Title: PREDICTING AND TREATING DIABETIC COMPLICATIONS

Figure 1, Bimodlg Distribution of Diabetic Retinopathy

Abstract: Compositions and methods for diagnosing, predicting risk of, and/or treating diabetic microvascular complications in diabetic subjects.
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Predicting and Treating Diabetic Complications

RELATED APPLICATIONS
This application claims the priority benefits of the U.S. Provisional application No.61/371,021 filed August 5, 2010.

TECHNICAL FIELD
This invention relates to compositions and methods for diagnosing or predicting risk of diabetic microvascular complications (DMC), and to compositions and methods for treating DMCs.

BACKGROUND
Diabetic microvascular complications are the major cause of vision loss, diabetic retinopathy and diabetic nephropathy in this country and is a major cause of morbidity and mortality in diabetic patients (Geraldes & King, Circ Res; 106(8):13 19-31 (2010)). Hyperglycemia is a major cause of microvascular complications. (No authors listed, N. Engl J Med., 329: 977-986 (1993)). Hyperglycemia has been shown to induce apoptosis in multiple vascular complications (Mitzutani et al, J. Clin. Invest. 97: 2883-2890 (1996); Busik et al. Diabetes. 57: 1952-1965 (2008); Geraldes et al, Nat. Med, 15(11): 1298-306 (2009); Brownlee M. NJfare;414(6865):813-20 (2001)). However there has been relatively little success in translating these basic findings into effective therapies. Thus, there is a need to identify compounds that modulate the toxic effects of hyperglycemia in diabetic subjects and their use as an effective therapy in treating diabetic microvascular complications.
SUMMARY

It is an object of the invention to provide methods of diagnosing or predicting the risk of developing diabetic microvascular complications (DMC) in a subject.

In one aspect, the invention provides methods of diagnosing or predicting the risk of developing diabetic microvascular complications (DMC) in a subject. The methods include evaluating activity, levels or expression of one or more agents listed in Table 3 (B3GNT1, SEZ6, APLPl, CST3, A2M, PTGDS) and Table 5 (APOA1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNDPAI) in a sample from the subject; wherein increased or decreased levels, activity or expression as compared to a control indicates an increased or decreased risk or the presence of a DMC.

In some embodiments, the method includes evaluating the level of expression of genes of one or more agents listed in Table 3 (B3GNT1, SEZ6, APLPl, CST3, A2M, PTGDS) and Table 5 (APOA1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNDPAI). In some embodiments, the method includes evaluating the activity of one or more agents listed in Table 3 (B3GNT1, SEZ6, APLPl, CST3, A2M, PTGDS) and Table 5 (APOA1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNDPAI). In some embodiments, the method includes evaluating protein levels of one or more agents listed in Table 3 (B3GNT1, SEZ6, APLPl, CST3, A2M, PTGDS) and Table 5 (APOA1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNDPAI).

In some embodiments, the method includes samples from the plasma, urine, vitreous, aqueous fluid or kidney tissue of a subject.

The invention described herein also include methods to ameliorate the risk of developing DMC in a subject. In some embodiments, the method includes administering to the subject a therapeutically effective amount of one or more compounds that increase or mimics the levels, the activity or the expression of the agents disclosed in Table 3 (B3GNT1, SEZ6, APLPl, CST3, A2M, PTGDS) and Table 5 (APOA1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNDPAI). In some embodiments, the compositions include a peptide, an antibody or a small molecule.

In another aspect, the invention provides methods for modulating hyperglycemic toxic effects in a subject. In some embodiments, the method includes administering to the subject a therapeutically effective amount of one or more compounds that increase or mimics the levels, the activity or the expression of the agents disclosed in
Table 3 (B3GNT1, SEZ6, APLP1, CST3, A2M, PTGDS) and Table 5 (AP0A1BP, HPX, GSTT1, HPRT1, GATM, AGMAT, CS, GNPDAl). In some embodiments, the compositions include a peptide, an antibody or a small molecule.

As used herein, diabetic microvascular complications include, but are not limited to, diabetic retinopathy (DR) or diabetic nephropathy.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

**DESCRIPTION OF DRAWINGS**

FIG. 1 is a bar graph showing the bimodal distribution of diabetic retinopathy in the Medalist subject population.

FIG. 2 is a line graph showing the rate of DR worsening in eyes with and without PDR at the final visit.

FIG. 3 is a bar graph showing the prevalence of DR status (no DR or mild DR, moderate to severe DR, and proliferative DR ((PDR) advanced disease)) among the subjects described in Example 3.

FIG. 4 is a western blot showing protein levels of PTGDS and CST3 in vitreous of Medalists without PDR versus in vitreous from Medalists with PDR.

FIG. 5 is a western blot of PTGDS expressed in BRP and BREC cells.

FIG. 6 is a bar graph showing the over expression of PTGDS against control GFP in transfected BREC cultured in high glucose.

FIG. 7 is a chart showing vascular complications in the subjects disclosed in Examples 3.

FIG. 8 is a Table summarizing the clinical characteristics of the subjects disclosed in Examples 3.
DETAILED DESCRIPTION

DR is damage that occurs to the retina of the eye in diabetics. Symptoms of DR range from blurred vision to loss of vision. Moreover, DR is a major cause of vision loss in developed countries. Understanding the causal role of vascular endothelial growth factor (VEGF) in the development of the proliferative DR (PDR) and vascular permeability has identified inhibitors of VEGF as effective treatments for diabetic neovascularization and macular edema. However, mechanisms by which hyperglycemia propagates the progression of DR are still not fully established. The only proven way to help prevent the initiation and progression of DR is good glycemic control. Several reasons may be responsible for the difficulty in identifying causal factors and effective treatments for the early stages of DR. These include challenges in obtaining retinal tissues in various stages of DR, lack of validated biomarkers and the absence of a salient rodent model. Recent therapeutic advances using inhibitors of VEGF for diabetic macular edema and neovascular lesions of DR are substantial. However, there remains a critical need to evaluate retinal tissue at all stages of DR to identify potential therapeutic targets and to validate potential markers of incidence and progression of DR in diabetic individuals.

DN is kidney disease or damage that occurs in diabetics. DN is a major cause of sickness and death in persons with diabetes. It is the leading cause of long-term kidney failure and end-stage kidney disease in the United States, and often leads to the need for dialysis or kidney transplantation.

Methods of Diagnosis

The present disclosure provides, inter alia, methods and compositions for diagnosing and predicting risk of developing diabetic retinopathy (DR) and or diabetic nephropathy (DN).

The methods include obtaining a sample from a subject, e.g., a sample of vitreous, aqueous, plasma, kidney tissue, or urine, and evaluating the presence and/or level of a biomarker described herein (e.g., in Table 3 or 5) in the sample, and comparing the presence and/or level with one or more references, e.g., a control reference that represents a normal level of the protein, e.g., a level in an unaffected subject, and/or a disease reference that represents a level of the proteins associated with DR or DN, e.g., a level in a subject having DR or DN or an increased (high) likelihood of developing DR or DN. The presence and/or level of a protein can be
evaluated using methods known in the art, e.g., using quantitative immunoassay methods. In some embodiments, high throughput methods, e.g., protein or gene chips as are known in the art (see, e.g., Ch. 12, Genomics, in Griffiths et al, Eds. Modern genetic Analysis, 1999, W. H. Freeman and Company; Ekins and Chu, Trends in Biotechnology, 1999, 17:217-218; MacBeath and Schreiber, Science 2000, 289(5485): 1760-1763; Simpson, Proteins and Proteomics: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 2002; Hardiman, Microarrays Methods and Applications: Nuts & Bolts, DNA Press, 2003), can be used to detect the presence and/or level of a biomarker listed herein (e.g., in Table 3 or 5).

In some embodiments, once it has been determined that a person has DR or DN, or has an increased risk of developing DR or DN, then a treatment, e.g., as known in the art or as described herein, can be administered.

The compositions disclosed herein can include agents that detect or bind (e.g., that detect or bind specifically) to a biomarker described herein (e.g., in Table 3 or 5). Such agents can include, but are not limited to, for example, antibodies, antibody fragments, and peptides. In some instances, the compositions can be in the form of a kit. Such kits can include one or more agents that can detect or bind (e.g., that detect or bind specifically) to one or more biomarkers described herein (e.g., one or more of the biomarkers disclosed in Tables 3 or 5) and instructions for use.

Methods of Treatment

As used herein, "treatment" means any manner in which one or more of the symptoms of DR and/or DN are ameliorated or otherwise beneficially altered. As used herein, amelioration of the symptoms of the disorder refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with treatment by the compositions and methods of the present invention. For example, with DR, treatment can include lessening of any symptom associated with DR, including, but not limited to, blurred or distorted vision or difficulty reading, floaters, reduced vision, vision loss, pain, and/or bleeding in the eye. Similarly, with DN, treatment can include lessening of any symptom associated with DN, including, but not limited to, changes in appetite, change in sleep, protein in serum, weakness, and/or nausea.

In some embodiments, the present disclosure provides methods for treating DR and/or DN in a subject (e.g., a subject with diabetes (e.g., type I and/or type II
diabetes) by administering to the subject one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more, including all) of the factors disclosed in Table 3 (for DR) and/or Table 5 (for DN). Accordingly, the present disclosure includes administering peptides consisting of, consisting essentially of, or comprising the amino acid sequences associated with any one or more of the accession numbers disclosed in Table 3 and/or Table 5. For example, useful amino acid sequences can have 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to any one or more of the accession numbers disclosed in Table 3 and/or Table 5.

The present disclosure also contemplates use of nucleic acid sequences that encode amino acid sequences consisting of, consisting essentially of, or comprising the amino acid sequences associated with any one or more of the accession numbers disclosed in Table 3 and/or Table 5. For example, useful nucleic acid sequences can encode an amino acid sequence with 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to any one or more of the accession numbers disclosed in Table 3 and/or Table 5.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, or 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The determination of percent identity between two amino acid sequences is accomplished using the BLAST 2.0 program. Sequence comparison is performed using an ungapped alignment and using the default parameters (Blossom 62 matrix, gap existence cost of 11, per residue gapped cost of 1, and a lambda ratio of 0.85). The mathematical algorithm used in BLAST programs is described in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997).
Useful amino acid sequences can also consist of, consisting essentially of, or comprising the amino acid sequences associated with any one or more of the accession numbers disclosed in Table 3 and/or Table 5 with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or any range between any of the aforementioned integers, or more than 100) conservative amino acid substitutions. Conservative amino acid substitutions are known in the art.

In some embodiments, useful peptides can include modified peptides that possess at least a portion of the activity (e.g., biological activity) of the unmodified peptide. For example, modified peptides can retain 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the activity (e.g., biological activity) of the unmodified peptide, e.g., the unmodified version of the amino acid sequences associated with any one or more of the proteins disclosed in Table 3 and/or 5.

In some embodiments, useful peptides can include peptides that include a fragment of any one or more of the factors disclosed in Table 3 and/or 5.

In some embodiments, treatment can include administering one or more of B3GNT1 N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase (e.g., IPI00009997 and IPI00009997.1), SEZ6 Isoform 3 of Seizure protein 6 homolog precursor (e.g., UniProt accession no. IP100748395), APLP2 Isoform 1 of Amyloid-like protein 2 precursor (e.g., EMBL-EBI accession no. IPI00031030 and IPI00031030.1), CST3:CST2 Cystatin-C precursor (e.g., EMBL-EBI accession no. IPI00032293 and IPI00032293.1), A2M Alpha-2-macroglobulin precursor (e.g., EMBL-EBI accession no. IPI00478003 and IPI00478003.1), IGLVI-40:40 VI-13 protein (Fragment) (e.g., EMBL-EBI accession no. IPI00789259) and PTGDS.

In other embodiments, treatment can include administering one or more of IPI00009305 (GNPDA1 Glucosamine-6-phosphate isomerase and UniProt P46926.107), IPI00873466 (HPRT1 Uncharacterized protein HPRT1 and NP_003881.2), IPI00893316 (GSTT1 Glutathione S-transferase theta 1 and UniProt C9JA47.9), IPI00848298 (APOA1BP Isoform 2 of Apolipoprotein A-I-binding protein precursor and EMBL-EBI accession no.IPI00848298.1), IPI00022488 (HPX Hemopexin precursor and EMBL-EBI accession no. IPI00022488.1), IPI00305360 (AGMAT Agmatinase, mitochondrial precursor and UniProt Q9B6E5.88) and IPI00025366 (CS Citrate synthase, mitochondrial precursor and IPI00025366.4 to a subject.)
The present disclosure also includes fusion proteins comprising any one or more amino acid sequence disclosed in Table 3 and/or 5 in combination with a moiety that increase stability of the fusion protein in vivo (e.g., polyethylene glycol (PEG)) and/or that increases transport of the fusion protein to the therapeutic target (e.g., the eye and/or the kidney).

The present disclosure also contemplates the use of gene therapy methods, e.g., to administer a nucleic acid encoding one or more of the factors (e.g., protective factors) disclosed herein to a subject. For example, nucleic acids encoding a polypeptide disclosed in Table 3 or 5, or an active fragment thereof, or a nucleic acid encoding a protein that increases the expression, level or activity, of one or more of the factors disclosed in Tables 3 and/or 5 can be incorporated into a gene construct to be used as a part of a gene therapy protocol.

The invention includes targeted expression vectors for in vivo transfection and expression of a polynucleotide that encodes a polypeptide disclosed in Table 3 or 5, or an active fragment thereof, in particular cell types, for cells of the eye and/or kidney. Expression constructs of such components can be administered in any effective carrier, e.g., any formulation or composition capable of effectively delivering the component gene to cells in vivo. Approaches include insertion of the gene in viral vectors, including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered naked or with the help of, for example, cationic liposomes (lipofectamine) or derivatized (e.g., antibody conjugated), polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaP04 precipitation carried out in vivo.

A preferred approach for in vivo introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, e.g., a cDNA. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid.

Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes in vivo, particularly into humans. These vectors provide efficient delivery of genes into cells,
and the transferred nucleic acids are stably integrated into the chromosomal DNA of
the host. The development of specialized cell lines (termed "packaging cells") which
produce only replication-defective retroviruses has increased the utility of retroviruses
for gene therapy, and defective retroviruses are characterized for use in gene transfer
for gene therapy purposes (for a review see Miller, *Blood* 76:271 (1990)). A
replication defective retrovirus can be packaged into virions, which can be used to
infect a target cell through the use of a helper virus by standard techniques. Protocols
for producing recombinant retroviruses and for infecting cells in vitro or in vivo with
such viruses can be found in Ausubel, et al., eds., *Current Protocols in Molecular
standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP,
pWE and pEM which are known to those skilled in the art. Examples of suitable
packaging virus lines for preparing both ecotropic and amphotropic retroviral systems
include pCSrip, pCres, p2 and pA1mt. Retroviruses have been used to introduce a
variety of genes into many different cell types, including epithelial cells, in vitro
and/or in vivo (see for example Eglitis, et al. (1985) Science 230:1395-1398; Danos
150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT
Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO
89/05345; and PCT Application WO 92/07573).

Another viral gene delivery system useful in the present methods utilizes
adenovirus-derived vectors. The genome of an adenovirus can be manipulated, such
that it encodes and expresses a gene product of interest but is inactivated in terms of
its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner et
al, BioTechniques 6:616 (1988); Rosenfeld et al, Science 252:431-434 (1991); and
Rosenfeld et al, Cell 68:143-155 (1992). Suitable adenoviral vectors derived from
the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3,
or Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances, in that they are not capable of infecting non-dividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld et al., (1992) supra). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situ, where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al., supra; Haj-Ahmand and Graham, J. Virol. 57:267 (1986).

Yet another viral vector system useful for delivery of nucleic acids is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al., Curr. Topics in Micro, and Immunol.158:97-129 (1992). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al., Am. J. Respir. Cell. Mol. Biol. 7:349-356 (1992); Samulski et al, J. Virol. 63:3822-3828 (1989); and McLaughlin et al, J. Virol. 62:1963-1973 (1989). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al, Mol. Cell. Biol. 5:3251-3260 (1985) can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al, Proc. Natl. Acad. Sci. USA 81:6466-6470 (1984); Tratschin et al, Mol. Cell. Biol. 4:2072-2081 (1985); Wondisford et al, Mol. Endocrinol. 2:32-39 (1988); Tratschin et al, J. Virol. 51:611-619 (1984); and Flotte et al, J. Biol. Chem. 268:3781-3790 (1993).

In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to cause expression of a nucleic acid compound described herein (e.g., a nucleic acid encoding a a polypeptide disclosed in Table 3 or 5, or an active fragment thereof, and/or a nucleic acid that increases the expression
and/or activity of a polynucleotide that encodes a polypeptide disclosed in Table 3 or 5) in the tissue of a subject. Typically non-viral methods of gene transfer rely on the normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In some embodiments, non-viral gene delivery systems can rely on endocytic pathways for the uptake of the subject gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes. Other embodiments include plasmid injection systems such as are described in Meuli et al, J. Invest. Dermatol. 116(1):131-135 (2001); Cohen et al., Gene Ther. 7(22): 1896-905 (2000); or Tarn et al, Gene Ther. 7(21): 1867-74 (2000).

In some embodiments, a gene encoding a factor described herein, e.g., a polynucleotide that encodes a polypeptide disclosed in Table 3 or 5, or an active fragment thereof, is entrapped in liposomes bearing positive charges on their surface (e.g., lipofectins), which can be tagged with antibodies against cell surface antigens of the target tissue (Mizuno et al, No Shinkei Geka 20:547-551 (1992); PCT publication WO91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

In clinical settings, the gene delivery systems for the therapeutic gene can be introduced into a subject by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the target cells will occur predominantly from specificity of transfection, provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited, with introduction into the subject being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by stereotactic injection (e.g., Chen et al, PNAS USA 91: 3054-3057 (1994)).

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant
cells, e.g., retroviral vectors, the pharmaceutical preparation can comprise one or more cells, which produce the gene delivery system.

In some embodiments, the present disclosure includes the use of agonists of any one or more of the factors disclosed in Tables 3 and/or 5. In some embodiments, suitable agonists can increase the expression and/or activity of one or more of the factors disclosed in Tables 3 and/or 5, e.g., by about 2-fold, 3-fold, 4-fold, 5-fold, or more.

Many of the factors disclosed herein are referenced by the International Protein Index (IPI) number assigned to them. The sequences associated with each of the disclosed IPI numbers are publically available and can be obtained and/or viewed, for example, using the European Institute for Bioinformatics website available at world wide web address ebi.ac.uk. Other suitable websites are also known in the art.

**Subject Selection**

The term "subject" is used throughout the specification to describe an animal, human or non-human, to whom treatment according to the methods of the present invention is provided.

The methods disclosed herein can include selecting a subject for treatment. For example, a subject can be selected if the subject has or is at risk for developing DR and/or DN. In some instances, a subject can be selected if the subject has or is at risk for developing type I and/or type II diabetes. In some instances, a subject can be selected if the subject is taking or will take insulin, e.g., to treat diabetes.

**Routes of Administration**

One or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more, including all) of the factors disclosed herein (e.g., disclosed in Tables 3 and/or 5) can be administered alone or in or as pharmaceutical compositions (disclosed below) using any mode of administration, e.g., including any mode of administration that results in a therapeutically effective level in the eye (for DR) and/or the kidney (for DN). In some instances, a therapeutically effective level is an amount or level of that results in one or more of the symptoms of DR and/or DN being ameliorated or otherwise beneficially altered. For the treatment of DR, an exemplary route of administration can include local administration into or onto the eye. For the treatment of DN, an exemplary route of administration can include local
administration to the kidney. Other exemplary modes of administration suitable for either DR or DN include, but are not limited to, oral, parenteral, inhalation (e.g., as a spray), topical, rectal, nasal, buccal, vaginal, and/or via an implanted reservoir.

The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrasynovial, intrarticular, intraarterial, intracranial injection or infusion techniques. Alternatively or in addition, the present invention may be administered according to any of the Food and Drug Administration approved methods, for example, as described in CDER Data Standards Manual, version number 004 (which is available at fda.gov/cder/dsm/DRG/drg00301.htm). Where application over a period of time is advisable or desirable, the compositions of the invention can be placed in sustained released formulations (e.g., hydrogels) or implantable devices (e.g., implantable pumps).

**Pharmaceutical Compositions**

One or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more, including all) of the factors disclosed herein (e.g., disclosed in Tables 3 and 5) can be formulated in or as pharmaceutical compositions. Such pharmaceutical compositions can contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form.

In some cases, pharmaceutical compositions containing one or more factors can be formulated according to the intended method of administration.

Pharmaceutical compositions containing one or more factors can be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. The nature of the pharmaceutical compositions for administration is dependent on the mode of administration and can readily be determined by one of ordinary skill in the art. In addition, methods for making such formulations are well known and can be found in, for example, Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990. In some embodiments, the pharmaceutical composition is sterile or sterilizable.

Pharmaceutical compositions featured in the invention can contain carriers or excipients, many of which are known to skilled artisans. Excipients that can be used
include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and
bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids,
polypeptides (for example, serum albumin), EDTA, sodium chloride, liposomes,
mannitol, sorbitol, water, and glycerol.

In some embodiments, pharmaceutical compositions can be presented in unit
dosage form, for example, in ampoules or in multi-dose containers, with an added
preservative. The compositions may take such forms as suspensions, solutions or
emulsions in oily or aqueous vehicles, and may contain formulatory agents such as
suspending, stabilizing and/or dispersing agents. Alternatively, the composition may
be in powder form for constitution with a suitable vehicle, for example, sterile
pyrogen-free water, before use. Agents that enhance delivery into a cell can be used
as well, e.g., liposomes or micelles.

In addition to the formulations described previously, the compositions can also
be formulated as a depot preparation. Thus, for example, the compositions can be
formulated with suitable polymeric or hydrophobic materials (for example as an
emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble
derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions can also take the form of tablets or capsules
prepared by conventional means with pharmaceutically acceptable excipients such as
binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or
hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline
cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium
stearate, talc or silica); disintegrants (for example, potato starch or sodium starch
glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets can
be coated by methods well known in the art. Liquid preparations for oral
administration may take the form of, for example, solutions, syrups or suspensions, or
they may be presented as a dry product for constitution with water or other suitable
vehicle before use. Such liquid preparations may be prepared by conventional means
with pharmaceutically acceptable additives such as suspending agents (for example,
sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents
(for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily
esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example,
methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also
contain buffer salts, flavoring, coloring and sweetening agents as appropriate.
Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Toxicity and therapeutic efficacy of the compounds and pharmaceutical compositions described herein can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Polypeptides or other compounds that exhibit large therapeutic indices are preferred.

Data obtained from cell culture assays and further animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

The formulations and routes of administration can be tailored to the disease or disorder being treated, and for the specific human being treated. A subject can receive a dose of the agent once or twice or more daily for one week, one month, six months, one year, or more. The treatment can continue indefinitely, such as throughout the lifetime of the human. Treatment can be administered at regular or irregular intervals (once every other day or twice per week), and the dosage and timing of the administration can be adjusted throughout the course of the treatment. The dosage can remain constant over the course of the treatment regimen, or it can be decreased or increased over the course of the treatment.

Generally the dosage facilitates an intended purpose for both prophylaxis and treatment without undesirable side effects, such as toxicity, irritation or allergic response. Although individual needs may vary, the determination of optimal ranges
for effective amounts of formulations is within the skill of the art. Human doses can readily be extrapolated from animal studies (Katocs et al, Chapter 27 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990). Generally, the dosage required to provide an effective amount of a formulation, which can be adjusted by one skilled in the art, will vary depending on several factors, including the age, health, physical condition, weight, type and extent of the disease or disorder of the recipient, frequency of treatment, the nature of concurrent therapy, if required, and the nature and scope of the desired effect(s) (Nies et al., Chapter 3, In: Goodman & Gilman's "The Pharmacological Basis of Therapeutics", 9th Ed., Hardman et al, eds., McGraw-Hill, New York, N.Y., 1996).

Subject Evaluation

The methods can also include monitoring or evaluating the subject during and after treatment to determine the efficacy of the treatment, and, if necessary, adjusting treatment (e.g., by altering the composition, by increasing the dose of a single administration of the composition, by increasing the number of doses of the composition administered per day, and/or by increasing the number of days the composition is administered) to improve efficacy. Monitoring or evaluating the subject can include identifying a suitable marker of disease prior to commencing treatment and optionally recording the marker, and comparing the identified or recorded marker to the same marker during and/or after treatment. Suitable markers can include one or more symptoms of the subject's disease. Adjustment of treatment would be recommended where the marker is a symptom of disease and comparison of the marker during or after treatment with the marker prior to treatment revealed either no change in the marker or an increase in the marker. Conversely, adjustment of treatment may not be required using the same markers where an increase in the marker is observed.
EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

METHODS AND MATERIALS:

**Proteomics**

Vitreous and retinal samples, separated by 1 dimensional SDS-PAGE gels, were fractionated into 40 slices of 1.5-mm width. Gel slices were individually digested with trypsin and each slice individually analyzed by capillary liquid chromatography, nanospray ionization, and tandem mass spectroscopy using LTQ 2-dimensional linear ion trap mass spectrometer (Thermo Fisher). Assignment of MS/MS data was performed using X!Tandem (version 2006.09.15, The Global Proteome Machine Organization) search against the International Protein Index (IPI) human sequence database and a randomized version of the same IPI database generated by a Perl script, decoy.pl (Matrix Science, London, UK). The default X!Tandem search parameters were used, except for the following: a maximum valid expectation value of 0.1; potential residue mass modification of +16.0 Da for oxidized methionine and +71.0 Da for acrylamide alkylated cysteine; spectrum parameters including a fragment monoisotopic mass error of ±0.4 Da and a precursor monoisotopic mass error of ±0.5 Da. Resultant matches were entered and compiled into a MySQL relational database and proteomics computational analyses was performed using the Hypertext Preprocessor-based interface according the algorithm we recently reported (12 - (FILLIN)). This label free proteomics method provided a semi-quantitative analysis while maximizing detection sensitivity.

**Statistical analysis/ power**

The statistical analysis was carried out as follows. Briefly, the median number of peptide recognitions between those classified as unaffected and affected using the ETDRS criteria was tested using the Kruskal-Wallis test for significance (p<0.1). A non-parametric test was used due to small sample size. No correction was made for multiple comparisons. Fold change was calculated as the mean of a peptide detected across the unaffected group divided by the mean of a peptide of the affected group.
Example 1

Evidence that diabetic retinal pathologies can be halted has been documented by the Joslin 50-Year Medalist Study (Keenan et al, Diabetes Care. 2007 Aug;30(8): 1995-7. Epub 2007 May 16). Population characteristics for these subjects are shown in Table 1.

Table 1. Characteristics of Medalist Study participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (n), mean ± std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>47.0% (192)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3% ±1.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.2 ± 7.4</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>11.0 ± 6.5</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>56.2 ± 5.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 5.1</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.07 ± 0.12</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>HDLc (mmol/L)</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>Insulin dose (u/kg)</td>
<td>0.46 ± 0.2</td>
</tr>
<tr>
<td>Family History</td>
<td>Any DM T1DM</td>
</tr>
<tr>
<td></td>
<td>29.7 (122)</td>
</tr>
<tr>
<td></td>
<td>12.9 (53)</td>
</tr>
<tr>
<td>DR3</td>
<td>38.8 (116)</td>
</tr>
<tr>
<td>DR4</td>
<td>52.0 (156)</td>
</tr>
<tr>
<td>DR3 or DR4</td>
<td>93.7 (295)</td>
</tr>
<tr>
<td>DR3/4</td>
<td>39.1 (118)</td>
</tr>
<tr>
<td>IA2 or GAD</td>
<td>29.7 (111)</td>
</tr>
<tr>
<td>IA2</td>
<td>14.9 (56)</td>
</tr>
<tr>
<td>GAD</td>
<td>18.4 (69)</td>
</tr>
<tr>
<td>PDR</td>
<td>55 (163)</td>
</tr>
<tr>
<td>Microalbuminuria (ACR&lt;7.91)</td>
<td>13.1 (45)</td>
</tr>
<tr>
<td>Neuropathy (MNSI&gt;2)</td>
<td>60.6 (183)</td>
</tr>
<tr>
<td>CVD</td>
<td>48.3 (160)</td>
</tr>
</tbody>
</table>

This study characterizes a number of individuals collectively referred to herein as 'Medalists' who have lived for 50 or more years with type 1 diabetes (TIDM) by clinical exam, medical history and extensive chemistries.

Retinal pathology was documented by dilated fundus examination and fundus photography. The degree of renal disease was assessed through measurements of albumin, creatinine, cystatin C and estimated glomerular filtration rate (eGFR). After characterizing over 500 Medalists, a bimodal distribution of DR was observed with
50% of the Medalists having none to moderate nonproliferative DR and 50% having quiescent PDR (Fig. 1 and Table 1). Surprisingly, standard risk factors, including duration of diabetes and HbAIC levels, were not associated with severity.

A subset of 97 Medalists has been followed with multiple ophthalmic examinations. Analysis of DR progression showed that 47.4% of eyes without proliferative DR (PDR) at baseline progressed to PDR, with a median time to progression to PDR of 38.4 years. The majority of Medalists who did not progress to PDR developed mild to moderate non-proliferative DR (NPDR) over a median follow-up of 20.6 years. Interestingly, of the eyes that did not develop PDR, all but one eye stopped progressing after 17 years of follow-up (Fig. 2). Over the last two years we have been collaborating with the network for Pancreatic Organ Donors with Diabetes (nPOD) to procure tissues from Medalists after they have passed away. Organ donations have been obtained from 11 Medalists, including whole eyes, kidneys, pancreas and skin. During their initial study visit we obtained specimens of DNA, serum, plasma, and urine.

Example 2

In this example, the proteins expressed in Medalists with no-to-mild DR was compared with (or versus or against) those with proliferative diabetic retinopathy (PDR).

Samples were taken from eight Medalist eyes: three with no to moderate NPDR, four with PDR, and one ungradeable (due to scatter laser performed for non-diabetic pathology) as determined by grading of fundus photographs. Retinal pathology was assessed using standard methods. For proteomic analysis, retinal and vitreous specimens were isolated using mass spectrometry methods as previously described (Gao et al, J Proteome Res. 2008;7:2516-25; Gao et al, Nat Med. 2007;13:181-8). Initial analysis of these eight eyes by mass spectrometry identified over 450 proteins.

Based on a minimum 1.5 fold increase and significance level of at least 0.1, 26 proteins were found to be higher in the vitreous of Medalists who have no to moderate NPDR compared to those with PDR. Interestingly, pathway analysis indicated the following categories of proteins: anti-oxidative stress properties, including but not limited to GPX3; lipid metabolism, including but not limited to ASHA (acid ceramidase) and APOE; adhesion molecules, including but not limited to CLSTN1,
SPARCL1, CDH2, SPP1 and NRCAM; anti-angiogenesis or protease inhibitors, including but not limited to SERPINF1 (PEDF), A2M (alpha-2-macroglobulin), PCSKIN, CST2 (cystatin), DKK3 and WIF1; Ig family, including but not limited to, rheumatoid factor C6 light chain, IGLVI-40 Ig kappa chain V-IV region Len and IGHM; complement, including but not limited to, C5 Complement and C6 Complement; Basement membrane, including but not limited to, B3GNT1: UDP-GlcNac:betaGal beta-1, 3-N-acetylglucosaminyltransferase; Prostaglandin enzyme, including but not limited to, PTGS: Prostaglandin D2 synthetase; and G-proteins, including but not limited to APLP2: amyloid beta (A4) precursor-like protein 2.

Table 2. Protective genes or proteins in Eye vitreous

<table>
<thead>
<tr>
<th>G-Protein</th>
<th>amyloid beta (A4) precursor-like protein 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>APLP2</td>
<td>G-protein coupled receptor protein signaling pathway</td>
</tr>
<tr>
<td>Prostaglandin Enzyme</td>
<td>prostaglandin D2 synthase 21kDa (brain)</td>
</tr>
<tr>
<td>PTGDS</td>
<td>glutathione-independent prostaglandin D synthase, PGH2-&gt;PGD2</td>
</tr>
<tr>
<td>associate with Basement Membrane</td>
<td>also fatty acid biosynthetic process</td>
</tr>
<tr>
<td>B3GNT1</td>
<td>UDP-GlcNAc:betaGal beta-1, 3-N-acetylglucosaminyltransferase 1</td>
</tr>
<tr>
<td>Complement</td>
<td>type II transmembrane protein, integral to Golgi membrane</td>
</tr>
<tr>
<td>C5</td>
<td>Complement C5</td>
</tr>
<tr>
<td>C6</td>
<td>Complement C6</td>
</tr>
<tr>
<td>A2M</td>
<td>Alpha-2-macroglobulin</td>
</tr>
<tr>
<td>Growth/tumor suppressor gene</td>
<td>a protease inhibitor and cytokine transporter</td>
</tr>
<tr>
<td>DKK3</td>
<td></td>
</tr>
<tr>
<td>WIF1</td>
<td></td>
</tr>
<tr>
<td>Ig superfamily</td>
<td>Rheumatoid factor</td>
</tr>
<tr>
<td>C6 light chain</td>
<td></td>
</tr>
<tr>
<td>IGLVI-40</td>
<td>Ig kappa chain V-IV region Len</td>
</tr>
<tr>
<td>IGHM</td>
<td></td>
</tr>
<tr>
<td>Cytokine</td>
<td>FAM3C</td>
</tr>
<tr>
<td>SERPINF I(PEDF)</td>
<td></td>
</tr>
<tr>
<td>A2M</td>
<td></td>
</tr>
<tr>
<td>Adhesion protein</td>
<td>CLSTN1</td>
</tr>
<tr>
<td>SPARCL1</td>
<td></td>
</tr>
<tr>
<td>CDH2</td>
<td>cadherin 2, type 1, N-cadherin</td>
</tr>
</tbody>
</table>
SPP1: secreted phosphoprotein 1
ECM-receptor interaction, Focal adhesion, Toll-like receptor signaling pathway,
Signaling by PDGF, Integrin cell surface interactions, osteoblast differentiation

NRCAM
APP

Protease inhibition
A2M: Alpha-2-macroglobulin
a protease inhibitor and cytokine transporter
SERPINF I (PEDF)
PCS KIN
CST3: cystatin C
CPE: Carboxypeptidase E
APP: serine-type endopeptidase inhibitor activity

Lipid metabolism
ASAH1: Acid ceramidase
APOE

Oxidative Stress
GPX3

Not grouped
ABI3BP
CUTA
SEZ-6

Example 3: Identification of Factors that Protect Against DR

The prevalence of DR in the Medalists evaluated is shown in Fig. 3. Candidate factors that protect against DR were identified by comparing the levels or expression of factors in individuals with DR and those without DR. From the 26 factors listed in Table 2, five factors were identified as having a statistically significant difference in expression between the individuals with DR and those without DR. Table 3 lists the five factors. Data was analyzed using the Kruskal Wallis test.

Table 3 - Protective Factors for DR

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fold Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3GNT1</td>
<td>1/0</td>
<td>0.006687</td>
</tr>
<tr>
<td>SEZ6</td>
<td>10.73</td>
<td>0.014282</td>
</tr>
<tr>
<td>APLP1</td>
<td>9.1</td>
<td>0.018053</td>
</tr>
<tr>
<td>CST3</td>
<td>2.45</td>
<td>0.042357</td>
</tr>
<tr>
<td>A2M</td>
<td>2.09</td>
<td>0.028351</td>
</tr>
<tr>
<td>PTGDS</td>
<td>3</td>
<td>0.0771</td>
</tr>
</tbody>
</table>
Example 4

We tested the protein levels of two of the sample factors listed in Table 3 by Western Blot. 10 micrograms of protein from each sample were loaded onto a 4-20% gel (Biorad) and transferred to a PVDF membrane (Immobilon-P). CystatinC and PTGDS (abeam, 1:2000), Endophilin and Tenascin R (santa cruz 1:1000) were used for immunodetection. Equal amounts of protein were loaded from each sample, band density was analyzed using Image J, and p values represent student t test. The results are shown in Fig. 4.

Example 5

In this example we tested whether PTDGS and CST3 are expressed in vascular cells using as an in vitro model BRP and BREC. In our experiments, we detected PTGDS in both BRP and BREC by western blot (Fig. 5). We also confirmed that CST3 is detected in mouse retina protein extracts by western blot (data not shown).

Example 6

The following experiment illustrates the functional importance of the protective factors identified in the present invention. Here we show that increasing expression of PTGDS can alter the adverse effects of high glucose levels on endothelial cells.

Bovine Retinal Endothelial cells (BREC) were isolated from Jersey Cow eyes using standard techniques. BREC's were maintained in EMB media using 10% FBS (Lonza). PTGDS pCMV plasmids (Oigene) were transiently transfected into BREC's with FUGENE HD (Promega) following the manufacturer's instructions.

BREC were transfected with either GFP (control) or pCMV Entry PTGDS plasmid (Origene), and IXIO-4 cells per well were plated on 24 well plates. The following day, their media was replaced by 1%FBS media containing either 5mMGlucose (Low Glucose) or 26 mM Glucose (High Glucose) overnight. The following day, the BrdU was added to these same media and proliferation was measured 20 hours later following the manufacturer's protocol (Roche). Overexpression of PTGDS was confirmed by SDS-PAGE using anti-DDK (Origene) at the end of the experiment.
Fig. 6 shows that BREC over expressing PTGDS proliferated more than the control GFP transfected REC when cultured in high glucose. This illustrates that an agent identified herein, including but not limited to the illustrative example using PTGDS, may protect retinal endothelial cells from the effects of high glucose.

Example 7

Nine kidney samples from the Medalists were analyzed by mass spectrometry as previously described (Gao et al, J Proteome Res. 2008;7:2516-25; Gao et al. Nat Med. 2007;13:181-8). Using the results from the proteomic analyses and correlated pathology derived from the renal glomeruli, we found that the expression of 14 proteins are significantly (1.5 fold) greater in kidneys without disease vs. those with class II and III levels of nephropathy. The proteins are listed in Table 4.

| Table 4. Protective genes or proteins in Renal Glomeruli |
|-----------------|----------------------------------------------------------|
| **Lipid Metabolism** | Isoform 2 of Apolipoprotein A-1-binding protein precursor interacts with apolipoprotein A-1 (apoA-1), the major apolipoprotein of high-density lipoproteins (HDLs) |
| **Oxidation reduction/ Oxidative Stree** | Isoform 1 of MOSC domain-containing protein 2, mitochondrial precursor (mt) |
| MOSC2 | Hemopexin precursor |
| HPX | glycoprotein, binds heme |
| GPX3 | Glutathione peroxidase 3 precursor |
| GSTT1 | Glutathione S-transferase theta 1 |
| PRDX1 | peroxiredoxin 1 |
| HADH | antioxidant enzymes, reduce hydrogen peroxide and alkyl hydroperoxides |
| SCCPDH | Isoform 2 of Hydroxacyl-coenzyme A dehydrogenase, mitochondrial precursor (mt) |
| | also fatty acid metabolism |
| | oxidoreductase activity (mt) |
| **Purine metabolism** | hypoxanthine phosphoribosyltransferase 1 |
| HPRT1 | collagen, type XVIII, alpha 1 |
| **Basement membrane** | Glycine amidinotransferase (L-arginine:glycine amidinotransferase) |
| COL18A1 | Metabolism of amino acids and derivatives |
| **Mitochondrial metabolism/enzymes** | agmatine ureohydrolase (agmatinase) |
| MOSC2 | Metabolism of amino acids and derivatives |
| HADH | citrate synthase |
| SCCPDH | GATM | Glycine amidinotransferase (L-arginine:glycine amidinotransferase) |
| | Metabolism of amino acids and derivatives |
| | AGMAT | agmatine ureohydrolase (agmatinase) |
| | CS | Metabolism of amino acids and derivatives |
Glucose metabolism
GNPDA1 glucosamine-6-phosphate deaminase 1
Amino sugar and nucleotide sugar metabolism

Example 8: Identification of Factors that Protect Against DR

Complications observed in Medalists are shown in FIG. 7 and a summary of clinical characteristics in Medalists is shown in FIG. 8.

Candidate factors that protect against DN were identified by comparing the levels or expression of factors in individuals with DN and those without DN. From the 14 factors listed in Table 4, eight factors were identified as having a statistically significant difference in expression between the individuals with DN and those without DN. Table 5 lists the eight factors. Data was analyzed using the Kruskal Wallis test.

Table 5 - Protective Factors for DR

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fold Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNPDA1</td>
<td>6.60</td>
<td>0.02</td>
</tr>
<tr>
<td>HPRT1</td>
<td>4.09</td>
<td>0.02</td>
</tr>
<tr>
<td>GSTT1</td>
<td>3.48</td>
<td>0.03</td>
</tr>
<tr>
<td>APOA1BP</td>
<td>2.69</td>
<td>0.01</td>
</tr>
<tr>
<td>HPX</td>
<td>2.44</td>
<td>0.02</td>
</tr>
<tr>
<td>AGMAT</td>
<td>2.35</td>
<td>0.03</td>
</tr>
<tr>
<td>CS</td>
<td>2.13</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Example 9

The protective factors identified from the kidneys of Medalist patients are secondarily screened using (intraocular) aqueous fluid and plasma from a population of both type 1 and 2 diabetic patients who are having cataract extraction, to determine the levels of biomarkers in more readily accessible fluids such as plasma, and correlate the levels in plasma to those in intraocular fluids. We will obtain medical history, physical exam, and blood samples to characterize the non-Medalists in the same manner as the Medalist participants. Using this case-control approach, the differential presence of candidates can be assessed in the aqueous, plasma and potentially urine across the stages of DR.
OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.
WHAT IS CLAIMED IS:

1. A method of diagnosing or predicting the risk of developing diabetic microvascular complications (DMC) in a subject, the method comprising: evaluating activity, levels or expression of one or more agents listed in Tables 3 or 5 in a sample from the subject; wherein increased or decreased levels, activity or expression as compared to a control indicates an increased or decreased risk or the presence of a DMC.

2. The method of claim 1, wherein the evaluating step comprises one of more of: (a) evaluating the level of expression of genes of one or more agents listed in Tables 3 or 5; (b) evaluating the activity of one or more agents listed in Tables 3 or 5 and (c) evaluating protein levels of one or more agents listed in Tables 3 or 5.

3. The method claim 1 or 2, wherein the sample comprises plasma, urine, vitreous, aqueous fluid, or kidney tissue.

4. A method for modulating hyperglycemic toxic effects in a subject, comprising identifying a subject having or at risk for hyperglycemic toxic effects, and administering to the subject in need thereof a therapeutically effective amount of one or more compounds that increase or mimics the levels, the activity or the expression of the agents disclosed in Tables 3 or 5.

5. A method for ameliorating DMC in a subject, comprising: identifying a subject having or at risk for a DMC, and administering to the subject in need thereof a therapeutically effective amount of one or more compounds that increase or mimics the levels, the activity or the expression of the agents disclosed in Tables 3 or 5.

6. The method of claim 5, wherein the DMC is diabetic retinopathy (DR) or diabetic nephropathy (DN).

7. The method of claims 4-6, wherein the compound is selected from the group consisting of a peptide, an antibody and a small molecule.
Figure 1. Bimodal Distribution of Diabetic Retinopathy

- Quiescent PDR
- PDR with HRC
- Severe NPDR
- Moderate NPDR
- Questionable DR
- No DR

N = 188

No to Moderate Severe NPDR

NPDR

PDR

Percentage of all patients
Figure 2. Rate of DR Worsening in Eyes With and Without PDR at Final Visit

Eyes with PDR at endpoint
N = 54
(54 worsened)

Eyes without PDR at endpoint
N = 94
(38 worsened)

Log rank p < 0.0001
* No sig difference b/t HbA1c

FIG. 2
FIG. 4
<table>
<thead>
<tr>
<th>Nephro Class</th>
<th>PDR</th>
<th>Sex</th>
<th>A1c (%)</th>
<th>Agedx (years)</th>
<th>Dur (years)</th>
<th>Cause of Death</th>
<th>HTN</th>
<th>ACR (mcg/mg)</th>
<th>eGFR (ml/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Mild</td>
<td>F</td>
<td>6.7</td>
<td>23</td>
<td>57</td>
<td>MI</td>
<td>Y</td>
<td>5.4</td>
<td>48.4</td>
<td></td>
</tr>
<tr>
<td>I Mod</td>
<td>M</td>
<td>5.6</td>
<td>7</td>
<td>50</td>
<td>Cancer</td>
<td>N</td>
<td>4.3</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>I PDR</td>
<td>M</td>
<td>6.6</td>
<td>4</td>
<td>72</td>
<td>Cardiac arrest</td>
<td>Y</td>
<td>3.5</td>
<td>47.2</td>
<td></td>
</tr>
<tr>
<td>IIA No</td>
<td>M</td>
<td>7.2</td>
<td>16</td>
<td>72</td>
<td>Organ failure</td>
<td>N</td>
<td>18.3</td>
<td>67.9</td>
<td></td>
</tr>
<tr>
<td>IIA No</td>
<td>M</td>
<td>7.1</td>
<td>27</td>
<td>51</td>
<td>Cancer</td>
<td>No</td>
<td>13.3</td>
<td>76.9</td>
<td></td>
</tr>
<tr>
<td>IIA Mild</td>
<td>M</td>
<td>9.0</td>
<td>5</td>
<td>79</td>
<td>Cardiac arrest</td>
<td>Y</td>
<td>23.1</td>
<td>61.4</td>
<td></td>
</tr>
<tr>
<td>IIA Mild</td>
<td>F</td>
<td>8.8</td>
<td>1</td>
<td>57</td>
<td>Stroke</td>
<td>Y</td>
<td>36.9</td>
<td>54.3</td>
<td></td>
</tr>
<tr>
<td>IIA PDR</td>
<td>F</td>
<td>7.3</td>
<td>7</td>
<td>64</td>
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FIG. 7
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/1, G01 N 33/00, A61 K 48/00 (201 1.01)
USPC - 514/44A

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: 514/44A, 435/7.92, 514/44R; IPC: C12N 5/1, G01N 33/00, A61K 48/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC : 435/7.92, 514/44R (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST - PUBLISPT,USOC,EPAB,JPAB; Dialog Classic Files - 654, 652, 349, 348, 35, 65, 155; USPTO Web Page; Google Scholar; Search terms — diagnosis, risk assessment, amelioration, hyperglycemic microvascular complications, diabetic retinopathy/nepropathy, plasma, expression levels, markers, gstrl, administration, small molecules, controls

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim</th>
</tr>
</thead>
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<td>Y</td>
<td>US 201001604142 A1 (GERALDES et al.) 24 June 2010 (24.06.2010) para [0005], [0010], [0012], [0014], [0025], [0029]</td>
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<td>Y</td>
<td>US 20080107755 A1 (LYONS et al.) 08 May 2008 (08.05.2008) para [0025], [0037], [0088], [0136], [0141], [0191], [0256], [0282], [0311], [0340], [0342], [0379]-0381, [0397], [0398], [0408]</td>
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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
03 December 2011 (03.12.2011)

Date of mailing of the international search report
23 DEC 2011

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

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