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(54) **Title**: METHODS FOR TREATING AND DIAGNOSING GLUCOSE METABOLIC SYNDROME

(57) **Abstract**: What are described are methods of screening for or detecting defects in glucose metabolism in subjects, including screening for therapeutic molecules for treating diseases in glucose metabolism.

METHODS FOR TREATING AND DIAGNOSING GLUCOSE METABOLIC SYNDROME

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/220,382, filed on June 25, 2009, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

The technical area of the description pertains to methods of screening for or detecting defects in glucose metabolism in subjects, including screening for therapeutic molecules for treating diseases in glucose metabolism.

BACKGROUND

This invention relates to compositions and methods useful for delaying or ameliorating human diseases associated with glucose metabolic syndrome. Abnormalities of glucose metabolism are the most common errors of carbohydrate metabolism. Disorders associated with elevated levels of plasma glucose have been classified into three categories: diabetes mellitus (DM) type 1, DM type 2 and maturity onset diabetes of youth (MODY), a subtype of DM 2. Both nutrition and the genetic response to nutrients play a role in the etiology of diabetes mellitus type II.

Glucose metabolic syndrome includes glycogen storage disease (GSD). GSD is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types. GSD has two classes of cause: genetic and acquired. Genetic GSD is caused by any inborn error of metabolism. Many animals utilize the hexosamine signaling pathway as a means to detect and respond to environmental nutrient supply. This nutrient sensing pathway works by converting a small percent of absorbed glucose to N-acetylglucosamine. N-acetylglucosamine is then transferred to hydroxyl groups of serine and threonine residues of a variety of substrates, creating an O-linked N-acetylglucosamine (O-glcNAc) modification.

At present there are few reliable methods for diagnosis of a genetic predisposition for abnormalities in glucose metabolism or GSD prior to major changes in health, including diabetes and obesity. The search for genetic markers linked to diabetes and obesity has proven difficult.

There remains a need for identifying genetic targets for diagnosis and therapy in detecting, preventing or treating glucose metabolic syndromes, including GSD.

SUMMARY

One aspect of the invention is a method for identifying a therapeutic compound that restores normal glycogen accumulation, said method comprising:

a) providing a nematode or an isolated nematode cell having impaired expression of a nematode gene;

b) contacting said nematode or isolated nematode cell with a candidate compound; and

c) comprising the step of measuring glycogen distribution; and

d) selecting for a compound that changes levels of glycogen accumulation.

Further information regarding the following referenced C. elegans genes can be found at http://www.wormbase.org a universal repository database containing reference sequence information for all C. elegans genes.

An embodiment of the invention is the method for identifying a therapeutic compound, in which the nematode gene is selected from the group consisting of B0491.5, B0495.4, C02F5.9, C05C8.7, C06H2.1, C09G5.6, C09H10.3, C15C7.5, C16A3.5, C18E9.4, C23H4.1, C27A2.2, C29E4.8, C30C11.2, C33D3.1, C34B2.8, C34E10.6, C46G7.1, C47B2.4, C50D2.1, C52E4.4, C53A5.1, C53A5.6, C53B7.4, C53D5.6, C54G4.8, C56G2.6, CD4.6, D1014.3, F01G10.1, F02E8.1, F10E7.7, F11G11.10, F14H8.2, F20G4.1, F23B12.5, F23H11.5, F25B4.6, F25H5.5, F26E4.6, F26E4.9, F26H9.6, F29C4.2, F29G6.3, F29G9.3, F32A7.6, F32D1.2, F33A8.1, F36A2.7, F40F4.6, F45H10.2, F46F11.5, F49C12.12, F49C12.13, F54D7.2, F54F2.1, F54H12.6, F55A3.3, F55A3.7, F56H1.4, F59C6.5, H15N14.2, H18N23.2, H28O16.1, H37A05.1, K02D10.5, K03A1.1, K04G7.4, K05C4.1, K07A12.3, K07D8.1, K08D12.1, K08E3.5, LLC1.3, M01F1.5, R10E11.2, R53.4, T02H6.11, T05D4.4, T10D4.3, T13C5.1, T14B4.6, T14B4.7, T20H4.5, T23H2.5, T26A5.3, T28D9.10, VW02B12L.1, VW02B12L.1, W02F12.5, W09C5.8, Y105E8A.9, Y106G6H.3, Y110A2AL.8, Y116A8C.35, Y37D8A.14, Y45G12B.1, Y46G5A.31, Y47D3B.7, Y47G6A.23, Y48B6A.3, Y57E12AL.5, Y57G11C.12, Y57G11C.24, Y71F9AL.17, Y71F9B.4, Y71H2B.10, Y82E9BR.15, Y82E9BR.3, ZC328.3, ZK328.5, ZK430.8, AC7.1, C04F6.1, C23G10.4, C23H3.4, C30B5.6, C36B1.4, C56C10.3, F23F1.8, F26D11.13, F27C1.7, F35H10.4, F38A5.5, F38E11.5, F39H11.5, F53G12.3, F55A12.7, F56C11.1, F57B9.10, F57B9.2, F59E10.3, H19M22.2, K07D4.3, K08E5.3, K11D9.2, R03E1.2,

R10E11.1, R12E2.3, T01H3.1, T14F9.1, T14G10.5, T20F5.2, W09B6.1, Y111B2A.14, Y25C1A.5, Y39B6A.12, Y49E10.1, Y55H10A.1, Y71D11A.5, ZK1058.3, ZK180.4, and ZK970.4, K10C2.4 and R05D11.3, and wherein the impaired expression of the nematode gene decreases glycogen levels.

Another embodiment is the method for identifying a therapeutic compound, in which the nematode gene is selected from the group wherein the nematode gene is selected from the group consisting of B0047.4,B0393.1,C05D2.1, C27H6.2, C34F11.3, C47D12.6, F22B5.2, F22D6.3, F26D10.3, F39B2.4, F42A8.1, F54D5.11, F55A12.8, F55B11.2, K06A1.6, K08E3.6, R06F6.1, R74.1, T01G9.6, T02G5.9, T03F1.9, T04A8.14, T11G6.1, T28F3.2, W07E6.4, Y110A7A.8, Y39G10AR.7, Y39G10AR.8, Y48G1A.4, Y57G11C.19, Y80D3A.1, Y87G2A.5, Y95B8A.10, ZK1127.5, K04E7.2C48E7.2, D2089.1, F14B4.3, F19F10.9, F55F8.3, K06A5.4, T23D8.3, T23D8.9, W07E6.1, Y48G1A.4, Y54E10A.15, C37C3.6, F38A6.1, Y39B6A.33, Y53C10A.3, and ZC168.5, and wherein the impaired expression of the nematode gene increases glycogen levels.

Another embodiment is the method for identifying a therapeutic compound, in which the nematode gene is selected from the group wherein the nematode gene is selected from the group consisting of B0336.2, C03D6.1, C03D6.3, C12C8.3, , C14C10.4, C16A3.4, D1054.15, F02D10.5, F13D12.7, F26E4.8, F36F2.3, F44A6.2, F48E8.5, F54A3.3, F54C9.1, F54E7.2, F57B10.1, F58E2.9, K01G5.7, M7.1, R06A10.2, R07E4.6, R144.2, T04A8.7, T10B5.5, T21B10.7, T28F12.2, W03C9.4, W04A4.5, W06H3.3, Y110A7A.11, Y116A8C.42, Y32F6A.3, Y41D4B.19, Y41D4B.19, Y48G1A.5, Y55F3AR.3, Y55F3AR.3, Y6B3A.1, Y71F9AM.5, Y71G12B.11, Y71G12B.11, ZK1151.2, ZK1290.3, ZK652.1, C29A12.3, C37H5.5, F28B3.7, F32D1.10, F53F4.11, F56A3.3, F59A2.1, K07C5.4, T19B4.2, T23D8.4, T27B1.2, Y39B6A.14, and Y9C9A.8, and wherein the impaired expression of the nematode gene causes abnormal glycogen distribution.

Another embodiment is the for identifying a therapeutic compound, in which the nematode gene also changes O-glcNAc modification of proteins, preferably selected from the group consisting of F26D10.3, F55F8.3, F59A2.1, W07E6.1, Y110A7A.8, Y39B6A.14, Y39B6A.33, Y87G2A.5, B0495.4, C56C10.3, F26H9.6, H15N14.2, H28)16.1, and W09B6.1.

Another embodiment is the method for identifying a therapeutic compound further comprising the step of measuring the level of free glucose. A further embodiment is a method in which the nematodes are subject to a glucose challenge.

Another embodiment is the method for identifying a therapeutic compound, in which the nematode is *C. elegans*.

Another embodiment is the method for identifying a therapeutic compound, in which the compound is a candidate compound for treating glycogen storage disease or glucose metabolic syndrome. A further embodiment is a method, in which the disease is diabetes or obesity.

Another embodiment is the method for identifying a therapeutic compound, in which the nematode gene expression is impaired by siRNA.

Another embodiment is the method for detecting defects in glycogen synthesis or accumulation of glucose in the blood, comprising isolating cells of a subject, and measuring genetic expression of at least one human gene in the cells, wherein said human gene is a homolog of the nematode gene, preferably wherein the human gene is identified by isolating DNA from the nematode gene, and contacting said DNA with isolated DNA from a human cell under hybridization conditions that provide detection of DNA sequences have about 70% or greater nucleic acid sequence identity to the nematodes gene. Another embodiment of the method is wherein genetic expression of a multiplicity of human genes is measured, preferably by changes in levels of mRNA, including by a microarray. Another embodiment is wherein the genetic expression is a measure of the response of a subject to a therapeutic for treating a glucose metabolic syndrome. Another embodiment is wherein genetic expression of at least one human gene is measured by contacting protein of a sample from a subject with at least one antibody to measure expression levels of the human gene, preferably by an ELISA.

Another embodiment is the method for detecting defects in glucose metabolism in which the genetic expression is a measure of the response of a subject to a therapeutic for treating a glucose metabolic syndrome.

The invention also includes kits for use in the diagnosis of a glucose metabolic syndrome, or a propensity for associated diseases, in a patient. One such kit includes a PCR primer complementary to an AMP deaminase nucleic acid sequence and instructions for diagnosing glucose metabolic syndrome or a propensity for associated diseases.

Yet another aspect of the invention features a transgenic, non-human animal, such as a mouse or a nematode, whose germ cells and somatic cells contain a transgene coding for a mutant polypeptide, for example, a mutant polypeptide that is derived from a human counterpart gene. In another preferred embodiment, the transgene includes a knockout mutation.

In related aspects, the invention features cells (for example, cells isolated from a mammal, such as mouse, human, or nematode cells) isolated from the transgenic animals described above.

Another aspect of the invention features a method of screening for a compound that increases the storage or utilization of glycogen. This method includes (a) exposing a non-human transgenic animal whose germ cells and somatic cells contain a transgene coding for a mutant polypeptide to a candidate compound, and (b) determining the activity of the polypeptide in the transgenic animal. An increase in polypeptide activity, as compared to untreated controls, is indicative of a compound that is capable of increasing polypeptide activity. In preferred embodiments, the compound can be used to treat glucose metabolic syndrome, e.g., obesity.

In a related aspect, the invention features a method of screening for a compound that correct the dysregulation of glycogen levels. This method includes (a) exposing a non-human transgenic animal whose germ cells and somatic cells contain a transgene coding for a protein regulating glycogen storage to a candidate compound, and (b) determining the affect of the candidate compound on glycogen in the transgenic animal. A change in glycogen levels, as compared to untreated controls, is indicative of a compound that is capable of correcting glycogen levels. In preferred embodiments, the compound can be used to treat a glucose metabolic syndrome, including glycogen storage disease, obesity, or atherosclerosis.

Also included in the invention is a method of screening for a compound that is capable of ameliorating or delaying a glucose metabolic syndrome. This method involves (a) exposing a transgenic, non-human animal whose germ cells and somatic cells contain a transgene coding for protein regulating glycogen storage to a candidate compound, and (b) monitoring the blood glucose level of the animal. A compound that promotes maintenance of a physiologically acceptable level of blood glucose in the animal, as compared to untreated controls, is indicative of a compound that is capable of ameliorating or delaying a glucose metabolic syndrome. In a preferred embodiment, the compound can be used to treat Type II diabetes.

Another method of screening for a compound that is capable of ameliorating or delaying obesity is also included in the invention. This method involves (a) exposing a transgenic, non-human animal whose germ cells and somatic cells contain a transgene coding for a protein regulating glycogen storage to a candidate compound, and (b) monitoring the adipose tissue of the animal. A compound that promotes maintenance of a physiologically acceptable level of adipose tissue in the animal, as compared to untreated controls, is indicative of a compound that is capable of ameliorating or delaying obesity.

A related method of the invention can be used for screening for a compound that is capable of ameliorating or delaying atherosclerosis. This method involves (a) exposing a

transgenic, non-human animal whose germ cells and somatic cells contain a transgene coding for a mutant AMP deaminase polypeptide to a candidate compound, and (b) monitoring the adipose tissue of the animal. A compound that promotes maintenance of a physiologically acceptable level of adipose tissue in the animal, as compared to untreated controls, is indicative of a compound that is capable of ameliorating or delaying atherosclerosis.

In another aspect, the invention includes a method for identifying a modulatory compound that is capable decreasing the activity of a gene that regulates glycogen storage. This method involves (a) providing a cell expressing the regulatory gene, and (b) contacting the cell with a candidate compound. A decrease in the gene expression following contact with the candidate compound identifies a modulatory compound. In preferred embodiments, the compound can be used to treat a glucose metabolic syndrome, including glycogen storage disease or obesity. This method can be carried out in an animal, such as a nematode.

In a related aspect, the invention includes a method for the identification of a modulatory compound that is capable of increasing the expression of a regulatory gene. This method involves (a) providing a cell expressing the regulatory gene, and (b) contacting the cell with a candidate compound. An increase in expression of the regulatory gene following contact with the candidate compound identifies a modulatory compound. In preferred embodiments, the compound can be used to treat a glucose metabolic syndrome, including glycogen storage disease or obesity. In other preferred embodiments, the compound is capable of increasing expression of the regulatory gene. This method can be carried out in an animal, such as a nematode.

DETAILED DESCRIPTION

Glycogen storage disease is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types. GSDs are genetic disorders. Glycogen storage disease includes GSD type I (von Gierke's diseasehttp://en.wikipedia.org/wiki/Glucose-6-phosphatase), GSD type II (Pompe's disease), GSD type III (Cori's disease or Forbe's disease),GSD type IV (Andersen disease), GSD type V (McArdle disease), GSD type VI (GSD type VIII, GSD type X, and Hers' disease), GSD type VII (Tarui's disease), GSD type IX, GSD type XI (Fanconi-Bickel syndrome), and GSD type 0.

We identified *C. elegans* metabolic genes critical for glucose metabolism. Among these crucial genes, we determined that certain specific genes are key regulatory components

that regulate glucose metabolism, and levels of glycogen. We discovered that dysregulation of AMP deaminase leads to accumulation of xylitol.

These results indicate that specific nematode genes are excellent candidate genes for human disease associated with glucose metabolic syndrome, e.g., glycogen storage disease, diabetes, obesity, and atherosclerosis. Our findings indicate that the human homologues of these regulatory genes may mediate regulation of glucose or glycogen storage in normal people and may be defective or deregulated in diabetics.

By "glucose metabolic syndrome" is meant any condition in which blood sugar levels are inappropriately elevated or lack normal metabolic regulation. Examples of such conditions include, without limitation, Type I diabetes, Type II diabetes, and gestational diabetes, glycogen storage disease and may be associated with obesity and atherosclerosis.

By "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

By "regulatory gene" or "gene that regulates glycogen storage" is meant:

(I) A *C. elegans* gene selected from the group consisting of B0491.5, B0495.4, C02F5.9, C05C8.7, C06H2.1, C09G5.6, C09H10.3, C15C7.5, C16A3.5, C18E9.4, C23H4.1, C27A2.2, C29E4.8, C30C11.2, C33D3.1, C34B2.8, C34E10.6, C46G7.1, C47B2.4, C50D2.1, C52E4.4, C53A5.1, C53A5.6, C53B7.4, C53D5.6, C54G4.8, C56G2.6, CD4.6, D1014.3, F01G10.1, F02E8.1, F10E7.7, F11G11.10, F14H8.2, F20G4.1, F23B12.5, F23H11.5, F25B4.6, F25H5.5, F26E4.6, F26E4.9, F26H9.6, F29C4.2, F29G6.3, F29G9.3, F32A7.6, F32D1.2, F33A8.1, F36A2.7, F40F4.6, F45H10.2, F46F11.5, F49C12.12, F49C12.13, F54D7.2, F54F2.1, F54H12.6, F55A3.3, F55A3.7, F56H1.4, F59C6.5, H15N14.2, H18N23.2, H28O16.1, H37A05.1, K02D10.5, K03A1.1, K04G7.4, K05C4.1, K07A12.3, K07D8.1, K08D12.1, K08E3.5, LLC1.3, M01F1.5, R10E11.2, R53.4, T02H6.11, T05D4.4, T10D4.3, T13C5.1, T14B4.6, T14B4.7, T20H4.5, T23H2.5, T26A5.3, T28D9.10, VW02B12L.1, VW02B12L.1, W02F12.5, W09C5.8, Y105E8A.9, Y106G6H.3, Y110A2AL.8, Y116A8C.35, Y37D8A.14, Y45G12B.1, Y46G5A.31, Y47D3B.7, Y47G6A.23, Y48B6A.3, Y57E12AL.5, Y57G11C.12, Y57G11C.24, Y71F9AL.17, Y71F9B.4, Y71H2B.10, Y82E9BR.15, Y82E9BR.3, ZC328.3, ZK328.5, ZK430.8, AC7.1, C04F6.1, C23G10.4, C23H3.4, C30B5.6, C36B1.4, C56C10.3, F23F1.8, F26D11.13, F27C1.7, F35H10.4, F38A5.5, F38E11.5, F39H11.5, F53G12.3, F55A12.7, F56C11.1, F57B9.10, F57B9.2, F59E10.3, H19M22.2, K07D4.3,

K08E5.3, K11D9.2, R03E1.2, R10E11.1, R12E2.3, T01H3.1, T14F9.1, T14G10.5, T20F5.2, W09B6.1, Y111B2A.14, Y25C1A.5, Y39B6A.12, Y49E10.1, Y55H10A.1, Y71D11A.5, ZK1058.3, ZK180.4, and ZK970.4, K10C2.4 and R05D11.3,

in which impaired expression of the regulatory gene decreases glycogen levels;

(II) A *C. elegans* gene selected from the group consisting of B0047.4,B0393.1,C05D2.1, C27H6.2, C34F11.3, C47D12.6, F22B5.2, F22D6.3, F26D10.3, F39B2.4, F42A8.1, F54D5.11, F55A12.8, F55B11.2, K06A1.6, K08E3.6, R06F6.1, R74.1, T01G9.6, T02G5.9, T03F1.9, T04A8.14, T11G6.1, T28F3.2, W07E6.4, Y110A7A.8, Y39G10AR.7, Y39G10AR.8, Y48G1A.4, Y57G11C.19, Y80D3A.1, Y87G2A.5, Y95B8A.10, ZK1127.5, K04E7.2C48E7.2, D2089.1, F14B4.3, F19F10.9, F55F8.3, K06A5.4, T23D8.3, T23D8.9, W07E6.1, Y48G1A.4, Y54E10A.15, C37C3.6, F38A6.1, Y39B6A.33, Y53C10A.3, and ZC168.5

in which the impaired expression of the regulatory gene increases glycogen levels; or

(III) A *C. elegans* gene selected from the group consisting of B0336.2, C03D6.1, C03D6.3, C12C8.3, , C14C10.4, C16A3.4, D1054.15, F02D10.5, F13D12.7, F26E4.8, F36F2.3, F44A6.2, F48E8.5, F54A3.3, F54C9.1, F54E7.2, F57B10.1, F58E2.9, K01G5.7, M7.1, R06A10.2, R07E4.6, R144.2, T04A8.7, T10B5.5, T21B10.7, T28F12.2, W03C9.4, W04A4.5, W06H3.3, Y110A7A.11, Y116A8C.42, Y32F6A.3, Y41D4B.19, Y41D4B.19, Y48G1A.5, Y55F3AR.3, Y55F3AR.3, Y6B3A.1, Y71F9AM.5, Y71G12B.11, Y71G12B.11, ZK1151.2, ZK1290.3, ZK652.1, C29A12.3, C37H5.5, F28B3.7, F32D1.10, F53F4.11, F56A3.3, F59A2.1, K07C5.4, T19B4.2, T23D8.4, T27B1.2, Y39B6A.14, and Y9C9A.8.

in which the impaired expression of the regulatory gene causes abnormal glycogen distribution.

By "regulatory protein" or "protein that regulates glycogen storage" is meant a protein encoded by a regulatory gene.

By "substantially pure" is meant a preparation which is at least 60% by weight (dry weight) the compound of interest. Preferably the preparation is at least 75%, more preferably

at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "isolated DNA" is meant DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By a "substantially identical" nucleic acid is meant a nucleic acid sequence which encodes a polypeptide differing only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the function of the polypeptide (assayed, e.g., as described herein). Preferably, the encoded sequence is at least 75%, more preferably 85%, and most preferably 95% identical at the amino acid level to the sequence of comparison. If nucleic acid sequences are compared a "substantially identical" nucleic acid sequence is one which is at least 85%, more preferably 90%, and most preferably 95% identical to the sequence of comparison. The length of nucleic acid sequence comparison will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

By "means for detecting" is meant any one or a series of components that sufficiently indicate a detection event of interest. Such means involve at least one label that may be assayed or observed, including, without limitation, radioactive, fluorescent, and chemiluminescent labels.

By "hybridization techniques" is meant any detection assay involving specific interactions (based on complementarity) between nucleic acid strands, including DNA-DNA, RNA-RNA, and DNA-RNA interactions. Such hybridization techniques may, if desired, include a PCR amplification step.

By a "modulatory compound", as used herein, is meant any compound capable of either decreasing gene expression (i.e., at the level of transcription, translation, or post-translation) or decreasing mRNA or protein levels or activity.

By "complementation" is meant an improvement of a genetic defect or mutation. Complementation is generally accomplished by expressing the wild-type version of the protein in a host cell or animal bearing a mutant or inactive version of the gene.

Cloning Regulatory Gene Sequences

The isolation of human gene nucleic acid sequences for a gene that regulates glycogen storage is made possible using the known *C. elegans* sequences and standard techniques. In particular, using all or a portion of the regulatory gene sequence, one may readily design oligonucleotide probes, including degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either strand of the DNA.

Using such motifs, may be isolated from sequence databases. Alternatively, such sequences may be used to design degenerate oligonucleotide probes to probe large genomic or cDNA libraries directly. General methods for designing and preparing such probes are provided. These oligonucleotides are useful for regulatory gene isolation, either through their use as probes for hybridizing to complementary sequences of the gene or as primers for various polymerase chain reaction (PCR) cloning strategies. If a PCR approach is utilized, the primers are optionally designed to allow cloning of the amplified product into a suitable vector.

Hybridization techniques and procedures are well known to those skilled in the art and are described. If desired, a combination of different oligonucleotide probes may be used for the screening of the recombinant DNA library. The oligonucleotides are, for example, labeled with radioactive phosphate using methods known in the art, and the detectably-labeled oligonucleotides are used to probe filter replicas from a recombinant DNA library. Recombinant DNA libraries may be prepared according to methods well known in the art, for example or may be obtained from commercial sources.

For detection or isolation of closely related gene sequences, high stringency hybridization conditions may be employed; such conditions include hybridization at about 42 degree C. and about 50% formamide; a first wash at about 65 degree C., about 2X-SSC, and 1% SDS; followed by a second wash at about 65 degree C. and about 0.1% SDS, 1X-SSC. Lower stringency conditions for detecting the regulatory genes having less sequence identity

to the nematode genes described herein include, for example, hybridization at about 42 degree C. in the absence of formamide; a first wash at about 42 degree C., about 6X-SSC, and about 1% SDS; and a second wash at about 50 degree C., about 6X-SSC, and about 1% SDS.

As discussed above, gene sequence-specific oligonucleotides may also be used as primers in PCR cloning strategies. Such PCR methods are well known in the art and are described. Again, sequences corresponding to conserved regions in a gene sequence (for example, those regions described above) are preferred for use in isolating mammalian counterpart gene sequences. Such probes may be used to screen cDNA as well as genomic DNA libraries.

Sequences obtained are then examined to identify those sequences having the highest amino acid sequence identity to the *C. elegans* gene sequence.

For example, addition of a counterpart human gene to an insulin responsive cell line (e.g., the 3T3-L1 cell line) may accentuate insulin responsiveness. Similarly genetic transformation of such a cell line with wild type or dominantly activated versions of a cAMP deaminase gene may alter metabolism. Such perturbations of metabolic control are stringent tests of candidate genes as homologues of the nematode gene.

In addition, if that mammalian candidate homologue acts in a metabolic control pathway, and is expressed in similar metabolic control tissues (liver, adipose), it is likely to function homologously to proteins expressed from *C. elegans* genes. Addition of a wild type or activated protein can confer on cell lines altered metabolic phenotypes. Thus supplying protein activity to such a cell line can alter its metabolism.

Screening Systems for Identifying Therapeutics

A number of screening procedures for identifying therapeutic compounds (e.g., anti-diabetic and anti-obesity pharmaceuticals or both) can be used in human patients. In particular, compounds that down regulate or rescue expression of a nematode gene or its human homolog are considered useful in the invention. In general, the screening methods of the invention involve screening any number of compounds for therapeutically active agents by employing any number of in vitro or in vivo experimental systems. Exemplary methods useful for the identification of such compounds are detailed below.

The methods of the invention simplify the evaluation, identification, and development of active agents for the treatment and prevention of impaired glucose tolerance conditions, such as diabetes and obesity. In general, the screening methods provide a facile means for

selecting natural product extracts or compounds of interest from a large population which are further evaluated and condensed to a few active and selective materials. Constituents of this pool are then purified and evaluated in the methods of the invention to determine their anti-diabetic or anti-obesity activities or both.

Test Extracts and Compounds

In general, novel drugs for the treatment of glucose metabolic syndrome conditions are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources. In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their anti-diabetic and anti-obesity activities should be employed whenever possible.

When a crude extract is found to have anti-diabetic or anti-obesity activities or both, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having anti-diabetic or anti-obesity activities. The same in vivo and

in vitro assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogenous extracts are known in the art. If desired, compounds shown to be useful agents for the treatment of pathogenicity are chemically modified according to methods known in the art. Compounds identified as being of therapeutic value are subsequently analyzed using any standard animal model of diabetes or obesity known in the art.

There now follow examples of high-throughput systems useful for evaluating the efficacy of a molecule or compound in treating (or preventing) a glucose metabolic syndrome.

Other Screening Assays

Other drug screening assays may also be performed using either *C. elegans* worms or mammalian cell cultures. If desired, such assays may include the use of reporter gene constructs.

For example, evaluation of the effects of test compounds on glycogen levels in mutant *C. elegans* strains expressing particular human homologs of the regulatory genes (i.e., humanized *C. elegans*) represent useful screening methods. Expression of the human homologs in *C. elegans* is accomplished according to standard methods and, if desired, such genes may be operatively linked to a gene promoter obtained from *C. elegans*. Such promoters include, without limitation, the endogenous *C. elegans* regulatory gene promoter.

Additionally, mammalian tissue culture cells expressing homologs to *C. elegans* regulatory genes may be used to evaluate the ability of a test compound or extract to modulate glycogen storage or glucose metabolism. Because the signaling pathways from the ligands, receptors, kinase cascades, and downstream transcription factors are conserved from man to worm, test compounds or extracts that inhibit or activate the worm signaling proteins should also inhibit or activate their respective human homolog.

Reporter Gene Construct

In one particular example, the invention involves the use of a reporter gene that is expressed under the control of a *C. elegans* gene promoter, e.g., a promoter that includes the enhancer element, such as a *C. elegans* regulatory gene promoter. Other equivalent enhancer elements may also be used in the invention. The enhancer element is cloned upstream of any standard reporter gene, e.g., the luciferase or green fluorescent protein (GFP) reporter genes.

In preferred embodiments, the GFP reporter gene is used in *C. elegans*. In other preferred embodiments, either the GFP or the luciferase reporter genes may used in a mammalian cell based assay. The reporter gene construct is subsequently introduced into an appropriate host (e.g., *C. elegans* or a mammalian cell) according to any standard method known in the art. Analysis of reporter gene activity in the host organism or cell is determined according to any standard method, e.g., those methods described herein. Such reporter gene (and host cell systems) are useful for screening for drugs that modulate glucose metabolism or levels of glycogen.

C. elegans

In one working example, the above-described reporter gene construct is introduced into wild-type *C. elegans* according to standard methods known in the art. If the enhancer element is operational, then it is expected that reporter gene expression is turned on. Alternatively, in mutants carrying the above-described reporter gene construct, reporter gene activity is turned off.

Using this on/off distinction, test compounds or extracts are evaluated for the ability to disrupt the signaling pathways described herein. In one working example, cAMP deaminase mutant worms carrying the reporter gene construct are used to assay the expression of the reporter gene. The majority of worms expressing the reporter gene will have defective glycogen storage. Such mutants are selected using methods described in the Examples, and reporter gene expression in the test compound or extract is assayed according to standard methods, e.g., worms are examined to reveal the presence of reporter gene expression, e.g., GFP. Candidate compounds that suppress the regulatory gene phenotype or turn on reporter gene expression, i.e., activate signals in the absence of regulatory gene receptor are considered useful in the invention.

Mammalian Cells

Mammalian insulin-responsive cell lines are also useful in the screening methods of the invention. Here reporter gene constructs (for example, those described above) are introduced into the cell line to evaluate the ability of a test compound or extract to modulate signaling pathways associated with metabolic control using a second construct expressing a *C. elegans* regulatory gene or its corresponding human homologs. Exemplary cell lines include, but are not limited to, mouse 3T3, L6, and L1 cells. Introduction of the constructs into such cell lines is carried out according to standard methods well known in the art.

To test a compound or extract, it is added to the cell line, and reporter gene expression is monitored. Compounds that induce reporter gene expression in the absence of regulatory gene signaling are considered useful in the invention. Such compounds may also turn the cells into adipocytes, as insulin does.

Compounds identified in mammalian cells may be tested in other screening assays described herein, and, in general, test compounds may be assayed in multiple screens to confirm involvement in signaling of a regulatory gene.

Metabolic control by protein expressed by a regulatory gene may be tested using any known cell line, e.g., those described herein.

In Vitro Screening Methods

A variety of methods are also available for identifying useful compounds in in vitro assays. In one particular example, test compounds are screened for the ability to activate the phosphorylation of proteins encoded by regulatory genes in vitro. In these assays, the protein encoded by a regulatory gene is preferably tagged with a heterologous protein domain, for example, the mycepitope tag domain(s)., and are incubated with the C-terminal kinase domain of the regulatory protein. Phosphorylation of the Smad proteins is preferably detected by immunoprecipitation using antibodies specific to the tag, followed by scintillation counting. Test compounds may be screened in high throughout microtiter plate assays. A test compound that effectively stimulates the phosphorylation of cAMP deaminase is considered useful in the invention. Using these same general assays, compounds that activate the phosphorylation of cAMP deaminase by AKT or GSK-3 may also be identified.

Modulatory Compounds

Our experimental results facilitate the isolation of compounds useful in the treatment of impaired glucose metabolic syndrome that change glucose metabolism or glycogen levels in *C. elegans* and described above. Exemplary methods for the isolation of such compounds now follow.

Antagonists

As discussed above, useful therapeutic compounds include those which down regulate the expression or activity of a regulatory gene or protein. To isolate such compounds, regulatory gene expression is measured following the addition of candidate antagonist molecules to a culture medium of the regulatory gene-expressing cells, or cells treated to

inhibit expression of the regulatory gene. Alternatively, the candidate antagonists may be directly administered to animals (for example, nematodes or mice) and used to screen for their effects on the expression of the regulatory gene.

The expression of the regulatory gene is measured, for example, by standard Northern blot analysis using a nucleic acid sequence (or fragment thereof) of the regulatory gene as a hybridization probe. The level of expression of the regulatory gene in the presence of the candidate molecule is compared to the level measured for the same cells, in the same culture medium, or in a parallel set of test animals, but in the absence of the candidate molecule. Preferred modulators for anti-diabetic or anti-obesity purposes are those which cause a change in expression of the regulatory gene.

Alternatively, the effect of candidate modulators on expression or activity may be measured at the level of regulatory protein production using the same general approach in combination with standard immunological detection techniques, such as Western blotting or immunoprecipitation with a regulatory gene-specific antibody. Again, useful anti-diabetic or anti-obesity therapeutic modulators are identified as those which produce a change in production of the regulatory gene. Antagonists may also affect activity of the regulatory protein without any effect on expression level. Phosphorylation state may be monitored by standard Western blotting using antibodies specific for phosphorylated amino acids. In addition, if the regulatory protein is transcription factors, reporter genes bearing operably linked regulatory protein binding sites may be used to directly monitor the effects of antagonists on regulatory gene activity.

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (e.g., an extract or supernatant obtained from cells). In a mixed compound assay, expression of the regulatory gene is tested against progressively smaller subsets of the candidate compound pool until a single compound or minimal compound mixture is demonstrated to modulate regulatory gene expression.

Candidate regulatory gene antagonists include peptide as well as non-peptide molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured).

Antagonists found to be effective at the level of cellular expression of regulatory genes or activity may be confirmed as useful in animal models (for example, nematodes or mice). For example, the compound may ameliorate the glucose metabolic syndrome, e.g., diabetic symptoms of mouse models for Type II diabetes (e.g., a db mouse model), mouse models for Type I diabetes, or models of streptozocin-induced β cell destruction.

A molecule which promotes a decrease in expression of a regulatory gene or regulatory protein activity is considered particularly useful in the invention; such a molecule may be used, for example, as a therapeutic to decrease the level or activity of native, cellular regulatory protein and thereby treat a glucose metabolic syndrome in an animal (for example, a human).

If desired, treatment with an antagonist of the invention may be combined with any other anti-diabetic or anti-obesity therapies.

Agonists

Also as discussed above, useful therapeutic compounds are those which up regulate the expression of the regulatory gene or activity of regulatory protein. To isolate such compounds, expression of these genes is measured following the addition of candidate agonist molecules to a culture medium of regulatory gene-expressing cells. Alternatively, the candidate agonists may be directly administered to animals (for example, nematodes or mice) and used to screen for effects on expression of the regulatory gene.

Regulatory gene-expression is measured, for example, by standard Northern blot analysis using all or a portion of one of these genes as a hybridization probe. The level of expression of the regulatory gene in the presence of the candidate molecule is compared to the level measured for the same cells, in the same culture medium, or in a parallel set of test animals, but in the absence of the candidate molecule. Preferred modulators for anti-diabetic or anti-obesity purposes are those which cause an increase in expression of a regulatory gene.

Alternatively, the effect of candidate modulators on expression may be measured at the level of regulatory protein production using the same general approach in combination with standard immunological detection techniques, such as Western blotting or immunoprecipitation with an appropriate antibody. Again, the phosphorylation state of these polypeptides is indicative of regulatory protein activity and may be measured on Western blots. Useful anti-diabetic or anti-obesity modulators are identified as those which produce an increase in regulatory protein production.

Candidate modulators may be purified (or substantially purified) molecules or may be one component of a mixture of compounds (e.g., an extract or supernatant obtained from cells). In a mixed compound assay, expression of a regulatory gene is tested against progressively smaller subsets of the candidate compound pool until a single compound or minimal compound mixture is demonstrated to modulate expression of a regulatory gene.

Alternatively, or in addition, candidate compounds may be screened for those which agonize native or recombinant regulatory protein activities. In one particular example, phosphorylation of a regulatory protein may be activated by agonists.

Candidate regulatory gene agonists include peptide as well as non-peptide molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured).

Agonists found to be effective at the level of cellular expression of a regulatory gene or activity of a regulatory protein may be confirmed as useful in animal models (for example, nematodes or mice).

A molecule which promotes an increase in expression of a regulatory gene or activity of a regulatory protein is considered particularly useful in the invention; such a molecule may be used, for example, as a therapeutic to increase the level or activity of these native, cellular genes and thereby treat glucose metabolic syndrome.

If desired, treatment with a regulatory gene agonist may be combined with any other anti-diabetic or anti-obesity therapies.

Animal Model Systems

Compounds identified as having activity in any of the above-described assays are subsequently screened in any number of available diabetic or obesity animal model systems, including, but not limited to ob, db, agouti mice, or fatty rats. Test compounds are administered to these animals according to standard methods. Additionally, test compounds may be tested in mice bearing knockout mutations in the insulin receptor, IGF-1 receptor, IR-related receptor, or any of the genes described herein. Compounds can also be tested using any standard mouse or rat model of Type I diabetes, e.g., a streptozin ablated pancreas model.

In one particular example, the invention involves the administration of regulatory protein or its homolog as a method for treating diabetes or obesity. Evaluation of the effectiveness of such a compound is accomplished using any standard animal model, for example, the animal diabetic model systems described above. Because these mouse diabetic models are also associated with obesity, they provide preferred models for human obesity associated Type II diabetes as well. Such diabetic or obese mice are administered *C. elegans* or a human counterpart to a regulatory protein according to standard methods well known in the art. Treated and untreated controls are then monitored for the ability of the compound to ameliorate the symptoms of the disease, e.g., by monitoring blood glucose, glycogen levels,

ketoacidosis, and atherosclerosis. Normalization of blood glucose and insulin levels is taken as an indication that the compound is effective at treating a glucose metabolic syndrome.

Therapy

Compounds of the invention, including but not limited to, a regulatory protein and its homologs, and any antagonist or agonist therapeutic agent identified using any of the methods disclosed herein, may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients. Although intravenous administration is preferred, any appropriate route of administration may be employed, for example, parenteral, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for antagonists or agonists of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

Regulatory molecules are administered at any appropriate concentration.

Pedigree Analysis and Genetic Testing

The discovery described herein that regulatory proteins are involved in glucose metabolism enables assays for genetic testing to identify those individuals with

predispositions toward the development of glucose metabolic syndrome, such as glycogen storage disease, diabetes or obesity, by determining the presence of a mutation found in a human gene having identity to any of the *C. elegans* regulatory genes. In one embodiment, the development of this testing method requires that the individual be a member of a family that has multiple affected and unaffected members carrying one mutation from the list of above-listed genes. Those skilled in the art will understand that a diabetic or obesity phenotype may be produced by mutations of regulatory genes found on different chromosomes, and that low resolution genetic mapping of the diabetic condition in single family pedigrees will be sufficient to favor regulatory genes over others as causing the glucose metabolic syndrome in a particular pedigree. In one particular example, mutations associated with glucose metabolic syndrome may be found in different genes in, for example, the regulatory gene signaling pathway in each pedigree. In addition, because mutations in a common pathway can show complex genetic interactions, multiple regulatory gene mutations may segregate in single pedigress. These mutations can behave recessively in some genetic backgrounds and dominantly in others.

Those skilled in the art further understand that, to determine disease linkage with a chromosomal marker, it may be necessary to evaluate the association of inheritance patterns of several different chromosomal markers (for example, from the collection of highly polymorphic mapped DNA allelic variants) in the genomic DNAs of family members and of the clinically affected individuals. Methods commonly used in determining segregation patterns of human genetic diseases are well known in the art. In addition, methods are known in the art for determining whether individuals in a family are useful for providing information to determine co-segregation of an allele with a glucose metabolic syndrome trait, including altered glycogen levels.

Here, fragments of genomic DNA (e.g., RFLP fragments) are prepared from each of the available members of the family, and each distinctive DNA allelic variant of the polymorphic chromosome marker within the family is evaluated to determine which polymorphisms (i.e., chromosomal region) is linked with the glucose metabolic syndrome phenotype within a particular family. It is preferred that the parents of the marker individual be heterozyous for a DNA allelic variant so that the segregation pattern of the DNA allelic variant linked with the diabetic or obese phenotype in the marker can be recognized. The inheritance of the diabetic phenotype can be judged to be dominant or recessive, depending on the pattern of inheritance. Most diabetes is dominantly inherited, and therefore inbred pedigrees are generally not necessary in the etiology of the diabetic condition.

With respect to Type II diabetes, the highest rate of this kind of diabetes in the world is found in American Indians of the Pima tribe. Such families are useful for mapping one particular cause of diabetes, but, in general, diabetes is caused by mutations in a variety of genes. Thus, by testing for low resolution linkage to a candidate regulatory gene mutation, and then by sequencing the particular linked regulatory gene in affected and unaffected individuals, a particular regulatory gene mutation can be associated with a particular diabetic pedigree.

Human regulatory gene homologues are mapped to chromosome regions using standard methods. The regulatory gene regions are PCR amplified and compared between affected and unaffected DNA samples. Mutations detected in affected individuals are expected to (but need not) map to conserved domains of the regulatory genes. Because it is known that not all carriers of known diabetes-inducing mutations show metabolic defects, we expect that some non-diabetic non-glucose intolerant family members will carry the same regulatory gene mutation as affected family members. For this reason, a correlation of affected family members with a regulatory gene mutation is more important than a correlation of nonaffected with no mutation. Those skilled in the art will know that phenotypic classification of affected and unaffected individuals can greatly enhance the power of this genetic analysis. In addition, other mutations in the same regulatory gene are expected in some but not all diabetic pedigrees. For dominant diabetic inheritance, the affected individuals carry a regulatory gene mutation as well as a normal allele. For recessive diabetic inheritance, individuals carry two regulatory gene mutations that may be identical or two independent mutations in the same gene.

It is routine in the art of genetic counseling to determine risk factors given the presence of a closely linked molecular genetic marker in the genomic DNA of the individual and when combined with the additional understanding provided by the pedigree of the individual in the family. For example, a risk factor may be calculated for an individual in a regulatory gene chromosome family in a manner similar to those described for assessing the risk of other commonly known genetic diseases that are known to run in families, e.g., Huntington's disease and cystic fibrosis.

Once mutations in regulatory genes are associated with diabetes in a pedigree analysis, diagnostic PCR sequencing of these regulatory genes can be used to diagnose glucose intolerant, prediabetic, diabetic, obesity, and atherosclerotic conditions. Preferably, the regulatory gene regions are PCR amplified from patients and mutations detected in the regulatory genes using standard DNA sequencing or oligonucleotide hybridization

techniques. The use of such gene sequences or specific antibody probes to the products of these sequences provide valuable diagnostics, particularly in view of the likelihood there exist two classes of type II diabetics: those with defects in the TGF-beta signaling genes, and those with defects in insulin signaling genes. Such genetic tests will influence whether drugs that affect regulatory gene or signals associated with regulatory proteins are prescribed.

To carry out the above analysis (as well as the other screening, diagnostic, and therapeutic methods described herein), mammalian homologs corresponding to the *C. elegans* regulatory genes are isolated as described above. Again, standard hybridization or PCR cloning strategies are employed, preferably utilizing conserved regulatory gene motifs for probe design followed by comparison of less conserved sequences flanking these motifs. Exemplary motifs for these genes are as follows:

In other embodiments, the invention includes any protein which possesses the requisite level of amino acid sequence identity (as defined herein) to regulatory gene sequence; such homologs include other substantially pure naturally-occurring mammalian regulatory proteins as well as allelic variants; natural mutants; induced mutants; proteins encoded by DNA that hybridizes to the regulatory gene DNA sequence or degenerate conserved domains of regulatory proteins (e.g., those described herein) under high stringency conditions; and proteins specifically bound by antisera directed to a regulatory protein.

The invention further includes analogs of any naturally-occurring regulatory proteins. Analogs can differ from the naturally-occurring protein by amino acid sequence differences which do not destroy function, by post-translational modifications, or by both. Modifications include in vivo and in vitro chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring regulatory protein by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis. Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., .beta. or gamma. amino acids.

In addition to full-length polypeptides, the invention also includes fragments of a regulatory protein. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids.

Fragments of such regulatory protein can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

For certain purposes, all or a portion of the regulatory protein sequence may be fused to another protein (for example, by recombinant means). In one example, the regulatory protein may be fused to the green fluorescent protein, GFP. Such a fusion protein is useful, for example, for monitoring the expression level of the regulatory protein in vivo (for example, by fluorescence microscopy) following treatment with candidate or known agonists or antagonists to the regulatory protein or gene expression.

The methods of the invention may be used to diagnose or treat any condition related to glucose metabolic syndrome or obesity in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is diagnosed or treated, the polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

The methods of the invention may be used to diagnose or treat any condition related to glucose metabolic syndrome or obesity in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is diagnosed or treated, the regulatory protein, nucleic acid, or antibody employed is preferably specific for that species.

EXAMPLES

Example 1

Glycogen storage tests

Whole *Caenorhabditis elegans* nematodes can be assessed for glycogen storage by exposing live animals to iodine vapor or a diluted (1:10-1:20) Lugol's iodine solution (2% I_2 in 4% KI). Both methods require 30-60s to fully react with the glycogen in the worm. The color of stained glycogen in the animal is usually dark red, but can be crimson or purple/black, depending upon the branching of the glycogen in the animal. A negative reaction has a yellow/brown color, which is the same color as a dilute iodine solution. Adult worms stained with iodine primarily store glycogen anterior to the posterior bulb of the pharynx, near the dorso-rectal ganglion, in the most proximal two oocytes of the gonad arm and in early embryos. Glycogen is also occasionally observed in the hypodermis and near the bend in the gonad.

To identify RNAi phenotypes that were altered by hexosamine signaling, all of the '+' and '-' clones from the original screen were tested with N2 as well as oga-1 (for '+' phenotypes) or ogt-1 (for '–' phenotypes). For each RNAi strain, both N2 and either oga-1 or ogt-1 were assayed simultaneously for rescue of the glycogen phenotype by iodine staining.

Example 2

Glucose challenge tests:

C. elegans were fed glucose by incubating the animals in a glucose solution, or by growing the animals on solid media seeded with bacteria and supplemented with glucose. Animals incubated for 48 hours in a 5% glucose solution gave a very strongly positive glycogen storage reaction, while those in a 0% glucose solution did not. Animals grown on glucose-supplemented plates have at least twice the level of internal free glucose when compared to animals grown on normal media.

Example 3

Glycogen storage mutants

87% of the genes in the *C. elegans* genome were screened by knockdown with RNAi technology for gene products that alter glycogen storage by assaying animals with the glycogen storage test described above. Those genes which displayed the same phenotype in 3 of 4 independent, blinded trials were considered for further analysis. The table below lists each of the genes tested by their assigned sequence code (CDS), as well as the phenotype for glycogen storage as determined by the screen.

The phenotypes fall into the following categories:

1. Less glycogen than normal (-), much less glycogen than normal (--).

2. More glycogen than normal (+), much more glycogen than normal (++).

3. Glycogen appears in the normal locations, but has an abnormal appearance (ab). If the glycogen appears outside the normal locations, the glycogen is not widely distributed. This phenotype can be accompanied by either more (ab+) or less (ab-) glycogen overall.

4. Glycogen appears widely distributed in the body of the worm (B). This probably indicates hypodermal or gut storage of glycogen. The phenotype is often accompanied by an overall increase in glycogen storage (B+).

Less Glycogen than normal

	D1014.3	F55A3.3	T28D9.10
B0491.5	F01G10.1	F55A3.7	VW02B12L.1
B0495.4	F02E8.1	F56H1.4	VW02B12L.1
C02F5.9	F10E7.7	F59C6.5	W02F12.5
C05C8.7	F11G11.10	H15N14.2	W09C5.8
C06H2.1	F14H8.2	H18N23.2	Y105E8A.9
C09G5.6	F20G4.1	H28O16.1	Y106G6H.3
C09H10.3	F23B12.5	H37A05.1	Y110A2AL.8
C15C7.5	F23H11.5	K02D10.5	Y116A8C.35
C16A3.5	F25B4.6	K03A1.1	Y37D8A.14
C18E9.4	F25H5.5	K04G7.4	Y45G12B.1
C23H4.1	F26E4.6	K05C4.1	Y46G5A.31
C27A2.2	F26E4.9	K07A12.3	Y47D3B.7
C29E4.8	F26H9.6	K07D8.1	Y47G6A.23
C30C11.2	F29C4.2	K08D12.1	Y48B6A.3
C33D3.1	F29G6.3	K08E3.5	Y57E12AL.5
C34B2.8	F29G9.3	LLC1.3	Y57G11C.12
C34E10.6	F32A7.6	M01F1.5	Y57G11C.24
C46G7.1	F32D1.2	R10E11.2	Y71F9AL.17
C47B2.4	F33A8.1	R53.4	Y71F9B.4
C50D2.1	F36A2.7	T02H6.11	Y71H2B.10
C52E4.4	F40F4.6	T05D4.4	Y82E9BR.15
C53A5.1	F45H10.2	T10D4.3	Y82E9BR.3
C53A5.6	F46F11.5	T13C5.1	ZC328.3
C53B7.4	F49C12.12	T14B4.6	ZK328.5
C53D5.6	F49C12.13	T14B4.7	ZK430.8
C54G4.8	F54D7.2	T20H4.5	
C56G2.6	F54F2.1	T23H2.5	
CD4.6	F54H12.6	T26A5.3	

	F35H10.4	R12E2.3
	F38A5.5	T01H3.1
	F38E11.5	T14F9.1
	F39H11.5	T14G10.5
Much less glycogen than normal	F53G12.3	T20F5.2
	F55A12.7	W09B6.1
AC7.1	F56C11.1	Y111B2A.14
C04F6.1	F57B9.10	Y25C1A.5
C23G10.4	F57B9.2	Y39B6A.12
C23H3.4	F59E10.3	Y49E10.1
C30B5.6	H19M22.2	Y55H10A.1
C36B1.4	K07D4.3	Y71D11A.5
C56C10.3	K08E5.3	ZK1058.3
F23F1.8	K11D9.2	ZK180.4
F26D11.13	R03E1.2	ZK970.4
F27C1.7	R10E11.1	

More glycogen than normal

B0047.4
B0393.1
C05D2.1
C27H6.2
C34F11.3
C47D12.6
F22B5.2
F22D6.3
F26D10.3
F39B2.4
F42A8.1
F54D5.11
F55A12.8
F55B11.2
K06A1.6
K08E3.6
R06F6.1
R74.1
T01G9.6
T02G5.9
T03F1.9
T04A8.14
T11G6.1
T28F3.2
W07E6.4
Y110A7A.8
Y39G10AR.7
Y39G10AR.8
Y48G1A.4
Y57G11C.19
Y80D3A.1
Y87G2A.5
Y95B8A.10
ZK1127.5

Much more glycogen than normal

K04E7.2

Abnormal glycogen distribution	F54C9.1	Y110A7A.11
	F54E7.2	Y116A8C.42
B0336.2	F57B10.1	Y32F6A.3
C03D6.1	F58E2.9	Y41D4B.19
C03D6.3	K01G5.7	Y41D4B.19
C12C8.3	M7.1	Y48G1A.5
C14C10.4	R06A10.2	Y55F3AR.3
C16A3.4	R07E4.6	Y55F3AR.3
D1054.15	R144.2	Y6B3A.1
F02D10.5	T04A8.7	Y71F9AM.5
F13D12.7	T10B5.5	Y71G12B.11
F26E4.8	T21B10.7	Y71G12B.11
F36F2.3	T28F12.2	ZK1151.2
F44A6.2	W03C9.4	ZK1290.3
F48E8.5	W04A4.5	ZK652.1
F54A3.3	W06H3.3	

Less glycogen -- Abnormal distribution

K10C2.4

R05D11.3

More glycogen -- Abnormal distribution

C37C3.6

F38A6.1

Y39B6A.33

Y53C10A.3

ZC168.5

Normal glycogen levels -- Widely distributed

C29A12.3

C37H5.5

F28B3.7

F32D1.10

F53F4.11

F56A3.3

F59A2.1

K07C5.4

T19B4.2

T23D8.4

T27B1.2

Y39B6A.14

Y9C9A.8

More glycogen -- Widely distributed

C48E7.2

D2089.1

F14B4.3

F19F10.9

F55F8.3

K06A5.4

T23D8.3

T23D8.9

W07E6.1

Y48G1A.4

Y54E10A.15

Example 4

AMP deaminase, and results obtained from that mutant/knockdown.

Ten genotypes identified in the screen were given the glucose challenge test described above wherein worms are fed additional glucose on plates. Upon challenge, the level of free glucose in wild-type worms increased to 2.2 times the value in unchallenged worms. The level of free glucose in worms treated with RNAi against AMP deaminase (CDS C34F11.3) only increased 1.7 times the wild-type value. In addition, these animals accumulated large quantities of the sugar alcohol xylitol. Xylitol is not found in wild-type animals treated with glucose or in AMP deaminase knockdown animals that are not treated with glucose. Both loss of function mutations in AMP deaminase and treatment with xylitol have previously been associated with improved glucose homeostasis in humans.

AMP deaminase, DNA coding sequence (SEQ ID NO: __)

atgaaaaccgtcgacagtgaactattcgactcgggaccattcaagaaaaa
ctttgcgttcggaaatgcagaccactcgacagcttcaccattcgaaatct
caaccactccgattgaagttaacgaatctcgtgctcacaaaactattgag
atctcgaaccgagcacgcctggaatctcaaaacgacggatgtctgtctcc
atcatcactgaaatcggtgctcgacgagattcaaacggagccaagatgca
agtcaccacagcatattgatctgcacactttcagagatgctgttgatgtg
aactaccaacggatggctatcactggagaagaactcagtggagtacctct
ggaggatctcaaaaccgcttccggacatctcatcgaggctcttcatctcc
gctcgaagtacatggaacgtattggaaatcagttcccatcaacgactcgc
aacttcctcagtggacattacccggcgaacttgccaaagcatcgtgtcaa
gaatacggaaacaaccgtccagacatcattcaatccaccagatccgccaa
aggatcattggggaaaaaatgatccgctgccaaagtatgagaaaatctat
catttgagacgtaatcgtggagtgactgaaatttgcaatgacgatggctc
aattgatcagcaattcaaaaatgttaatgtgacgaaggaggagttcttaa
atgacactgaaaagctgacagcaatgattgttgatggtccacttaaatca
ttctgcttccgtcgactttcctaccttgaaaacaagtttcaacttcatgt
tcttctcaacgaacttcgtgagcttcatgagcagaaggggtgtcgcatc
gagacttctataacatcagaaaggtagacactcacatccacgccgcatca
tcaatgaatcagaaacatttgctccgtttcatcaagaagaagataaagac
tgaagctgacacggttgttctgaataacaatggaacaaaggttacaatga
aggaagtattcaagaagatgggaattgatgcctacgatttgtcagttgat
atgcttgatgttcatgctgatcgtaatacattccatcgtttcgataagtt
caacacaaagtataatccagttggagaatctactctccgcgagattttca
tcaaaactgacaattatgtcggtggaaaatactttgccgatttgctcaag
gaggtgttgtcggacctcgaggatagcaagtatcagcacgcggagccaag
attgtcgatttacggaaggagcaagaacgaatgggacaaccttgcgaaat

gggcgctcacacatgatgtatggagcccgaacgctagatggctcgttcag
attcctcgactctacgacgtctatcgtgcaaagaatatggtaaagaattt
tgacgatatgcttgataacctgttcaccccgttattcgaagtcaccaacg
atccatcgtctcatccagagcttcatctcttccttcagcaaatttccgga
attgattcagttgatgatgagagcaagcatgaatttgttaatttcgatcg
ttcaacaccttgcccaccagagtacactgacttggagaatccaccataca
actattatctcttctacatgtaccgcaacatttgcgccttgaatgcattc
cgccgtgctcgtggactcaacacctttgcacttcgtccacattgtggaga
ggctggccacgtttcgcatctgctcaccggatacctgaccagtgaaagta
ttgctcacggaattcttctccgcaaggtgccagttcttcagtatttgtat
tatttgacgcaaattggaatcgccatgtcaccactgtccaacaattcgtt
gttcatcagttaccagcggaatcctttgccagaatatctgcaaaaaggac
tgaatgtctctctgtccactgatgatccactccagttccactacaccaag
gaagcattgatggaagaattctcgattgctgctcaagtttggaagctcag
ctcatgtgatatgtgtgagctcgcgagaaatagtgtcatgcagagtggat
ttgaagataaggtcaaaattcactggctcggaccaaattacaaagaagaa
ggagttcttggcaacgatatccaccgtaccaatgttccagatattcgtgt
cagcttccgtcacgaggctctggttgatgagctttacaacctcttccgtg
tacaaaatactctcaaacagtag

AMP deaminase protein sequence (SEQ ID NO: __)

MKTVDSELFD SGPFKKNFAF GNADHSTASP FEISTTPIEV
NESRAHKTIE ISNRARLESQ NDGCLSPSSL KSVLDEIQTE
PRCKSPQHID LHTFRDAVDV NYQRMAITGE ELSGVPLEDL
KTASGHLIEA LHLRSKYMER IGNQFPSTTR NFLSGHYPAN
LPKHRVKNTE TTVQTSFNPP DPPKDHWGKN DPLPKYEKIY
HLRRNRGVTE ICNDDGSIDQ QFKNVNVTKE EFLNDTEKLT
AMIVDGPLKS FCFRRLSYLE NKFQLHVLLN ELRELHEQKG
VSHRDFYNIR KVDTHIHAAS SMNQKHLLRF IKKKIKTEAD
TVVLNNNGTK VTMKEVFKKM GIDAYDLSVD MLDVHADRNT
FHRFDKFNTK YNPVGESTLR EIFIKTDNYV GGKYFADLLK
EVLSDLEDSK YQHAEPRLSI YGRSKNEWDN LAKWALTHDV
WSPNARWLVQ IPRLYDVYRA KNMVKNFDDM LDNLFTPLFE
VTNDPSSHPE LHLFLQQISG IDSVDDESKH EFVNFDRSTP
CPPEYTDLEN PPYNYYLFYM YRNICALNAF RRARGLNTFA
LRPHCGEAGH VSHLLTGYLT SESIAHGILL RKVPVLQYLY
YLTQIGIAMS PLSNNSLFIS YQRNPLPEYL QKGLNVSLST
DDPLQFHYTK EALMEEFSIA AQVWKLSSCD MCELARNSVM
QSGFEDKVKI HWLGPNYKEE GVLGNDIHRT NVPDIRVSFR
HEALVDELYN LFRVQNTLKQ

The DNA sequence between the oligonucleotide probes is determined, and those sequences having the highest degree of homology are selected. Once isolated, these sequences are then tested in a *C. elegans* AMP deaminase mutant or mouse model as described above for the ability to functionally complement the mutation or ameliorate the glucose metabolic syndrome phenotype.

Example 5

Hexosamine signaling regulates glycogen storage and glucose uptake in C. elegans:

Glycogen storage in the nematode was altered by changing the nutrient supply. Nematodes placed in buffer for 24 hrs gradually lose their glycogen, while those placed in a 5% (w/v) glucose solution accumulate glycogen for at least 48 hrs. Nematode *oga-1* and *ogt-1* mutant animals lack the ability to remove and add O-glcNAc modifications to proteins, respectively. When exposed to the same conditions, *ogt-1* animals were found to be more capable than wild type or *oga-1* nematodes to synthesize glycogen in the presence of glucose.

The o-glcNAc modifications of these mutants were assayed by immunofluorescence microscopy using an RL2 antibody, which binds primarily to O-glcNAc modified nuclear pore proteins. The results showed that *oga-1* was more highly O-glcNAc modified in the presence of glucose, while *ogt-1* did not have increased RL2 staining in glucose. Overall, these results show that glucose activates the hexosamine biosynthesis in the glycogen synthesis pathway, which is at least partially controlled by the activity of *oga-1* and *ogt-1*. Changes in the level of O-glcNAc modification of proteins were verified by western blot.

In the presence of glucose, the luminal membrane of the intestine of *oga-1* animals became very reactive to RL2 antibody, suggesting that the O-glcNAc modifications may alter glycogen synthesis by changing the uptake of glucose from the lumen. Under these conditions, *ogt-1* mutants takes up an average of five-fold more radioactive glucose than wild-type in four hours, consistent with O-glcNAc modification affecting the uptake of glucose. These results show that the hexosamine signaling pathway acts to detect glucose in the environment and regulate glucose uptake and glycogen synthesis.

Example 6

Genes that modify both glycogen storage and the hexosamine signaling pathway:

Some of the mutants identified in our RNAi screen that have altered glycogen likely have altered hexosamine signaling. To show this RNAi hits that had a (+/G) or (-) in wild-

type backgrounds, were compared to the same RNAi in either an *oga-1* (for +/G RNAi) or *ogt-1* (for – RNAi) background, i.e., RNAi glycogen phenotypes that were rescued through the presence of the additional mutation in *oga-1* or *ogt-1*. Fourteen RNAi clones were found to be rescued in these mutants , eight from the *oga-1* phenotypes, and six from the *ogt-1* phenotypes (Table 1).

Suppressed by oga-1		
Target	Gene	Description
F26D10.3	*hsp-1*	Molecular chaperones HSP70/HSC70
F55F8.3		Subunit of the 18S rRNA processing complex
F55A2.1	*npp-9*	Ran-binding protein
W07E6.1	*nol-1*	tRNA and rRNA cytosine-C5-methylase
Y110A7A.8	*prp-6*	mRNA splicing factor PRP31
Y39B6A.14	*arx-3*	BOA-1 homolog
Y39B6A.33		
Y87G2A.5	*vrs-2*	Valyl-tRNA synthetase

Suppressed by ogt-1		
Target	Gene	Description
B0495.4	*nhx-2*	Sodium/hydrogen exchanger protein
C56C10.3	*VPS32.1*	
F26H9.6	*rab-5*	Ras GTPase
H15N14.2	*nsf-1*	AAA+-type ATPase
H28O16.1	*atp-37*	F0F1-type ATP synthase, alpha subunit
W09B6.1	*pod-2*	Acetyl-CoA carboxylase

Among the hits are a number of genes implicated in the regulation of glucose uptake. For example:

- npp-9 (F59A2.1) is a homolog of RanBP2, deficiency of which results in defects in glucose catabolism in mammalian models;
- pod-2 (W09B6.1), which encodes acetyl-CoA carboxylase, is known to influence insulin secretion in rodents;
- rab-5 (F26H9.6) and nsf-1 (H15N14.2) have both been shown to control the cycling of glucose transporters to the cell surface in mammals;
- nsf-1 and VPS-32.1 (C56C10.3) are also needed for glucose derepression in yeast, a sodium-proton exchanger, is induced in the kidney in response to insulin in mammals.

Another major class of targets from this screen was genes involved with ribosomal biogenesis and export. Six of the eight genes rescues by *oga-1* are directly implicated in this process, strongly implying an interaction between ribosome biogenesis and glucose

metabolism that may operate through hexosamine signaling, e.g., hsp-1, shuttles to the nucleus during environmental stress, and has glucose-regulated O-glcNAc lectin activity.

What is claimed is:

1. A method for identifying a therapeutic compound that restores normal glycogen accumulation, said method comprising:

a) providing a nematode or an isolated nematode cell having impaired expression of a nematode gene;

b) contacting said nematode or isolated nematode cell with a candidate compound;

c) measuring glycogen distribution; and

d) selecting for a compound that changes levels of glycogen accumulation.

2. The method of claim 1, wherein the nematode gene is selected from the group consisting of B0491.5, B0495.4, C02F5.9, C05C8.7, C06H2.1, C09G5.6, C09H10.3, C15C7.5, C16A3.5, C18E9.4, C23H4.1, C27A2.2, C29E4.8, C30C11.2, C33D3.1, C34B2.8, C34E10.6, C46G7.1, C47B2.4, C50D2.1, C52E4.4, C53A5.1, C53A5.6, C53B7.4, C53D5.6, C54G4.8, C56G2.6, CD4.6, D1014.3, F01G10.1, F02E8.1, F10E7.7, F11G11.10, F14H8.2, F20G4.1, F23B12.5, F23H11.5, F25B4.6, F25H5.5, F26E4.6, F26E4.9, F26H9.6, F29C4.2, F29G6.3, F29G9.3, F32A7.6, F32D1.2, F33A8.1, F36A2.7, F40F4.6, F45H10.2, F46F11.5, F49C12.12, F49C12.13, F54D7.2, F54F2.1, F54H12.6, F55A3.3, F55A3.7, F56H1.4, F59C6.5, H15N14.2, H18N23.2, H28O16.1, H37A05.1, K02D10.5, K03A1.1, K04G7.4, K05C4.1, K07A12.3, K07D8.1, K08D12.1, K08E3.5, LLC1.3, M01F1.5, R10E11.2, R53.4, T02H6.11, T05D4.4, T10D4.3, T13C5.1, T14B4.6, T14B4.7, T20H4.5, T23H2.5, T26A5.3, T28D9.10, VW02B12L.1, VW02B12L.1, W02F12.5, W09C5.8, Y105E8A.9, Y106G6H.3, Y110A2AL.8, Y116A8C.35, Y37D8A.14, Y45G12B.1, Y46G5A.31, Y47D3B.7, Y47G6A.23, Y48B6A.3, Y57E12AL.5, Y57G11C.12, Y57G11C.24, Y71F9AL.17, Y71F9B.4, Y71H2B.10, Y82E9BR.15, Y82E9BR.3, ZC328.3, ZK328.5, ZK430.8, AC7.1, C04F6.1, C23G10.4, C23H3.4, C30B5.6, C36B1.4, C56C10.3, F23F1.8, F26D11.13, F27C1.7, F35H10.4, F38A5.5, F38E11.5, F39H11.5, F53G12.3, F55A12.7, F56C11.1, F57B9.10, F57B9.2, F59E10.3, H19M22.2, K07D4.3, K08E5.3, K11D9.2, R03E1.2, R10E11.1, R12E2.3, T01H3.1, T14F9.1, T14G10.5, T20F5.2, W09B6.1, Y111B2A.14, Y25C1A.5, Y39B6A.12, Y49E10.1, Y55H10A.1, Y71D11A.5, ZK1058.3, ZK180.4, and

ZK970.4, K10C2.4 and R05D11.3,
and wherein the impaired expression of the nematode gene decreases glycogen levels.

3. The method of claim 1, wherein the nematode gene is selected from the group wherein the nematode gene is selected from the group consisting of B0047.4,B0393.1,C05D2.1, C27H6.2, C34F11.3, C47D12.6, F22B5.2, F22D6.3, F26D10.3, F39B2.4, F42A8.1, F54D5.11, F55A12.8, F55B11.2, K06A1.6, K08E3.6, R06F6.1, R74.1, T01G9.6, T02G5.9, T03F1.9, T04A8.14, T11G6.1, T28F3.2, W07E6.4, Y110A7A.8, Y39G10AR.7, Y39G10AR.8, Y48G1A.4, Y57G11C.19, Y80D3A.1, Y87G2A.5, Y95B8A.10, ZK1127.5, K04E7.2C48E7.2, D2089.1, F14B4.3, F19F10.9, F55F8.3, K06A5.4, T23D8.3, T23D8.9, W07E6.1, Y48G1A.4, Y54E10A.15, C37C3.6, F38A6.1, Y39B6A.33, Y53C10A.3, and ZC168.5
and wherein the impaired expression of the nematode gene increases glycogen levels.

4. The method of claim 1, wherein the nematode gene is selected from the group wherein the nematode gene is selected from the group consisting of B0336.2, C03D6.1, C03D6.3, C12C8.3, , C14C10.4, C16A3.4, D1054.15, F02D10.5, F13D12.7, F26E4.8, F36F2.3, F44A6.2, F48E8.5, F54A3.3, F54C9.1, F54E7.2, F57B10.1, F58E2.9, K01G5.7, M7.1, R06A10.2, R07E4.6, R144.2, T04A8.7, T10B5.5, T21B10.7, T28F12.2, W03C9.4, W04A4.5, W06H3.3, Y110A7A.11, Y116A8C.42, Y32F6A.3, Y41D4B.19, Y41D4B.19, Y48G1A.5, Y55F3AR.3, Y55F3AR.3, Y6B3A.1, Y71F9AM.5, Y71G12B.11, Y71G12B.11, ZK1151.2, ZK1290.3, ZK652.1, C29A12.3, C37H5.5, F28B3.7, F32D1.10, F53F4.11, F56A3.3, F59A2.1, K07C5.4, T19B4.2, T23D8.4, T27B1.2, Y39B6A.14, and Y9C9A.8.
and wherein the impaired expression of the nematode gene causes abnormal glycogen distribution.

5. The method according to any of claims 1-4, wherein impaired expression of the nematode gene also changes O-glcNAc modification of proteins.

6. The method of claim 5, wherein the nematode gene is selected from the group wherein the nematode gene is selected from the group consisting of F26D10.3, F55F8.3, F59A2.1, W07E6.1, Y110A7A.8, Y39B6A.14, Y39B6A.33, Y87G2A.5, B0495.4, C56C10.3, F26H9.6, H15N14.2, H28)16.1, and W09B6.1.

7. The method of any of claims 1 to 6, further comprising the step of measuring the level of free glucose.

8. The method of claim 7, wherein the nematodes are subject to a glucose challenge.

9. The method of 7, wherein the nematode is *C. elegans*.

10. The method of claim 7, where in the compound is a candidate compound for treating glycogen storage disease or glucose metabolic syndrome in human patients.

11. The method of claim 10, wherein the disease is diabetes or obesity.

12. The method of claim 7, wherein the nematode gene expression is impaired by siRNA.

13. A method for detecting defects in glycogen synthesis or accumulation of glucose in the blood, comprising isolating cells of a subject, and measuring genetic expression of at least one human gene in the cells, wherein said human gene is a homolog of the nematode gene of any of claims 1-12.

14. The method of claim 13, wherein the human gene is identified by isolating DNA from the nematode gene, and contacting said DNA with isolated DNA from a human cell under hybridization conditions that provide detection of DNA sequences have about 70% or greater nucleic acid sequence identity to the nematodes gene.

15. The method of claim 13, wherein genetic expression of a multiplicity of human genes is measured.

16. The method of claim 13,wherein genetic expression is measured by changes in levels of mRNA

17. The method of claim 13, wherein the genetic expression is measured by a microarray.

18. The method of claim 13, wherein the genetic expression is a measure of the response of a subject to a therapeutic for treating a glucose metabolic syndrome.

19. The method of claim 13, wherein genetic expression of at least one human gene is measured by contacting protein of a sample from a subject with at least one antibody to measure expression levels of the human gene.

20. The method of claim 13, wherein the protein is measured by an ELISA.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/39760

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/02 (2010.01)
USPC - 435/29
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)
USPC: 435/29

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)
Databases: PubWEST DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ; Google Scholar, Google Patents
Search Terms: glycgen, accumulate, nematode, worm, elegans, metabolic syndrome, mellitus, O-linked N-acetylglucosamine, or O-glcNAc, F26D10.3, F55F8.3, F59A2.1, W07E6.1, Y11OA7A.8, Y39B6A.14, Y39B6A.33, (other genes), Frazier, Roth

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0158879 A1 (RUVKUN et al.) 12 August 2004 (12.08.2004) esp: abstract, paras [0007]-[0010], [0012], [0028], [0063], [0064], [0122], [0123], [0125], [0127], [0140]-[0149[, [0197], Tables VI, VIIIB, X.	1-6
Y	US 6,861,256 B2 (RUVKUN et al.) 1 March 2005 (01.03.2005) esp: abstract, col 2 ln 1-31, col 9 ln 16-21, col 9 ln 29-33, col 9 ln 42-46, col 30 ln 5-20.	1-6
A	US 6,627,746 B1 (DOBERSTEIN et al.) 30 September 2003 (30.09.2003) entire document.	1-6
A	US 2005/0260652 A1 (RUVKUN et al.) 24 November 2005 (24.11.2005) entire document.	1-6
A	US 2004/0250299 A1 (VERWAERDE et al.) 9 December 2004 (09.12.2004) entire document.	1-6
A	US 2005/0229260 A1 (FEICHTINGER et al.) 13 October 2005 (13.10.2005) entire document.	1-6
A	US 2006/0293325 A1 (CHANG et al.) 28 December 2006 (28.12.2006) entire document.	1-6
A	US 2005/0171027 A1 (SINCLAIR et al.) 4 August 2005 (04.08.2005) entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 September 2010 (05.09.2010)	16 SEP 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/39760

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.