the outer DRs. Cui et al. (J. Mol Biol 318:1221-1235) investigated the roles of the DR elements in transposition. Within the 1286-bp element, the essential regions are contained in the intervals bounded by coordinates 229-586, 735-765, and 939-1066, numbering in base pairs from the extreme 5′ end of the element. These regions may contain sequences that are necessary for transposase binding or that are needed to maintain proper spacing between binding sites.

[0093] Transposons are bracketed by terminal inverted repeats that contain binding sites for the transposase. Elements of the IR/R subgroup of the Tc1/mariner superfamily have a pair of transposase-binding sites at the ends of the 200-250 bp long inverted repeats (IRs) (Izsvak, et al. 1995). The binding sites contain short, 15-20 bp direct repeats (DRs). This characteristic structure can be found in several elements from evolutionarily distant species, such as Minos and S elements in flies (Franz and Savakis, 1991; Merriman et al, 1995), Quetzal elements in mosquitoes (Ke et al, 1996), Txr elements in frogs (Lam et al, 1996) and at least three Tc1-like transposon subfamilies in fish (Ivies et al., 1996), including SB [Sleeping Beauty] and are herein incorporated by reference.

[0094] Whereas Tc1 transposons require one binding site for their transposase in each IR, Sleeping Beauty requires two direct repeat (DR) binding sites within each IR, and is therefore classified with Tc3 in an IR/DR subgroup of the Tc1/mariner superfamily (96,97). Sleeping Beauty transposes into TA dinucleotide sites and leaves the Tel/mariner characteristic footprint, i.e., duplication of the TA, upon excision. The non-viral plasmid vector contains the transgene that is flanked by IR/DR sequences, which act as the binding sites for the transposase. The catalytically active transposase may be expressed from a separate (trans) or same (cis) plasmid system. The transposase binds to the IR/DRs, catalyzes the excision of the flanked transgene, and mediates its integration into the target host genome.

[0095] Naturally occurring mobile genetic elements, known as retrotransposons, are also candidates for gene transfer vehicles. This mutagenesis method generally involves the delivery of a gene trap.

[0096] Retrotransposons are naturally occurring DNA elements which are found in cells from almost all species of animals, plants and bacteria which have been examined to date. They are capable of being expressed in cells, can be reverse transcribed into an extrachromosomal element and reintegrate into another site in the same genome from which they originated.

[0097] Retrotransposons may be grouped into two classes, the retrovirus-like LTR retrotransposons, and the non-LTR elements such as human L1 elements, *Neurospora* TAD elements (Kinsey, 1990, Genetics 126:317-326), I factors from *Drosophila* (Bucheton et al., 1984, Cell 38:153-163), and R2Bm from *Bombyx mori* (Luan et al., 1993, Cell 72: 595-605). These two types of retrotransposon are structurally different and also retrotranspose using radically different mechanisms.

[0098] Unlike the LTR retrotransposons, non-LTR elements (also called polyA elements) lack LTRs and instead end with polyA or A-rich sequences. The LTR retrotransposition mechanism is relatively well-understood; in contrast, the mechanism of retrotransposition by non-LTR retrotransposons has just begun to be elucidated (Luan and Eickbush, 1995, Mol. Cell. Biol. 15:3882-3891; Luan et al., 1993, Cell 72:595-605). Non-LTR retrotransposons can be subdivided into sequence-specific and non-sequence-specific types. L1 is of the latter type being found to be inserted in a scattered manner in all human, mouse and other mammalian chromosomes.

[0099] Some human L1 elements (also known as a LINES) can retrotranspose (express, cleave their target site, and reverse transcribe their own RNA using the cleaved target site as a primer) into new sites in the human genome, leading to genetic disorders.

[0100] In some embodiments mutations are used for assessing the frequency with which selected cells undergo insertional mutagenesis for the generation of genetically modified animals and the like. Engineered L1 elements can also be used as retrotransposon mutagens. Sequences can be introduced into the L1 that increases its mutagenic potential or facilitates the cloning of the interrupted gene. DNA sequences useful for this application of the invention include marker DNAs, such as GFP, that are specifically engineered to integrate into genomic DNA at sites which are near to the endogenous genes of the host organism. Other potentially useful DNAs for delivery are regulatory DNA elements, such as promoter sequences, enhancer sequences, retroviral LTR elements and repressors and silencers. In addition, genes which are developmentally regulated are useful in the invention.

Viral Mutagenesis Methods

[0101] Viral vectors are often created using a replication defective virus vector with a genome that is partially replaced by the genetic material of interest (e.g., gene trap, selectable marker, and/or a therapeutic gene). The viral vector is produced by using a helper virus to provide some of the viral components that were deleted in the replication defective virus, which results in an infectious recombinant virus whose genome encodes the genetic material of interest. Viral vectors can be used to introduce an insertion mutation into the rat's genome. Integration of the viral genetic material is often carried out by the viral enzyme integrase. Integrase brings the ends of viral DNA together and converts the blunt ends into recessed ends. Integrase creates staggered ends on chromosomal DNA. The recessed ends of the viral DNA are then joined with the overhangs of genomic DNA, and the single-stranded regions are repaired by cellular mechanisms. Some recombinant virus vectors are equipped with cell uptake, endosomal escape, nuclear import, and expression mechanisms allowing the genetic material of interest to be inserted and expressed in the rat's genome. The genetic material introduced via viral vectors can genetically modify the rat's genome but is not limited to disrupting a gene, inserting a gene to be expressed, and by delivery of interfering RNA. Viral vectors can be used in multiple methods of delivery. The most common mode of delivery is the microinjection of a replication deficient viral vector (e.g., retroviral, adenoviral) into an early embryo (1-4 day) or a onecell pronuclear egg. After viral vector delivery, the embryo is cultured in vitro and transferred to recipient rats to create genetically modified progeny.

[0102] In some embodiments, insertion mutations can be created by delivery of a gene trap vector into the rat genome. The gene trap vector consists of a cassette that contains selectable reporter tags. Upstream from this cassette is a 3′ splice acceptor sequence. Downstream from the cassette lays a termination sequence poly adenine repeat tail (polyA).

The splice accepter sequence allows the gene trap vector to be spliced into chromosomal mRNA. The polyA tail signals the premature interruption of the transcription. The result is a truncated mRNA molecule that has decreased function or is completely non-functional. The gene trap method can also be utilized to introduce exogenous DNA into the genome.

[0103] In some embodiment an enhancer trap is used for insertional mutagenesis. An enhancer trap is a transposable element vector that carries a weak minimal promoter which controls a reporter gene. When the transposable element is inserted the promoter drives expression of the reporter gene. The expression of the reporter gene also displays the expression patterns of endogenous genes. Enhancer trapping results in genetic modification and can be used for gain-of-function genetics. The Gal4-mediated expression system is an example of an enhancer trap.

[0104] Further included are one or more selectable marker genes. Examples of suitable prokaryotic marker genes include, but are not limited to, the ampicillin resistance gene, the kanamycin resistance gene, the gene encoding resistance to chloramphenicol, the lacZ gene and the like. Examples of suitable eukaryotic marker genes include, but are not limited to, the hygromycin resistance gene, the green fluorescent protein (GFP) gene, the neomycin resistance gene, the zeomycin gene, modified cell surface receptors, the extracellular portion of the IgG receptor, composite markers such as beta-geo (a lac/neo fusion) and the like.

[0105] In some embodiment, the gene trap an be integrated into the host genome and an integrating enzyme can be needed. Integrating enzymes can be any enzyme with integrating capabilities. Such enzymes are well known in the art and can include but are not limited to transposases, integrases, recombinases, including but not limited to tyrosine site-specific recombinases and other site-specific recombinases (e.g., cre), bacteriophage integrases, retrotransposases, and retroviral integrases.

[0106] The integrating enzymes can be any enzyme with integrating capabilities. Such enzymes are well known in the art and can include but are not limited to transposases (especially DDE transposases), integrases, tyrosine site-specific recombinases and other site-specific recombinases (e.g., cre), bacteriophage integrases, integrons, retrotransposases, retroviral integrases and terminases.

[0107] In some embodiments, the integrating enzyme is a transposase. It is understood and herein contemplated that the transposase of the composition is not limited and to any one transposase and can be selected from at least the group consisting of Sleeping Beauty (SB), Tn7, Tn5, mosl, piggyBac, Himar1, Hermes, Tol2, Pokey, Minos, S elements, P-elements, ICESt1, Quetzal elements, Tn916, maT, Tc1/mariner and Tc3.

[0108] Where the integrating enzyme is a transposase, it is understood that the transposase of the composition is not limited and to any one transposase and can be selected from at least the group consisting of Sleeping Beauty (SB), Tn7, Tn5, Tn916, Tc1/mariner, Minos and S elements, Quetzal elements, Txr elements, maT, mosI, piggyBac, Himar1, Hermes, Tol2, Pokey, P-elements, and Tc3. Additional transposases may be found throughout the art, for example, U.S. Pat. Nos. 6,225,121, 6,218,185 5,792,924 5,719,055, U.S. Patent Application No. 20020028513, and U.S. Patent Application No. 20020016975 and are herein incorporated by reference in their entirety. Since the applicable principal of the invention remains the same, the compositions of the invention can include transposases not yet identified.

[0109] In some cases, the integrating enzyme can be a bacteriophage integrase. Such integrase can include any bacteriophage integrase and can include but is not limited to lamda bacteriophage and mu bacteriophage, as well as Hong Kong 022 (Cheng Q., et al. Specificity determinants for bacteriophage Hong Kong 022 integrase: analysis of mutants with relaxed core-binding specificities. (2000) Mol Microbiol. 36(2):424-36), HP1 (Hickman, A. B., et al. (1997). Molecular organization in site-specific recombination: The catalytic domain of bacteriophage HP1 integrase at 2.7 A resolution. Cell 89: 227-237), P4 (Shoemaker, N B, et al. (1996). The *Bacteroides mobilizable* insertion element, NBU1, integrates into the 3′ end of a Leu-tRNA gene and has an integrase that is a member of the lambda integrase family. J Bacteriol. 178(12):3594-600), P1 (Li Y, and Austin S. (2002) The P1 plasmid in action: time-lapse photomicroscopy reveals some unexpected aspects of plasmid partition. Plasmid. 48(3):174-8), and T7 (Rezende, L. F., et al. (2002) Essential Amino Acid Residues in the Single-stranded DNA-binding Protein of Bacteriophage T7. Identification of the Dimer Interface. J. Biol. Chem. 277, 50643-50653). Integrase maintains its activity when fused to other proteins.

[0110] In some cases, the recombinase can be a Cre recombinase, Flp recombinase, HIN recombinase, or any other recombinase. Recombinases are well-known in the art. An extensive list of recombinases can be found in Nunes-Duby S E, et al. (1998) Nuc. Acids Res. 26(2): 391-406, which is incorporated herein in its entirety for its teachings on recombinases and their sequences.

[0111] In some cases, the retrotransposase can be a GATE retrotransposase (Kogan G L, et al. (2003) The GATE retrotransposon in *Drosophila melanogaster*: mobility in heterochromatin and aspects of its expression in germ line tissues. Mol Genet Genomics. 269(2):234-42).

[0112] Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination. These systems typically

rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

Zinc Finger Nucleases

[0113] In another method, a zinc finger nuclease creates site-specific deletions via double-stranded DNA breaks that are repaired by non-homologous end joining (NHEJ). Zinc finger nucleases may also be used to create an insertion mutation by combining the ZFN with a homologously integrating cassette to create an insertion in the genomic DNA. Therefore, this genetic modification method can be used for both targeted (site-specific) DNA insertions and targeted DNA deletions. In one embodiment, the method involves transformation of a cell with a nucleic acid or mRNA construct minimally comprising DNA encoding a chimeric zinc finger nuclease (ZFN), which can be used to create a DNA deletion. In another embodiment, a second DNA construct can be provided that will serve as a template for repair of the cleavage site by homologous recombination. In this embodiment, a DNA insertion may be created. The DNA insertion may contain a gene trap cassette. In some embodiment, this method can be combined with spermatogonial stem cell technology or embryonic stem cell technology, as mentioned above. In another embodiment, this method can be combined with mobile DNA technology. This technique can also be done directly in the rat embryo.

Nucleic Acid Modification Methods

[0114] In some embodiments, a random mutation is created with a chemical mutagen and then a screen is performed for insertions in a particular gene. Chemical mutagens such as methane-sulfonic acid ethylester (EMS), N-ethyl-N-nitrosourea (ENU), diepoxyoctane and UV/trimethylpsorlalen may be employed to create nucleic acid sequence mutations.

[0115] Sequence editing methods can also be used that involve the delivery of small DNA fragments, hybrid DNA/RNA molecules, and modified DNA polymers to create sequence mismatches and nucleic acid mutations. RNA/DNA hybrids are molecules composed of a central stretch of DNA flanked by short RNA sequences that form hairpin structures. The RNA/DNA hybrids can produce single base-pair substitutions and deletions resulting in nucleotide mutations. Some other sequence editing examples include triplex forming oligonucleotides, small fragment homologous replacement, single stranded DNA oligonucleotides, and adeno-associated virus (AAV) vectors.

[0116] In some embodiments, RNA interference may be used to alter the expression of a gene. In another genetic modification method, the delivery of a transgene encoding a dominant negative protein may alter the expression of a target gene.

Vector Delivery Methods

[0117] A nucleic acid vector may be introduced into one or more cells using any of a variety of techniques known in the art such as, but not limited to, microinjection, combining the nucleic acid fragment with lipid vesicles, such as cationic lipid vesicles, particle bombardment, electroporation, DNA condensing reagents (e.g., calcium phosphate, polylysine or polyethyleneimine) or incorporating the nucleic acid fragment into a viral vector and contacting the viral vector with the cell. Where a viral vector is used, the viral vector can include any of a variety of viral vectors known in the art including viral vectors selected from the group consisting of a retroviral vector, an adenovirus vector or an adeno-associated viral vector.

[0118] DNA or other genetic material may be delivered through viral and non-viral vectors. These vectors can carry exogenous DNA that is used to genetically modify the genome of the rat. For example Adenovirus (AdV), Adeno-associated virus (AAV), and Retrovirus (RV) which contain LTR regions flanking a gene trap, transgene, cassette or interfering RNA are used to integrate and deliver the genetic material. Another delivery method involves non-viral vectors such as plasmids used for electroporation and cationic lipids used for lipofection. The non-viral vectors usually are engineered to have mechanisms for cell uptake, endosome escape, nuclear import, and expression. An example would be a non-viral vector containing a specific nuclear localization sequence and sequence homology for recombination in a targeted region of the genome.

[0119] There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

[0120] The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

[0121] Thus, the compositions can comprise, in addition to the disclosed non-viral vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposome, or polymersomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. Am. J. Resp. Cell. Mol. Biol. 1:95-100 (1989); Feigner et al. Proc. Natl. Acad. Sci USA 84:7413-7417 (1987); U.S. Pat. No. 4,897,355. Furthermore, the vector can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

[0122] In the methods described above, which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, Md.), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, Wis.), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered in vivo by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, Calif.) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, Ariz.).

[0123] These vectors may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue and are incorporated by reference herein (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K. D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other specific cell types.

Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid-mediated drug targeting to colonic carcinoma), receptor-mediated targeting of DNA through cell specific ligands, lymphocyte-directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells in vivo.

[0124] Nucleic acids that are delivered to cells which are to be integrated into the host cell genome typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

Methods of Screening of the Invention

[0125] Such animal model may for instance be of major interest for industrial validation of current and future treatments against T2D and Alzheimer's disease.

[0126] Therefore, also disclosed herein is a method of screening a compound for therapeutic use in the treatment of T2D and/or Alzheimer's disease, using the animal of the invention.

[0127] Also disclosed is the use of said animal for assessing potential side-effects of treatment of T2D and/or Alzheimer's disease. Said treatment may include, for example, administration of therapeutic compounds that act on APP accumulation, as described below.

[0128] The compound to be screened for therapeutic use against T2D and/or Alzheimer's disease may be used for preventing or treating T2D and/or Alzheimer's disease. Such compound may be any kind of compound that may act on T2D and/or Alzheimer's disease. It may for instance decrease accumulation of APP and/or decrease accumulation of neurotoxic metabolites derived from APP (Aβ42 and βCTF) for example. The compound to be screened for therapeutic use against T2D and/or Alzheimer's disease should preferably display a low toxicity.

[0129] The screening may for instance include the steps of administering a compound to be screened to the animal disclosed herein, waiting for a certain period of time, optionally repeating the administration, measuring blood glucose, the accumulation of APP and/or neurotoxic metabolites, or a combination thereof, and selecting the compound according to its effect on blood glucose and/or the accumulation of APP and/or neurotoxic metabolites. For example, if the compound tested allows a decrease of the accumulation of APP and/or neurotoxic metabolites, it could be select as potential therapeutic drug against Alzheimer's disease.

[0130] Alternatively, the disclosed animal may also be for use for studying the mechanism of T2D and/or Alzheimer's disease. Another embodiment concerns the use of an animal having T2D and Alzheimer's disease for studying the mechanism of the disease, said animal being obtained by the method disclosed herein. For instance, such an animal can be useful for understanding the physio-pathology or the molecular mechanism involved in T2D and Alzheimer's disease.

[0131] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

EXAMPLES

Example 1:

[0132] FIG. 1 shows two schemes for producing animal models having type 2 diabetes and Alzheimer's disease.

[0133] FIG. 2 shows colony founders with APP and PS1 transgenes and resulting glucose.

[0134] FIGs. 3A to 3C show amyloid burden in brain of T2D-/- rats at 6 months (FIG. 3A), T2D+/- rats at 6 months (FIG. 3B), and T2D+/- rats at 9 months (FIG. 3C). Propidium Iodide (PI) in red. F1-Fluoro-2,5-bis[(E)-3-carboxy-4-hydroxystyryl]benzene solution (FSB)in blue. Rats never spontaneously develop AD. T2D-AD$^{+/-}$ shows amyloid burden at 6 mos. Tg344AD$^{+/-}$ shows amyloid burden at 16 months.

[0135] FIGs. 4A to 4C show cognitive performance of T2D-AD$^{+/-}$ and T2D-AD$^{-/-}$ rats. FIG. 4A shows escape latency as a function of time for T2D-AD$^{+/-}$ (square) and T2D-AD$^{-/-}$ (circle) rats. FIGs. 4B and 4C show % use as a function of time for T2D-AD$^{-/-}$ (FIG. 4B) and T2D-AD$^{+/-}$ (FIG. 4C) rats.

[0136] FIG. 5 shows diabetic incidence (%) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats. Diabetes incidence is glucose > 350 mg/dL.

[0137] FIG. 6 shows non-fasting glucose levels (mg/dL) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats.

[0138] FIG. 7 shows insulin levels (ng/mL) as a function of time for T2D-AD$^{+/-}$ (dashed line) and T2D-AD$^{-/-}$ (solid line) rats.

[0139] FIGs. 8A to 8C show Metabolite dynamics of Pyr, Lac and Bic signals in representative control (FIG. 8A), T2D-AD$^{-/-}$ (FIG. 8B), and T2D-AD$^{+/-}$ (FIG. 8C) animals after an injection of hyperpolarized ~100mM [1-^{13}C]Pyr solution.

[0140] FIG. 9 is a comparison of spectra from three representative animals highlights the increase in lactate and reduction of bicarbonate production in a T2D-AD$^{-/-}$ animal compared to a control, which is further amplified in a T2D-AD$^{+/-}$ animal.

[0141] FIG. 10 shows Bic/Lac expression in control, T2D-AD$^{-/-}$, and T2D-AD$^{+/-}$, Tg344-AD$^{-/-}$, and Tg344-AD$^{+/-}$ rats.

Table 1.

	F-statistics	Df	N	P
Ctrl vs. T2D-AD$^{+/-}$	140	6	7	7.6×10^{-5}
Ctrl vs. T2D-AD$^{-/-}$	50	7	8	4.1×10^{-4}
T2D-AD$^{-/-}$ vs. T2D-AD$^{+/-}$	105	10	11	3.0×10^{-6}

Table 2.

	Average ± Std. Error
Control	0.194 ± 0.014
T2D-AD$^{-/-}$	0.111 ± 0.002
T2D-AD$^{+/-}$	0.064 ± 0.001
Tg344-AD$^{-/-}$	0.275
Tg344-AD$^{+/-}$	0.071

[0142] FIG. 11 shows astrocyte neuron lactate shuttle (ANLS).

[0143] Therefore, this example provides an animal model with T2D and AD transgenes. The Tg344AD and T2D-AD transgenes have the same transgene intercalation site.

[0144] In these rats, amyloid burden appears early (6mos). In addition, there is a cognitive performance: T2D-AD +/- poorer than T2D -/-. PDH activity decreases with T2D and further with T2D-AD. DCA appears to restore PDH activity. And, finally, AD and T2D have reciprocal effects.

Example 2:

[0145] FIG. 12 shows an agarose DNA gel electrophoresis following polymerase chain reaction of PS1, Prp, and APP genes. The far-left lane contains the DNA ladder. All rats (TgF344-AD -/-, TgF344-AD +/-, T2D-AD -/-, T2D-AD +/-, and T2D) contain a band at 799 bp, indicating the presence of the genomic Prp product. Only the TgF344-AD +/- and T2D-AD +/- rats contain the bands at 1,300 and 400 bp, indicating the presence of the PS1 and APP genes, respectively. The TgF344-AD -/- and T2D-AD -/- do not display the bands at 1,300 and 400 bp.

[0146] FIG. 13 shows a comparison of amyloid plaques in brain regions of TgF344-AD and T2D-AD rats. 9-month-old TgF344-AD -/- and T2D-AD -/- rat brains display no amyloid

plaques (panels A, B). Nine-month-old TgF344-AD +/- and T2D-AD +/- rat brains display amyloid plaques in hippocampus (red), cortex (green), and thalamus (panels C, D).

[0147] FIG. 14 shows quantitative analysis (ImageJ (NIH)) amyloid plaque distribution. TgF344AD +/- rat: hippocampus (2.92%±0.34%), cortex (1.11%±0.26%), and thalamus (0.12%±0.04%); T2D-AD +/- rats: hippocampus (5.39%±0.18%), cortex (2.65%±0.13%), and thalamus (0.32%±0.14%).

[0148] FIG. 15 shows non-fasting blood glucose concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 8 and for T2D-AD +/- rats, n = 10. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in non-fasting blood glucose concentrations at and after diabetes onset ($F_{7,3} = 15.44$, $p = 1.56\times10\text{-}5$). A two-tail t-test indicates a significant difference between non-fasting blood glucose concentrations in T2D-AD -/- and T2D-AD +/- rats at onset (*), at 1 month (**) and 2 months after onset (***). Data points are mean ± SEM.

[0149] FIG. 16 shows fasting blood glucose concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 7, and for T2D-AD +/- rats, n = 10. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in fasting blood glucose concentrations after diabetes onset ($F_{6,3} = 5.20$, $p = 0.009$). A two-tail t-test indicates a significant difference between fasting blood glucose concentrations in T2D-AD -/- and T2D-AD +/- rats 1 month after onset (*) and 2 months after onset (**). Data points are mean ± SEM.

[0150] Fig. 17 shows fasting insulin concentrations before and after diabetes onset. For T2D-AD -/- rats, n = 7, and for T2D-AD +/- rats, n = 8. One-way repeated measures ANOVA for T2D-AD -/- rats indicates a significant difference in fasting insulin concentrations before and after diabetes onset ($F_{6,3} = 6.82$, $p = 2.89\times10\text{-}3$). A two-tail t-test indicates a significant difference between fasting insulin concentrations in T2D-AD -/- and T2D-AD +/- rats at onset (*), at 1 month after onset (**), and at 2 months after onset (***). Data points are mean ± SEM.

[0151] FIG. 18 shows Barnes maze escape latencies. Escape latencies for the T2D-AD -/- and T2D-AD +/- rats for days 2-6 (n = 18 animals per genotype). T2D-AD +/- rats show no change, while the T2D-AD -/- rats decrease their escape latencies over time. T-test indicates a significant difference between T2D-AD -/- and T2D-AD +/- latencies on day 5 (*, $t(110) = -3.656$, $p = 3.950\times10\text{-}4$) and day 6 (**, $t(144) = -3.097$, $p = 2.345\times10\text{-}3$). Data points are shown as mean ± standard error of the mean (SEM).

[0152] FIG. 19 shows Barnes maze escape latencies for TgF344-AD rats for days 2-6. TgF344-AD -/- (n = 10). TgF344-AD +/- (n = 7). TgF344-AD -/- latencies for days 2-6 TgF344-AD +/- rats show no change, while the TgF344-AD -/- rats decrease their escape latencies over

time. T-test indicates a significant difference on day 5 (*, t(41) = -1.795, p = 0.040) and day 6 (**, t(61) = -2.028, p =0.023). Data points are shown as mean ± standard error of the mean (SEM).

[0153] FIGs. 20A and 20B show Barnes maze search strategies for T2D-AD -/- (FIG. 20A) T2D-AD +/- (FIG. 20B) rats. T2D-AD -/- rats used the direct search strategy much more than T2D-AD +/- rats. Direct strategy reflects cognitive skills. Chi-square analysis collapsing data across all trials revealed a significant difference in the distribution of search patterns between T2D-AD -/- and T2D-AD +/- rats ($p = 2.46\times10^{-4}$).

[0154] FIGs. 21A and 21B show Barnes maze search strategies for T2D-AD -/- (FIG. 20A) T2D-AD +/- (FIG. 20B) rats. TgF344AD -/- rats used the direct search strategy much more than TgF344AD +/- rats. Direct strategy reflects cognitive skills. Chi-square analysis collapsing data across all trials revealed a significant difference in the distribution of search patterns between Tg344-AD -/- and Tg344-AD +/- rats (p = 0.0172).

[0155] FIG. 22 shows Bic/lac ratio before and after dichloroacetate (DCA). Dichloroacetate activates pyruvate dehydrogenase. Control rats (n=3) displayed a bic/lac ratio of 0.164±0.012. Data not available for post DCA. T2D-AD -/- (n=8) displayed a bic/lac ratio of 0.075±0.005. After DCA (n=2) bic/lac ratios increased to 0.207±0.014. T2D-AD +/- rats displayed (n=7) a bic/lac ratio of 0.042±0.004, almost 50% lower than the T2D-AD -/- rats. Post-DCA (n=2) the bic/lac ratio increased to 0.263±0.068. The Tg344-AD -/- rats (n=1) displayed a bic/lac ratio of 0.275. Post DCA bic/lac ratio jumps to 0.653. Tg344-AD +/- rat (n=1) displayed a bic/lac ratio of 0.071. Post-DCA (n=1) rat showed a bic/lac ratio of 0.361.

[0156] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

[0157] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A rodent model of type 2 diabetes with Alzheimer's disease comprising:
   (a) insulin resistance,
   (b) a defect in pancreatic beta-cell function but normal leptin signaling,
   (c) transgenic hemizygous human amyloid precursor protein (*APP*) expression, and
   (d) transgenic human presenilin-1 (*PS1*) expression.

2. The rodent model of claim 1, produced by a process comprising cross-breeding a first diabetic rodent having insulin resistance and a defect in pancreatic beta-cell function but normal leptin signaling with a second rodent containing hemizygous human amyloid precursor protein (*APP*) and presenilin-1 (*PS1*) alleles.

3. The rat model of claim 2, wherein the second rodent is a TgF344AD$^{+/-}$ rat.

4. The rat model of claim 2 or 3, wherein the first rodent is a UCD_T2DM Sprague Dawley rat.

5. The rat model of claim 4, wherein the first rodent is produced by a process comprising cross-breeding a polygenic obesity rodent model with a rodent having a defect in pancreatic beta-cell function but normal leptin signaling.

6. The rat model of claim 5, wherein the first rodent is produced by a process comprising cross-breeding the polygenic obesity rodent model with a with Zucker diabetic fatty (ZDF)-lean rat.

Scheme 1

T2D

Inject into nuclei

+ = 2 human AD genes
APP (APPsw)
Presenilin-1 (PS1ΔE9)

T2D -AD +/-
T2D -AD -/-

Scheme 2

T2D x TgF344-AD +/-

Cross breed

T2D -AD +/-
T2D -AD -/-

FIG. 1

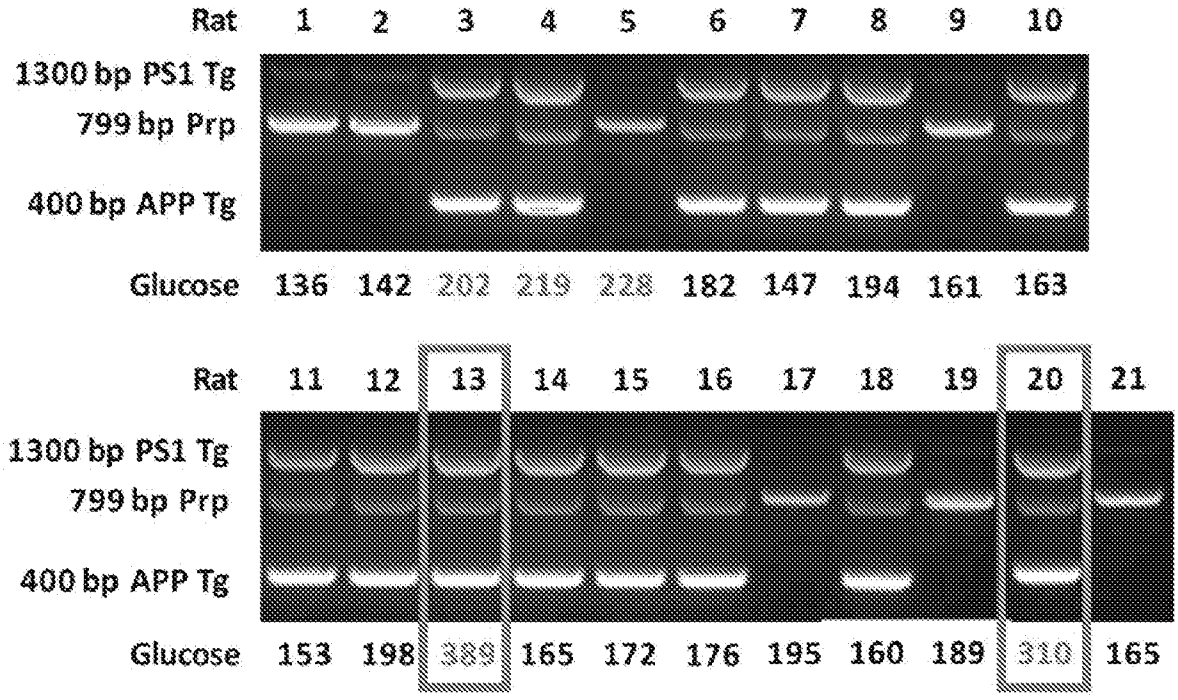

FIG. 2

T2D -/- 6mos

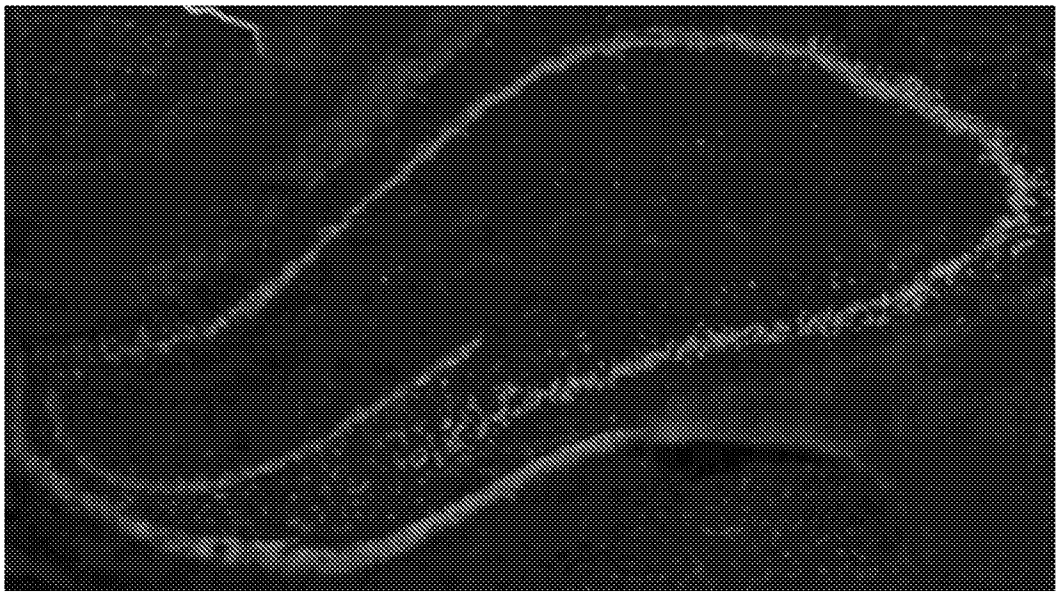

FIG. 3A

T2D +/- 6mos

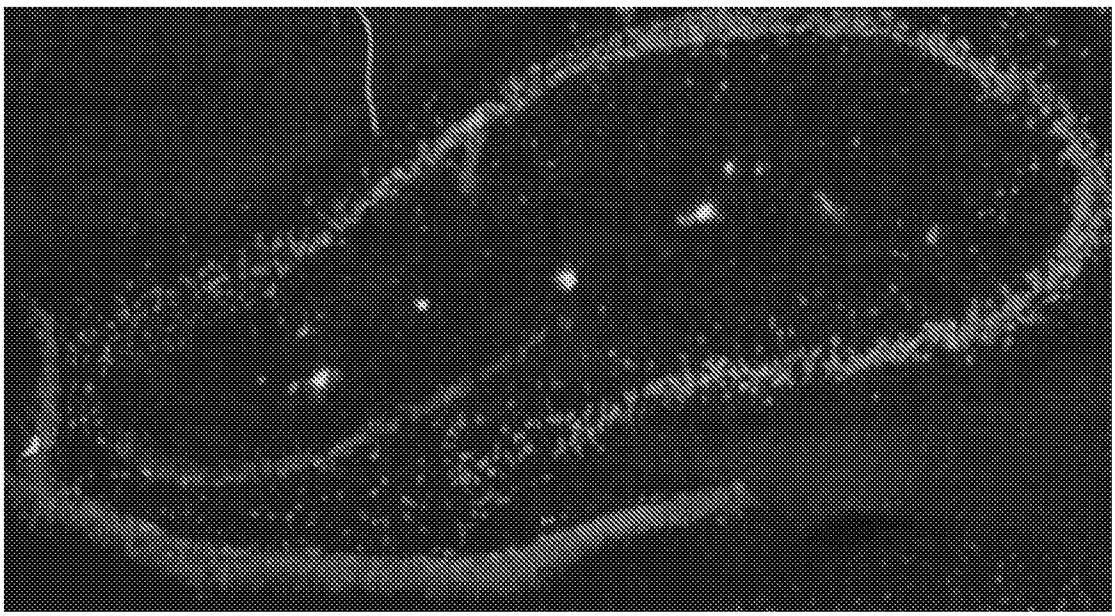

FIG. 3B

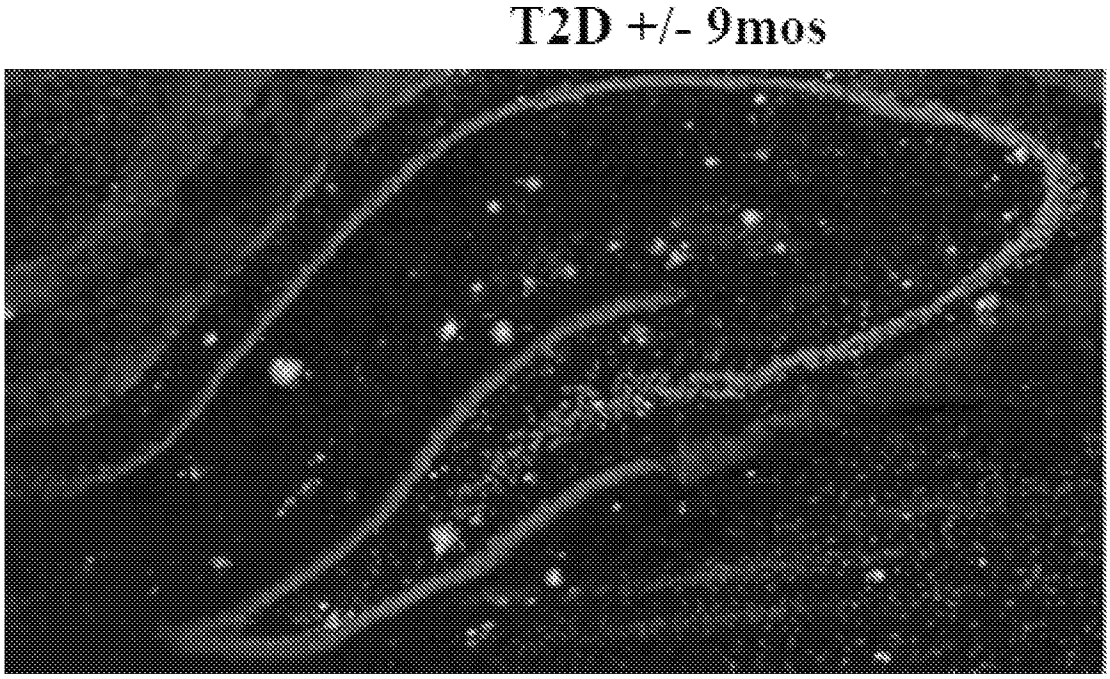
T2D +/- 9mos

FIG. 3C

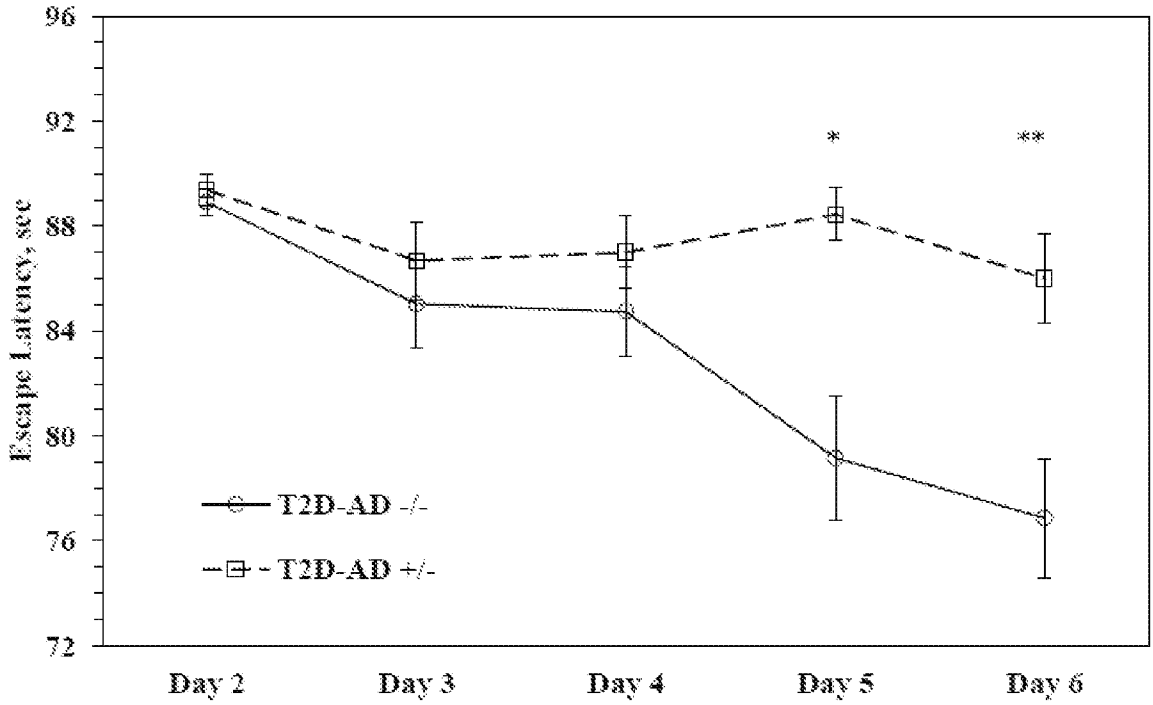
96
92
88
84
80
76
72
Escape Latency, sec
Day 2
Day 3
Day 4
Day 5
Day 6
*
**
T2D-AD -/-
T2D-AD +/-

FIG. 4A

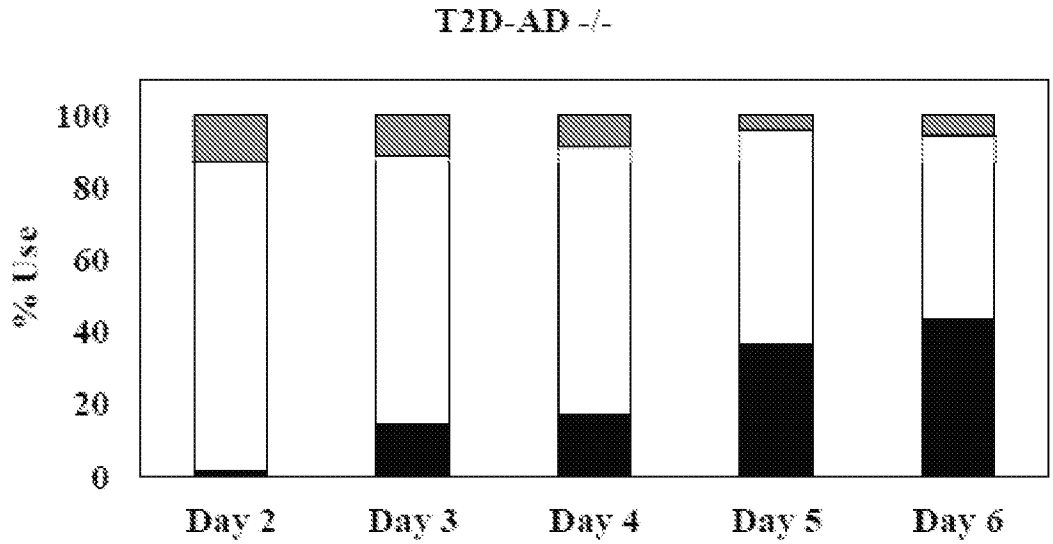

FIG. 4B

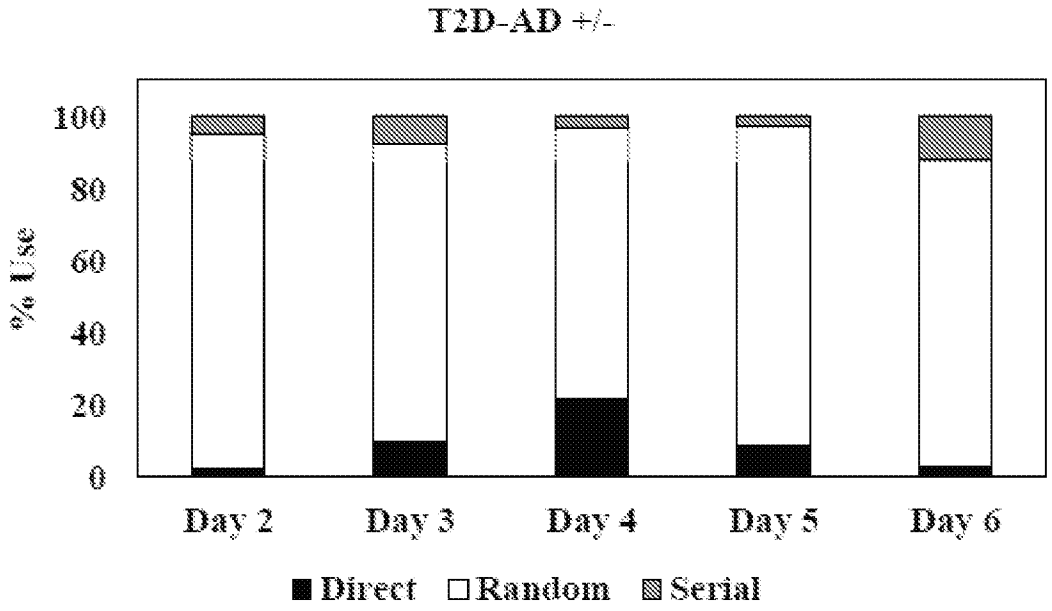
T2D-AD +/-
100
80
60
40
20
0
% Use
Day 2
Day 3
Day 4
Day 5
Day 6
Direct
Random
Serial

FIG. 4C

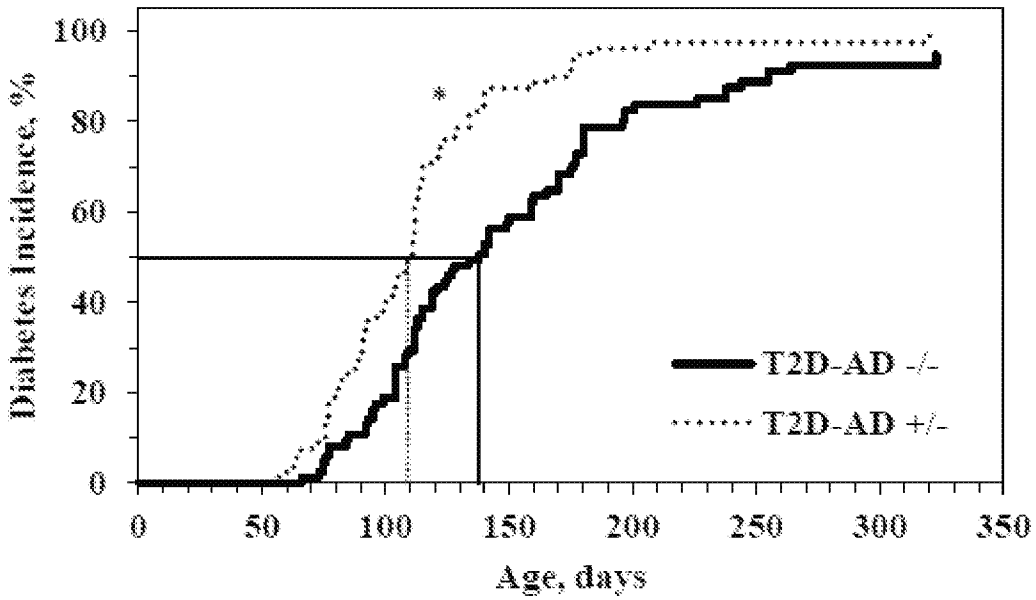
100
80
60
40
20
0
Diabetes Incidence, %
*
T2D-AD -/-
T2D-AD +/-
0
50
100
150
200
250
300
350
Age, days

FIG. 5

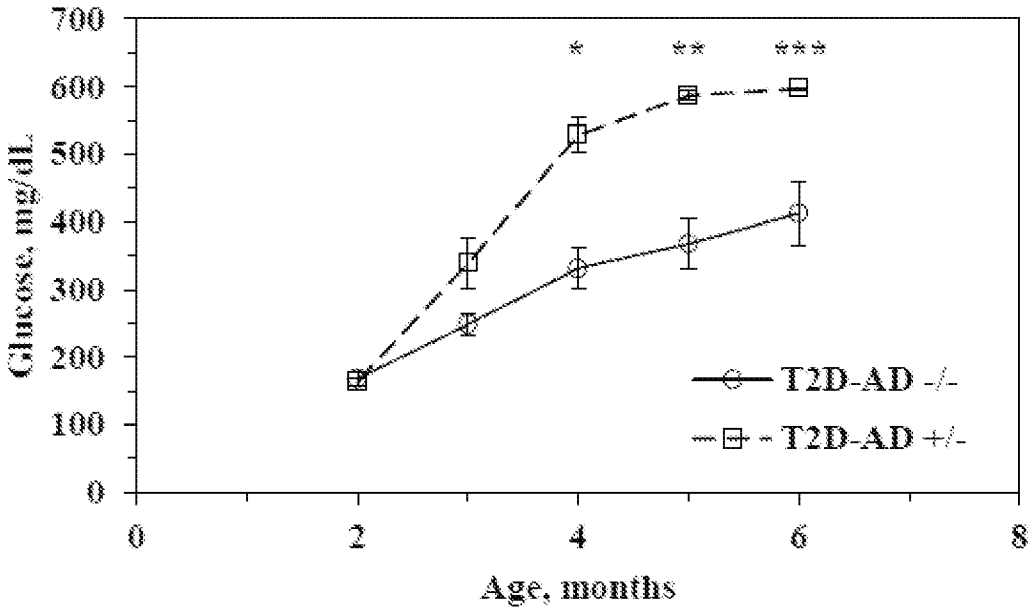
700
600
500
400
300
200
100
0
Glucose, mg/dL
0
2
4
6
8
Age, months
*
**
***
T2D-AD -/-
T2D-AD +/-

FIG. 6

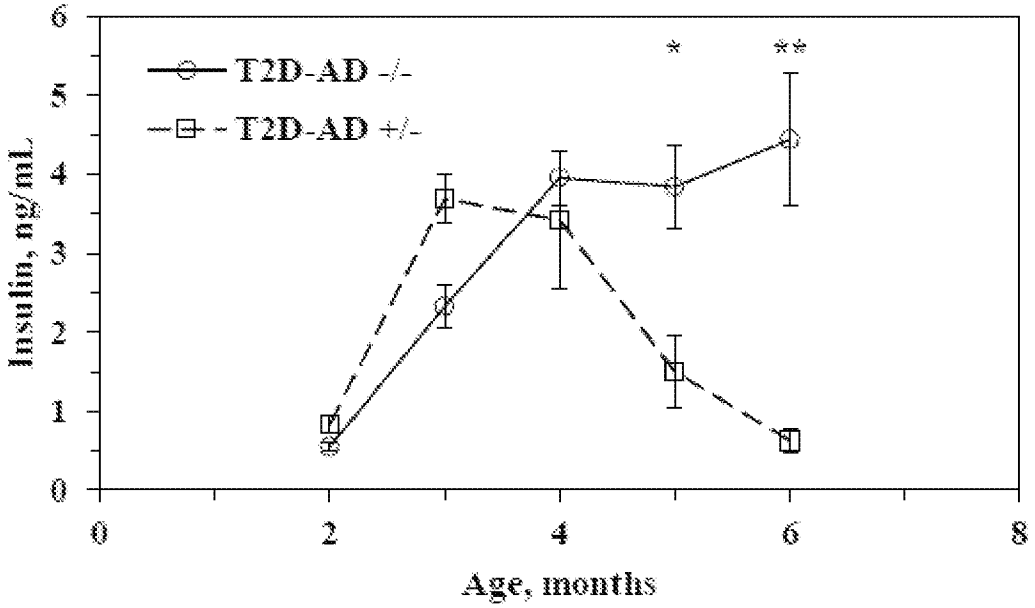
6
5
4
3
2
1
0
Insulin, ng/mL
0
2
4
6
8
Age, months
*
**
T2D-AD -/-
T2D-AD +/-

FIG. 7

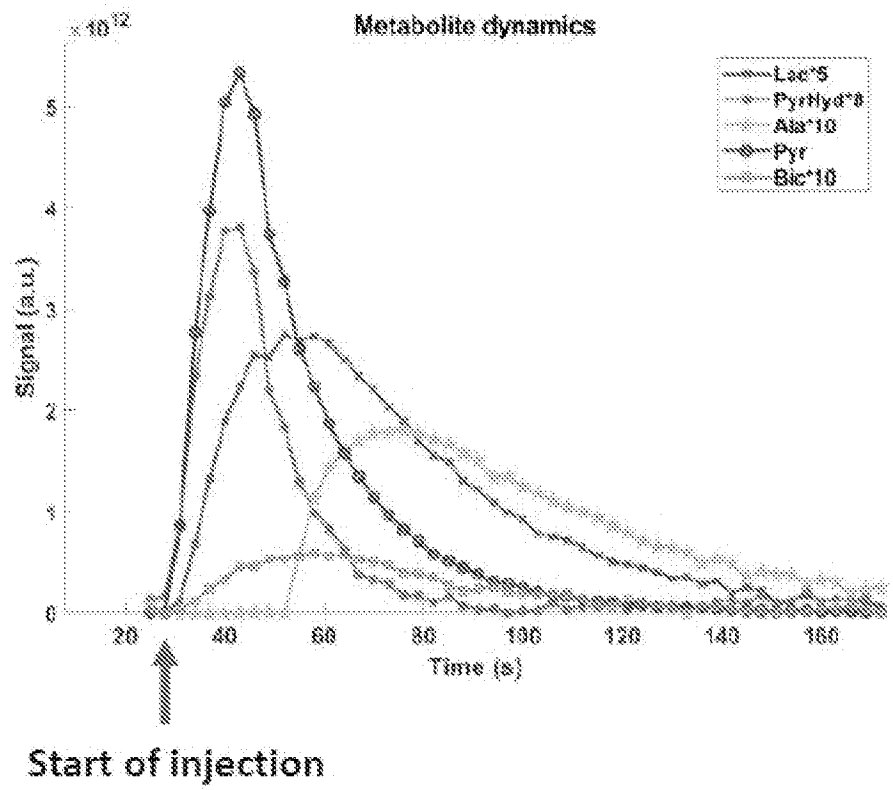
Metabolite dynamics
Lac*5
PyrHyd*8
Ala*10
Pyr
Bic*10
Signal (a.u.)
Time (s)
Start of injection

FIG. 8A

T2D-AD -/-

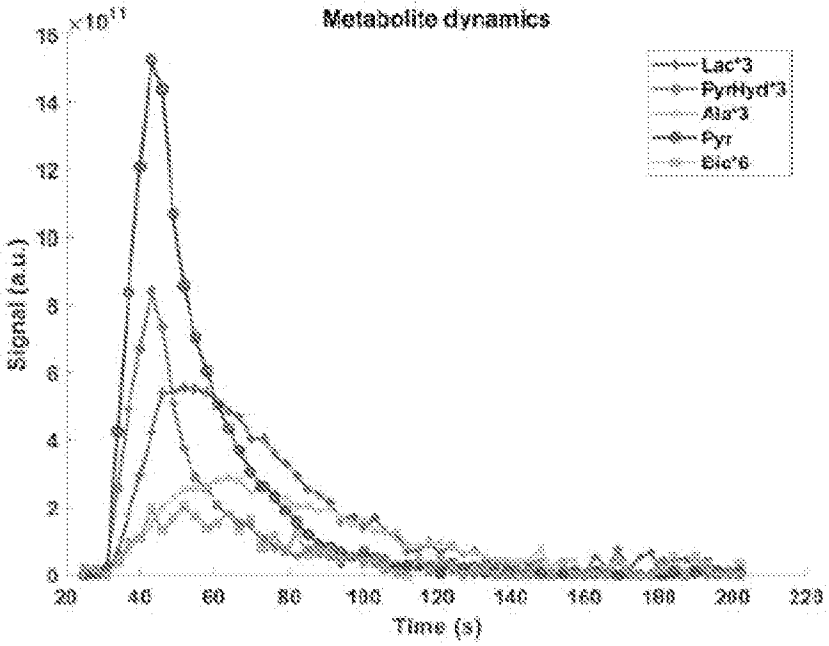
Metabolite dynamics
×10^11
Signal (a.u.)
Time (s)
Lac*3
PyrHyd*3
Ala*3
Pyr
Bic*6

FIG. 8B

T2D-AD +/-

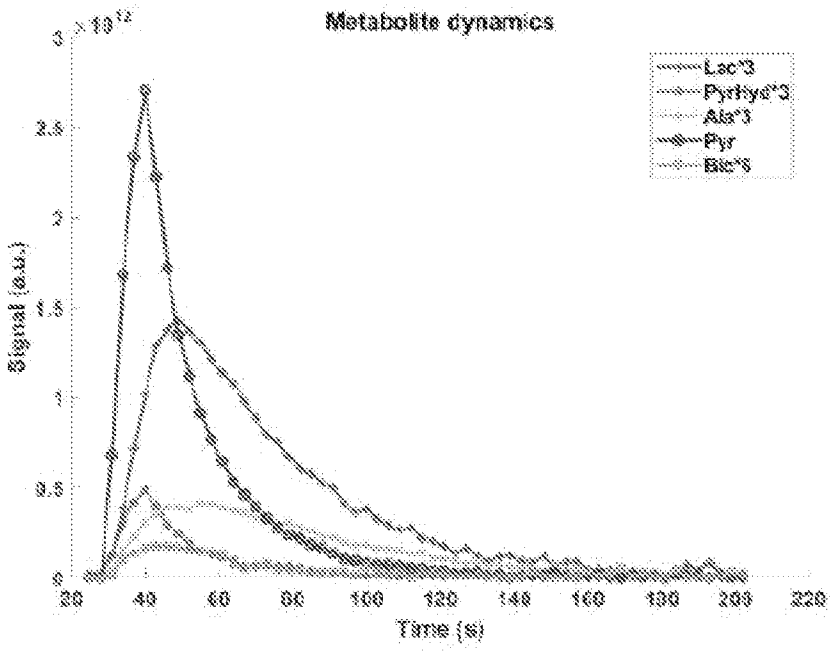
Metabolite dynamics
Lac*3
Pyr*3
Ala*3
Pyr
Bic*3
Signal (a.u.)
Time (s)

FIG. 8C

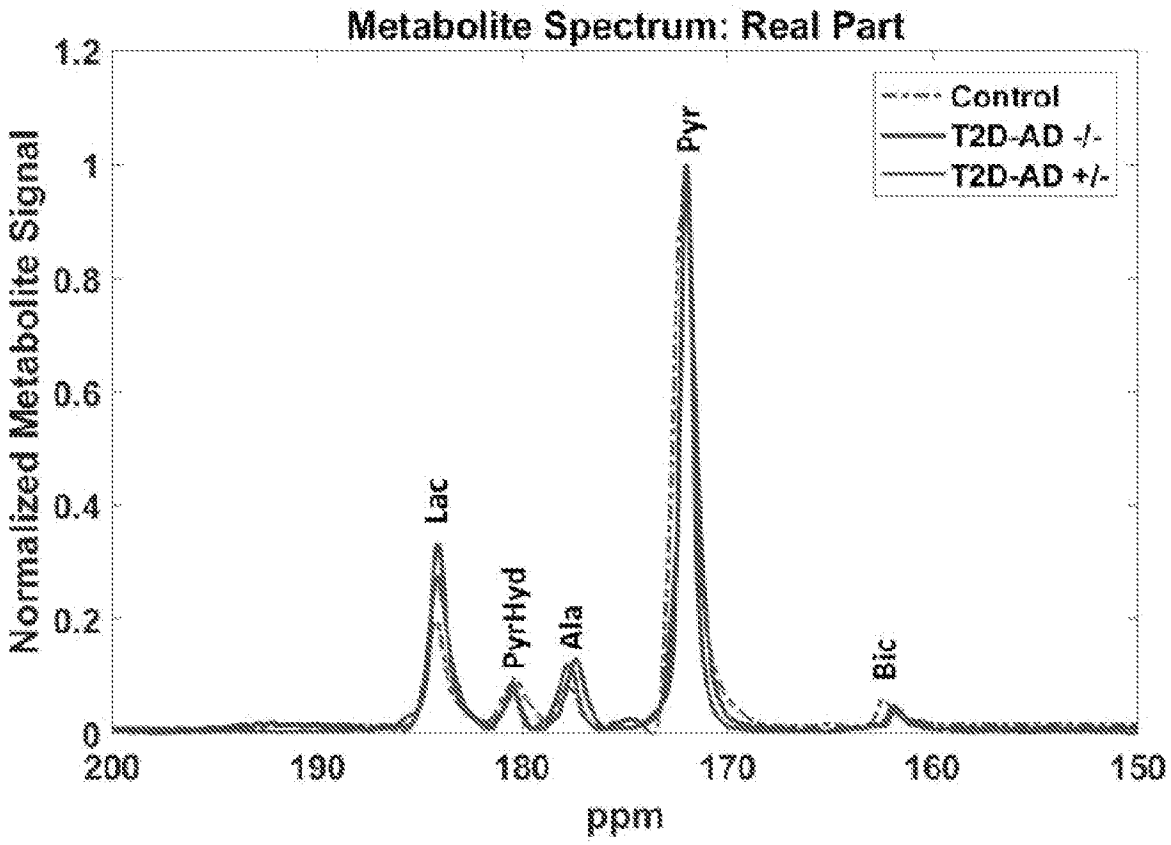
Metabolite Spectrum: Real Part
Normalized Metabolite Signal
ppm
Control
T2D-AD -/-
T2D-AD +/-
Lac
PyrHyd
Ala
Pyr
Bic
0
0.2
0.4
0.6
0.8
1
1.2
200
190
180
170
160
150

FIG. 9

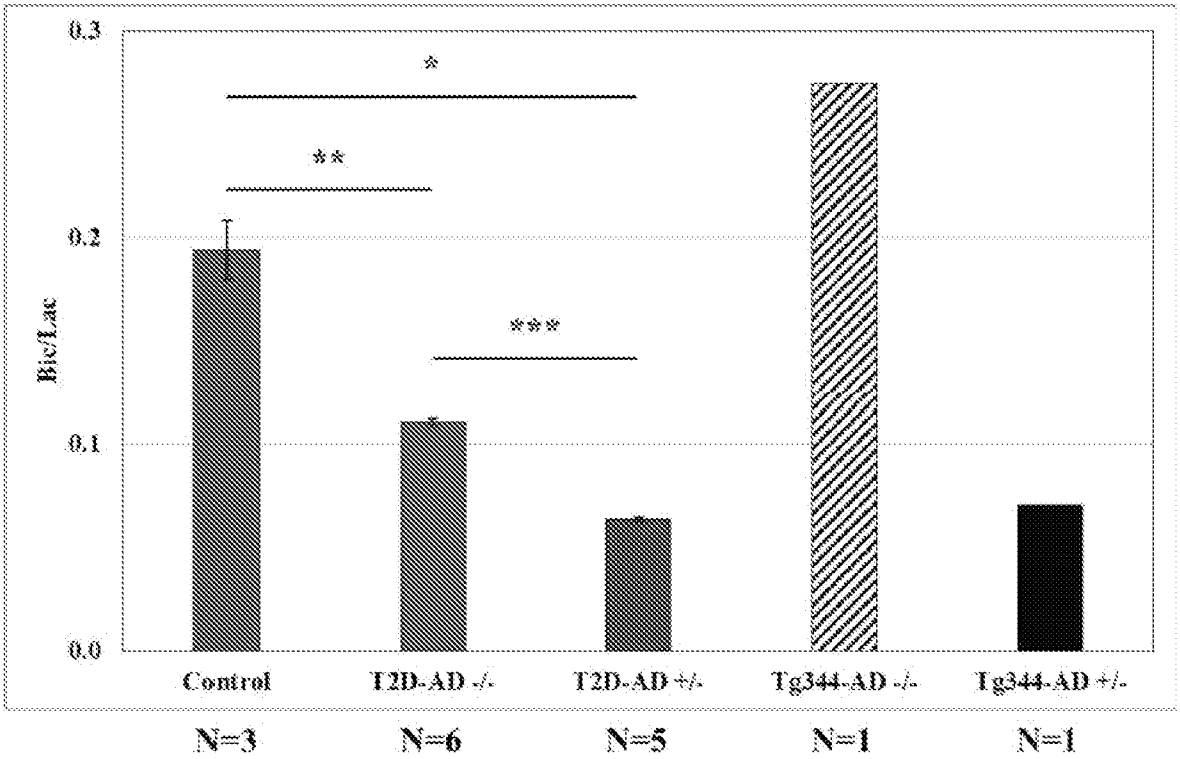
0.3
0.2
0.1
0.0
Bic/Lac
*
**
***
Control
T2D-AD -/-
T2D-AD +/-
Tg344-AD -/-
Tg344-AD +/-
N=3
N=6
N=5
N=1
N=1

FIG. 10

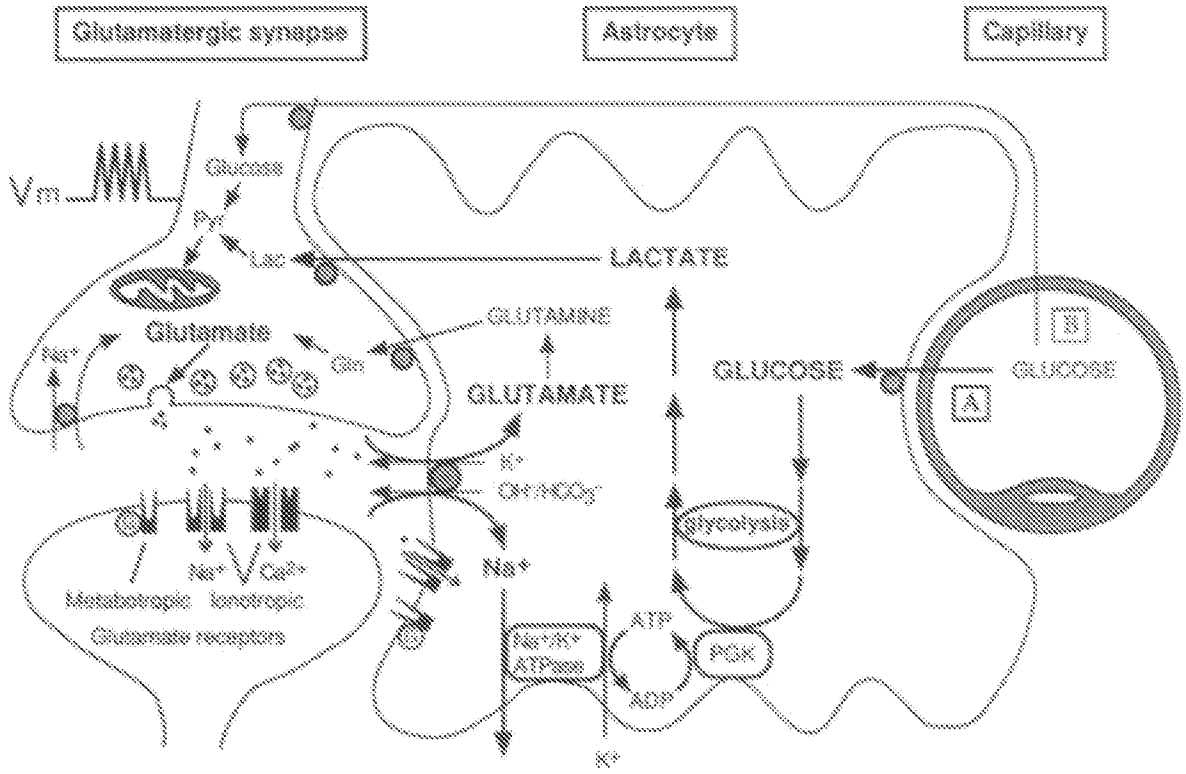
Glutamatergic synapse
Astrocyte
Capillary
Vm
Glucose
Pyr
Lac
LACTATE
Glutamate
Gln
GLUTAMINE
GLUTAMATE
GLUCOSE
GLUCOSE
B
A
K+
OH-/HCO3-
Glycolysis
Na+
Na+ Ca2+
Metabotropic Ionotropic
Glutamate receptors
Na+/K+ ATPase
ATP
PGK
ADP
K+

FIG. 11

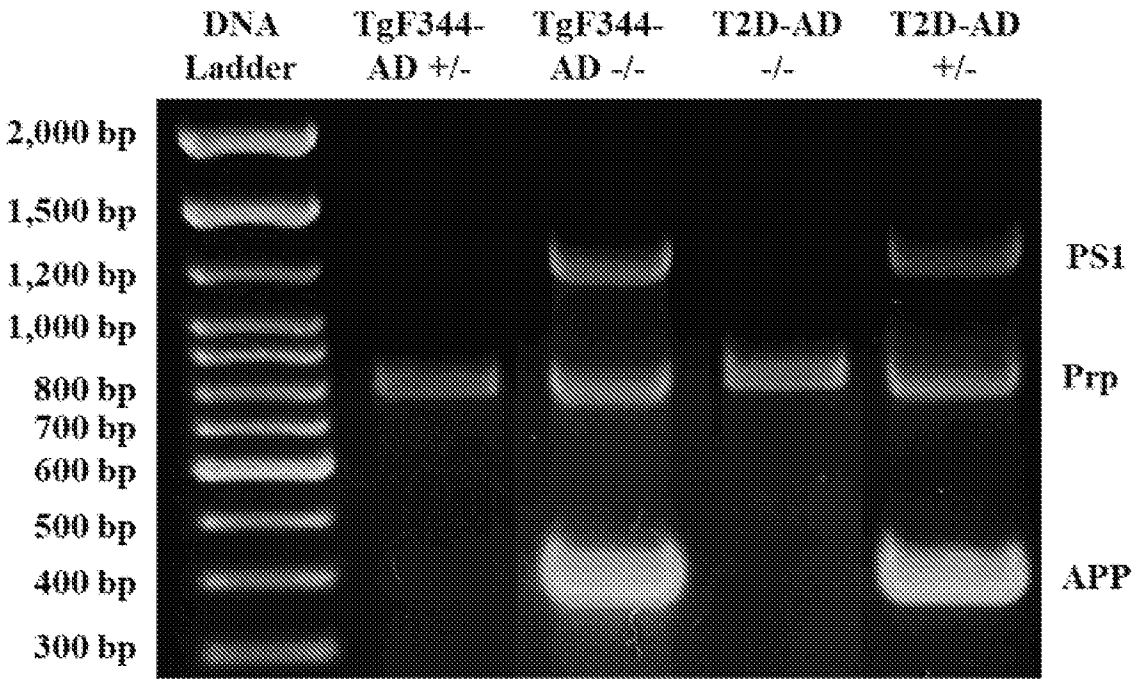
DNA Ladder
TgF344-AD +/-
TgF344-AD -/-
T2D-AD -/-
T2D-AD +/-
2,000 bp
1,500 bp
1,200 bp
1,000 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
PS1
Prp
APP

FIG. 12

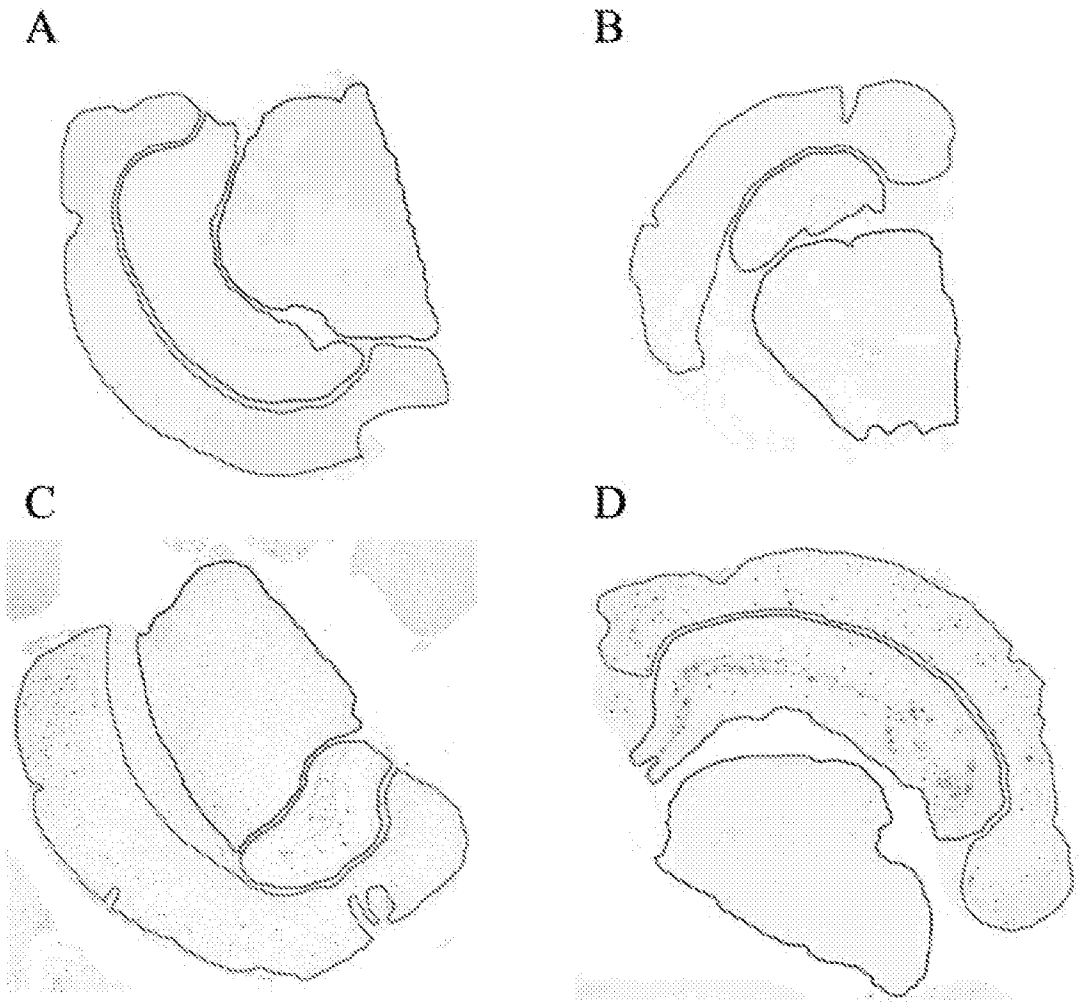

FIG. 13

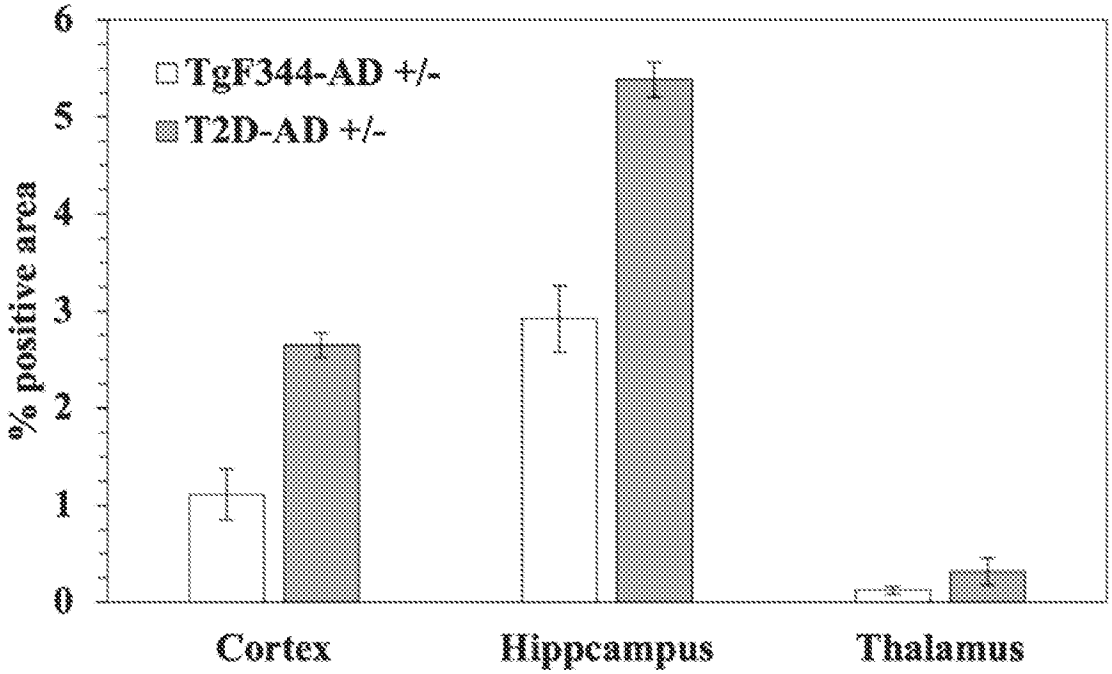
TgF344-AD +/-
T2D-AD +/-
% positive area
0
1
2
3
4
5
6
Cortex
Hippcampus
Thalamus

FIG. 14

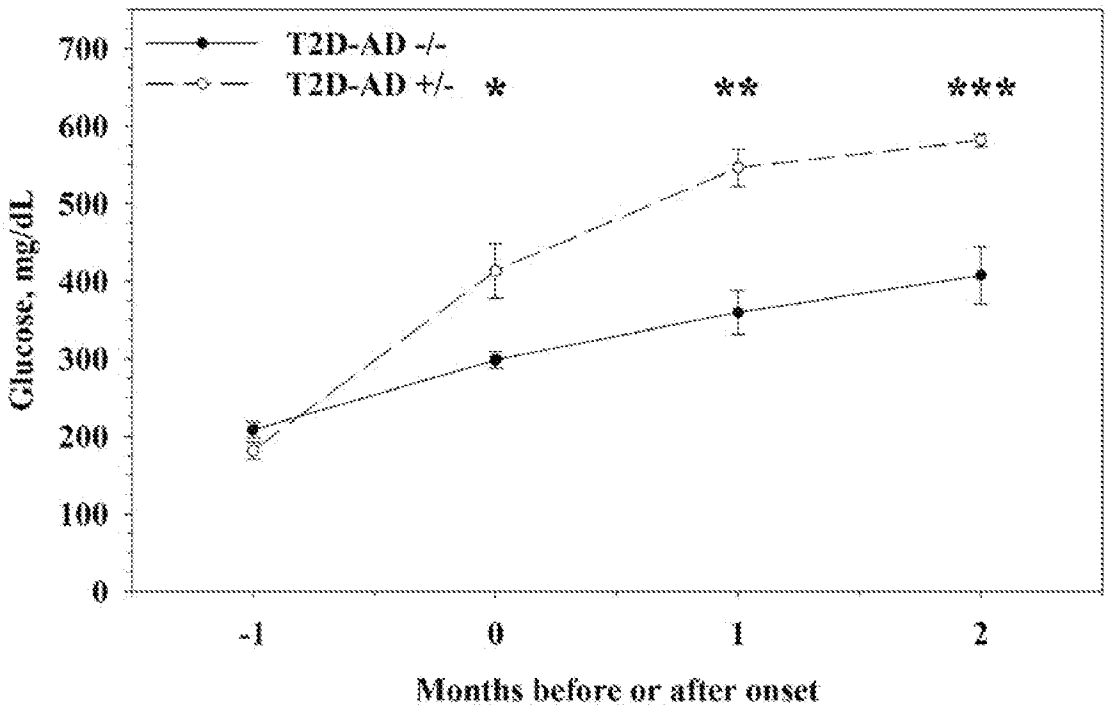
T2D-AD -/-
T2D-AD +/-
*
**
***
Glucose, mg/dL
0
100
200
300
400
500
600
700
-1
0
1
2
Months before or after onset

FIG. 15

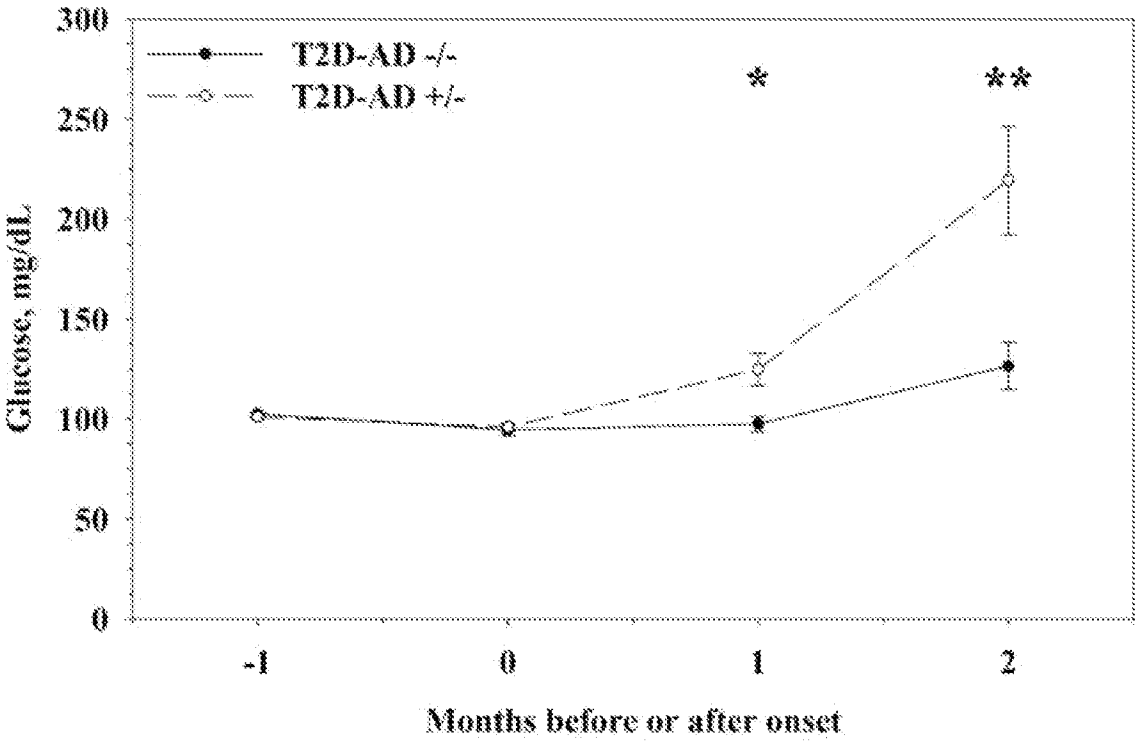
300
250
200
150
100
50
0
Glucose, mg/dL
T2D-AD -/-
T2D-AD +/-
*
**
-1
0
1
2
Months before or after onset

FIG. 16

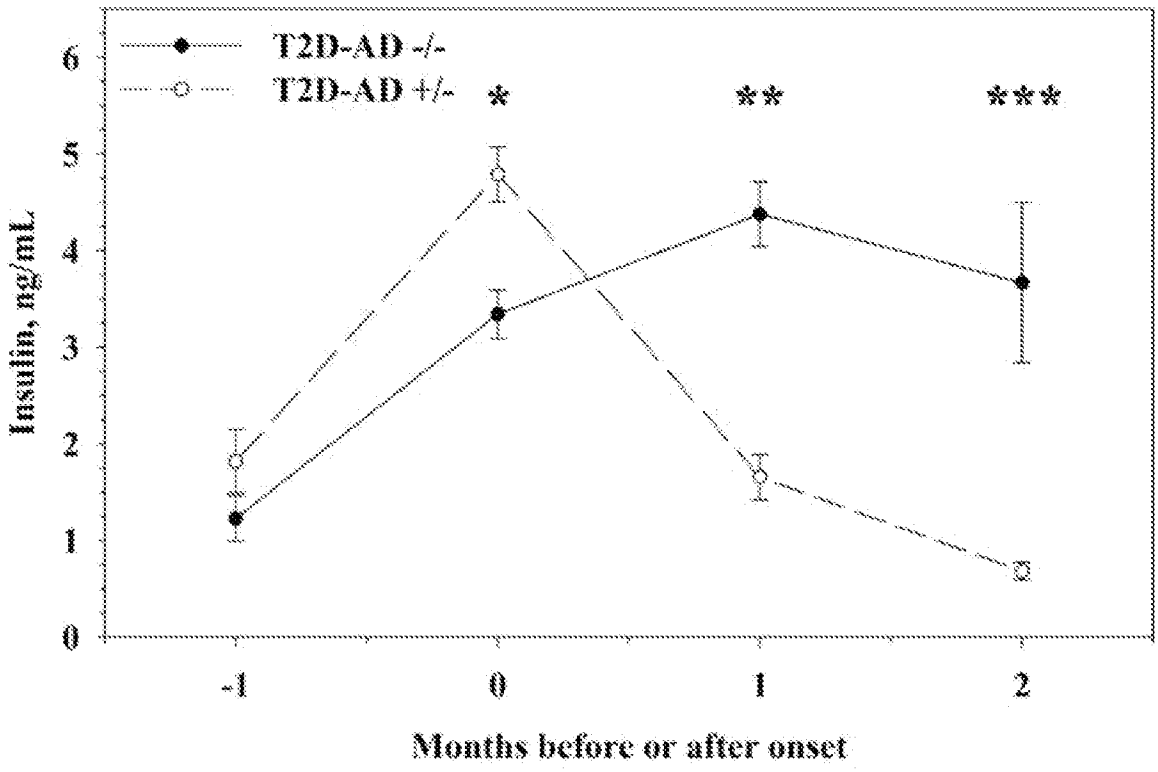
T2D-AD -/-
T2D-AD +/-
*
**
***
Insulin, ng/mL
0
1
2
3
4
5
6
-1
0
1
2
Months before or after onset

FIG. 17

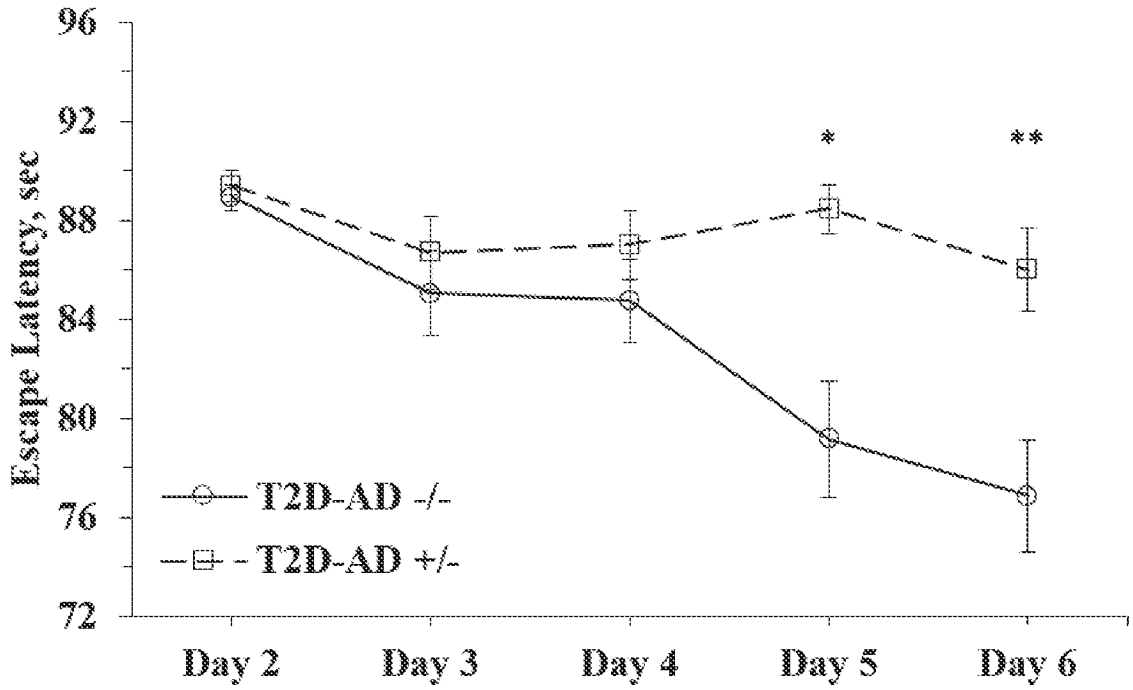
96
92
88
84
80
76
72
Escape Latency, sec
T2D-AD -/-
T2D-AD +/-
*
**
Day 2
Day 3
Day 4
Day 5
Day 6

FIG. 18

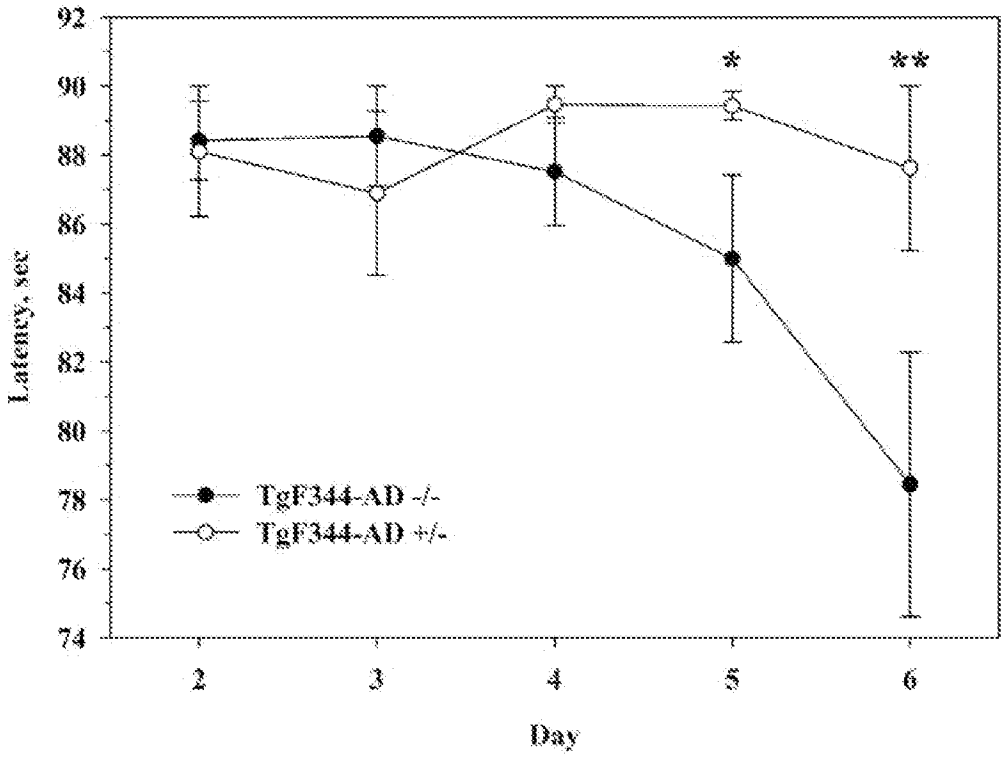
92
90
88
86
84
82
80
78
76
74
Latency, sec
2
3
4
5
6
Day
*
**
TgF344-AD -/-
TgF344-AD +/-

FIG. 19

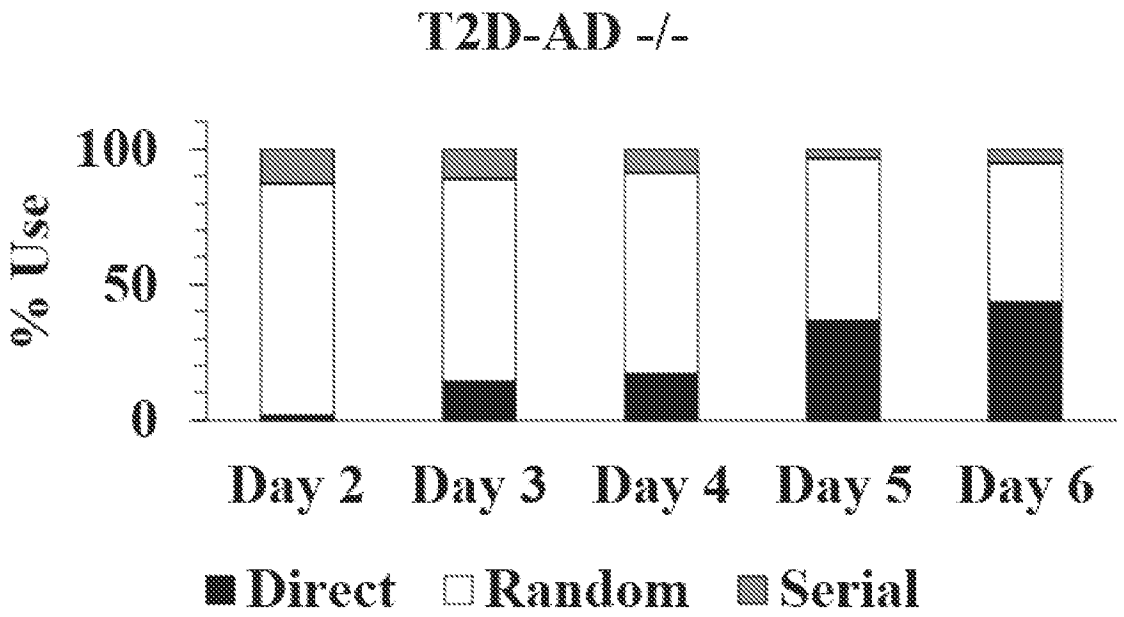

FIG. 20A

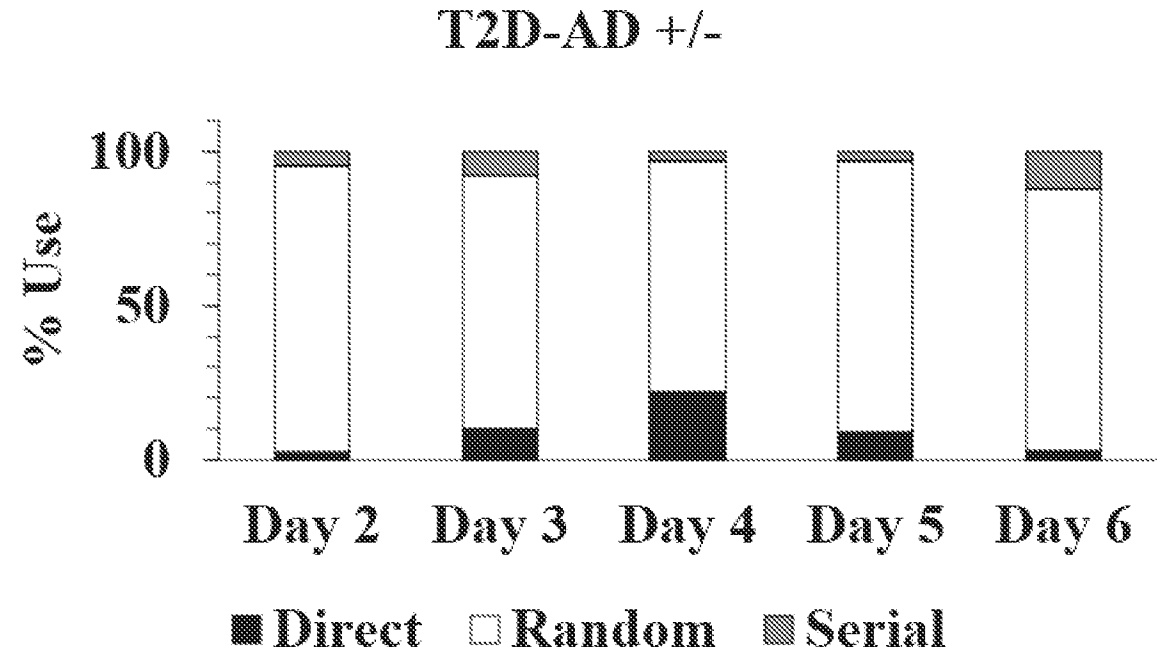

FIG. 20B

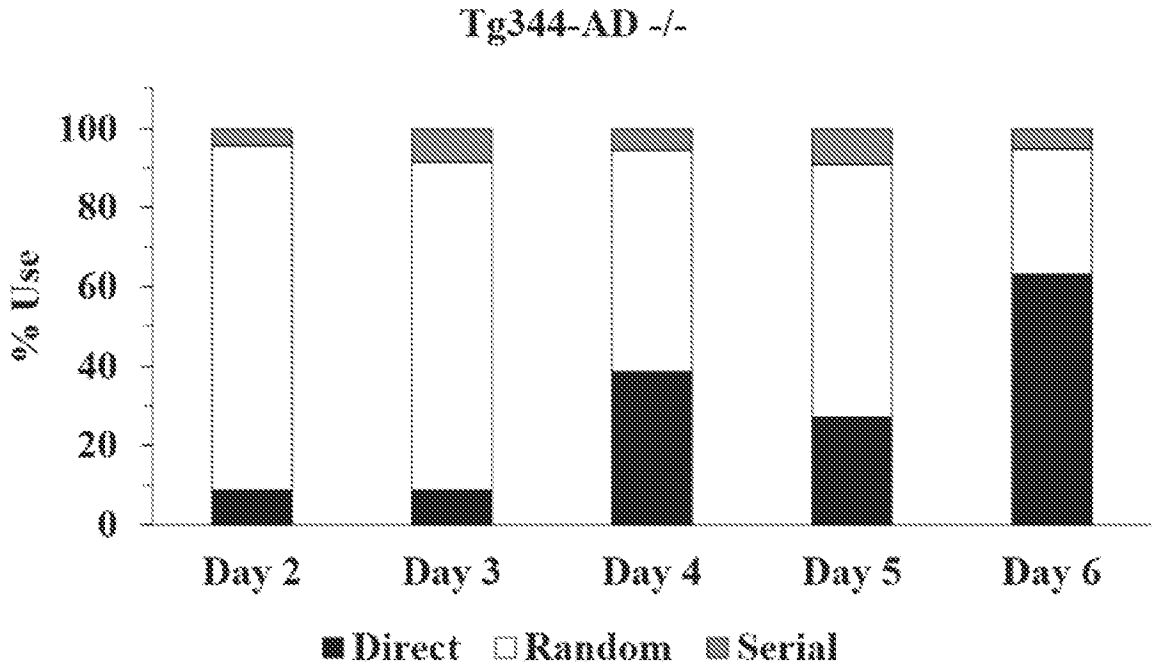

FIG. 21A

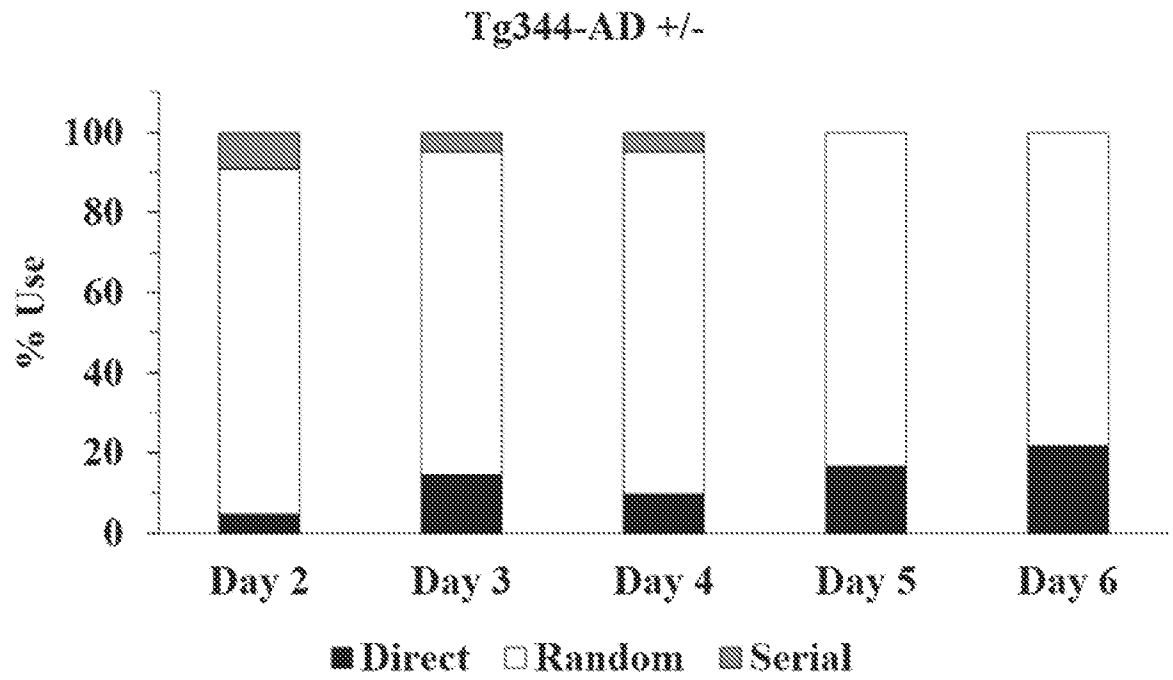

FIG. 21B

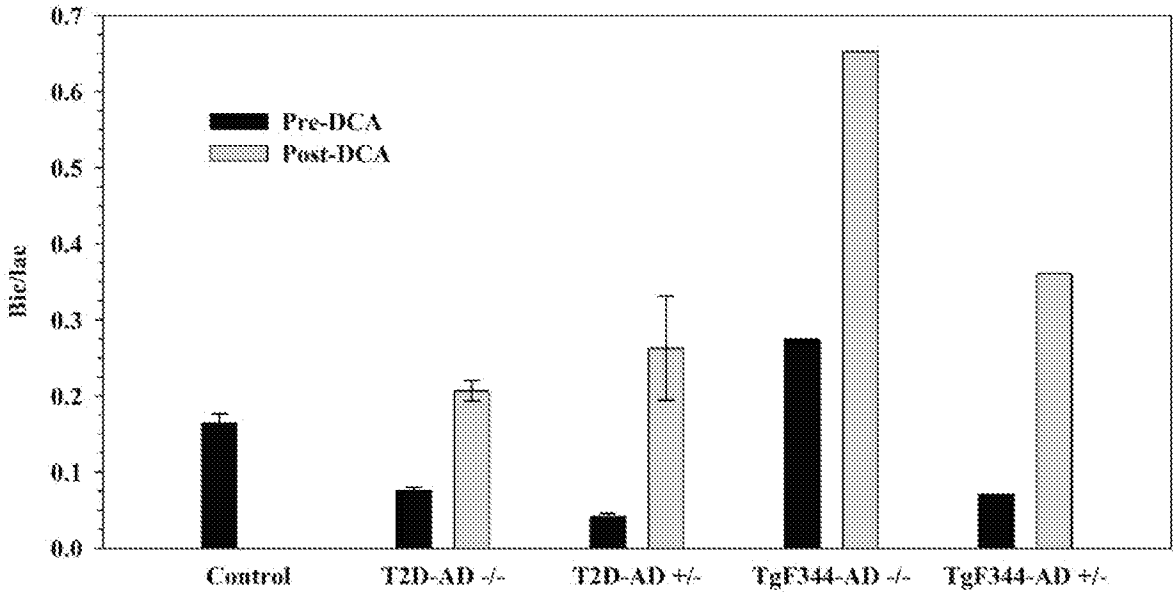
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bic/lac
Pre-DCA
Post-DCA
Control
T2D-AD -/-
T2D-AD +/-
TgF344-AD -/-
TgF344-AD +/-

FIG. 22

FIG. 23