Title: THE ISLET AMYLOID POLYPEPTIDE TOXIC OLIGOMER IS A BIOMARKER OF BRAIN ALTERATION IN TYPE-2 DIABETES MELLITUS

Abstract: Methods for predicting a propensity for brain disease in a diabetic or pre-diabetic individual determining the amount and/or molecular weight of islet amyloid polypeptide (IAPP) present a sample from the individual are provided. Further provided are methods for screening for agents that prevent or reduce the propensity for brain disease in an individual who has type diabetes or is pre-diabetic and a method of treating or preventing brain disease in an individual who has type 2 diabetes or is pre-diabetic.
THE ISLET AMYLOID POLYPEPTIDE TOXIC OLIGOMER IS A BIOMARKER OF BRAIN ALTERATION IN TYPE-2 DIABETES MELLITUS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present application claims priority to USSN 61/524,952, filed August 18, 2011, herein incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government Support under Grant No. HL030077, awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods for predicting the propensity for brain disease in individuals having diabetic conditions comprising the determination of the amount of islet amyloid polypeptide (IAPP) in the brain, reducing the propensity for brain disease, and methods for screening for agents that reduce the propensity for brain disease.
BACKGROUND OF THE INVENTION

[0004] Diabetes mellitus, or diabetes, is a chronic disease that is characterized by impaired glucose regulation. Diabetes can be divided into two clinical syndromes, type 1 diabetes mellitus and type 2 diabetes mellitus (T2D). In type 1 diabetes, previously called juvenile-onset or insulin-dependent, insulin production is absent due to autoimmune pancreatic β-cell destruction. Although the pathogenesis of autoimmune β-cell destruction is not completely understood, it is believed to involve interactions between susceptibility genes, autoantigens, and environmental factors. Type 1 diabetes generally develops in childhood or adolescence and accounts for about 10% of all cases of diabetes.

[0005] In type 2 diabetes, previously called adult-onset or non-insulin-dependent, insulin production may or may not be inadequate, but the body is unable to utilize the insulin that is present to normalize glucose levels in the body. It is caused by a combination of poorly understood genetic and acquired risk factors, including high-fat diet, lack of exercise, and aging. Type 2 diabetes accounts for about 90% of the cases of diabetes around the world, and is estimated to affect more than 220 million people worldwide. Although it more commonly occurs in adults, type 2 diabetes is now becoming more common in children.

[0006] Chronic diabetes can lead to long-term complications affecting various organs, especially the heart, blood vessels, eyes, kidneys, and nerves. Signs of severe brain dysfunction often occur in patients with obesity and insulin resistance/hyperinsulinemia. To date, however, there is no brain disease diagnostic method or treatment specific to diabetics.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention provides for methods for predicting a propensity for brain disease in an individual. In some embodiments, the method comprises determining the amount of islet amyloid polypeptide (IAPP) oligomer in a sample from the individual; and predicting the propensity for brain disease in the individual based on the determined amount of IAPP oligomer, wherein an elevated amount of IAPP oligomer compared to normal levels indicates an increased propensity for brain disease.
In some embodiments, the sample is a blood sample. In some embodiments, the individual has type 2 diabetes. In some embodiments, the individual is pre-diabetic.

In some embodiments, the determining step comprises contacting a reagent that specifically binds IAPP oligomers with the sample; and detecting the amount of IAPP oligomers bound by the reagent. In some embodiments, the reagent is an antibody. In some embodiments, the reagent (e.g., the antibody) is linked to a solid support (e.g., as a "capture reagent").

In some embodiments, the detecting step comprises contacting a detecting antibody that binds IAPP oligomers with the IAPP oligomers bound with the reagent; and quantifying the binding of the detection antibody to the bound IAPP oligomers. In some embodiments, the detection antibody is detectably labeled.

In some embodiments, the method further comprises, prior to determining the amount of islet amyloid polypeptide (IAPP) oligomer in a sample from an individual, extracting blood from the individual.

In some embodiments, wherein it is determined that the individual has a propensity for brain disease, the method further comprises designing a treatment plan to reduce the propensity for brain disease in the individual. In some embodiments, the method further comprises administering at least one medication to the individual that reduces the propensity for brain disease.

The present invention also provides for methods for predicting a propensity for brain disease in an individual. In some embodiments, the method comprises (a) determining the amount of islet amyloid polypeptide (IAPP) oligomer bound to amyloid beta (Aβ) in a sample from the individual; and (b) predicting the propensity for brain disease in the individual based on the determined amount of IAPP oligomer bound to Aβ, wherein an elevated amount of IAPP oligomer bound to Aβ compared to normal levels of IAPP oligomer bound to Aβ indicates an increased propensity for brain disease.

In some embodiments, the sample is a blood sample. In some embodiments, the individual has type 2 diabetes. In some embodiments, the individual is pre-diabetic.
In some embodiments, wherein the determining step comprises contacting a reagent that specifically binds IAPP oligomers with the sample; and contacting a reagent that specifically binds Aβ with the sample, and detecting the amount of IAPP oligomers bound by the reagent that specifically binds Aβ or detecting the amount of Aβ bound by the reagent that specifically binds IAPP oligomers.

In some embodiments, the reagent is an antibody. In some embodiments, the method further comprises, prior to determining the amount of islet amyloid polypeptide (IAPP) oligomer in a sample from an individual, extracting blood from the individual. In some embodiments, wherein it is determined that the individual has a propensity for brain disease, the method further comprises designing a treatment plan to reduce the propensity for brain disease in the individual. In some embodiments, the method further comprises administering at least one medication to the individual that reduces the propensity for brain disease.

The present invention also provides for kits for predicting a propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic. In some embodiments, the kit comprises a solid support operably linked to a reagent that specifically binds IAPP oligomers.

In some embodiments, the reagent is an antibody. In some embodiments, the kit further comprises a detection antibody that binds to IAPP oligomers when the oligomers are bound to the reagent. In some embodiments, the detection antibody is detectably labeled. In some embodiments, the solid support comprises a sensor operably linked to one or more nanoparticles, wherein the one or more nanoparticles are conjugated to an antibody that specifically binds IAPP oligomers.

The present invention further provides for screening for agents that prevent or reduce the propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic. In some embodiments, the method comprises screening a plurality of agents for the ability:

to enhance excretion of IAPP oligomers from the body and/or
to block or interfere with the formation of IAPP oligomers.

In some embodiments, the method further comprises identifying at least one agent from the plurality that enhances excretion of IAPP oligomers from the body and/or blocks or interferes
with the formation of IAPP oligomers; and administering the identified agent to an animal and measuring the ability of the agent to reduce the rate of brain disease. In some embodiments, the animal is an animal model for diabetes. In some embodiments, the animal has diabetes or is pre-diabetic.

5 [0021] In another embodiment, a method of treating or preventing brain disease in an individual who has type 2 diabetes or is pre-diabetic is described. The method comprises administering an effective amount of a compound that has the ability to i) enhance excretion of IAPP oligomers from the body, ii) block or interfere with the formation of IAPP oligomers, or iii) block or interfere with the function of IAPP oligomers. In some embodiments, the compound is a polymer-based membrane sealant. In some embodiments, the polymer-based membrane sealant blocks or interferes with the function of IAPP oligomers by restoring membranes damaged by IAPP oligomers.

10 [0022] In another embodiment, the IAPP oligomers are larger molecular weight IAPP oligomers. In some embodiments, the larger molecular weight IAPP oligomers are 32 kDa. In other embodiments, the larger molecular weight IAPP oligomers are 64 kDa.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1 illustrates the detection of IAPP oligomers in serum samples. (A) Representative western blot with anti-IAPP primary antibody of serum samples from patients with overweight/obesity (OW/OB) vs. lean (L) controls. (B) Intensity analysis of the high molecular weight bands revealed significantly larger IAPP oligomer levels in OW/OB (N=7; P=0.02) and DM (N=6; P=0.002) groups than in the L control group (N=7). Each western blot experiment was performed 4 times. (C) Western blot with anti-IAPP primary antibody showing 4 kD and 16 kD of serum samples from patients with type 2 diabetes and BMI>25.

20 [0024] Figure 2 illustrates significantly increased accumulation of larger IAPP oligomers in Alzheimer's disease ("AD") individuals than age matched humans without Alzheimer's disease.
Figure 3 illustrates accumulation of IAPP oligomers in patients with AD. (A)-(D) IAPP deposits in brains from patients with AD. (E) IAPP deposits in pancreas from patients with AD. (F) IAPP deposits in heart from patients with AD.

Figure 4 illustrates accumulation of IAPP. (A) and (B) IAPP deposits in brain from patient with type 2 diabetes with vascular dementia.

Figure 5 illustrates accumulation of IAPP oligomers in brains of control.

Figure 6 illustrates accumulation and co-localization of IAPP and Aβ in the human brain.

Figure 7 illustrates that IAPP binds to Aβ in the human brain.

Figure 8 illustrates accumulation of IAPP oligomers in the heart of HIP rats. (A) Dot blots with the anti-IAPP antibody comparing total IAPP level in HIP vs. UCD-T2DM rats. Dots on the left show positive controls using recombinant human (hIAPP) and rat (rIAPP); 5 ng for both. The antibody binds rIAPP with about 10x higher affinity than hIAPP. (B) Representative western blot with anti-IAPP primary antibody on ventricular myocyte lysates from pre-diabetic HIP rats, and left ventricle protein homogenates from pre-diabetic (PD) and diabetic (DM) HIP rats. High molecular weight IAPP bands are evident in all groups, indicating that IAPP accumulates in the heart starting from pre-diabetes. (C) Representative western blot with the anti-IAPP primary antibody of serum samples from HIP rats.

Figure 9 illustrates IAPP deposition in HIP rat brain.

Figure 10 illustrates relative mRNA levels in the HIP rat brain.

Figure 11 illustrates activated perivascular macrophages in HIP rats.

Figure 12 illustrates microglia in HIP rats.

Figure 13 illustrates elevated levels of the pro-inflammatory cytokines IL-6 and TNF-α, and reduced levels of the anti-inflammatory cytokine IL-10 in HIP rats.

Figure 14 illustrates activated soluble RAGE in isolated cardiac myocytes.
Figure 15 illustrates reduced expression of SERCA in HIP rat brains.

Figure 16 illustrates altered expression of proteins involved in mitochondrial fission and fusion in the HIP rat brain.

Figure 17 illustrates incubation of cardiac myocytes with exogenous IAPP oligomers (hIAPP) and Poloxamer 188 reduces the alteration of Ca cycling.

Figure 18 illustrates distribution of sarcolemma defect depths derived from AFM data. Density of thin sarcolemma patches is higher on cardiac myocytes incubated with IAPP oligomers. Incubation of cardiac myocytes with PI88 and IAPP oligomers prevents sarcolemma damage.

Figure 19 illustrates effect of diabetes and hyperglycemia on IAPP toxic oligomer formation.

DETAILED DESCRIPTION OF THE INVENTION

Introduction


[0044] The present invention surprisingly demonstrates that islet amyloid polypeptide ("IAPP") oligomer, a toxic amyloidogenic entity formed intracellularly in pancreatic β-cells, is present in significantly increased levels in the brain of subjects with AD. The present invention
also surprisingly demonstrates that IAPP oligomer is present in significantly increased levels in
the blood of pre-diabetic and diabetic subjects. The discovery that IAPP oligomers accumulate
in the brain provides evidence for IAPP oligomers as an early pathogenic molecular link among
obesity, diabetes and AD. It is assumed that lack of insulin accelerates cerebrovascular alteration
in AD. Likewise, elevated blood insulin levels, e.g. hyperinsulinemia, a common occurrence in
patients with insulin resistance and obesity, are considered harmful to the brain. In AD,
hyperinsulinemia is linked to exacerbated extracellular Aβ accumulation attributed to altered γ-
secretoase activity or increased Aβ secretion from neurons. However, induced hyperinsulinemia
in AD patients can demonstrate memory improvement, suggesting that it is not the elevated
insulin levels, but conditions secondary to hyperinsulinemia, that play a significant role in the

Accordingly, methods of predicting a propensity for brain disease in pre-diabetic and/or
diabetic subjects by determining the amount of IAPP oligomer present, and methods of reducing
propensity for brain disease in said subjects, are provided. The present invention further
provides kits for detecting IAPP and methods for identifying agents that interfere with IAPP
oligomer formation and/or enhance excretion of IAPP oligomers from the body.

**Definitions**

"Islet amyloid polypeptide" or "IAPP" or "amylin" is a 37-amino acid peptide hormone
that is co-expressed and co-secreted with insulin by pancreatic β-cells. IAPP is a major
component of amyloid deposits in pancreatic islets of patients with type 2 diabetes mellitus. See,
able to form "oligomers," intermediate structures comprising more than one monomer of IAPP
which in turn can lead to the formation of either "amyloid fibrils," IAPP oligomers arranged in a
β-pleated sheet structure that appear as non-branching fibrils by electron microscopy, or "toxic
oligomers," soluble oligomers that include spherical particles and curvilinear "protofibrils" and
which can induce cell death. Kayed et al, *Science* 300:486-489 (2003); Haataja et al,
*Endocrine Rev.* 29:303-316 (2008). As used herein, "toxic oligomers" comprise at least an
octamer of IAPP. Without being bound to a particular theory, it is believed that IAPP toxic
oligomers are not simply "pre"-amyloid fibrils, but are an off-amyloid fibril pathway form of
The term "amyloid beta" or "Aβ" refers to amyloid beta peptides processed from the amyloid beta precursor protein (APP).

The term "brain disease" or "brain alteration" or "central nervous system disease" or "neurodegenerative disease" refers to any disease that originates in an individual's central nervous system or diseases where central nervous systemic disease is the major clinical manifestation of the disease. Examples of brain disease include, but are not limited to, Amyotrophic Lateral Sclerosis, Multiple Sclerosis (MS), Alzheimer's Disease (AD), Traumatic Brain Injury, Stroke, Ischemic Brain Disease, Psychiatric Disorders, and reversible or metabolic encephalopathies, including hepatic encephalopathy, hypoxia, and drug or toxin-related encephalopathies.

The term "propensity" as used herein refers to an increased susceptibility to experiencing brain disease in a population or subpopulation of individuals. A predisposition can be measured in comparison to a general or unstratified population.

The term "diabetes mellitus" or "diabetes" refers to a disease or condition that is generally characterized by metabolic defects in production and utilization of glucose which result in the failure to maintain appropriate blood sugar levels in the body. Diabetes may be classified as type 1 diabetes (generally due to the absence of insulin production due to autoimmune destruction of pancreatic β-cells) or type 2 diabetes (T2D; generally due to existing insulin levels in the body that are inadequate to normalize plasma glucose levels, and believed to primarily result from a condition known as "insulin resistance," in which there is a decreased biological response to normal concentrations of circulating insulin). In some cases, diabetes may also be caused by any number of other conditions, including pregnancy. The present invention can be used with regard to any form of diabetes, to the extent that the diabetes is characterized by the presence of IAPP oligomers.

A "pre-diabetic individual," when used to compare with a sample from a patient, refers to an adult with a fasting blood glucose level greater than about 110 mg/dl but less than about 126 mg/dl or a 2 hour post-load glucose (PG) reading of greater than about 140 mg/dl but less than about 200 mg/dl. A "diabetic individual," when used to compare with a sample from a patient, refers to an adult with a fasting blood glucose level greater than about 126 mg/dl or a 2 hour PG reading of greater than about 200 mg/dl.
"Antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab')_2, a dimer of Fab which itself is a light chain joined to V_H-C_H by a disulfide bond. The F(ab')_2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab')_2 dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, Paul (Ed.) Fundamental Immunology, Third Edition, Raven Press, NY (1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv).

As used herein, "specific binding," when referring to antibody binding, refers to a binding reaction which is determinative of the presence of soluble IAPP oligomers, or toxic oligomers, in the presence of other IAPP species (e.g., soluble low molecular weight oligomers or amyloid fibrils). Thus, under designated immunoassay conditions, the specified antibodies bind to the soluble IAPP oligomers of the present invention but do not significantly bind to soluble low molecular weight IAPP species or amyloid fibrils. "Low molecular weight IAPP,"
as used herein, refers to IAPP species that are less than about 40 kD, which corresponds to the approximate size of an IAPP octamer. Accordingly, a soluble IAPP oligomer, or toxic oligomer, of the present invention has a molecular weight of at least about 40 kD and includes oligomers that are octamers or larger, while low molecular weight IAPP species include IAPP monomers, dimers, and tetramers. Typically, a specific or selective reaction will be at least twice the background signal or noise and more typically more than about 10 to 100 times background or more. In some embodiments, an antibody that specifically binds soluble IAPP oligomers binds to the soluble IAPP oligomers at least about 10-fold, about 100-fold, about 200-fold, about 500-fold, or about 1000-fold or more than it binds low molecular weight IAPP species or amyloid fibrils.

[0056] The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Cassol et al. (1992); Rossolini et al, Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0057] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins (e.g., antigens), wherein the amino acid residues are linked by covalent peptide bonds.
The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, e.g., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but which functions in a manner similar to a naturally occurring amino acid.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid.
acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(see, e.g., Creighton, *Proteins* (1984)).

**[0061]** The term "effective amount" means an amount of a compound according to the invention which, in the context of which it is administered or used, is sufficient to achieve the desired effect or result.

**[0062]** The term "compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, e.g., protein, oligopeptide (e.g., from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide, oligonucleotide, etc., to be tested for the capacity to directly or indirectly modulation tumor cell proliferation. The term "function of IAPP oligomers" refers to the toxicity associated with IAPP oligomers, which can include, but is not limited to, membrane destabilization.
The term "larger molecular weight IAPP oligomers" refers to IAPP oligomers that have a molecular weight of about 32 or 64 kDa, and are made up mostly of IAPP octamers and 16-mers, respectively.

The term "smaller molecular weight IAPP oligomers" refers to IAPP oligomers that have a molecular weight of about 12 or 16 kDa, and are made up mostly of IAPP trimers and tetramers, respectively.

The term "polymer-based membrane sealant" refers to a synthetic surfactant having the ability to be inserted into a cell membrane to affect the membrane surface pressure in a manner that repairs or prevents damage resulting from membrane permeabilization.

Methods for Predicting Propensity for Brain Disease in Individuals Having Diabetic Conditions

In one aspect, the invention provides for a method for predicting a propensity for brain disease in an individual who has diabetes or is pre-diabetic, the method comprising determining the amount of islet amyloid polypeptide (IAPP) oligomer in a sample from the individual; and predicting the propensity for brain disease based on the determined amount of IAPP oligomer, wherein an elevated amount of IAPP compared to normal levels indicates an increased propensity for brain disease.

Predicting a propensity for brain disease involves determining the amount of IAPP oligomer in a patient or patient sample and then comparing the level to a baseline or range. Typically, the baseline value is representative of levels of IAPP oligomer in a healthy person not suffering from, or likely to develop, brain disease, as measured using a biological sample such as a blood sample, other fluid sample, or tissue sample (such as brain tissue). Variations of levels of IAPP oligomer from the baseline range (e.g., levels of IAPP oligomer that are higher than the baseline level) indicate that the patient has an increased propensity or risk of developing brain disease or an increased risk of its recurrence.

In some embodiments, the propensity in pre-diabetic individuals is measured by evaluating the molecular weight of the IAPP oligomer. An accumulation of larger molecular weight IAPP oligomers, e.g. approximately about 32 or 64 kDa, is indicative of a high propensity of brain disease.
In some embodiments, the comparing step involves computer-based calculations and tools. The tools are advantageously provided in the form of computer programs that are executable by a general purpose computer system (referred to herein as a "host computer") of conventional design. The host computer may be configured with many different hardware components and can be made in many dimensions and styles (e.g., desktop PC, laptop, tablet PC, handheld computer, server, workstation, mainframe). Standard components, such as monitors, keyboards, disk drives, CD and/or DVD drives, and the like, may be included. Where the host computer is attached to a network, the connections may be provided via any suitable transport media (e.g., wired, optical, and/or wireless media) and any suitable communication protocol (e.g., TCP/IP); the host computer may include suitable networking hardware (e.g., modem, Ethernet card, WiFi card). The host computer may implement any of a variety of operating systems, including UNIX, Linux, Microsoft Windows, MacOS, or any other operating system.

Computer code for implementing aspects of the present invention may be written in a variety of languages, including PERL, C, C++, Java, JavaScript, VBScript, AWK, or any other scripting or programming language that can be executed on the host computer or that can be compiled to execute on the host computer. Code may also be written or distributed in low level languages such as assembler languages or machine languages.

The host computer system advantageously provides an interface via which the user controls operation of the tools. In the examples described herein, software tools are implemented as scripts (e.g., using PERL), execution of which can be initiated by a user from a standard command line interface of an operating system such as Linux or UNIX. Those skilled in the art will appreciate that commands can be adapted to the operating system as appropriate. In other embodiments, a graphical user interface may be provided, allowing the user to control operations using a pointing device. Thus, the present invention is not limited to any particular user interface.

Scripts or programs incorporating various features of the present invention may be encoded on various computer readable media for storage and/or transmission. Examples of suitable media include magnetic disk or tape, optical storage media such as compact disk (CD) or DVD (digital versatile disk), flash memory, and carrier signals adapted for transmission via wired, optical, and/or wireless networks conforming to a variety of protocols, including the Internet.
In some embodiments, the methods comprise recording a result relating to the propensity for brain disease determined from an individual. Any type of recordation is contemplated, including electronic recordation, e.g., by a computer.

**Diabetic Conditions Subject to the Methods**

The methods of the present invention find use in any subject, human or non-human animal (e.g., pig, horse, birds including domestic birds, or other animals, especially those used in animal models such as mouse, rat, ferret, or non-human primate) having a diabetic condition. Diabetic conditions include, for example, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, pre-diabetes, hyperglycemia, and metabolic syndrome.

In some embodiments, the subject has type 2 diabetes. Type 2 diabetes is generally characterized by metabolic defects in production and utilization of glucose which result in the failure to maintain appropriate blood sugar levels in the body. A subject having type 2 diabetes may or may not also exhibit diabetic complications, such as damage to the nerves, blood vessels, heart, feet, kidneys, and eyes. In some embodiments, the subject is pre-diabetic. Pre-diabetes is generally characterized by impaired glucose tolerance, and frequently, although not always, precedes the onset of diabetes in a subject.

A diagnosis of diabetes or pre-diabetes can be made using any of a number of assays known in the field. Examples of assays for diagnosing or categorizing an individual as diabetic or pre-diabetic include, but are not limited to, a glycosylated hemoglobin (HbAlc) test, a connecting peptide (C-peptide) test, a fasting plasma glucose (FPG) test, an oral glucose tolerance test (OGTT), and a casual plasma glucose test. Thresholds for identifying or diagnosing an individual as pre-diabetic or diabetic using the above-described assays are readily ascertainable to one of skill in the art. For example, using the FPG test, a subject is diagnosed as having diabetes if the subject has a fasting blood glucose level greater than about 126 mg/dl or a 2 hour post-load glucose reading of greater than about 200 mg/dl; a subject is diagnosed as having pre-diabetes using the FPG test if the subject has a fasting blood glucose level greater than about 110 mg/dl but less than about 126 mg/dl or a 2 hour post-load glucose reading of greater than about 140 mg/dl but less than about 200 mg/dl.
Methods of Detecting IAPP Oligomers

[0077] In some embodiments, the step of determining the amount of IAPP oligomers in a sample comprises contacting a reagent that specifically binds IAPP oligomers to the sample, and detecting the amount of IAPP oligomers bound by the reagent. In some embodiments, the detecting step comprises contacting a detection antibody that binds IAPP oligomers to the IAPP oligomers bound to the reagent; and quantifying the binding of the detection antibody to the bound IAPP oligomers. In some embodiments, the detecting step comprises determining whether IAPP is bound to Aβ.

[0078] IAPP oligomers can be detected using any of a number of well-known immunological binding assays (see, e.g., U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also Asai Methods in Cell Biology Volume 37: Antibodies in Cell Biology, Academic Press, Inc. NY (1993); Stites, supra. Immunological binding assays (or immunoassays) typically utilize a "capture agent" to specifically bind to and often immobilize the analyte (e.g. IAPP oligomers). In some embodiments, the capture agent is a moiety that specifically binds to the analyte. The antibody may be produced by any of a number of means well known to those of skill in the art and as described above. The capture agent can also be, for example, a non-antibody protein having affinity for IAPP oligomers. Examples of non-antibody affinity proteins include, but are not limited to, avimers, adnectins (see, e.g., U.S. Pat. No. 6,818,418), and anticalins (see, e.g., Beste et al., Proc. Natl. Acad. Sci. U.S.A. 96(5):1898-1903 (1999)).

[0079] Immunoassays also often utilize a labeling agent to bind specifically to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/protein complex.

[0080] In some embodiments, the labeling agent is a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second antibody can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.
Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G, can also be used as the label agents. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (see, generally, Kronval, et al. J. Immunol, 111:1401-1406 (1973); and Akerstrom, et al. J. Immunol, 135:2589-2542 (1985)).

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. The incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

Immunoassays can be used to determine cellular status such as oxidative and inflammatory damage, or lipolysis. For example, immunostaining can be used to determine expression levels of the pro-inflammatory cytokines IL-6, IL-10, and TNF-a; proteins involved in mitochondrial fission, such as Fis1, and DRP1; and mitochondrial fusion, such as Mfn2 and OPAL.

Additionally, co-staining can be used to show co-localization of two proteins, for example, to show co-localization of IAPP and another protein including, but not limited to, Aβ in a tissue such as brain.

Non-Competitive Assay Formats

Immunoassays for detecting IAPP oligomers from biological samples, such as blood and brain tissue, may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured protein or analyte is directly measured. In one preferred "sandwich" assay, for example, the capture agent (e.g., antibodies specific for the IAPP oligomers of the invention) can be bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture the IAPP oligomers present in the test sample. The IAPP oligomers of the invention thus immobilized are then bound by a labeling agent, such as a second labeled antibody specific for the polypeptide. Alternatively, the second antibody may
lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the
species from which the second antibody is derived. The second can be modified with a
detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as
enzyme-labeled streptavidin.

[0086] Non-competitive assays of the present invention can be based on the ability of IAPP to
be immobilized by a capture agent, such as an anti-IAPP antibody, and then using labeling, such
as a secondary labeled antibody, to detect the bound IAPP oligomers.

Competitive Assay Formats

[0087] In competitive assays, the amount of protein or analyte present in the sample is
measured indirectly by measuring the amount of an added (exogenous) protein or analyte
displaced (or competed away) from a specific capture agent (e.g., antibodies specific for IAPP
oligomers of the invention) by the protein or analyte present in the sample. The amount of
immunogen bound to the antibody is inversely proportional to the concentration of immunogen
present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a
solid substrate. The amount of analyte may be detected by providing a labeled analyte molecule.
It is understood that labels can include, e.g., radioactive labels as well as peptide or other tags
that can be recognized by detection reagents such as antibodies.

[0088] In a competitive assay, IAPP oligomers can be measured indirectly by measuring the
amount of an exogenous protein or analyte competed away from IAPP antibodies by IAPP
oligomers present in a sample. The amount of IAPP bound to the antibody will be inversely
proportional to the concentration of IAPP present in the sample.

Other Assay Formats

[0089] In some embodiments, dot blot or western blot (immunoblot) analysis is used to detect
and quantify the presence of IAPP oligomers of the invention in a sample. The technique
generally comprises separating sample proteins by gel electrophoresis on the basis of molecular
weight, transferring the separated proteins to a suitable solid support (such as, e.g., a
nitrocellulose filter, a nylon filter, or a derivatized nylon filter) and incubating the sample with
the antibodies that specifically bind the IAPP oligomers. For example, antibodies are selected
that specifically bind to the IAPP oligomers of the invention on the solid support. These
antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the antibodies against the IAPP oligomers of interest.

[0090] IAPP-specific immunoreactivity can be determined in T2D patients and pre-diabetic individuals compared to non-diabetic, non-obese individuals. IAPP can be detected in any fluid or tissue in the body, including, but not limited to, blood and/or brain tissue. IAPP content and size distribution in tissues can be compared using immunoreactivity assays, such as western blot, between diabetic or pre-diabetic patients against control subjects without diabetes. Such immunoassays can be used to detect the presence of IAPP deposits in other tissues such as the pancreas, blood vessels, and heart of diabetic individuals to determine if IAPP accumulation is greater in diabetic or pre-diabetic patients as compared to control subjects.

[0091] In some embodiments, non-antibody antigen binding molecules are used in assays to detect and/or quantify the presence of IAPP oligomers of the invention in a sample. Exemplary non-antibody antigen binding molecules include, without limitation, antibody mimics that use non-immunoglobulin protein scaffolds, including adnectins, avimers, anticalins, single chain polypeptide binding molecules, and antibody-like binding peptidomimetics.

[0092] In some embodiments, immunoprecipitation can be used to detect and quantify the presence of IAPP oligomers. Immunoprecipitation is the technique of precipitating an antigen out of solution using an antibody specific to that antigen. The process can be used to identify protein complexes present in cell extracts by targeting a protein believed to be in the complex. The complexes are brought out of solution by insoluble antibody-binding proteins isolated initially from bacteria, such as Protein A and Protein G. The antibodies can also be coupled to sepharose beads that can easily be isolated out of solution. After washing, the precipitate can be analyzed using mass spectrometry, Western blotting, or any number of other methods for identifying constituents in the complex.

[0093] Immunoprecipitation can be used, for example, to determine the co-localization and physical interaction of IAPP and other proteins. For example, immunoprecipitation can be used to determine the physical interaction between IAPP and Β in a brain. In some embodiments, immunoprecipitation can be used to determine the interaction between IAPP and Β in brains of individuals with both type 2 diabetes and AD binding can be demonstrated by
immunoprecipitation of IAPP from brains of AD and T2D patients and then exposure of the IP to the anti-Aβ antibody on western blot. Such data can discriminate a control group from groups of patients with T2D and AD based on the presence of IAPP bound to Aβ in brain extracts.

**Antibodies Against Oligomers**


[0095] Methods for producing polyclonal and monoclonal antibodies that react specifically with a protein of interest are known to those of skill in the art (see, e.g., Coligan, supra; and Harlow and Lane, supra; Stites et al, supra and references cited therein; Goding, supra; and Kohler and Milstein *Nature*, 256:495-497 (1975)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors (see, Huse et al, supra; and Ward et al, supra). For example, in order to produce antisera for use in an immunoassay, the protein of interest or an antigenic fragment thereof, is isolated as described herein. For example, a recombinant protein is produced in a transformed cell line. An inbred strain of mice or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen.

[0096] Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of $10^4$ or greater are selected and tested for their cross-reactivity against non-IAPP proteins, using a competitive binding immunoassay. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a $K_D$ of at least about 0.1 mM, more usually at least about 1 µM, preferably at least about 0.1 µM or better, and most preferably, 0.01 µM or better.
For preparation of antibodies, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, *Immunology* (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks *et al.*, *Bio/Technology* 10:779-783 (1992); Lonberg *et al.*, *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, e.g., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker *et al.*, *EMBOJ.* 10:3655-3659 (1991); and Suresh *et al.*, *Methods in Enzymology* 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (see, e.g., U.S. Patent No. 4,676,980, WO 91/00360; WO 92/200373; and EP 03089).

A number of IAPP oligomers of the invention comprising immunogens may be used to produce antibodies specifically or selectively reactive with the proteins of interest. Recombinant protein is an exemplary immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure form. Synthetic peptides made using the protein sequences described herein may also be used as an immunogen for the
production of antibodies to the protein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells and purified as generally described supra. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated for subsequent use in immunoassays to measure the protein.

[0099]  Methods of production of IAPP oligomer-specific antibodies are known to those of skill in the art. See, e.g., Kayed et al, Science 300:486-489 (2003). In brief, a molecular mimic of soluble oligomers is synthesized that mimics the structural organization of Aβ in micellar oligomers by attaching the C-terminus of synthetic Aβ peptides to colloidal gold particles via a thioester bond. The molecular mimics, which are of the same approximate size as the naturally formed oligomeric intermediates and which have the same β-sheet secondary structure and properties as determined by circular dichroism, are then mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the soluble oligomers. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (see, Harlow and Lane, supra). Specificity of the anti-oligomer antibody can be determined by testing for the lack of reactivity of the antibody with monomeric protein or fibrillar deposits.

[00100]  Once IAPP specific antibodies are available, the protein can be measured by a variety of immunoassay methods with qualitative and quantitative results available to the clinician. For a review of immunological and immunoassay procedures in general, see, Stites, supra. Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in Maggio Enzyme Immunoassay, CRC Press, Boca Raton, Florida (1980); Tijssen, supra; and Harlow and Lane, supra.

[00101]  The particular label or detectable group used in an assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the IAPP antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most labels useful in such methods can be applied to the present
invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (e.g., Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (e.g., $^3$H, $^{125}$I, $^{35}$S, $^{14}$C, or $^{32}$P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads.

[00102] The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, the ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[00103] Non-radioactive labels are often attached by indirect means. The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorescent compound. A variety of enzymes and fluorescent compounds can be used with the methods of the present invention and are well-known to those of skill in the art (for a review of various labeling or signal producing systems which may be used, see, e.g., U.S. Patent No. 4,391,904).

[00104] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Alternatively, simple colorimetric labels may be detected directly by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[00105] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case,
antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need to be labeled and the presence of the target antibody is detected by simple visual inspection.

**Samples for Detection**

[00106] Samples for detection of IAPP oligomer may be obtained from any tissue or fluid from a human or non-human animal including, but not limited to, plasma and serum. In some embodiments, the sample is a blood sample. In some embodiments, the sample is brain tissue.

**Reducing the Propensity for Brain Disease**

[00107] In some embodiments, wherein it is determined that an individual has a propensity for brain disease, the method further comprises designing a treatment plan to reduce the propensity for brain disease in the individual. In some embodiments, the method further comprises administering at least one medication to the individual that reduces the propensity for brain disease. In some embodiments an individual can prevent brain disease by reinforcing cell membranes before said membranes are damaged by toxic IAPP oligomers.

[00108] The duration of treatment for brain disease can vary: it may be as short as about 3 or 6 months, or may be as long as about 18 months, 2 years, 5 years, 10 years, or longer. In some cases, the treatment may last the remainder of a patient's natural life. Effectiveness of the treatment may be assessed during the entire course of administration of the treatment after a certain time period, e.g., about every 3 months or every 6 months for about an 18-month treatment plan. In other cases, effectiveness may be assessed about every 9 or 12 months for a longer treatment course. The administration schedule (dose and frequency) of a treatment may be adjusted accordingly for any subsequent administration. Alternatively, the treatment that is administered (e.g., type of medication) may be adjusted accordingly for any subsequent administration.

[00109] In some embodiments, a treatment plan comprises administering one or more medications that relieve or alleviate the symptoms and/or causes of brain disease. In some embodiments, once there is a determination that the level of IAPP toxic oligomers that are present in a sample, such as blood or brain tissue, at levels higher than normal levels (e.g., levels of IAPP toxic oligomers in control samples), the method further comprises designing a treatment
plan for the administration of, and subsequently administering, a treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers. In some embodiments, the method further comprises designing a treatment plan for the administration of, and subsequently administering, one or more of the following treatments: intravenous delivery of a membrane sealant that can seal damaged sarcolemma and improve calcium cycling or restore calcium cycling back to normal levels in cardiac myocytes; administration of a solubilizer of oligomers; administration of insulin to reduce the demand of insulin and IAPP production on pancreatic β-cells; and administration of one or more insulin sensitizing drugs that increase the uptake of glucose by cells and decrease blood glucose levels. In some embodiments, a membrane sealant comprising a poloxamer such as Poloxamer 188 (PI 88) is administered. In some embodiments, the insulin that is administered comprises a recombinant human insulin or insulin analog that is rapid-acting, short-acting, intermediate-acting, or long-acting. In some embodiments, the insulin-sensitizing drug that is administered comprises a biguanide (e.g., metformin) or a thiazolidinedione (e.g., troglitazone, rosiglitazone, and pioglitazone).

[00110] In some embodiments, the treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers is provided in combination with another therapeutic agent for relieving or alleviating the causes and/or symptoms of brain disease, such as an Angiotensin-Converting Enzyme (ACE) inhibitor, an angiotensin receptor blocker, a beta blocker, a diuretic, a positive inotrope, or a vasodilator. Accordingly, in some embodiments, the treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers is administered to a patient who is also being treated with an ACE inhibitor such as a sulfhydryl-containing ACE inhibitor, e.g., captopril or zofenopril; a dicarboxylate-containing ACE inhibitor, e.g., enalapril, ramipril, quinapril, perindopril, lisinopril, or benazepril; and a phosphonate-containing ACE inhibitor such as fosinopril. In other embodiments, the treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers is administered to a patient that is being treated with an angiotensin receptor blocker such as candesartan, losartan, irbesartan, valsartan, olmesartan, telmisartan, or eprosartan; or a beta blocker such as bisoprolol, carvedilol, and metoprolol. In some embodiments, the treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers is administered to a patient who is being treated with a diuretic, such as a loop diuretics (e.g., furosemide, bumetanide); a thiazide diuretics (e.g., hydrochlorothiazide, chlorthalidone, chlorthiazide); a potassium-sparing diuretic (e.g., amiloride); and/or spironolactone or eplerenone. As understood in the art, a patient may be
treated with various combinations of such agents in addition to receiving a treatment that relieves, alleviates, or counteracts the activity of the IAPP toxic oligomers.

[00111] Treatments to reduce the propensity for brain disease may be administered in a wide variety of oral, parenteral and topical dosage forms. Thus, the treatments to reduce the propensity for brain disease can be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally; by inhalation, for example, intranasally; or transdermally.

Methods of Screening for Agents that Reduce the Propensity for Brain Disease

[00112] In another aspect, the invention provides for a method for screening for agents that prevent or reduce the propensity for brain disease in an individual who has a diabetic condition, such as type 2 diabetes or pre-diabetes, the method comprising screening a plurality of agents for the ability: to enhance excretion of IAPP oligomers from the body and/or to block or interfere with the formation of IAPP oligomers.

[00113] In some embodiments, the method further comprises identifying at least one agent from the plurality that enhances excretion of IAPP oligomers from the body and/or blocks or interferes with the formation of IAPP oligomers; and administering the identified agent to an animal and measuring the ability of the agent to reduce the rate of brain disease.

Agents That Reduce the Propensity for Brain Disease

[00114] The agents that reduce the propensity for brain disease may comprise agents that enhance the excretion of IAPP oligomers, e.g., by solubilizing the IAPP oligomers, or alternatively, agents that block or interfere with the formation of IAPP toxic oligomers, e.g., by blocking monomers from forming intermediate IAPP oligomers or by blocking intermediate IAPP oligomers from forming toxic oligomers. The agents screened for enhancing the excretion of IAPP oligomers or for blocking or interfering with IAPP oligomer formation can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Typically, test compounds will be small chemical molecules and peptides. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are
many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland) and the like.

[00115] In some embodiments, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds. Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[00116] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (e.g., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[00117] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al., Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., PCT Publication WO 93/20242), random bio-oligomers (e.g., PCT Publication No. WO 92/00091), benzodiazepines (e.g., U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., Proc. Nat. Acad. Sci. USA 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara et al., J. Amer. Chem. Soc. 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., J. Amer. Chem. Soc. 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen et al., J. Amer. Chem. Soc. 116:2661 (1994)), oligocarbamates (Cho et al., Science 261:1303 (1993)), and/or peptidyl phosphonates (Campbell et al., J. Org. Chem. 59:658 (1994)), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra),

[00118] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

Methods of Screening

[00119] A number of different screening protocols can be utilized to identify agents that enhance the excretion of IAPP oligomers block or interfere with the formation of IAPP toxic oligomers in cells, particularly mammalian cells, and especially human cells. In general terms, the screening methods involve screening a plurality of agents to identify an agent that enhance the excretion of IAPP oligomers by, e.g., breaking down or solubilizing IAPP oligomers, or that block or interfere with the formation of IAPP toxic oligomers by, e.g., binding to an IAPP monomer or an IAPP oligomer.

[00120] For screening for agents that enhance the excretion of IAPP oligomers, any cell binding IAPP oligomers can be used. For screening for agents that block or interfere with the formation of IAPP oligomers, any cell binding IAPP monomers or oligomers can be used. In some embodiments, the cells are eukaryotic cell lines (e.g., CHO or HEK293) transformed to express and/or bind IAPP monomers or oligomers. In some embodiments, a cell that endogenously binds or expresses IAPP monomers or oligomers is used in screens.
Polypeptide Binding Assays

[00121] For screening for agents that block or interfere with the formation of IAPP oligomers, preliminary screens can be conducted by screening for agents capable of binding to IAPP monomers or oligomers, as at least some of the agents so identified are likely to block or interfere with the formation of IAPP oligomers. Binding assays are also useful, e.g., for identifying endogenous proteins that interact with IAPP oligomers. For example, antibodies or other molecules that bind IAPP oligomers can be identified in binding assays.

[00122] Binding assays usually involve contacting an IAPP monomer or oligomer with one or more test agents and allowing sufficient time for the protein and test agents to form a binding complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation or co-migration on non-denaturing SDS-polyacrylamide gels, and co-migration on Western blots [see, e.g., Bennet, J.P. and Yamamura, H.I. (1985) "Neurotransmitter, Hormone or Drug Receptor Binding Methods," in Neurotransmitter Receptor Binding (Yamamura, H. I., et al, eds.), pp. 61-89). Other binding assays involve the use of mass spectrometry or NMR techniques to identify molecules bound to an IAPP monomer or oligomer or displacement of labeled substrates. The IAPP monomers or oligomers utilized in such assays can be naturally expressed, cloned or synthesized.

[00123] In mammalian or yeast two-hybrid approaches [see, e.g., Barrel, P.L. et. al. Methods Enzymol, 254:241 (1995)] can be used to identify polypeptides or other molecules that interact or bind when expressed together in a host cell.

Oligomerization Assay

[00124] The effect of an agent on the formation of IAPP oligomers can be screened using an oligomerization assay. As a non-limiting example, a thioflavin T (TFT) fluorescence assay can be used to measure the ability of IAPP to form oligomers. See Lin et al., J. Clin. Endocrinol. Metab. 90:6678-6686 (2005); Meier et al., Am. J. Physiol. Endocrinol. Metab. 291:E1317-E1324 (2006), incorporated herein by reference for all purposes. Briefly, IAPP monomer and the agent to be screened are incubated with thioflavin T, a dye known to preferentially bind amyloid fibrils, and fluorescence is measured at multiple time points to measure IAPP oligomerization.
Using the TFT assay, if a solution of agent and IAPP monomer exhibited less fluorescence signal than a control solution (e.g., a solution of IAPP monomer alone), then that agent would be identified as blocking or interfering with the formation of IAPP oligomers.

**Oligomer Excretion Assay**

The effect of an agent on enhancing the excretion of IAPP oligomers can be screened *in vivo*, for example by administering an agent to an animal expressing IAPP oligomers and measuring the levels of IAPP oligomers that are excreted from the animal, *e.g.* in a bodily fluid such as urine. The levels of excreted IAPP oligomers can be measured using an immunoassay as described herein, such as by dot blot or Western blot analysis using A11 antibody. Using such an assay, if the administration of an agent to the animal resulted in increased levels of IAPP excreted by the animal as compared to a control animal (*e.g.*, an animal not administered the agent), then that agent would be identified as enhancing the excretion of IAPP oligomers.

**Two-Step Screen**

In some embodiments, the method of screening for agents comprises screening a plurality of agents for the ability to enhance excretion of IAPP oligomers from the body and/or to block or interfere with the formation of IAPP oligomers, and further comprises administering the identified agent to an animal and measuring the ability of the agent to reduce the rate of brain disease. Agents that are identified by any of the foregoing screening methods can be administered to an animal that serves as a model for human diabetic conditions or brain disease, then the ability of the agent to reduce the rate of brain disease in that animal is measured. For example, if the animal serves as a model for human diabetic conditions, the ability of the agent to reduce the rate of brain disease in the animal can be measured by any known test for diabetic conditions, such as the HbAlc test, the C-peptide) test, the FPG test, the OGTT test, and/or the casual plasma glucose test. If the animal serves as a model for brain disease, the ability of the agent to reduce the rate of brain disease in the animal can be measured by, for example, echocardiography, MRI, micromanometer conductance catheters, or by measuring calcium transient amplitudes in cardiac myocytes. The animal models utilized in such screens generally are mammals of any kind. Specific examples of suitable animals include, but are not limited to, primates, mice and rats.
Compositions, Kits, and Integrated Systems

[00127] The invention compositions, kits and integrated systems for practicing the methods described herein using IAPP polypeptides of the invention, antibodies, etc.

[00128] The invention provides assay compositions for use in solid phase assays; such compositions can include, for example, one or more IAPP polypeptides immobilized on a solid support, and a labeling reagent. In each case, the assay compositions can also include additional reagents that are desirable for hybridization. Modulators of activity of an IAPP polypeptide of the invention can also be included in the assay compositions.

[00129] In some embodiments, the solid support comprises a sensor operably linked to one or more nanoparticles, wherein the one or more nanoparticles are conjugated to an antibody that specifically binds IAPP oligomers. As used herein, the term "nanoparticle" refers to a defined particle of typically about 5 to 5000, or more typically about 5 to 500 atoms. Typically, the nanoparticles have dimensions of less than about 150 nanometers. In some embodiments, nanoparticles may be made from such materials as metal, such as silver or gold; semiconductor material; carbon; or biological materials such as nucleic acids or peptides.

[00130] The invention also provides kits for predicting the propensity for brain disease in an individual who has a diabetic condition such as type 2 diabetes or pre-diabetes. The kits typically include a probe which comprises an antibody that specifically binds to oligomers or IAPP oligomers, and a label for detecting the presence of the probe. Kits optionally further include additional components such as instructions to practice a high-throughput method of assaying for an effect on activity of the IAPP oligomers of the invention, one or more containers or compartments (e.g., to hold the probe, labels, or the like), a control modulator of the activity of IAPP oligomers, a robotic armature for mixing kit components or the like.

[00131] The invention also provides integrated systems for high-throughput screening of potential modulators for an effect on the activity of an IAPP oligomer of the invention. The systems can include a robotic armature which transfers fluid from a source to a destination, a controller which controls the robotic armature, a label detector, a data storage unit which records label detection, and an assay component such as a microtiter dish comprising a well having a reaction mixture or a substrate comprising a fixed nucleic acid or immobilization moiety.
A number of robotic fluid transfer systems are available, or can easily be made from existing components. For example, a Zymate XP (Zymark Corporation; Hopkinton, MA) automated robot using a Microlab 2200 (Hamilton; Reno, NV) pipetting station can be used to transfer parallel samples to 96 well microtiter plates to set up several parallel simultaneous binding assays.

Optical images viewed (and, optionally, recorded) by a camera or other recording device (e.g., a photodiode and data storage device) are optionally further processed in any of the embodiments herein, e.g., by digitizing the image and storing and analyzing the image on a computer. A variety of commercially available peripheral equipment and software is available for digitizing, storing and analyzing a digitized video or digitized optical image.

One conventional system carries light from the specimen field to a cooled charge-coupled device (CCD) camera, in common use in the art. A CCD camera includes an array of picture elements (pixels). The light from the specimen is imaged on the CCD. Particular pixels corresponding to regions of the specimen (e.g., individual hybridization sites on an array of biological polymers) are sampled to obtain light intensity readings for each position. Multiple pixels are processed in parallel to increase speed. The apparatus and methods of the invention are easily used for viewing any sample, e.g., by fluorescent or dark field microscopic techniques.

The following examples are offered to illustrate, but not to limit, the claimed invention.

EXAMPLE

IAPP toxic oligomers are present in blood from T2DM humans

Blood samples were collected from type 2 diabetes mellitus ("T2DM"; N=5), overweight (Body Mass Index > 25; N=5)) and lean healthy subjects (N=5) enrolled in an epidemiological study on cardiovascular diseases at the University of California Davis Medical Center. Using an antibody specific for toxic oligomers (Al 1), (Kayed et al, Science 300:486-489 (2003)), toxic oligomers in serum samples from T2DM patients and from overweight individuals were detected (Fig. 1). Oligomer-specific immunoreactivity was significantly abundant in T2DM patients and obese (BMI > 32) individuals compared to non-diabetic, non-
obese (BMI < 28) individuals. This correlates with larger bands corresponding to high molecular weight IAPP species (~ 25 kDa and ~ 50 kDa) that can be seen on western blots using an anti-IAPP antibody (Fig. 1). The average anti-IAPP specific immunoreactivity signal derived by the integration of bands at 25 kDa and 50 kDa was about 40% larger for T2DM and obese individuals (OW/OB) than for control individuals (Fig. 1). The anti-IAPP specific immunoreactivity signal derived by the integration of bands at 4 kDa (monomer) and 16 kDa (tetramer) is about 40% larger in T2D and overweight/obesity (BMI>25) than in a control (Fig. 1C).

IAPP toxic oligomers are present in brains of individuals with Alzheimer's disease

The presence of IAPP oligomers in human brain tissues was determined. IAPP content and size distribution in brain samples was compared from patients with Alzheimer's disease (AD) (N=10) vs. control (age matched humans without AD) (N=8). Significantly increased accumulation of large IAPP oligomers was found in AD brains vs. control brains (Fig. 2).

Immunohistochemistry using an anti-IAPP antibody showed large IAPP deposits (marked by arrows) in brains from patients with AD (Fig. 3(A)-(D)) in patients having type 2 diabetes with vascular dementia (Fig. 4A,B; N=8). Similar amylin deposits were present in the pancreas (Fig. 3E) and heart (Fig. 3F) of type 2 diabetes patients. IAPP deposits were significantly larger in blood vessels and vicinities (Fig. 3A,D and 4B), which is consistent with an IAPP oligomer influx from the circulation. Brain samples from patients in a control group (>75 yo, without AD and type 2 diabetes) show significantly lower levels of IAPP oligomers (Fig. 2) and less frequent IAPP plaques (Fig. 5A-D). Moderate IAPP accumulation in control samples is likely a result of the advanced age of the group and thus the highly probable state of insulin resistance.

IAPP toxic oligomers co-localize and bind Aβ in the brain

The ability of IAPP to co-localize and bind Aβ in the brain was test. Co-staining of IAPP and amyloid beta (Aβ) shows that IAPP and Aβ co-localize on large areas of the brain (Figure 6, arrows). Furthermore, IAPP and Aβ physically interact in brains of individuals with both type 2 diabetes and AD (Fig. 7; WB-western blot, IP-immunoprecipitation). Binding is demonstrated by immunoprecipitation of IAPP from brains of AD and T2D patients and then
exposure of the IP to the anti-Aβ antibody on western blot. The amount of IAPP that immunoprecipitates Aβ is an additional marker of the onset of neurodegeneration in diabetes and obese patients. This data shows that the control group can be discriminated from groups of patients with T2D and AD based on the presence of IAPP bound to Aβ in brain extracts.

5 Accumulation of IAPP toxic oligomers in the brain in HIP rats

[00140] Accumulation of IAPP toxic oligomers in the brain can be determined using HIP rats, which overexpress human IAPP in pancreatic β-cells. Human IAPP is overexpressed because rodent IAPP is not amyloidogenic. The HIP rat has been well characterized with respect to IAPP toxic oligomer formation in pancreatic islets (Matveyenko A.V. and Butler P.C. ILAR Journal 47:225-233 (2006)). It has been shown that humans with T2DM and HIP rats share in common the formation of IAPP toxic oligomers in the secretory track of pancreatic β-cells, deficit in β-cell mass and islet amyloid (Gurlo T. et al. Am J Pathol. 176(2):861-9 (2010); Huang C.J. et al., J Biol Chem. 285:339-48 (2010)).

[00141] IAPP amyloid oligomers released from pancreatic islets circulate through the blood and accumulate in peripheral organs (heart), in HIP rats (Figure 8). In contrast, rats expressing same level of wild-type rat IAPP (UCD-T2DM rats) do not accumulate IAPP in peripheral organs. IAPP significantly accumulates only in HIP rat hearts, a consequence of human IAPP's amyloidogenicity (Figure 8A). Western blots on heart protein homogenates, cardiac myocyte lysates and blood serum from HIP rats (Figure 8B, C) show IAPP molecular weight bands that match those detected in human serum (Figure 1). In HIP rats, the IAPP oligomers circulate through the blood and start accumulating in the heart in the pre-diabetic state (Figure 8B, C). The oligomers likely attach to sarcolemma or enter the myocyte, as suggested by their presence in cardiac myocyte lysates (Figure 8B). Based on these data, IAPP amyloid oligomers likely cross the blood-brain barrier (BBB) and accumulate in the brain, in HIP rats. Therefore, the HIP can be used as a pre-diabetes and diabetes model to investigate IAPP oligomer accumulation in the brain.

[00142] Immunohistochemistry using an anti-IAPP antibody showed large IAPP deposits in the brain and pancreas of HIP rats in comparison to control rats (Fig. 9).
IAPP mRNA levels were determined. Q-PCR tests showed no presence of human IAPP mRNA in the HIP rat brain, indicating that cerebral IAPP accumulation is entirely from circulation (Fig. 10). Wild-type littermates served as non-insulin resistant control rats.

**IAPP buildup in the brain causes oxidative and inflammatory damage**

IAPP oligomers are membrane-permeant, alter calcium cycling in various cells, including neurons, and cause oxidative stress. IAPP oligomer-mediated cytotoxicity is similar to Aβ pathology. IAPP further participates in stimulating lipolysis, elevating plasma free fatty acid levels, activating the renin-angiotensin-aldosterone system, stimulating RAGE, and promoting inflammatory processes.

HIP rats were used to show activated perivascular macrophages (Fig. 11) and microglia (Fig. 12; EDI immunostaining), elevated levels of the pro-inflammatory cytokines IL-6 and TNF-a (Fig. 13), and reduced levels of the anti-inflammatory cytokine IL-10 (Fig. 13). These results suggest that circulating IAPP oligomers engage RAGE on the endothelia cells and trigger inflammation.

It is known that incubation of isolated of cardiac myocytes with IAPP (50 μM, 2h) (i.e. amylin) results in activation of soluble RAGE (Fig. 14). Cerebrovascular inflammation is a disruptor of normal synaptic function at the starting point of AD pathological progression. IAPP oligomers elevate calcium in cultured neurons, which is similar to the effect induced by Aβ oligomers. In HIP rat brains, reduced expression of SERCA was observed (Fig. 15), which is the protein responsible for calcium uptake in the endoplasmic reticulum (ER) in neurons. SERCA downregulation is a direct effect of activation of calcium-mediated hypertrophic and remodeling pathways in neurons.

HIP rat brains additionally show altered expression of proteins involved in mitochondrial fission (fission protein 1, Fisl, and dynamin-related protein 1, DRPl) and fusion (mitofusin 2, Mfn2, and optic atrophy 1, OPA1) in HIP rat brain (Fig. 16). These results show altered mitochondrial dynamics in HIP rats, similar to changes in mitochondrial dynamics observed in AD brains. Mitochondrial stress alters ATP production leading to neuron dysfunction and death. Because HIP rats lack Aβ accumulation, possible changes in insulin signaling in the brain of HIP rats likely effect IAPP oligomeric buildup.
Alzheimer's disease-like effects in cultured neurons

Previous studies have shown that exogenous IAPP oligomers alter calcium signaling in cultured neurons, which is similar to the Aβ oligomer-mediated effect in neurons (Kawahara et al, Biol. Chem. 2000: 275:14077-14083 (2000)). Interaction of IAPP oligomers with cultured neurons can therefore be shown to induce pathogenic effects similar to those triggered by Aβ in AD. Neurons and astrocytes can be isolated from HIP rats at different stages of their disease development, wherein intracellular calcium can be measured. One can expect elevated levels of intracellular calcium in pre-diabetic HIP rats as compared to age-matched controls. Elevated intracellular calcium levels alter neuron function and can trigger apoptosis (Green, K.N. and LaFerla, F.M. Neuron 59:190-194 (2008); Demuro et al. J Biol. Chem. 280:17294-17300 (2005)).

Reversal of IAPP oligomer-induced membrane damage

Poloxamer 188 is efficient in sealing damaged membranes (Collins J.M. et al, Biochim. Biophys. Acta 1768, 1238-1246 (2007) and has been shown to seal damaged neurons incubated with exogenous Aβ oligomers (Mina E.W., J Mol Biol 391, 577-585 (2009)). Oligomerization of Aβ peptides is associated with Alzheimer's disease, and Aβ and IAPP have the same molecular weight sizes and are about 40% identical at the amino acid level. Incubation of cardiac myocytes simultaneously with exogenous IAPP oligomers and poloxamer 188 (1:1 ratio, 50µM final concentration) reduced the level of alteration of Ca cycling (Fig. 17). This suggests that the polymer molecules either prevent the attachment of IAPP oligomers to sarcolemma or efficiently sealed damaged membranes. Atomic force microscopy approaches demonstrate that IAPP oligomers intercalate into sarcolemma and induce sarcolemma thinning (Fig. 18).

Discussion

activating the rennin-angiotensin-aldosterone system and in promoting the inflammatory process (Hayden M.R. and Tyagi S.C., *JOP* 3:86-108 (2002); Wendt T. *et al.*, *J Am Soc Nephrol* 14:1383-1395 (2003)). Although complications of T2DM are difficult to predict, it is increasingly clear that β-cell dysfunction and formation of IAPP oligomers result in a feed-forward process, whereby the secretion of these amyloidogenic entities in the blood causes additional damage in organs other than pancreas, including the brain (Fig. 19). Thus, IAPP oligomers, which are secondary products to an increased demand for insulin biosynthesis, could be pathogens of brain disease.

[00151] Similar amyloidogenic entities, e.g. Aβ oligomers, the molecular entities implicated in the development of Alzheimer's disease, are also extremely toxic (Selkoe, D. J., *Nature* 426:900-904 (2003); Haass C. *et al.*, *Nat. Rev. Mol. Cell Biol.* 8:101-112 (2007); Kayed R., *et al.* *Science* 300:486-489 (2003)). Data show that even nanomolar concentrations of preamyloid oligomers are able to kill mature neurons (Kirkitadze, M.D. *et al.*, *J Neurosci Res.* 69:567-577 (2002)). Previous data has shown that circulating IAPP oligomers induce cell toxicity by attachment to cell membranes and subsequent alteration of cell Ca2+ cycling. The interaction of IAPP oligomers with cultured neurons can be shown to induce similar pathogenic effects as those triggered by the Aβ oligomers in AD. Thus, it is possible that IAPP oligomers circulating through the blood in obesity and insulin resistance/pre-diabetes may compromise the blood-brain barrier (BBB), favoring toxic IAPP oligomer accumulation in the brain. IAPP oligomers likely alter neuron function and contribute to AD pathological progression in individuals having diabetes. IAPP oligomers therefore provide an early pathogenic molecular link between diabetes and brain disease, including AD.

[00152] IAPP mediated brain disease had remained unnoticed to date because 1) poor prognosis of brain disease in T2DM; 2) IAPP's amyloidogenity was considered to manifest only in pancreas (Westermark P. *et al.*, *Amyloid* 9:197-200 (2002)); and 3) rodent models bear only the non-amyloidogenic rat IAPP that does not form amyloids.

[00153] Accumulation of oligomeric IAPP in the brain also induces inflammatory and oxidative damage through various molecular mechanisms. IAPP oligomers likely engage RAGE on endothelia cells, activate astroglia, and trigger inflammation.
Promising therapies against IAPP oligomer toxicity may derive from, but is not limited to, the use of polymer molecules to either prevent the attachment of IAPP oligomers to sarcolemma or to efficiently seal damaged membranes. Results described herein show that PI88, for example, can attach to patches of thinner sarcolemma and provide protection to myocytes against the IAPP oligomer-induced membrane damage.

In summary, the present results suggest that IAPP oligomers contribute to brain disease, independently of hyperglycemia. As they circulate through the blood, these toxic entities may represent an effective target for diagnostic purposes and therapeutic strategies.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

INFORMAL SEQUENCE LISTING

SEQ ID NO:1
Human IAPP amino acid sequence
KCNTATCATQRLANFLVHSSNFGAILSSTNVGSNTY

SEQ ID NO:2
Rat IAPP amino acid sequence
KCNTATCATQRLANFLVRSSNNGPVLPPNTNVSNTY
WHAT IS CLAIMED IS:

1. A method for predicting a propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic, the method comprising:

   (a) determining the amount of islet amyloid polypeptide (IAPP) oligomer in a sample from the individual; and

   (b) predicting the propensity for brain disease in the individual based on the determined amount of IAPP oligomer, wherein an elevated amount of IAPP oligomer compared to normal levels indicates an increased propensity for brain disease.

2. The method of claim 1, wherein the sample is a blood sample.

3. The method of any of claims 1-2, wherein the individual has type 2 diabetes.

4. The method of any of claims 1-2, wherein the individual is pre-diabetic.

5. The method of any of claims 1-4, wherein the determining step comprises contacting a reagent that specifically binds IAPP oligomers with the sample; and detecting the amount of IAPP oligomers bound by the reagent.

6. The method of claim 5, wherein the detecting step comprises contacting a detection antibody that binds IAPP oligomers with the IAPP oligomers bound with the reagent; and quantifying the binding of the detection antibody to the bound IAPP oligomers.

7. The method of claim 6, wherein the detection antibody is detectably labeled.

8. The method of claim 5, wherein the reagent is an antibody.

9. The method of claim 1, wherein the method further comprises, prior to (a), extracting blood from the individual.

10. The method of any of claims 1-9, wherein it is determined that the individual has a propensity for brain disease, the method further comprising designing a treatment plan to reduce the propensity for brain disease in the individual.
11. A kit for predicting a propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic, the kit comprising a solid support operably linked to a reagent that specifically binds IAPP oligomers.

12. The kit of claim 11, wherein the reagent is an antibody.

13. The kit of claim 11 or 12, further comprising a detection antibody that binds to IAPP oligomers when the IAPP oligomers are bound to the reagent.

14. The kit of claim 13, wherein the detection antibody is detectably labeled.

15. The kit of claim 11 or 12, wherein the solid support comprises a sensor operably linked to one or more nanoparticles, wherein the one or more nanoparticles are conjugated to an antibody that specifically binds IAPP oligomers.

16. A method for screening for agents that prevent or reduce the propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic, the method comprising screening a plurality of agents for the ability:

- to enhance excretion of IAPP oligomers from the body and/or
- to block or interfere with the formation and/or function of IAPP oligomers.

17. The method of claim 16, further comprising identifying at least one agent from the plurality that enhances excretion of IAPP oligomers from the body and/or blocks or interferes with the formation of IAPP oligomers; and

administering the identified agent to an animal and measuring the ability of the agent to reduce the rate of brain disease.

18. The method of claim 17, wherein the animal is an animal model for diabetes.

19. The method of claim 18, wherein the animal has diabetes or is pre-diabetic.

20. A method of treating or preventing brain disease in an individual who has type 2 diabetes or is pre-diabetic, the method comprising
administering an effective amount of a compound that has the ability to
i) enhance excretion of IAPP oligomers from the body,
ii) block or interfere with the formation of IAPP oligomers, or
iii) block or interfere with the function of IAPP oligomers.

21. The method of claim 20, wherein the compound is a surfactant.

22. The method of claim 21, wherein the surfactant is a polymer-based membrane sealant.

22. The method of claim 22, wherein the polymer-based membrane sealant blocks or interferes with the function of IAPP oligomers by restoring membranes damaged by IAPP oligomers.

23. The method of claim 1, wherein the IAPP oligomers are larger molecular weight IAPP oligomers.

24. The method of claim 23, wherein the larger molecular weight IAPP oligomers are about 32 kDa.

25. The method of claim 23, wherein the larger molecular weight IAPP oligomers are about 64 kDa.

26. A method for predicting a propensity for brain disease in an individual who has type 2 diabetes or is pre-diabetic, the method comprising:

(a) determining the amount of islet amyloid polypeptide (IAPP) oligomer bound to amyloid beta (Aβ) in a sample from the individual; and

(b) predicting the propensity for brain disease in the individual based on the determined amount of IAPP oligomer bound to Aβ, wherein an elevated amount of IAPP oligomer bound to Aβ compared to normal levels of IAPP oligomer bound to Aβ indicates an increased propensity for brain disease.

27. The method of claim 26, wherein the sample is a blood sample.
28. The method of any of claims 26-27, wherein the individual has type 2 diabetes.

29. The method of any of claims 26-27, wherein the individual is pre-diabetic.

30. The method of any of claims 26-29, wherein the determining step comprises contacting a reagent that specifically binds IAPP oligomers with the sample; and contacting a reagent that specifically binds Aβ with the sample, and detecting the amount of IAPP oligomers bound by the reagent that specifically binds Aβ or detecting the amount of Aβ bound by the reagent that specifically binds IAPP oligomers.

31. The method of claim 30, wherein the reagent is an antibody.

32. The method of claim 26, wherein the method further comprises, prior to (a), extracting blood from the individual.

33. The method of any of claims 26-32, wherein it is determined that the individual has a propensity for brain disease, the method further comprising designing a treatment plan to reduce the propensity for brain disease in the individual.
FIGURE 1
FIGURE 2
FIGURE 5
FIGURE 6
FIGURE 7
FIGURE 8
FIGURE 9
FIGURE 10
FIGURE 12
FIGURE 13
FIGURE 14
FIGURE 15
FIGURE 16
FIGURE 17
FIGURE 18
FIGURE 19
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2012/051603

A.  CLASSIFICATION OF SUBJECT MATTER

G01N 33/68 (2006.01)
G01N 33/53 (2006.01)
G01N 33/15 (2006.01)
A61P 25/28 (2006.01)

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

B.  FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 33/15, 33/48, 33/53, 33/68, A61P 25/28

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

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

Rossiskaya meditsina, DWPI, EAPATIS, Esp@cenet, Medline, PAJ, PatSearch, RUPTO, USPTO, WIPO

C.  DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>RORIZ-FILHO J.S. et al. (Pre)diabetes, brain aging, and cognition. Biochimica et Biophysica Acta., 2009 May, 1792, pp. 432-443</td>
<td>1-19, 26-33</td>
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X Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 October 2012 (3.11.2012)

Date of mailing of the international search report

06 December 2012 (06.12.2012)

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<td>KAYED R. et al. Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. Molecular Neurodegeneration, 26 September 2007, 2:18. Retrieved from the Internet: <a href="">URL:http://www.molecularneurodegeneration.com/content/2/1/18</a>, pp. 1-11</td>
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