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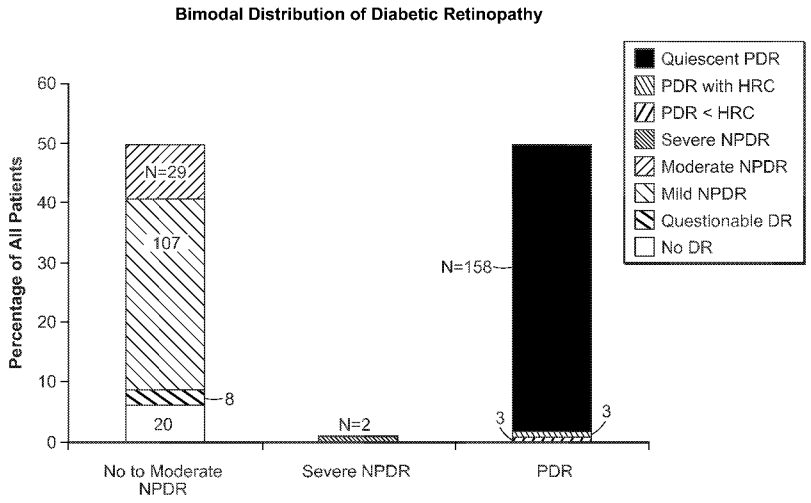

FIG. 1

(57) Abstract: Compositions and methods for diagnosing, predicting risk of, and/or treating diabetic retinopathy and/or diabetic nephropathy.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/63514

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; G01N 33/53 (2012.01)
USPC - 435/6.1, 7.1
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)
IPC(8): C12Q 1/68; G01N 33/53 (2012.01)
USPC: 435/6.1, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.11, 6.12, 7.92; 514/6.9, 7.3
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,EPAB,JPAB); Google Patents; Google Scholar.
Search Terms: biomarkers predict diabetic retinopathy, predicting retinopathy in diabetes patients, biomarkers protective for diabetic neuropathy, risk factors biomarkers retinopathy, age of onset type I diabetes, Biomarkers neuropathy diabetes, ARPC4, P4HB,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Gao et al. Characterization of the Vitreous Proteome in Diabetes without Diabetic Retinopathy and Diabetes with Proliferative Diabetic Retinopathy. Journal of Proteome Research. 2008, Vol 7(6), pages 2516-2525: abstract; pg 2517, col 1, para 2, para 4 to col 2, para 1, Table 2, Table 3	1-12
Y	Koopman, et al. Changes in Age at Diagnosis of Type 2 Diabetes Mellitus in the United States, 1988 to 2000. Ann. Fam. Med. January/February 2005. Vol 3(1), pages 60-63: Abstract	1-12
Y	Datta et al. Effect of GSTM1 and GSTT1 Double Deletions in the Development of Oxidative Stress in Diabetic Nephropathy Patients. Indian Journal of Biochemistry & Biophysics. April 2010, Vol 47, pages 100-103. abstract, pg 100, col 2, para 2	2-10, 12
Y	Li et al. Proteomic profile of primary isolated rat mesangial cells in high-glucose culture condition and decreased expression of PSMA6 in renal cortex of diabetic rats. Biochem. Cell Biol. 18 June 2010, Vol 88, pages 635-648; pg 646, col 1, para 1-2, Table 1, Fig. 5B	2-10, 12
Y	US 2010/0150920 A1 (GLASER) 17 June 2010 (17.06.2010) para [0004], [0006], [0013]-[0015]	10
A	Yamane et al. Proteome Analysis of Human Vitreous Proteins. Molecular & Cellular Proteomics. 15 September 2003, Vol 2, pages 1177-1187: pg 1178, col 2 para 1, pg 1181, col 2, para 2-pg 1182, col 1 para 1.	5

☐ 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
30 May 2012 (30.05.2012)	20 JUN 2012
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

INTERNATIONAL SEARCH REPORT

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

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

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

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

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

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-12, drawn to a method of diagnosing or predicting risk of developing a microvascular complication in a subject having diabetes; and a method for determining the prognosis of a microvascular complication in a subject having diabetes and a microvascular complication.

Group II: Claims 13-14, drawn to a method of determining the effectiveness of a treatment to prevent or treat a microvascular complication in a subject having diabetes and being being treated for diabetes and/or for the microvascular complication.

********** See Supplemental Sheet to continue **********

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/63514

Continuation of Box No. III, Observations where unity of invention is lacking:

Group III: Claims 15-32, drawn to a method for treating diabetic retinopathy (DR) or diabetic nephropathy (DN) in a subject; and A composition comprising a therapeutic agent that increases the level or activity of a protective factor of Table 6 or 8 or a therapeutic agent that decreases the level or activity of a risk factor of Table 7 or 9, and a pharmaceutically acceptable excipient.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I-II do not include the inventive concept of a method for treating diabetic retinopathy (DR) or diabetic nephropathy (DN) in a subject; and A composition comprising a therapeutic agent that increases the level or activity of a protective factor of Table 6 or 8 or a therapeutic agent that decreases the level or activity of a risk factor of Table 7 or 9, and a pharmaceutically acceptable excipient, as required by Group III.

Groups I and III do not include the inventive concept of a method of determining the effectiveness of a treatment to prevent or treat a microvascular complication in a subject having diabetes and being being treated for diabetes and/or for the microvascular complication, as required by Group II.

Groups II-III do not include the inventive concept of a method of diagnosing or predicting risk of developing a microvascular complication in a subject having diabetes; and a method for determining the prognosis of a microvascular complication in a subject having diabetes and a microvascular complication, as required by Group I.

Groups I-III share the technical feature of the protective factor for diabetic retinopathy or diabetic nephropathy listed in Table 6 or 8 and the risk factors for diabetic retinopathy or diabetic nephropathy listed in Table 7 or 9.

Groups I-II further share the technical feature of providing or obtaining a sample from the subject, determining the level of a biomarker listed in Table 6, 8, 7 or 9 in the sample from the subject; comparing the level of the biomarker to a reference level, wherein (i) a lower level of a biomarker of Table 6 or 8 in the sample as compared to a DR or DN indicates a first conclusion; and (ii) a higher level of Table 9 in the sample as compared to a DN reference level indicate a second conclusion.

However, these shared technical features do not represent a contribution over the prior art, specifically, the article titled 'Characterization of the Vitreous Proteome in Diabetes without Diabetic Retinopathy and Diabetes with Proliferative Diabetic Retinopathy' by Gao et al. (Journal of Proteome Research 2008, Vol 7, pages 2516-2525) (hereinafter 'Gao') teaches a method comprising:
--providing or obtaining a sample from a subject (pg 2517, col 1, para 3-'Vitreous fluid was obtained from individuals undergoing pars plana vitrectomy');
--determining the level of a biomarker listed in Table 6, 8, 7 or 9 in the sample from the subject (fig 1; Table 2-'RBP3', 'APLP2' Table 3-'A2M' are listed as protective factor for diabetic retinopathy (DR) in Table 6 of the Current Application; Table 3-'CFD' is listed as a risk factor for DR in Table 7 of the Current Application (note: Table 7 is mislabeled 'protective' instead of 'risk'); pg 2517, col 1, para 4 to col 2, para 1-'Proteomic analysis was performed on50 uL of undiluted vitreous from noDR (diabetes with no apparent diabetic retinopathy), PDR, and NDM subjects');
--comparing the level of the biomarkers to one or more reference levels (table 2-3), wherein:
----a subject with a microvascular complication such as DR has a lower level of one or more biomarker of Table 6 in the sample as compared to a DR reference level, wherein the reference level is the the level of a biomarker in a subject who has had diabetes and has not developed a microvascular complication such as DR (table 2-3- lower RBP3, APLP2 and A2M level in DR subjects compared to noDR subject; pg 2517, col 1, para 4 to col 2, para 1-'Proteomic analysis was performed on50 uL of undiluted vitreous from noDR (diabetes with no apparent diabetic retinopathy), PDR, and NDM subjects');
----a subject with a microvascular complication such as DR has a higher level of one or more biomarker of Table 7 as compared to a DR reference level, wherein the reference level is the the level of a biomarker in a subject who has had diabetes and has not developed a microvascular complication such as DR (table 3-higher CFD level in a subject with DR compared to noDR subject; pg 2517, col 1, para 4 to col 2, para 1-'Proteomic analysis was performed on50 uL of undiluted vitreous from noDR (diabetes with no apparent diabetic retinopathy), PDR, and NDM subjects').
Thus one of ordinary skill in the art would have found it obvious that said biomarkers could be used for diagnostic or prognostic purposes such as predict risking of developing a microvascular complication such as diabetic retinopathy in a subject having diabetes by comparing the levels of said biomarkers in a subject to a reference noDR subject or determining the effectiveness of a treatment to prevent or treat a microvascular complication such as DR in a subject by comparing the levels of said biomarkers before and during/after the treatment, as were commonly practiced in the art.

Further the article titled 'Effect of GSTM1 and GSTT1 Double Deletions in the Development of Oxidative Stress in Diabetic Nephropathy Patients' by Datta et al. (Indian Journal of Biochemistry & Biophysics. April 2010, Vol 47, pages 100-103) (hereinafter 'Datta') teaches that GSTT1 (listed as a protective factor in Table 8 of the Current Application) teaches that GSTT1 gene deletion is associated with diabetic nephropathy (DN) (abstract; pg 100, col 2, para 2), thus one of ordinary skill in the art would have known that a DN subject would have lower level of GSTT1 compare to a diabetic subject without DN.

Further the article titled 'Proteomic profile of primary isolated rat mesangial cells in high-glucose culture condition and decreased expression of PSMA6 in renal cortex of diabetic rats' by Li et al. (Biochem. Cell Biol. 18 June 2010, Vol 88, pages 635-648) (hereinafter 'Li') teaches that PDIA3 (listed in Table 9 of the Current Application) is differentially expressed in cells under conditions mimicking diabetic nephropathy (pg 646, col 1, para 1-2-'In the present study, rat mesangial cell proliferation was inhibited by a high glucose concentration, which indicated an effect of glucose on the mesangial cell cycle. Thus, our in vitro cell culture model of mesangial cells mimics a similar condition of diabetic nephropathy. In the current study, we identified 24 proteins that were differentially expressed in rat mesangial cells between normal and high glucose concentrations. Eleven of these proteins are associated with diabetes, including TCP1 (Sol et al. 2009), PDIA3'), thus one of ordinary skill in the art would have found it obvious to further study the relationship between said PDIA3 level and diabetic nephropathy to determine whether it is a protective factor or risk factor.

As said shared technical features were known or would have been obvious to one of ordinary skill in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.