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(54) **DIAGNOSTIC BIOMARKERS OF DIABETES**

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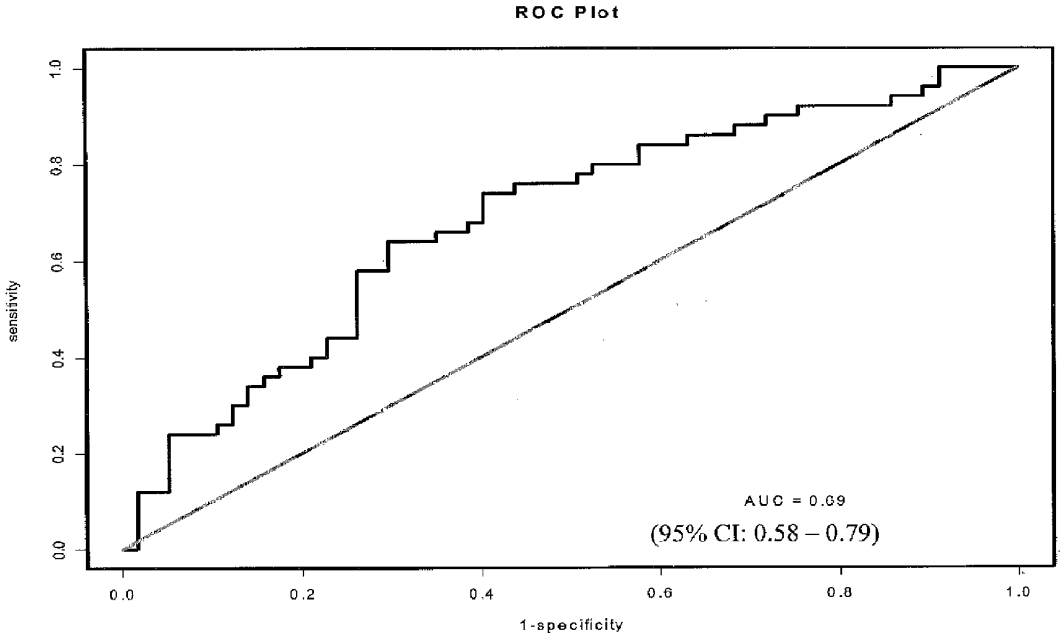
(57) **ABSTRACT**

Methods are disclosed for the identification of gene sets that are differentially expressed in PBMCs of patients diagnosed with a pre-diabetic disease state and overt type II diabetes. 3 gene and 10 gene signatures are shown to accurately predict a diabetic disease state in a patient. The application also described kits for the rapid diagnosis of diabetic disease states in patients at a point of care facility.



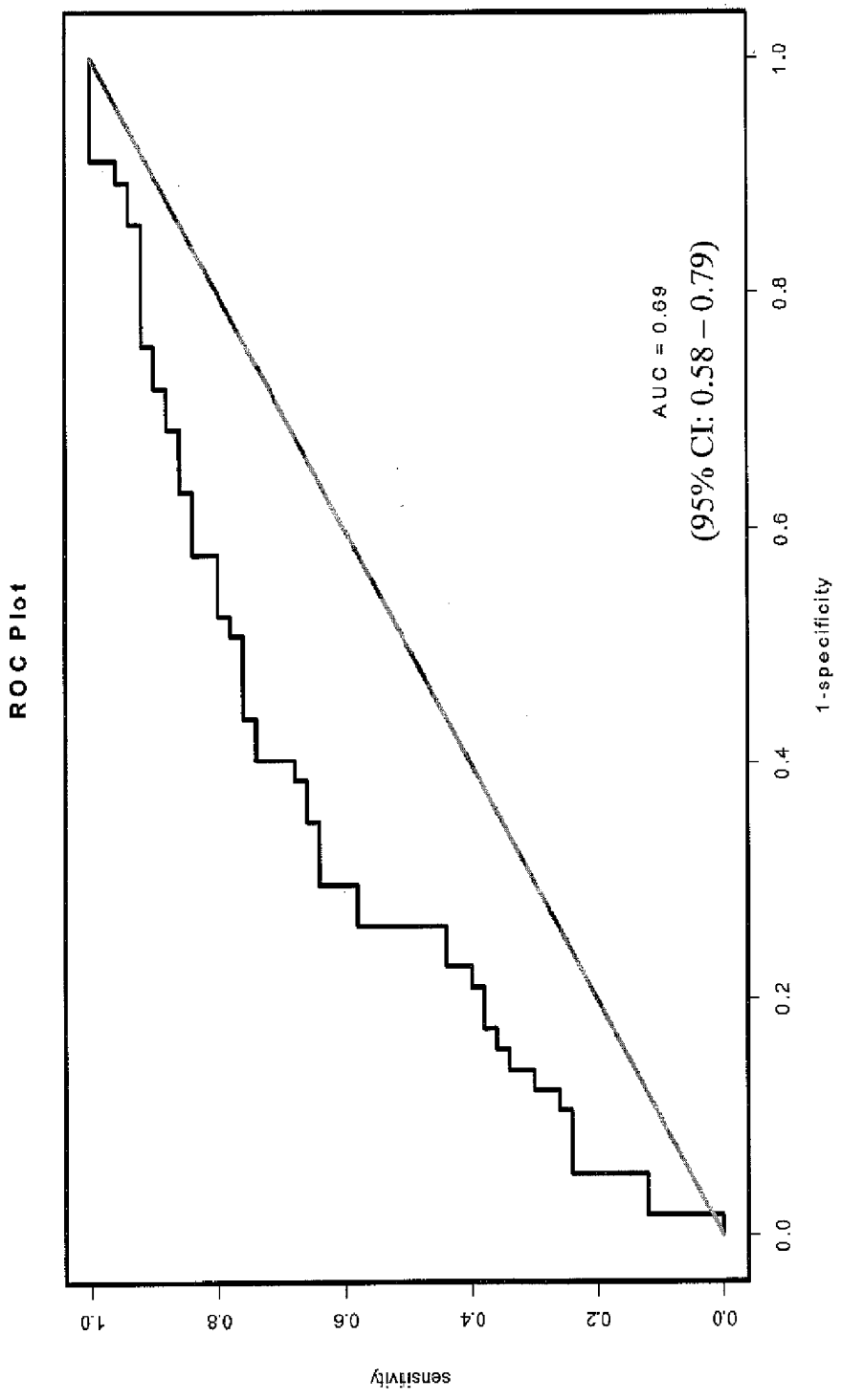

ROC Plot
AUC = 0.69
(95% CI: 0.58 – 0.79)
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 1:

FIGURE 2A:

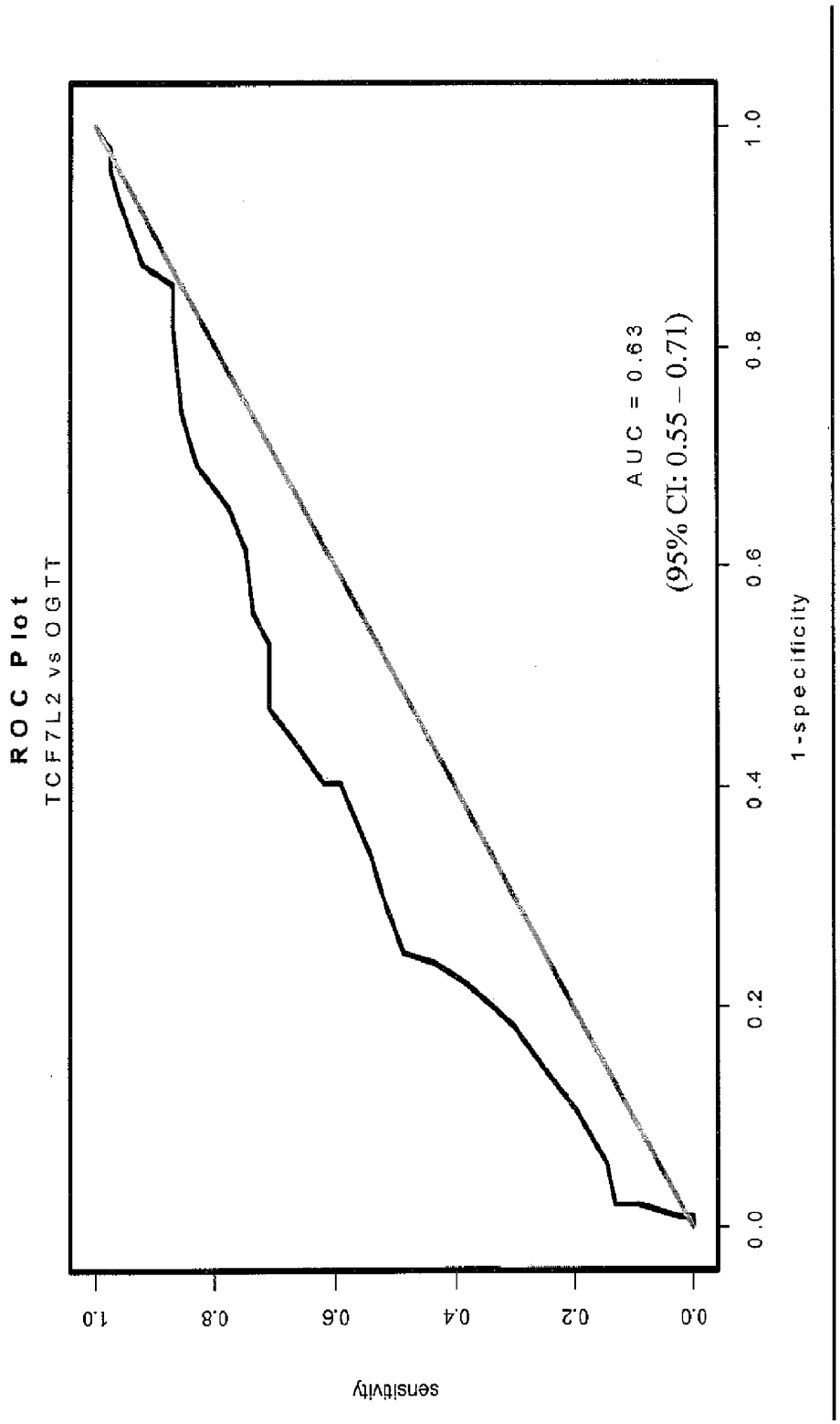

ROC Plot
TCF7L2 vs OGTT
AUC = 0.63
(95% CI: 0.55 – 0.71)
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 2B:

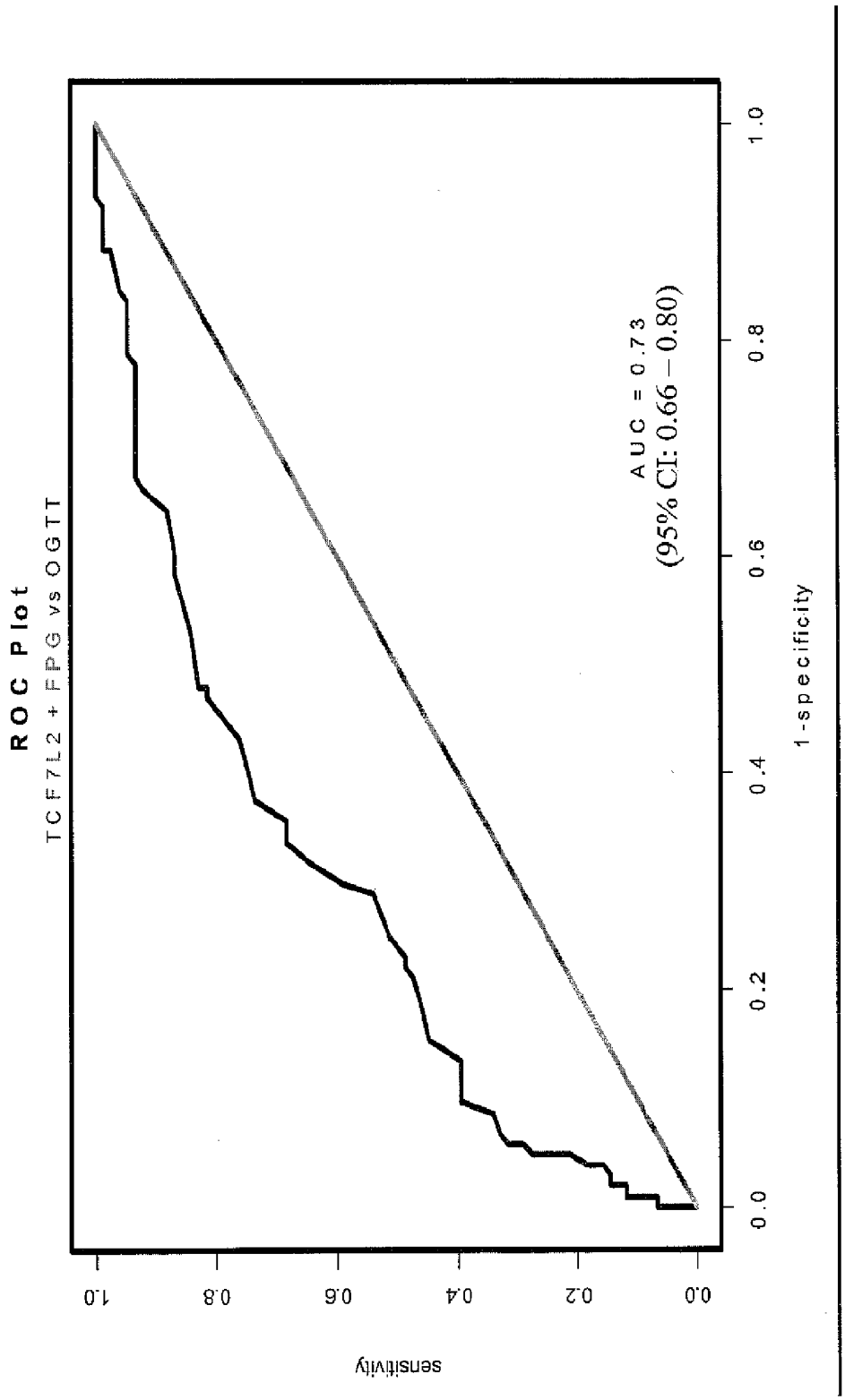

ROC Plot
TCF7L2 + FPG vs OGTT
AUC = 0.73
(95% CI: 0.66 – 0.80)
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 3:

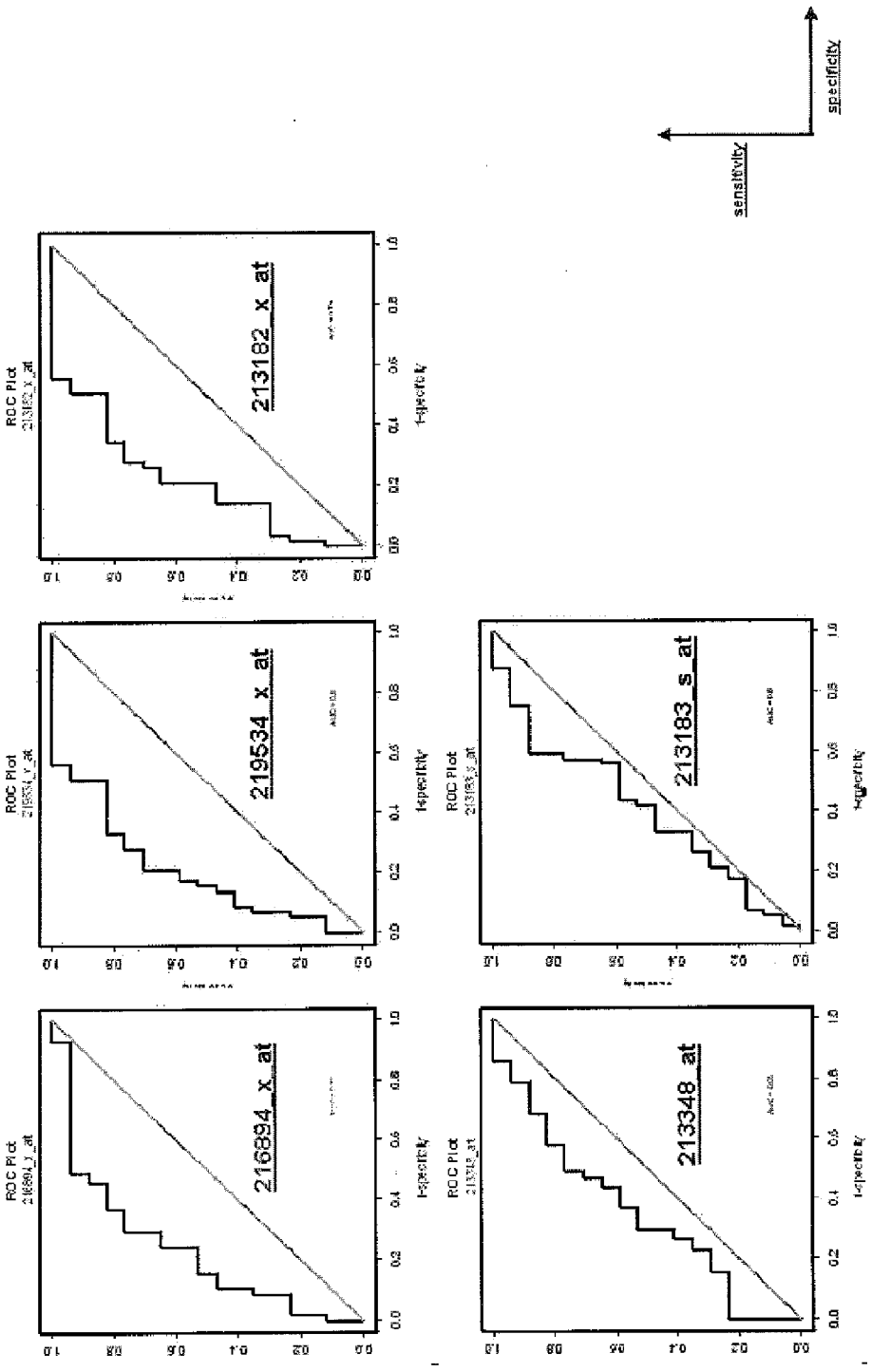

ROC Plot
213182_x_at
ROC Plot
219534_x_at
ROC Plot
213183_s_at
ROC Plot
216894_x_at
ROC Plot
213348_at
sensitivity
specificity

FIGURE 4A:

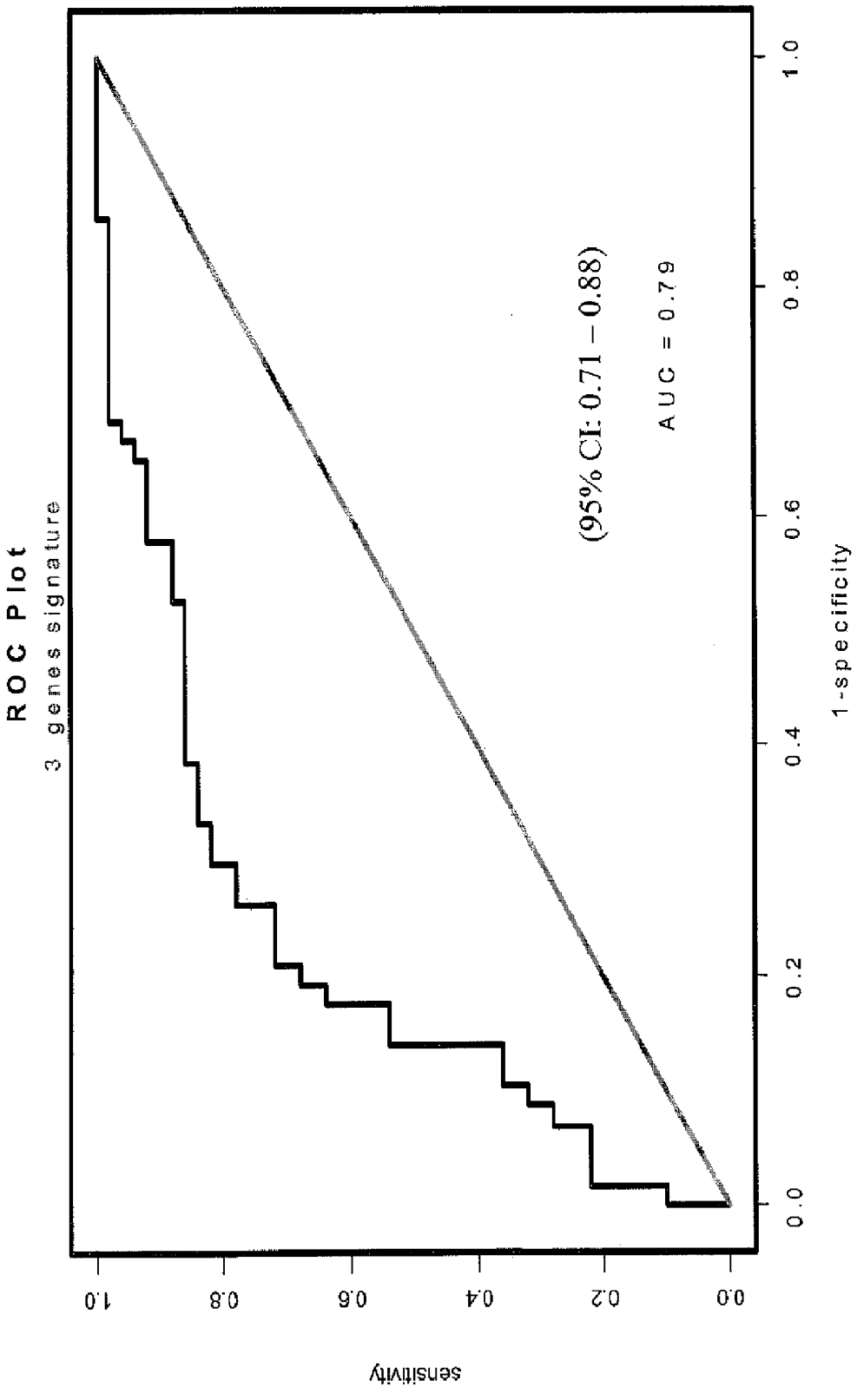

ROC Plot
3 genes signature
AUC = 0.79
(95% CI: 0.71 – 0.88)
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 4B:

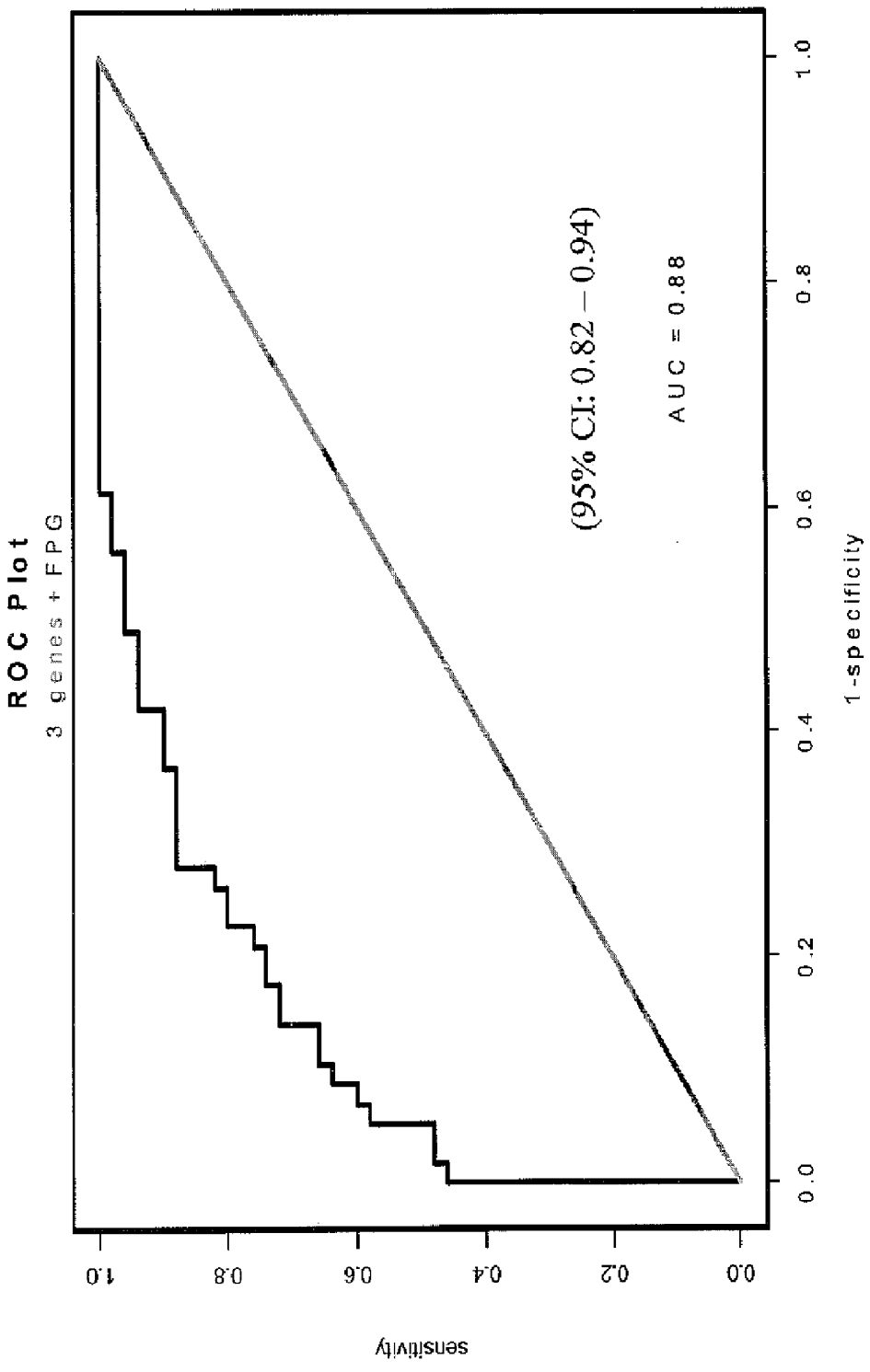
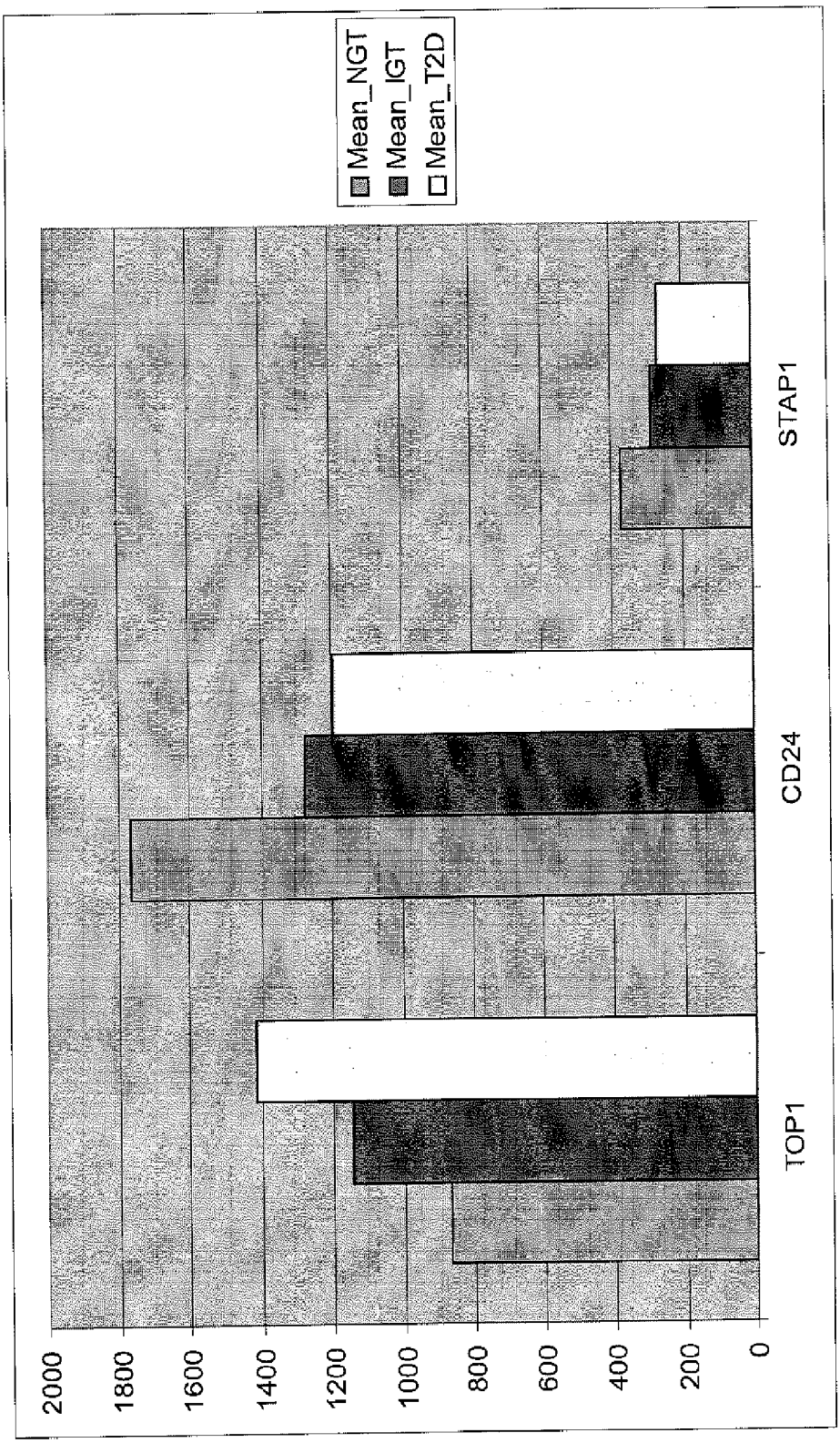

ROC Plot
3 genes + FPG
(95% CI: 0.82 – 0.94)
AUC = 0.88
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 4C:


Mean_NGT
Mean_IGT
Mean_T2D
2000
1800
1600
1400
1200
1000
800
600
400
200
0
TOP1
CD24
STAP1

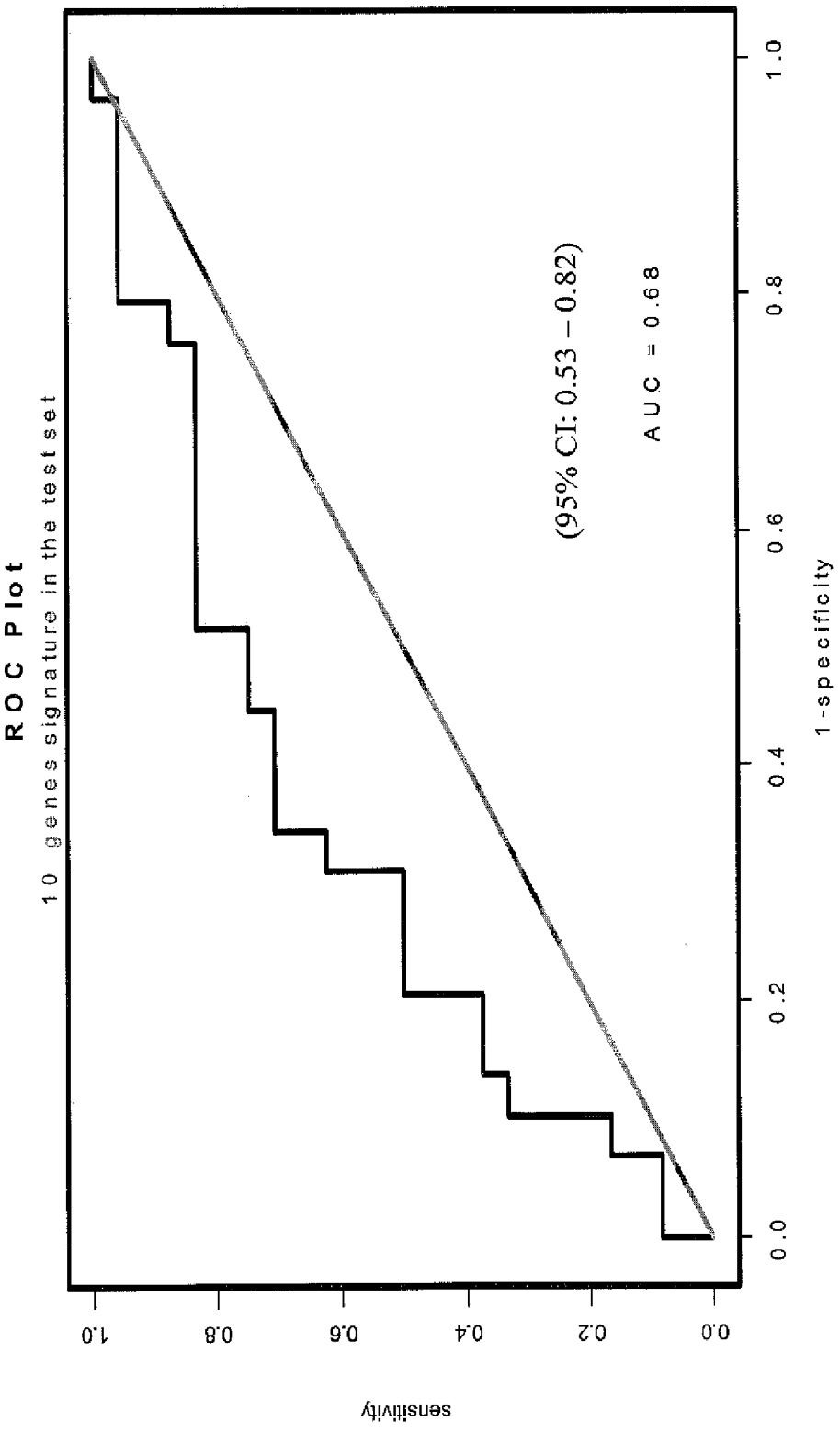

ROC Plot
10 genes signature in the test set
AUC = 0.68
(95% CI: 0.53 – 0.82)
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 5A:

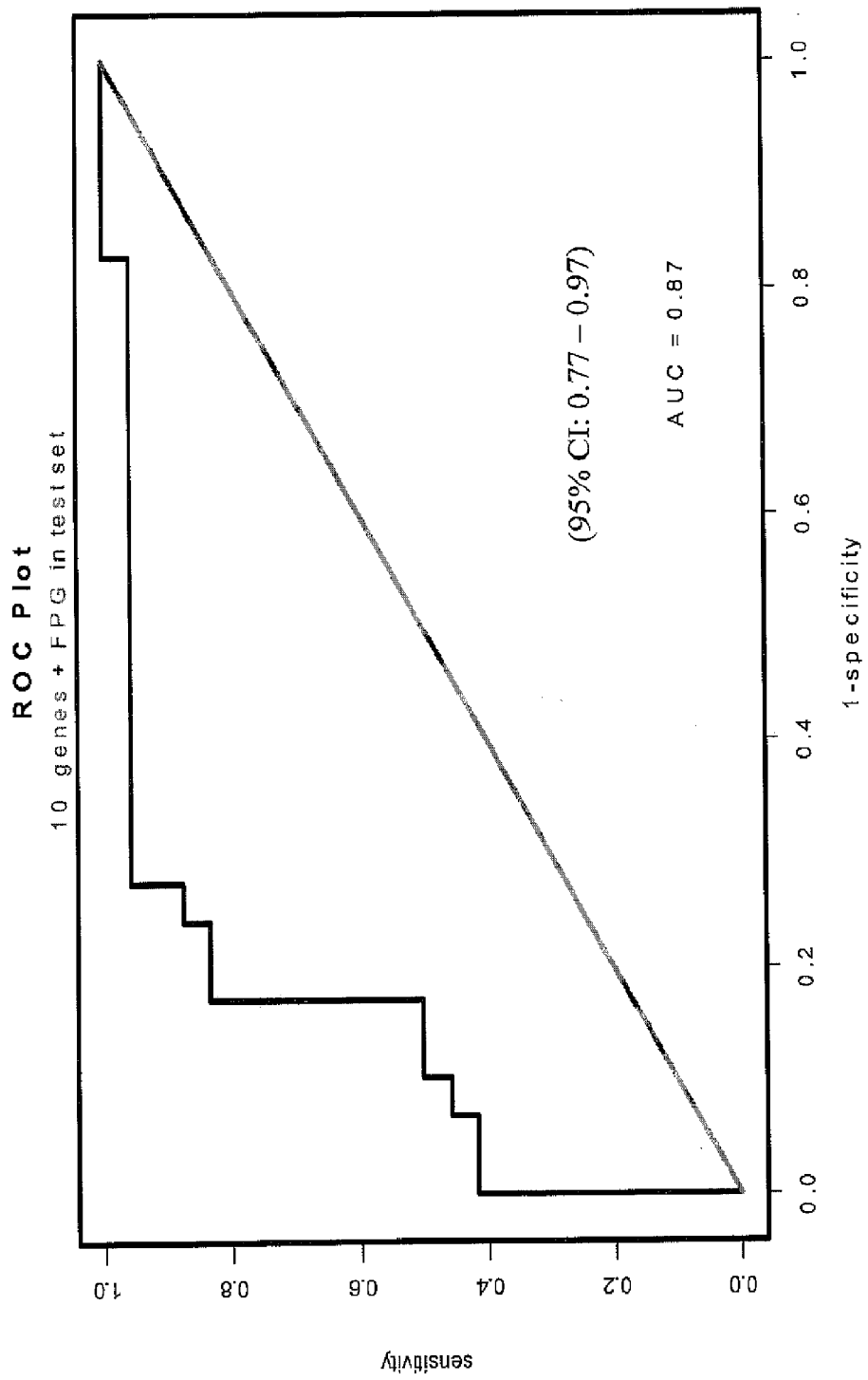
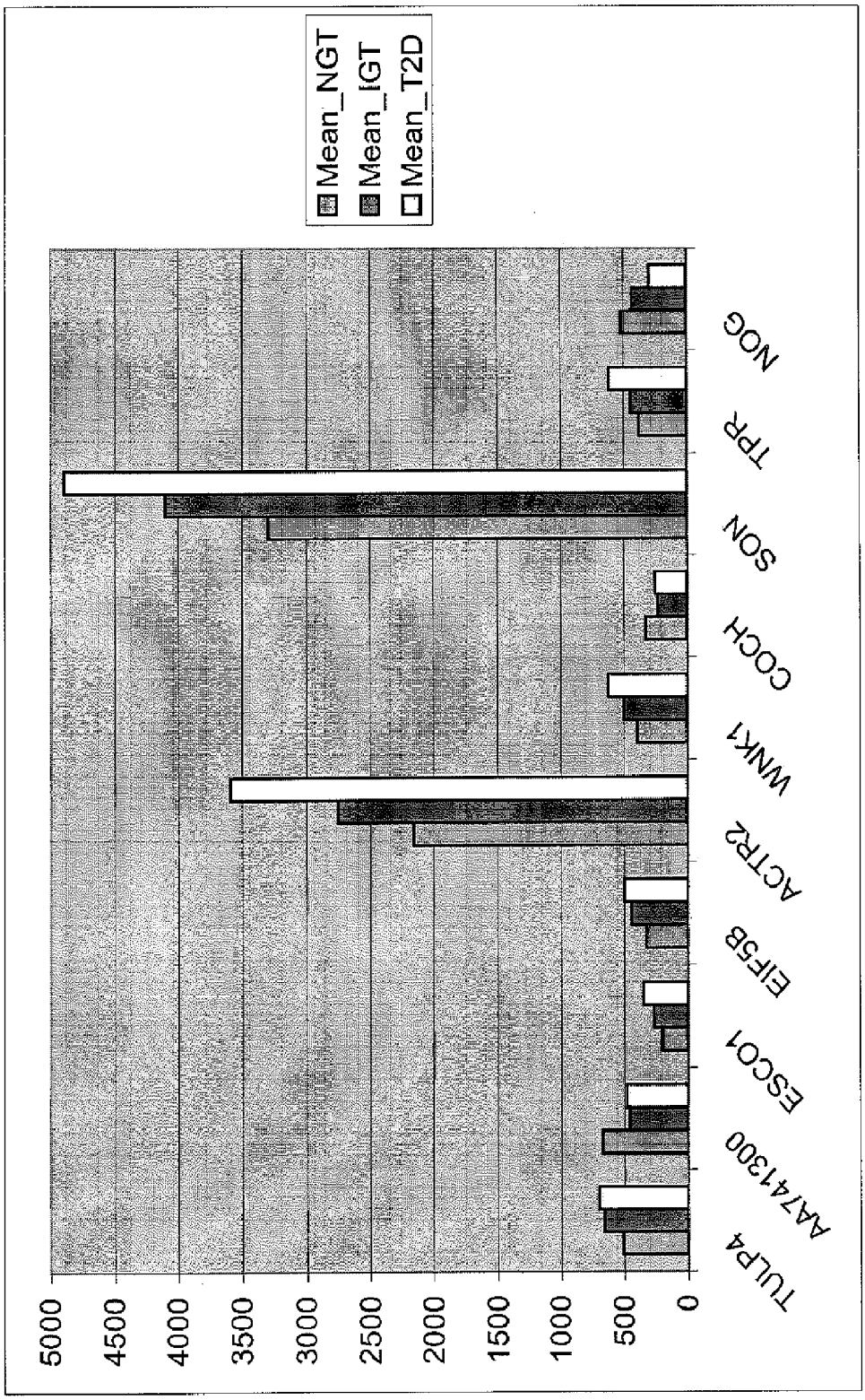

ROC Plot
10 genes + FPG in test set
(95% CI: 0.77 – 0.97)
AUC = 0.87
sensitivity
1-specificity
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 5B:

FIGURE 5C:


Mean_NGT
Mean_IGT
Mean_T2D
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
TULP4
AA741300
ESCO1
EIF5B
ACTR2
WNK1
COCH
SON
TPR
NOG

DIAGNOSTIC BIOMARKERS OF DIABETES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Ser. No. 60/987,540, filed on Nov. 13, 2007, which is hereby incorporated into the present application in its entirety.

FIELD OF THE APPLICATION

[0002] The application relates to the field of medical diagnostics and describes methods and kits for the point-of-care diagnosis of a diabetic disease state in a patient.

BACKGROUND OF THE INVENTION

[0003] Diabetes is a group of diseases marked by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Left untreated, it can cause many serious short term complications including symptoms of hypoglycemia, ketoacidosis, or nonketotic hyperosmolar coma. In the long term, diabetes is known to contribute to an increased risk of arteriosclerosis, chronic renal failure, retinal damage (including blindness), nerve damage and microvascular damage.

[0004] The spreading epidemic of diabetes in the developing world is predicted to have a profound impact on the healthcare system in the United States. A recent study by the Center for Disease Control and Prevention indicates the incidence of new diabetes cases in the U.S. nearly doubled in the last 10 years. As of 2007, at least 57 million people in the United States have pre-diabetes. Coupled with the nearly 24 million who already have diabetes, this places more than 25% of the U.S. population at risk for further complications from this disease. According to the American Diabetes Association, the estimated cost of diabetes in the United States in 2007 amounted to $174 billion with direct medical costs approaching $116 billion.

[0005] Although the etiology of diabetes appears to be multi-factorial in nature, increasing experimental evidence suggests the onset of obesity, especially abdominal obesity, disrupts immune and metabolic homeostasis and ultimately leads to a broad inflammatory response. The production of inflammatory cytokines in the adipose tissue, such as TNF alpha, then deregulates the immune response and a cell's ability to respond to insulin. Detection of an alteration in the transcriptional profiles of circulating immune cells, such as monocytes and macrophages, therefore provides a convenient avenue to diagnose the disease and monitor its progression before even the more overt signs of glucose intolerance become apparent.

[0006] For the forgoing reasons, there is an unmet need for rapid and accurate diagnostic assays for the diagnosis and monitoring of patients at risk of developing diabetes. In particular, there is an unmet need for diagnostic assays in a kit format that can be readily used at a point-of-care facility for the routine screening of patients for early onset diabetes.

SUMMARY OF THE APPLICATION

[0007] Methods are described for the determination of gene signature expression profiles that are diagnostic of pre-diabetic and diabetic disease states. The disclosure further pertains to diagnostic kits comprising reagents for the rapid measurement of gene signature expression profiles in a patient's blood sample. The kit format is cost-effective and convenient for use at a point-of-care facility.

[0008] In one embodiment, a method is described for the diagnosis of Diabetes Mellitus in a patient, the method comprising the steps of (a) providing a test sample taken from a patient, (b) measuring the gene expression profile of a gene signature comprising a gene selected from the group of TOP1, CD24 and STAP1 genes, (c) comparing the gene expression profile with a diagnostic gene expression profile of the gene signature, (d) determining a diabetic disease state in the patient based at least in part upon a substantial match between the gene expression profile and the diagnostic gene expression profile and (e) displaying the determination to a medical professional.

[0009] The determining step can be executed by a computer system running one or more algorithms selected from the group of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM). The determining step can also include analysis of the patient's metabolic disease profile.

[0010] The gene signature can include any two genes selected from the group of TOP1, CD24 and STAP1 genes or all three genes. In one embodiment, the gene signature can include one or more genes selected from the group of TOP1, CD24 and STAP1 genes and one or more genes selected from the genes listed in TABLES 1 or 6.

[0011] The patient can have a normal BMI. The diabetic disease state can be a pre-diabetic disease state or a Type 2 Diabetes disease state.

[0012] The test sample can be a blood sample or a test sample containing PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or CD 14^+ monocytes.

[0013] The measuring can involve real-time PCR, an immunochemical assay or a specific oligonucleotide hybridization.

[0014] In another embodiment, a method is described for the diagnosis of Diabetes Mellitus in a patient, the method comprising the steps of (a) providing a test sample taken from a patient, (b) measuring the gene expression profile of a gene signature comprising a gene selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes, (c) comparing the gene expression profile with a diagnostic gene expression profile of the gene signature, (d) determining a diabetic disease state in the patient based at least in part upon a substantial match between the gene expression profile and the diagnostic gene expression profile and (e) displaying the determination to a medical professional.

[0015] The determining step can be executed by a computer system running one or more algorithms selected from the group of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM). The determining step can also include analysis of the patient's metabolic disease profile.

[0016] The gene signature can include any two genes or any three genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In one aspect, the gene signature includes the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1,

COCH, SON, TPR and NOG genes. In one aspect, the gene signature includes the TOP1, CD24 and STAP1 genes in addition to at least one gene selected from the group of the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In another aspect, the gene signature includes one or more genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes and one or more genes selected from the genes listed in TABLES 1 or 6.

[0017] The patient can have a normal BMI. The diabetic disease state can be a pre-diabetic disease state or a Type 2 Diabetes disease state.

[0018] The test sample can be a blood sample or a test sample containing PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $CD14^+$ monocytes.

[0019] The measuring can involve real-time PCR, an immunochemical assay or a specific oligonucleotide hybridization.

[0020] In one embodiment, a method is described for the diagnosis of Diabetes Mellitus in a patient, the method comprising the steps of (a) providing a test sample taken from a patient, (b) measuring the gene expression profile of a gene signature comprising the TCF7L2 and CLC genes, (c) comparing the gene expression profile with a diagnostic gene expression profile of the gene signature, (d) determining a diabetic disease state in the patient based at least in part upon a substantial match between the gene expression profile and the diagnostic gene expression profile and (e) displaying the determination to a medical professional.

[0021] The determining step can be executed by a computer system running one or more algorithms selected from the group of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM). The determining step can also include analysis of the patient's metabolic disease profile.

[0022] The gene signature can include either the TCF7L2 or CLC gene. In one aspect, the gene signature includes one or more variants of the TC7L2 or CLC gene. In one aspect, the gene signature includes either the TCF7L2 or CLC gene and one or more genes selected from the genes listed in TABLES 1 or 6.

[0023] The patient can have a normal BMI. The diabetic disease state can be a pre-diabetic disease state or a Type 2 Diabetes disease state.

[0024] The test sample can be a blood sample or a test sample containing PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$**0** $CD11c^+$] or [Emr^+**0** $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or CD 14^+ monocytes.

[0025] The measuring can involve real-time PCR, an immunochemical assay or a specific oligonucleotide hybridization.

[0026] In one embodiment, a method is described for diagnosing a change in the diabetic, disease state of a patient comprising the steps of (a) providing a first test sample taken from a patient at a first time point, (b) measuring a first expression profile of a gene signature comprising a gene selected from the group of the TOP1, CD24 and STAP1 genes in the first test sample, (c) providing a second test sample taken from the patient at a second time point, (d) measuring a second expression profile of the gene signature in the second test sample, (e) comparing the first expression profile with the second expression profile, (f) determining a change in the diabetic disease state in the patient based at least in part upon a substantial difference between the first gene expression profile and the second gene expression profile, and (g) displaying the determination to a medical professional.

[0027] In one aspect, the determining step is executed by a computer system running one or more algorithms selected from the group of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM). In another aspect, the determining step also includes an analysis of the patient's metabolic disease profile.

[0028] The gene signature can include any two genes selected from the group of the TOP1, CD24 and STAP1 genes. In one aspect, the gene signature includes the TOP1, CD24 and STAP1 genes. In another aspect, the gene signature includes a gene selected from the group of the TOP1, CD24 and STAP1 genes and one or more genes selected from the genes listed in TABLES 1 or 6.

[0029] In one aspect, the time period between the first time point and the second time point is from 0 to 2 years or from ¼ to 2 years or from ½ to 2 years or from 2 to 5 years, or from 5 to 10 years or more.

[0030] A change in diabetic disease state can be indicative of a progression toward a pre-diabetic disease state or a Type II Diabetes disease state. In one aspect, the patient at the first time point gas a normal BMI.

[0031] The first and second test sample can he blood samples. In one aspect, the first and second test sample can a test sample containing PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $C14^+$ monocytes.

[0032] The measuring can involve real-time PCR, an immunochemical assay or a specific oligonucleotide hybridization.

[0033] In one embodiment, a method is described for diagnosing a change in the diabetic disease state of a patient comprising the steps of (a) providing a first test sample taken from a patient at a first time point, (b) measuring a first expression profile of a gene signature comprising a gene selected from the group of the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes in the first test sample, (c) providing a second test sample taken from the patient at a second time point, (d) measuring a second expression profile of the gene signature in the second test sample, (e) comparing the first expression profile with the second expression profile, (f) determining a change in the diabetic disease state in the patient based at least in part upon a substantial difference between the first gene expression profile and the second gene expression profile, and (g) displaying the determination to a medical professional.

[0034] In one aspect, the determining step is executed by a computer system running one or more algorithms selected from the group of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM). In another aspect, the determining step also includes an analysis of the patient's metabolic disease profile.

[0035] The gene signature can include any two genes selected from the group of the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes.

In another aspect, the gene signature includes any three genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In one aspect, the gene signature includes the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In another aspect, the gene signature includes one or more genes selected from the group of the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes and one or more genes selected from the genes listed in TABLES 1 or 6.

[0036] In one aspect, the time period between the first time point and the second time point is from 0 to 2 years or from ¼ to 2 years or from Y2 to 2 years or from 2 to 5 years, or from 5 to 10 years or more.

[0037] A change in diabetic disease state can be indicative of a progression toward a pre-diabetic disease state or a Type II Diabetes disease state. In one aspect, the patient at the first time point gas a normal BMI.

[0038] The first and second test sample can be blood samples. In one aspect, the first and second test sample can a test sample containing PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ CD11c] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $CD14^+$ monocytes.

[0039] The measuring can involve real-time PCR, an immunochemical assay or a specific oligonucleotide hybridization.

[0040] In another embodiment, a kit is described for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus. The kit includes (a) reagents for collecting a test sample from a patient; and (b) reagents for measuring the expression profile of a gene signature comprising the TCF7L2 and CLC genes or variants thereof in a patient's test sample.

[0041] Reagents in step (a) and (b) are sufficient for a plurality of tests. Reagents for collecting a test sample from a patient can be packaged in sterile containers.

[0042] The gene signature can include one or more of the genes selected from the group of TCF7L2 and CLC genes and one or more genes selected from the list of genes of TABLES 1 or 6.

[0043] The test sample can be a blood sample.

[0044] The kit can also include reagents for the isolation of PBMCs or reagents for the isolation of $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or reagents for the isolation of $CD14^+$ monocytes. The reagents for measuring the expression profile of a gene signature can be real-time PCR reagents, immunochemical assay reagents or for specific oligonucleotides hybridization.

[0045] In another embodiment, a kit is described for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus. The kit includes (a) reagents for collecting a test sample from a patient; and (b) reagents for measuring the expression profile of a gene signature comprising the TOP1, CD24 and STAP1 genes or variants thereof in a patient's test sample.

[0046] The gene signature can include any two genes selected from the group of the TOP1, CD24 and STAP1 genes. In another aspect, the gene signature includes one or more of the genes selected from the group of TOP1, CD24 and STAP1 genes. In another aspect, the gene signature includes one or more genes selected from the group of TOP1, CD24 and STAP1 genes and one or more genes selected from the list of genes of TABLES 1 or 6.

[0047] Reagents in step (a) and (b) are sufficient for a plurality of tests. Reagents for collecting a test sample from a patient can be packaged in sterile containers.

[0048] The test sample can be a blood sample.

[0049] The kit can also include reagents for the isolation of PBMCs or reagents for the isolation of $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or reagents for the isolation of $CD14^+$ monocytes. The reagents for measuring the expression profile of a gene signature can be real-time PCR reagents, immunochemical assay reagents or for specific oligonucleotides hybridization.

[0050] In another embodiment, a kit is described for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus. The kit includes (a) reagents for collecting a test sample from a patient; and (b) reagents for measuring the expression profile of a gene signature a gene or variant thereof selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes in a patient's test sample.

[0051] In one aspect, the gene signature comprises one or more genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In one aspect, the gene signature comprises two or more genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes. In one aspect, the gene signature comprises three or more genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes.

[0052] Reagents in step (a) and (b) are sufficient for a plurality of tests. Reagents for collecting a test sample from a patient can be packaged in sterile containers.

[0053] The gene signature can also include one or more genes selected from the group of TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes and one or more genes selected from the list of genes of TABLES 1 or 6.

[0054] The test sample can be a blood sample.

[0055] The kit can also include reagents for the isolation of PBMCs or reagents for the isolation of $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $C11c^+$] cells or reagents for the isolation of $CD14^+$ monocytes. The reagents for measuring the expression profile of a gene signature can be real-time PCR reagents, immunochemical assay reagents or for specific oligonucleotides hybridization.

[0056] It should be understood that this application is not limited to the embodiments disclosed in this Summary, and it is intended to cover modifications and variations that are within the scope of those of sufficient skill in the field, and as defined by the claims.

[0057] The previously described embodiments have many advantages, including novel gene signatures for the early diagnosis of a pre-diabetic disease state and the monitoring of patients who are at risk of developing diabetes or who have already acquired the disease. The disclosure also describes kits with reagents and instructions for the cost-effective and rapid testing of blood samples by medical personnel at a point-of-care facility.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] FIG. **1** depicts a ROC Curve Analysis of CLC Gene compared to OGTT in accordance with a first embodiment;

[0059] FIG. **2**A depicts a ROC Curve Analysis of TCF7L2 set 1 compared to OGTT according to a second embodiment;
[0060] FIG. **2**B depicts a ROC Curve Analysis of TCF7L2 set 1 compared to compared to OGTT vs. FPG according to a third embodiment;
[0061] FIG. **3** shows a ROC Curve Analysis of CDKN1C gene according to a fourth embodiment;
[0062] FIG. **4**A shows a ROC analysis of the 3-gene signature compared to OGTT according to a fifth embodiment;
[0063] FIG. **4**B depicts a ROC analysis of the 3-gene signature compared to FPG vs. OGTT according to a sixth embodiment;
[0064] FIG. **4**C depicts bar chart of the mean expression of the 3-gene signature according to a seventh embodiment;
[0065] FIG. **5**A shows a ROC analysis of the 10-gene signature compared to OGTT according to an eighth embodiment;
[0066] FIG. **5**B shows a ROC analysis of the 10-gene signature compared to FPG vs. OGTT according to a ninth embodiment; and
[0067] FIG. **5**C depicts bar chart of the mean expression of the 10-gene signature according to a tenth embodiment

DETAILED DESCRIPTION

[0068] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art. The following definitions are provided to help interpret the disclosure and claims of this application. In the event a definition in this section is not consistent with definitions elsewhere, the definition set forth in this section will control.
[0069] Furthermore, the practice of the invention employs, unless otherwise indicated, conventional molecular biological and immunological techniques within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature. See, e.g., Colignan, Dunn, Ploegh, Speicher and Wingfield "Current protocols in Protein Science" (1999-2008) Volume I and II, including all supplements (John Wiley & Sons Inc.); and Bailey, J. E. and Ollis, D. F., Biochemical Engineering Fundamentals, McGraw-Hill Book Company, NY, 1986; Ausubel, et aL, ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2008), including all supplements; Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); and Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989). ROC analysis is reviewed in "An Introduction to ROC Analysis" by Tom Fawcett, Pattern Recognition Letters 27 (2006) 861-874.
[0070] As used herein, "Diabetes Mellitus" refers to any disease characterized by a high concentration of blood glucose (hyperglycemia). Diabetes mellitus is diagnosed by demonstrating any one of the following: a fasting plasma glucose level at or above 126 mg/dL (7.0 mmol/1) or a plasma glucose at or above 200 mg/dL (11.1 mmol/l) two hours after a 75 g oral glucose load as in a glucose tolerance test or symptoms of hyperglycemia and casual plasma glucose at or above 200 mg/dL (11.1 mmol/l).
[0071] As used herein, diabetes refers to "type 1 diabetes" also known as childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes (IDDM) or "type 2 diabetes" also known as adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes (NIDDM) or others forms of diabetes include gestational diabetes, insulin-resistant type 1 diabetes (or "double diabetes"), latent autoimmune diabetes of adults (or LADA) and maturity onset diabetes of the young (MODY) which is a group of several single gene (monogenic) disorders with strong family histories that present as type 2 diabetes before 30 years of age.
[0072] As used herein, a "diabetic disease state" refers to a pre-diabetic disease state, intermediate diabetic disease states characterized by stages of the disease more advanced then the pre-diabetic disease state and to disease states characteristic of overt diabetes as defined herein, including type I or II diabetes.
[0073] As used herein, a "pre-diabetic disease state" is one where a patient has an impaired fasting glucose level and impaired glucose tolerance. An impaired fasting glucose is defined as a blood glucose level from 100 to 125 mg/dL (6.1 and 7.0 mmol/l) i.e. an impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL or 7.8 mmol/l, but not over 200, two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance.
[0074] As used herein, a "medical professional" is a physician or trained medical technician or nurse at a point-of-care facility.
[0075] A "point-of-care" facility can be at an inpatient location such as in a hospital or an outpatient location such as a doctor's office or a walk-in clinic. In one embodiment, the diagnostic assay may be distributed as a commercial kit to consumers together with instruments for the analysis of gene signature expression profile in a blood sample. In another embodiment, the commercial kit may be combined with instruments and reagents for the monitoring of blood glucose levels.
[0076] The term "blood glucose level" refers to the concentration of glucose in blood. The normal blood glucose level (euglycemia) is approximately 120 mg/dl. This value fluctuates by as much as 30 mg/dl in non-diabetics.
[0077] The condition of "hyperglycemia" (high blood sugar) is a condition in which the blood glucose level is too high. Typically, hyperglycemia occurs when the blood glucose level rises above 180 mg/dl.
[0078] As used herein, a "test sample" is any biological sample from a patient that contains cells that differentially express genes in response to a diabetic disease state. The biological sample can be any biological material isolated from an atopic or non-atopic mammal, such as a human, including a cellular component of blood, bone marrow, plasma, serum, lymph, cerebrospinal fluid or other secretions such as tears, saliva, or milk; tissue or organ biopsy samples; or cultured cells. Preferably the biological sample is a cellular sample that can be collected from a patient with minimal intervention. In a preferred embodiment, a test sample is a blood sample or a preparation of PBMCs (peripheral blood mononuclear cells) or CD14+ monocytes or CD11b+or CD11c+ or Emr^{+} cells.
[0079] The mammal may be a human, or may be a domestic, companion or zoo animal. While it is particularly contemplated the herein described diagnostic tools are suitable for use in medical treatment of humans, they are also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as non-human primates, felids, canids, bovids, and ungulates.
[0080] As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (e.g., mRNA, rRNA, tRNA, or snRNA)

through "transcription" of the gene (e.g., via the enzymatic action of an RNA polymerase), and for protein encoding genes, into protein through "translation" of mRNA.

[0081] "Gene expression profile" refers to identified expression levels of at least one polynucleotide or protein expressed in a biological sample.

[0082] The term "primer" as used herein refers to an oligonucleotide either naturally occurring (e.g. as a restriction fragment) or produced synthetically, which is capable of acting as a point of initiation of synthesis of a primer extension product which is complementary to a nucleic acid strand (template or target sequence) when placed under suitable conditions (e.g. buffer, salt, temperature and pH) in the presence of nucleotides and an agent for nucleic acid polymerization, such as DNA dependent or RNA dependent polymerase. A primer must be sufficiently long to prime the synthesis of extension products in the presence of an agent for polymerization. A typical primer contains at least about 10 nucleotides in length of a sequence substantially complementary or homologous to the target sequence, but somewhat longer primers are preferred. Usually primers contain about 15-26 nucleotides.

[0083] As used herein, a "gene signature" refers to a pattern of gene expression of a selected set of genes that provides a unique identifier of a biological sample. A gene signature is diagnostic of a diabetic disease state if the pattern of gene expression of the selected set of genes is a substantial match to a gene signature in a reference sample taken from a patient with a diabetic disease state. For purposes of this application, a "gene signature" may be a pre-determined combination of nucleic acid or polypeptide sequences (if the genes are protein-coding genes). Gene signatures may comprise genes of unknown function or genes with no open reading frames including, but not limited to, rRNA, UsnRNA, microRNA or tRNAs.

[0084] As used herein, a "diagnostic gene expression profile" refers to the gene expression profile of a gene signature in a biological sample taken from a patient diagnosed with a particular disease state. The disease state can be a diabetic disease state or a non-diabetic disease state. A "substantial match" between a test gene expression profile from the patient and a diagnostic gene expression profile characteristic of a diabetic disease state indicates the patient has a diabetic disease state. Alternatively, a "substantial match" between a test gene expression profile from the patient and a diagnostic gene expression profile characteristic of a non-diabetic disease state indicates the patient does not have a diabetic disease state.

[0085] As used herein, a "variant" of a gene means gene sequences that are at least about 75% identical thereto, more preferably at least about 85% identical, and most preferably at least 90% identical and still more preferably at least about 95-99% identified when these DNA sequences are, compared to a nucleic acid sequence of the prevalent wild type gene. In one embodiment, a variant of a gene is a gene with one or more alterations in the DNA sequence of the gene including, but not limited to, point mutations or single nucleotide polymorphisms, deletions, insertions, rearrangements, splice donor or acceptor site mutations and gene alterations characteristic of a pseudogenes. Throughout this application a gene implicitly includes both wild type and variants forms of the gene as defined herein.

[0086] As used herein, a "substantial match" refers to the comparison of the gene expression profile of a gene signature in a test sample with the gene expression profile of the gene signature in a reference sample taken from a patient with a defined disease state. The expression profiles are "substantially matched" if the expression of the gene signature in the test sample and the reference sample are at substantially the same levels. i.e., there is no statistically significant difference between the samples after normalization of the samples. In one embodiment, the confidence interval of substantially matched expression profiles is at least about 50% or from about 50% to about 75% or from about 75% to about 80% or from about 80% to about 85% or from about 85% to about 90% or from about 90% to about 95%. In a preferred embodiment, the confidence interval of substantially matched expression profiles is about 95% to about 100%. In another preferred embodiment, the confidence interval of substantially matched expression profiles is any number between about 95% to about 100%. In another preferred embodiment, the confidence interval of substantially matched expression profiles is about 95% or about 96% or about 97% or about 98% or about 99%, or about 99.9%.

[0087] As used herein, a "substantial difference" refers to the difference in the gene expression profile of a gene at one time point with the gene expression profile of the same gene signature at a second time point. The expression profiles are "substantially different" if the expression of the gene signature at the first and second time points are at different levels i.e. there is a statistically significant difference between the samples after normalization of the samples. In one embodiment, expression profiles are "substantially different" if the expression of the gene signature at the first and second time points are outside the calculated confidence interval. In one embodiment, the confidence interval of substantially different expression profiles is less than about 50% or less than about 75% or less than about 80% or less than from about 85% or less than about 90% or less than about 95%.

[0088] A 95% confidence interval CI is equal to AUC+1.96 x standard error of AUC, where AUC is the area under the ROC Curve.

[0089] As used herein, "ROC" refers to a receiver operating characteristic, or simply ROC curve, which is a graphical plot of the sensitivity vs. (1—specificity) for a binary classifier system as its discrimination threshold is varied.

[0090] As used herein, the terms "diagnosis" or "diagnosing" refers to the method of distinguishing one diabetic disease state from another diabetic disease state, or determining whether a diabetic disease state is present in an patient (atopic) relative to the "normal" or "non-diabetic" (non-atopic) state, and/or determining the nature of a diabetic disease state.

[0091] As used herein, "determining a diabetic disease state" refers to an integration of all information that is useful in diagnosing a patient with a diabetic disease state or condition and/or in classifying the disease. This information includes, but is not limited to family history, human genetics data, BMI, physical activity, metabolic disease profile and the results of a statistical analysis of the expression profiles of one or more gene signatures in a test sample taken from a patient. In the point-of-care setting, this information is analyzed and displayed by a computer system having appropriate data analysis software. Integration of the clinical data provides the attending physician with the information needed to determine if the patient has a diabetic condition, information related to the nature or classification of diabetes as well as information related to the prognosis and/or information useful in selecting

an appropriate treatment. In one embodiment, the diagnostic assays, described herein, provide the medical professional with a determination of the efficacy of the prescribed medical treatment

[0092] As used herein, a "metabolic disease profile" refers to any number of standard metabolic measures and other risk factors that can be diagnostic of a diabetic disease state including, but not limited to fasting plasma glucose, insulin, pro-insulin, c-peptide, intact insulin, BMI, waist circumference, GLP-1, adiponectin, PAI-1, hemoglobin Alc, HDL, LDL, VLDL, triglycerides, free fatty acids. The metabolic disease profile can be used to generate a superior model for classification equivalence to 2-hr OGTT.

[0093] A glucose tolerance test is the administration of glucose to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia. The glucose is most often given orally so the common test is technically an oral glucose tolerance test (OGTT).

[0094] The fasting plasma glucose test (FPG) is a carbohydrate metabolism test which measures plasma, or blood, glucose levels after a fast.. Fasting stimulates the release of the hormone glucagon, which in turn raises plasma glucose levels. In people without diabetes, the body will produce and process insulin to counteract the rise in glucose levels. In people with diabetes this does not happen, and the tested glucose levels will remain high.

[0095] As used herein, the body mass index (BMI), or Quetelet index, is a statistical measurement which compares a person's weight and height. Due to its ease of measurement and calculation, it is the most widely used diagnostic tool to identify obesity.

[0096] As used herein, NGT means Normal Glucose Tolerance, IGT means Impaired Glucose Tolerance and T2D means type 2 diabetes.

[0097] As used herein, $CD11c^+$, $CD11b^+$ and Emr^+ are cell surface markers of human monocyte/macrophage and myeloid cells and their precursors. In mice, the most commonly used monocyte/macrophage and myeloid cell surface markers are F4/80 and CD11b, although F4/80 and CD11b antibodies have been reported to react with eosinophils and dendritic cells and NK and other T and B cell subtypes, respectively (Nguyen, et al. (2007) J Biol Chem 282, 35279-35292; Patsouris, et al. (2008) Cell Metab. 8, 301-309). The F4/80 gene in mouse is the ortholog to the human Emr1 gene. The human ortholog for the mouse CD11c gene is ITGAX also called integrin, alpha X (complement component 3 receptor 4 subunit), SLEB6, OTTHUMP00000163299; leu M5, alpha subunit; leukocyte surface antigen p150,95, alpha subunit; myeloid membrane antigen, alpha subunit; p150 95 integrin alpha chain (Chromosome: 16; Location: 16p11.2 Annotation: Chromosome 16, NC_000016.8 (31274010 . . . 31301819) MIM: 151510, GeneID:3687). The human ortholog for the mouse CD11b gene is ITGAM or integrin, alpha M (complement component 3 receptor 3 subunit) also called CD11B, CR3A, MAC-1, MAC1A, MGC117044, MO1A, SLEB6, macrophage antigen alpha polypeptide; neutrophil adherence receptor alpha-M subunit (Chromosome: 16; Location: 16p11.2 Chromosome 16, NC_000016.8 (31178789 . . . 31251714), MIM: 120980, GeneID: 3684). $CD11c^+$, $CD11b^+$ and Emr^+ and $CD14^+$ cells can be purified from PBMCs by positive selection using the appropriate human blood cell isolation kit (StemCell Technologies). Purity of isolated cells populations (>85%) is then confirmed by flow cytometry staining of fluorescent-conjugated antibodies to the appropriate cell surface mnrker (BioLegend).

[0098] As used herein, "real-time PCR" refers to real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (Q-PCR/qPCR) or kinetic polymerase chain reaction. Real-time PCR is a laboratory technique based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of a specific sequence in a DNA sample.

[0099] As used herein, an immunochemical assay is a biochemical test that measures the concentration of a substance in a cellular extract using the reaction of an antibody or antibodies to its antigen. In this disclosure, the antigen is a protein expressed by anyone of the protein coding genes comprising a gene signature. In a preferred embodiment, the immunochemical assay is an Enzyme-Linked ImmunoSorbent Assay (ELISA).

[0100] As used herein, "specific oligonucleotide hybridization" refers to hybridization between probe sequences on a solid support such as a chip and cDNA sequences generated from transcripts within the patient's test sample. If the two nucleic acid sequences are substantially complementary, hybridization occurs which is directly proportional to the amount of cDNA sequences in the test sample. Detection of hybridization is then achieved using techniques well known in the art. Numerous factors influence the efficiency and selectivity of hybridization of two nucleic acids, for example, a nucleic acid member on a array, to a target nucleic acid sequence. These factors include nucleic acid member length, nucleotide sequence and/or composition, hybridization temperature, buffer composition and potential for steric hindrance in the region to which the nucleic acid member is required to hybridize. A positive correlation exists between the nucleic acid member length and both the efficiency and accuracy with which a nucleic acid member will anneal to a target sequence. In particular, longer sequences have a higher melting temperature (TM) than do shorter ones, and are less likely to be repeated within a given target sequence, thereby minimizing promiscuous hybridization. Hybridization temperature varies inversely with nucleic acid member annealing efficiency, as does the concentration of organic solvents, e.g., formamide, that might be included in a hybridization mixture, while increases in salt concentration facilitate binding. Under stringent annealing conditions, longer nucleic acids, hybridize more efficiently than do shorter ones, which are sufficient under more permissive conditions.

[0101] As used herein, the term "antibody" includes both polyclonal and monoclonal antibodies; and may be an intact molecule, a fragment thereof (such as Fv, Fd, Fab, Fab' and F(ab)'2 fragments, or multimers or aggregates of intact molecules and/or fragments; and may occur in nature or be produced, e.g., by immunization, synthesis or genetic engineering.

[0102] As used herein, all references to probes in Tables 1, 7, 8, 9, 10A and 10B refer to probe sets represented on the GeneChip Human Genome U133 Plus 2.0 Array.

[0103] The following description relates to certain embodiments of the application, and to a particular methodology for diagnosing a diabetic disease state in a patient. In particular, the application discloses a number of genes, including some which had not previously been considered to be associated

with a diabetic disease state, are differentially expressed in peripheral blood mononuclear cells (PMBC) from patients which have a diabetic or pre-diabetic disease state as compared to patients who do not have a diabetic disease state.

[0104] In one embodiment, genes that are differentially expressed in PBMCs of NGTs and T2Ds are identified using microarray analysis. Transcripts from PBMCs of NGT and T2D patients (in this example, a cohort of 107 patients) were initially screened using the Affymetrix Human Genome HG-U133Plus2 chip, according to the manufacturer's instructions. Approximately 200 differentially expressed genes were selected which had a False Discovery Rate, FDR<20%, fold change >1.7 between NGTs and T2Ds using the Significance Analysis of Microarray (SAM) program (see TABLE 1).

[0105] Methods of diabetes classification are now described by determining the differential expression of different combinations of diabetes susceptibility genes identified in the initial microarray screen (see TABLE 12A). Table 12A also includes the Genbank Accession Numbers of each of the selected genes.

[0106] Gene expression of diabetes susceptibility genes may be measured in a biological sample using a number of different techniques. For example, identification of mRNA from the diabetes-associated genes within a mixture of various mRNAs is conveniently accomplished by the use of reverse transcriptase-polymerase chain reaction (RT-PCR) and an oligonucleotide hybridization probe that is labeled with a detectable moiety.

[0107] First a test sample is collected from a patient. To obtain high quality RNA it is necessary to minimize the activity of RNase liberated during cell lysis. This is normally accomplished by using isolation methods that disrupt tissues and inactivate or inhibit RNases simultaneously. For specimens low in endogenous ribonuclease, isolation protocols commonly use extraction buffers contain detergents to solubilize membranes, and inhibitors of RNase such as placental ribonuclease inhibitor or vanadyl-ribonucleoside complexes. RNA isolation from more challenging samples, such as intact tissues or cells high in endogenous ribonuclease, requires a more aggressive approach. In these cases, the tissue or cells are quickly homogenized in a powerful protein denaturant (usually guanidinium isothiocyanate), to irreversibly inactivate nucleases and solubilize cell membranes. If a tissue sample can not be promptly homogenized, it must be rapidly frozen by immersion in liquid nitrogen, and stored at −80° C. Samples frozen in this manner must never be thawed prior to RNA isolation or the RNA will be rapidly degraded by RNase liberated during the cell lysis that occurs during freezing. The tissue must be immersed in a pool of liquid nitrogen and ground to a fine powder using mortar and pestle. Once powdered, the still-frozen tissue is homogenized in RNA extraction buffer. A number of kits for RNA isolation are now commercially available (Ambion, Quiagen).

[0108] As is well known in the art, cDNA is first generated by first reverse transcribing a first strand of cDNA from a template mRNA using a RNA dependent DNA polymerase and a primer. Reverse transcriptases useful according to the application include, but are not limited to, reverse transcriptases from HIV, HTLV-1, HTLV-II, FeLV, FIV, SIV, AMV, MMTV, MoMuLV and other retroviruses (for reviews, see for example, Levin, 1997, Cell 88:5-8; Verma, 1977, Biochim. Biophys. Acta 473:1-38; Wu et al., 1975, CRC Crit. Rev. Biochem. 3:289-347). More recently, a number of kits are now commercially available for RT-PCR reactions using thermostable reverse transcriptase, e.g. GeneAinp® Thermostable rTth Reverse Transcriptase RNA PCR Kit (Applied Biosystems).

[0109] "Polymerase chain reaction," or "PCR," as used herein generally refers to a method for amplification of a desired nucleotide sequence in vitro, as described in U.S. Pat. Nos. 4,683,202, 4,683,195, 4,800,159, and 4,965,188, the contents of which are hereby incorporated herein in their entirety. The PCR reaction involves a repetitive series of temperature cycles and is typically performed in a volume of 10-100 μl. The reaction mix comprises dNTPs (each of the four deoxynucleotides dATP, dCTP, dGTP, and dTTP), primers, buffers, DNA polymerase, and nucleic acid template. The PCR reaction comprises providing a set of polynucleotide primers wherein a first primer contains a sequence complementary to a region in one strand of the nucleic acid template sequence and primes the synthesis of a complementary DNA strand, and a second primer contains a sequence complementary to a region in a second strand of the target nucleic acid sequence and primes the synthesis of a complementary DNA strand, and amplifying the nucleic acid template sequence employing a nucleic acid polymerase as a template-dependent polymerizing agent under conditions which are permissive for PCR cycling steps of (i) annealing of primers required for amplification to a target nucleic acid sequence contained within the template sequence, (ii) extending the primers wherein the nucleic acid polymerase synthesizes a primer extension product.

[0110] Other methods of amplification include, but are not limited to, ligase chain reaction (LCR), polynucleotide-specific based amplification (NSBA).

[0111] Primers can readily be designed and synthesized by one of skill in the art for the nucleic acid region of interest. It will be appreciated that suitable primers to be used with the application can be designed using any suitable method. Primer selection for PCR is described, e.g., in U.S. Pat. No. 6,898,531, issued May 24, 2005, entitled "Algorithms for Selection of Primer Pairs" and U.S. Ser. No. 10/236,480, filed Sep. 5,. 2002; for short-range PCR, U.S. Ser. No. 10/341,832, filed Jan. 14, 2003 provides guidance with respect to primer selection. Also, there are publicly available programs such as "Oligo", LASERGENE®, primer premier 5 (available at the website of the company Premier Biosoft) and primer3 (available at the website of the Whitehead Institute for Biomedical Research, Cambridge, Mass., U.S.A). Primer design is based on a number of parameters, such as optimum melting temperature (Tm) for the hybridization conditions to be used and the desired length of the oligonucleotide probe. In addition, oligonucleotide design attempts to minimize the potential secondary structures a molecule might contain, such as hairpin structures and dimmers between probes, with the goal being to maximize availability of the resulting probe for hybridization. In a preferred embodiment, the primers used in the PCR method will be complementary to nucleotide sequences within the cDNA template and preferably over exon-intron boundaries.

[0112] In one embodiment, the PCR reaction can use nested PCR primers.

[0113] In one embodiment, a detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorexcein (6-FAM), 2',7'-dimethoxy-4',5'-

dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine(ROX), 6-carboxy-2',4',7',4,7-hexachlorolluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. 32P, 35S, 3H; as well as others. The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, or the like having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

[0114] In a particularly preferred embodiment the application utilizes a combined PCR and hybridization probing system so as to take advantage of assay systems such as the use of FRET probes as disclosed in US patents U.S. Pat. Nos. 6,140,054 and 6,174,670, the entirety of which are also incorporated herein by reference. In one of its simplest configurations, the FRET or "fluorescent resonance energy transfer" approach employs two oligonucleotides which bind to adjacent sites on the same strand of the nucleic acid being amplified. One oligonucleotide is labeled with a donor fluorophore which absorbs light at a first wavelength and emits light in response, and the second is labeled with an acceptor fluorophore which is capable of fluorescence in response to the emitted light of the first donor (but not substantially by the light source exciting the first donor, and whose emission can be distinguished from that of the first fluorophore). In this configuration, the second or acceptor fluorophore shows a substantial increase in fluorescence when it is in close proximity to the first or donor fluorophore, such as occurs when the two oligonucleotides come in close proximity when they hybridise to adjacent sites on the nucleic acid being amplified, for example in the annealing phase of PCR, forming a fluorogenic complex. As more of the nucleic acid being amplified accumulates, so more of the fluorogenic complex can be formed and there is an increase in the fluorescence from the acceptor probe, and this can be measured. Hence the method allows detection of the amount of product as it is being formed.

[0115] It will also be appreciated by those skilled in the art that detection of amplification can be carried out using numerous means in the art, for example using TaqMan™ hybridisation probes in the PCR reaction and measurement of fluorescence specific for the target nucleic acids once sufficient amplification has taken place. TaqMan Real-time PCR measures accumulation of a product via the fluorophore during the exponential stages of the PCR., rather than at the end point as in conventional PCR. The exponential increase of the product is used to determine the threshold cycle, CT, i.e. the number of PCR cycles at which a significant exponential increase in fluorescence is detected, and which is directly correlated with the number of copies of DNA template present in the reaction.

[0116] Although those skilled in the art will be aware that other similar quantitative "real-time" and homogenous nucleic acid amplification/detection systems exist such as those based on the TaqMan approach (see U.S. Pat. Nos. 5,538,848 and 5,691,146, the entire contents of which are incorporated herein by reference.), fluorescence polarisation assays (e.g. Gibson et al., 1997, Clin Chem., 43: 1336-1341), and the Invader assay (e.g. Agarwal et al., Diagn Mol Pathol 2000 September; 9(3): 158-164; Ryan D et al, Mol Diagn 1999 June; 4(2): 135-144). Such systems would also be adaptable for use in the described application, enabling real-time monitoring of nucleic acid amplification.

[0117] In another embodiment of the application, matrices or microchips are manufactured to contain an array of loci each containing a oligonucleotide of known sequence. In this disclosure, each locus contains a molar excess of selected immobilized synthetic oligomers synthesized so as to contain complementary sequences for desired portions of a diabetes susceptibility gene. Transcripts of diabetes susceptibility genes present in PBMC are amplified by RT-PCR and labeled, as described herein. The oligomers on the microchips are then hybridized with the labeled RT-PCR amplified diabetes susceptibility gene nucleic acids. Hybridization occurs under stringent conditions to ensure that only perfect or near perfect matches between the sequence embedded in the microchip and the target sequence will occur during hybridization. The resulting fluorescence at each locus is proportional to the expression level of the one or more diabetes susceptibility gene in the PBMCs.

[0118] In other embodiments of the application, gene signature expression profiles of protein-coding genes are determined using techniques well known in the art of immunochemistry including, for example, antibody-based binding assays such as ELISA or radioimmunoassays or protein arrays containing antibodies directed to the protein products of genes within a pre-determined signature as defined herein.

[0119] In one embodiment, the expression profiles of the TCF7L2 and CLC genes were analyzed in peripheral blood mononuclear cells from normal glucose tolerant and type 2 diabetic patients.

[0120] The human Charcot-Leyden crystal protein gene is expressed primarily in eosinophils. CLC is down regulated sequentially in PBMC of NGTs to IGTs to T2Ds. The mean signal intensities of its expression in microarray of the 107-patient cohort are listed In TABLE 2 below. Receiver operating characteristic (ROC) analysis demonstrated that the CLC gene expression level can be used to separate NGTs from IGTs/T2Ds.

TABLE 2

CLC gene expression in PBMCs isolated from NGTs, IGTs and T2Ds

NGT	IGT	T2D
1504	900	410

[0121] The performance of CLC gene in predicting the clinical status was further examined using a receiver operating characteristic (ROC) analysis. An ROC curve shows the relationship between sensitivity and specificity. That is, an increase in sensitivity will be accompanied by a decrease in specificity. The closer the curve follows the left axis and then the top edge of the ROC space, the more accurate the test. Conversely, the closer the curve comes to the 45-degree diagonal of the ROC graph, the less accurate the test. The area under the ROC is a measure of test accuracy. The accuracy of the test depends on how well the test separates the group being tested into those with and without the disease in question. An area under the curve (referred to as "AUC") of 1 represents a perfect test, while an area of 0.5 represents a less useful test. Thus, preferred genes and diagnostic methods of the present application have an AUC greater than 0.50, more preferred tests have an AUC greater than 0.60, more preferred tests have an AUC greater than 0.70.

[0122] The area under curve (AUC) was calculated as a measure of the performance of CLC gene in predicting patient status. Receiver operating characteristic (ROC) analysis of the CLC gene date demonstrated that the CLC gene expression level can be used to separate NGTs from IGTs/T2Ds (see FIG. **1**).

[0123] Genetic variants in the gene encoding for transcription factor-7-like 2 (TCF7L2) have been strongly associated with a risk for developing type 2 diabetes and impaired β-cell insulin function (see US 2006/0286588, the contents of which are hereby incorporated herein in their entirety). Genome-wide association studies implicate SNPs within TCF7L2 give the highest lifetime risk score for predicting type 2 diabetes progression compared to SNPs in other marker genes, including CDKAL1, CDKN2A/2B, FTO, IGF2BP2, and SLC30A8 (range of risk scores 1.12-1.20). TCF7L2 is widely expressed and this transcription factor is known to respond to developmental signals from members of the Wnt family of proteins. Functional and genetic studies point to a critical role for TCF7L2 in the development of the intestine and proglucagon gene expression in entero endocrine cells.

[0124] To ascertain if TCF7L2 and the CLC gene are diagnostic markers of diabetes, either individually or in combination, 180 subjects were recruited from the German population in association with the Institute for Clinical Research & Development (IKFE), in Mainz, Germany. Appropriate IRB approvals were obtained prior to patient sample collection. The inclusion criteria consisted of patients between 18-75 years and a body-mass index (BMI) 30 who had no previous diagnosis of diabetes, and the legal capacity and ability to understand the nature and extent of the clinical study and the required procedures. The exclusion criteria consisted of blood donation within the last 30 days, insulin dependent diabetes mellitus, lactating or pregnant women, or women who intend to become pregnant during the course of the study, sexually active women not practicing birth control, history of severe/ multiple allergies, drug or alcohol abuse, and lack of compliance to study requirements. AU clinical measurements including the 75 g-oral glucose tolerance test results (OGTT) were obtained using standard procedures.

[0125] Blood samples were drawn by venipuncture into CPT tubes (BD Biosciences). PBMCs were isolated according to manufacturer's protocol and the final cell pellet was resuspended in 1 ml of Trizol (Invitrogen), and stored at −80° C. Subsequently, total RNA was purified using manufacturer's protocol and resuspended in DEPC-treated ddH_2O. RNA quantification and quality was performed using the ND-1000 Spectrophotometer (NanoDrop) and reconfirmed by spectrophotometric quantitation with RiboGreen kit (Molecular Probes). The quality of RNA templates was measured by using the Bioanalyzer 2100 (Agilent Technologies).

[0126] First-strand cDNA synthesis was performed using 200 ng of total RNA from each patient PBMC sample using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Afterwards, the reaction mixture was diluted 10-fold with ddH_2O, and 4 μl was used as template in a 10 μl Taqman PCR reaction on the ABI Prism 7900HT sequence detection system. The reaction components consisted of 2× Taqman PCR master mix (Applied Biosystems), 0.9 μM of each primer, and 0.25 μM of fluorescent-labeled probe (Biosearch Technologies). Sequences for primer/probe sets used in RT-PCR Taqman assay are presented in Table 3.

[0127] Cycling conditions for reverse transcription step were as follows:

	Step 1	Step 2	Step 3	Step 4
Temp (C.°)	25	37	85	4
Time	10 min.	120 min.	5 sec.	∞

TABLE 3

Probe and Primer sequences used in Taqman assays for TCF7L2, CLC and ACTIN

Marker set #	5' > 3'-Sequence	Sequence Name	Comment
TCF7L2 set 1	ACCTGAGCGCTCCTAAGAAATG	TCF7L2_DL_F1	NOT over junction
	AGGGCCGCAGCAGTTATTC	TCF7L2_DL_R1	NOT over junction
	FAM-AGCGCGCTTTGGCCTTGATCAAC-BHQ1	TCF7L2_DL_Pro1	NOT over junction
TCF7L2 set 2	CGTCGACTTCTTGGTTACATTCC	TCF7L2_DL_F2	NOT over junction
	CACGACGCTAAAGCTATTCTAAAGAC	TCF7L2_DL_R2	NOT over junction
	FAM-CAGCCGCTGTCGCTCGTCACC-BHQ1	TCF7L2_DL_Pro2	NOT over junction
TCF7L2 set 3	GAAAGCGCGGCCATCAAC	TCF7L2_1564_U18	Over junction
	CAGCTCGTAGTATTTCGCTTGCT	TCF7L2_1644_L23	Over junction
	FAM-TCCTTGGGCGGAGGTGGCATG-BHQ1	TCF7L2_1586_P21	Over junction
CLC set 1	GCTACCCGTGCCATACACAGA	CLC_85_U21	Over junction
	GCAGATATGGTTCATTCAAGAAACA	CLC_185_L25	Over junction
	FAM-TTCTACTGTGACAATCAAAGGGCGACCA-BHQ1	CLC_127_P28	Over Junction
ACTIN	CCTGGCACCCAGCACAAT	B-actin-1F	Internal Control
	GCCGATCCACACGGAGTACTT	B-actin-1R	
	FAM-ATCAAGATCATTGCTCCTCCTGAGCGC-BHQ1	B-actin P	

[0128] Quantitative real-time RT-PCR by Taqman assay using two different primer/probe combinations specific for TCF7L2 and one prime/probe set for CLC, was performed on RNA isolated from PBMCs from individual patients. Ther-

mocycling profile for PCR step was as follows, 95° C. for 10 min, followed by 40 cycles of 95° C. (15 sec) and 60° C. (1 min). Ct values were calculated from the raw data using the software SDS version 2.1 (Applied Biosystems), with threshold set at 0.2 to 0.3. Run-to run reproducibility by Pearson correlation was $R^2$0.96-0.98 for the above-mentioned markers. The delta Ct (cycle threshold) value was calculated by subtracting the Ct of the housekeeping β-actin gene from the Ct of marker of interest, for instance, TCF7L2 (Ct TCF7L2-Ct actin). The value of $[2^{-(delta\ Ct)}\times 1000]$ was used to represent the expression of TCF7L2 relative to β-actin. The OGTT result was used as the true clinical status. Student's T-test was used for determining statistical significance between expression levels of gene markers using normalized Ct values.

[0129] Based on the 2-hr OGTT measurement and current ADA guidelines, of the 180 patients enrolled in the study, 104 patients were classified as NGT (Normal Glucose Tolerance), 49 patients as IGT (impaired glucose tolerance) and 27 patients were considered T2D (type 2 diabetes). Because the T2D subjects were diagnosed with diabetes for the first time in this study, the duration of the disease for each patient, and how long the PBMCs were sustained in a hyperglycemic microenvironment was unknown.

[0130] T-test analysis for expression levels of TCF7L2 and CLC normalized to β-actin for each patient, based on primer/probe sets values and separated by glucose tolerance, is depicted in Table 4. The NGT and IGT plus T2D patient groups had a statistically significant difference between expression levels by Student's T-test, with p-values=0.004; 0.021 and 0.022 for TCF7L2 set 1, set 3 and CLC, respectively. These results indicate differential expression of the TCF7L2 and CLC genes in PBMCs of pre-diabetic (IGT) patients or pre-diabetic and T2D patients combined together compared to NGT.

TABLE 4

Statistical difference in expression levels of TCF7L2 and CLC by Student's T-test

Sample n	T-test	Bactin Ct	Normalized TCF7L2_1	Normalized TCF7L2_2	Normalized TCF7L2_3	Normalized CLC
104/49	NGT/IGT	0.451	0.005	0.075	0.008	0.013
104/76	NGT/(IGT + T2D)	0.277	0.004	0.093	0.021	0.022
			Statistically significant differentiation			

[0131] Next, the performance of the TCF7L2 and CLC Taqman assays as a diagnostic tool for the classification of patients as normal or pre-diabetic/diabetic, compared to the 2-hr OGTT was assessed. Receiver Operating Characteristic (ROC) curves for each TCF7L2 and CLC primer/probe set normalized delta Ct value were generated (Table 5, FIGS. **2**A and **2**B). The AUC values for the TCF7L2 set 1 and CLC PCR assays were 0.63 and 0.61, respectively. Compared to the 2-hr OGTT classification, TCF7L2 set 1 expression from PBMCs can correctly classify a patient as being normal or pre-diabetic/diabetic with an AUC of 0.73 when used in conjunction with the FPG test (FIGS. **2**A and **2**B). CLC did not have an additive value to TCF7L2 set 1 and was not considered for the diagnostic algorithm. Additionally, exclusion of 14 patients that had FPG≧126mg/dL (also considered diabetic) did not change the performance of the assay.

TABLE 5

ROC curve AUC values for each marker probe-primer set and in combination with FPG values

	ROC AUC value	
Marker set #	Marker vs. OGTT	Marker + FPG vs. OGTT
TCF7L2 set 1	0.63	0.73
TCF7L2 set 2	0.59	ND
TCF7L2 set 3	0.61	0.72
CLC set 1	0.60	0.69

[0132] In one embodiment, each of the genes selected in the microarray analysis (see TABLE 1) may be combined with the performance of TCF7L2 set 1 to more closely match the 2-hr OGTT result.

[0133] In another embodiment, genes that are strongly associated with a risk of type 2 diabetes (see TABLE 6) may also be combined with the performance of TCF7L2 set 1 to more closely match the 2-hr OGTT result. In another embodiment, the genes of Table 6 in combination with one or more genes of TABLE 1 can be tested as described herein for gene signatures that are diagnostic of a diabetic disease state.

TABLE 6

Gene Symbols			
NOTCH2	IGF2BP2	LGR5	CDKN2A-2B
THADA	WFS1	FTO	HHEX-IDE
PPARG	KCNJ11	JAZF1	CDC123
ADAMTS9	TSPAN8	SLC30A8	CAMK1D

[0134] In another embodiment, the expression of CDKN1C, a member of the CIP/KIP family was also differentially expressed in PBMCs from NGTs and T2Ds.

[0135] The CIP/KIP family consists of three members, CDKN2A, CDKN2B and CDKN1C. All of the three members can inhibit the activity of CDK4, which plays a central role in regulating mammalian cell cycle. Islet β-cell replication plays an essential role in maintaining β-cell mass homeostasis. It has been known that CDK4 has an important role in the regulation of body weight and pancreatic β-cell proliferation. In mice, loss of the CDK4 gene resulted in insulin-deficient diabetes due to the reduction of β-cell mass whereas activation of CDK4 caused β-islet cell hyperplasia. Recently, genome-wide association studies of type 2 diabetes have revealed that nucleotide variation near CDKN2A and CDKN2B genes is associated with type 2 diabetes risk. In addition, over-expression of CDKN2A leads to decreased islet proliferation in aging mice and over-expression of

CDKN2B is related to islet hypoplasia and diabetes in murine models. CDKN1C is a maternally expressed gene located on chromosome 11p15.5 and is involved in the pathogenesis of Beckwith-Wiedemann syndrome (BWS), a disorder characterized by neonatal hyperinsulinemic hypoglycemia, as well as pre- and postnatal overgrowth. Recent studies also showed that CDKN1C is down-regulated by insulin and variants of CDKN1C may be associated to increased birth weights in type 2 diabetes patients. In addition to regulating the cell cycle, the CIP/KIP family plays an important role in other biological processes, such as apoptosis, transcription regulation, differentiation and cell migration. The expression of the three genes in the 107 patient cohort was analyzed. Only CDKN1C displayed differential expression among NGTs, IGTs and T2Ds (see TABLE 7). There are 5 probes expressing in PBMC for CDKN1C on the HG-U133Plus2 GeneChip. Each of them displayed differential expression between NGTs and IGTs/T2Ds (TABLE 7). ROC analysis showed that expression levels of the 5 probes can be used to separate NGTs from T2Ds (FIG. **3**).

TABLE 7

CDKN1C gene expression in PBMCs isolated from NGTs, IGTs and T2Ds

Gene	Probe	Expression in PBMC	Mean_NGT	Mean_IGT	Mean_T2D
CDKN1C	213182_x_at	Yes	935	1178	1784
	213183_s_at	Yes	531	712	624
	213348_at	Yes	2648	3246	3957
	216894_x_at	Yes	797	1030	1439
	219534_x_at	Yes	1092	1356	1973

[0136] In a person of ordinary skill in the art will recognize that the described embodiments provide a premise to investigate gene signatures as a diagnostic tool of diabetes. To investigate the underlying biological processes between normal subjects and pre-diabetes and diabetes patients, pathway analysis was conducted. Namely, the probes on HG-U133Plus2 chip were mapped to Gene Ontology Biological Process (GOBP) as described by Yu et al. BMC Cancer 7:182 (2007). Since genes with very low expression tend to have higher variations, genes whose mean intensity is less than 200 in the dataset were removed from pathway analysis. As a result, 21247 probes were retained. To identify pathways that have significant association with the development of pre-diabetes or diabetes, global test program was run by comparing NGT vs. IGT, NGT vs. T2D, or NGT vs. IGT+T2D. The pathways that have at least 10 probes and a significant p value ($p<0.05$) were identified for each comparison. There were 3 pathways that had consistent association with the patient outcomes through the three comparisons. They are B cell activation (GO0042113), humoral immune response (GO0006959), and DNA unwinding during replication (GO0006268). Among the 3 pathways, B cell activation and humoral immune response have dominantly negative association with diabetes (lower expression in IGT/T2D) whereas DNA unwinding during replication has positive association with diabetes (higher expression in IGT/T2D).

[0137] To build a pathway-based gene signature from the 3 key pathways, genes with a $p<0.05$ were pooled and sorted based on their statistical significance (z score from Global Test). If a gene has more than one probe in the list and their behaviors were consistent, the one with the highest significance was retained. If a gene has more than one probe in the list and their behaviors were opposite, all probes for this gene were removed. As a result, 14 unique genes were obtained (SEE TABLE 8 below).

TABLE 8

Pathway Significant Genes

PSID	Gene Symbol	Gene Title
208900_s_at	TOP1	topoisomerase (DNA) I
216379_x_at	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
222430_s_at	YTHDF2	YTH domain family, member 2
1554343_a_at	BRDG1	BCR downstream signaling 1
228592_at	MS4A1	membrane-spanning 4-domains, subfamily A, member 1

TABLE 8-continued

Pathway Significant Genes

PSID	Gene Symbol	Gene Title
216894_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
1558662_s_at	BANK1	B-cell scaffold protein with ankyrin repeats 1
205267_at	POU2AF1	POU domain, class 2, associating factor 1
205859_at	LY86	lymphocyte antigen 86
221969_at	PAX5	Paired box gene 5 (B-cell lineage specific activator)
207655_s_at	BLNK	B-cell linker
206126_at	BLR1	Burkitt lymphoma receptor 1, GTP binding protein (chemokine (C—X—C motif) receptor 5)
206983_at	CCR6	chemokine (C-C motif) receptor 6
204946_s_at	TOP3A	topoisomerase (DNA) III alpha
214252_s_at	CLN5	ceroid-lipofuscinosis, neuronal 5

[0138] To build a signature using genes with relatively high variation, 10 genes with a CV>0.25 were retained. To determine the optimal number of genes for a signature, combination of top 2-10 genes were examined in the dataset. The result indicated that the top 3 genes gave the best performance in the prediction of patients' outcomes. The 3 genes, TOP1, CD24 and STAP1 below in TABLE 9.

TABLE 9

3 gene expression in PBMCs isolated from NGTs, IGTs and T2Ds

Top 3 genes from pathway analysis

Probe	Gene Symbol	Gene Title
208900_s_at	TOP1	topoisomerase (DNA) I
216379_x_at	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
1554343_a_at	STAP1	signal transducing adaptor family member 1

The mean expression of the top 3 genes in subgroups

Probe	Gene	Mean_NGT	Mean_IGT	Mean_T2D
208900_s_at	TOP1	868	1145	1418
216379_x_at	CD24	1767	1274	1194
1554343_a_at	STAP1	373	283	265

[0139] The ROC analysis of the 3-gene signature in the 107-patient cohort (FIG. **4**A and **4**B) demonstrates this signature can separate NGTs from IGTs/T2Ds. A histogram depicting the mean expression of the genes is shown in FIG. **4**C.

[0140] To remove non-informative genes, only genes that had 10 or more presence calls in the cohort were retained. The 107-patient cohort was then divided into a 54-patient training set and a 53-patient test set. Based on OGTT classification, there are 28 NGTs, 17 IGTs and 9 T2Ds in the training set whereas there are 29 NGTs, 16 IGTs and 8 T2Ds in the test set. To identify genes that have differential expression between NGT and IGT+T2D patients, Significant Analysis of Microarray (SAM) program was performed. Genes were selected if the False Discovery Rate (FDR) is lower than 20%. As a result, 235 genes were selected. To further narrow down the gene list, genes with the fold-change larger than 1.5 between the two groups, and the average intensity of the gene in the dataset is larger than 200 were retained. As a result, 17 probe sets were obtained. Among them, 4 were probes representing hemoglobin gene. Considering that hemoglobin has extremely high expression in red blood cells, the 4 probes were removed to eliminate possible contamination. To determine the optimal number of genes as a signature, performance of combination of the top genes were examined from 2 to 13 in the training set. The result indicated that the top 10 genes gave the best performance based on the area under curve (AUC) (see Table 10).

TABLE 10

A: 10 gene expression in PBMCs isolated from NGTs, IGTs and T2Ds

Probe	Symbol	Title
239742_at	TULP4	Tubby like protein 4
244450_at	AA741300	Weakly similar to ALU8_HUMAN ALU SUBFAMILY SX SEQUENCE
235216_at	ESCO1	establishment of cohesion 1 homolog 1
201026_at	EIF5B	eukaryotic translation initiation factor 5B
200727_s_at	ACTR2	ARP2 actin-related protein 2 homolog
211993_at	WNK1	WNK lysine deficient protein kinase 1
205229_s_at	COCH	coagulation factor C homolog, cochlin
201085_s_at	SON	SON DNA binding protein
1557227_s_at	TPR	translocated promoter region (to activated MET oncogene)
231798_at	NOG	Noggin

TABLE 10-continued

B: The mean expression of the top 10 genes in subgroups

Probe	Gene	Mean_NGT	Mean_IGT	Mean_T2D
239742_at	TULP4	514	659	702
244450_at	AA741300	674	461	482
235216_at	ESCO1	199	262	351
201026_at	EIF5B	330	440	500
200727_s_at	ACTR2	2153	2751	3590
211993_at	WNK1	397	505	625
205229_s_at	COCH	330	231	250
201085_s_at	SON	3300	4103	4900
1557227_s_at	TPR	378	445	616
231798_at	NOG	515	430	302

[0141] To further evaluate the gene signature, patient outcomes in the test set were determined. Prediction of pre-diabetes and diabetes using plasma fasting glucose (FPG) levels was also examined. To investigate the complementary effect between the gene signature and FPG levels, combination of these two predictors were used to predict the patient outcomes. A comparison of ROC analyses among using FPG, or 10-gene signature, or combination of FPG and 10-gene signature in the test set is depicted in FIG. **5**. It demonstrates that the 10-gene signature can independently separate NTGs from IGTs/T2Ds, and the FPG and the 10-gene signature are complementary for better prediction (see FIGS. **5**A and **5**B). The mean expression signals of the 10 genes in the 107-patient cohort are shown in the table and bar chart in FIG. **5**C.

[0142] The statistical analysis of the clinical data identified a 3 gene and 10 signature that are differentially expressed in NGTs and T2D.

[0143] In another embodiment, a diagnostic assay is described for the point-of-care classification of normal versus pre-diabetes/ diabetes or for the prediction of progression to pre-diabetes/ diabetes over a defined period time, e.g. from ½ to 2 years or from 2 to 5 years, or from 5 to 10 years or more.

[0144] Alternatively gene expression profiles are determined by detection of the protein encoded by the mRNA, for example using ELISA or proteomic array. All of these methods are well known in the art.

[0145] The disclosure herein also provides for a kit format which comprises a package unit having one or more reagents for the diagnosis of a diabetic disease state in a patient. The kit may also contain one or more of the following items: buffers, instructions, and positive or negative controls. Kits may include containers of reagents mixed together in suitable proportions for performing the methods described herein. Reagent containers preferably contain reagents in unit quantities that obviate measuring steps when performing the subject methods.

[0146] The kit may include sterile needles and tubes/ containers for the collection of a patient's blood. Collection tubes will typically contain certain additives e.g. heparin to inhibit blood coagulation

[0147] Kits may also contain reagents for the measurement of a gene signature expression profile in a patient's sample. As disclosed herein, gene signatures expression profiles may be measured by a variety of means known in the art including RT-PCR assays, oligonucleotide based assays using microchips or protein based assays such as ELISA assays.

[0148] In a preferred embodiment, gene signature expression profiles are measured by real-time RT-PCR.

[0149] In one embodiment of the application, the kit comprises primers of the amplification and detection of gene signature expression profiles in a patient's blood sample. Primers may have a sequence that is complementary to any one of the diabetes susceptibility genes as defined herein including TOP1, CD24, STAP1, TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR, NOG genes or any one of the genes listed in Tables 1 or 6.

[0150] Examples of primer sequences used for the real-time RT-PCR of diabetes susceptibility genes are disclosed in Tables 12B and 12C.

[0151] In a preferred embodiment, the kit reagents are designed to function with the 7500 Fast Dx Real-Time PCR Instrument by Applied Biosystems, which is a PCR-based technology that was approved by the FDA's Office of In Vitro Diagnostics (FDA-OIVD).

[0152] In yet another embodiment, the kit includes a microchip comprising an array of hybridization probes for the 3 gene (TOP1, CD24 and STAP1) or 10 gene (TULP4, AA741300, ESCO1, EIF5B, ACTR2; WNK1, COCH, SON, TPR and NOG) signatures. In another aspect, the microchips may further comprise an array of one or more hybrization probes for one or more of the genes listed in Tables 1 or 6.

[0153] In a preferred embodiment, the microchips are designed to function with Affymetrix GeneChipDx technology that can measure, in parallel, the gene expression of 1 to more than 55,000 mRNAs. FDA-OIVD this platform for use with the AmpliChip P450 product from Roche Molecular Diagnostics and the Pathwork Diagnostics Tissue of Origin test.

TABLE 1

Probe	Gene Symbol	Gene Title
218659_at	ASXL2	additional sex combs like 2 (*Drosophila*)
230528_s_at	MGC2752	hypothetical protein MGC2752
211921_x_at	PTMA	prothymosin, alpha (gene sequence 28) /// prothymosin, alpha (gene sequence 28)
209102_s_at	HBP1	HMG-box transcription factor 1
239946_at	KIAA0922	KIAA0922 protein
226741_at	TMEM85	Transmembrane protein 85
239742_at	TULP4	Tubby like protein 4
202844_s_at	RALBP1	ralA binding protein 1
237768_x_at	TAF15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68 kDa
202373_s_at	RAB3GAP2	RAB3 GTPase activating protein subunit 2 (non-catalytic)
223413_s_at	LYAR	hypothetical protein FLJ20425
222371_at	PIAS1	Protein inhibitor of activated STAT, 1
244450_at	MAK	Male germ cell-associated kinase
201024_x_at	EIF5B	eukaryotic translation Initiation factor 5B
202615_at	GNAQ	Guanine nucleotide binding protein (G protein), q polypeptide
222621_at	DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1
212774_at	ZNF238	zinc finger protein 238
238883_at	THRAP2	Thyroid hormone receptor associated protein 2
223130_s_at	MYLIP	myosin regulatory light chain interacting protein
225445_at	—	Transcribed locus
235601_at	MAP2K5	Mitogen-activated protein kinase kinase 5
209258_s_at	CSPG6	chondroitin sulfate proteoglycan 6 (bamacan)
1557238_s_at	SETD5	SET domain containing 5
202927_at	PIN1	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1
1568618_a_at	GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)
222417_s_at	SNX5	sorting nexin 5
208836_at	ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide
202738_s_at	PHKB	phosphorylase kinase, beta
224872_at	KIAA1463	KIAA1463 protein
235200_at	ZNF561	Zinc finger protein 561
235216_at	ESCO1	establishment of cohesion 1 homolog 1 (*S. cerevisiae*)
201026_at	EIF5B	eukaryotic translation initiation factor 5B
208095_s_at	SRP72	signal recognition particle 72 kDa
244457_at	ITPR2	Family with sequence similarity 20, member C
216563_at	ANKRD12	Ankyrin repeat domain 12
211983_x_at	ACTG1	actin, gamma 1
227854_at	FANCL	Fanconi anemia, complementation group L
1552343_s_at	PDE7A	phosphodiesterase 7A
221548_s_at	ILKAP	integrin-linked kinase-associated serine/threonine phosphatase 2C
215772_x_at	SUCLG2	succinate-CoA ligase, GDP-forming, beta subunit
229010_at	CBL	Cas-Br-M (murine) ecotropic retroviral transforming sequence
226879_at	MGC15619	hypothetical protein MGC15619
1556451_at	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2
225490_at	ARID2	AT rich interactive domain 2 (ARID, RFX-like)
214055_x_at	BAT2D1	BAT2 domain containing 1
32069_at	N4BP1	Nedd4 binding protein 1
235457_at	MAML2	mastermind-like 2 (*Drosophila*)
217985_s_at	BAZ1A	bromodomain adjacent to zinc finger domain, 1A
229399_at	C10orf118	chromosome 10 open reading frame 118
208994_s_at	PPIG	peptidyl-prolyl isomerase G (cyclophilin G)
202656_s_at	SERTAD2	SERTA domain containing 2
241917_at	FCHSD2	FCH and double SH3 domains 2
238807_at	ANKRD46	Ankyrin repeat domain 46
204415_at	G1P3	interferon, alpha-inducible protein (clone IFI-6-16)

TABLE 1-continued

Probe	Gene Symbol	Gene Title
240176_at	LOC391426	Similar to ENSANGP00000004103
233284_at	—	—
232583_at	—	—
200772_x_at	PTMA	prothymosin, alpha (gene sequence 28)
239721_at	UBE2H	Ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)
218607_s_at	SDAD1	SDA1 domain containing 1
204160_s_at	ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)
243303_at	ECHDC1	Enoyl Coenzyme A hydratase domain containing 1
225266_at	ZNF652	Zinc finger protein 652
220072_at	CSPP1	centrosome and spindle pole associated protein 1
234196_at	TMCC3	Transmembrane and coiled-coil domain family 3
222616_s_at	USP16	ubiquitin specific peptidase 16
201274_at	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5
238714_at	RAB12	RAB12, member RAS oncogene family
204563_at	SELL	selectin L (lymphocyte adhesion molecule 1)
1557239_at	BBX	Bobby sox homolog (*Drosophila*)
232510_s_at	DPP3	dipeptidylpeptidase 3
235653_s_at	THAP6	THAP domain containing 6
200727_s_at	ACTR2	ARP2 actin-related protein 2 homolog (yeast)
221564_at	HRMT1L1	HMT1 hnRNP methyltransferase-like 1 (*S. cerevisiae*)
211993_at	WNK1	WNK lysine deficient protein kinase 1 /// WNK lysine deficient protein kinase 1
201114_x_at	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7
233089_at	QRSL1	glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1
212991_at	FBXO9	F-box protein 9
227770_at	VPS4A	Vacuolar protein sorting 4A (yeast)
222111_at	FAM63B	Family with sequence similarity 63, member B
1558604_a_at	—	MRNA; clone CD 43T7
205229_s_at	COCH	coagulation factor C homolog, cochlin (*Limulus polyphemus*)
219130_at	FLJ10287	hypothetical protein FLJ10287
241262_at	—	—
202412_s_at	USP1	ubiquitin specific peptidase 1
225092_at	RABEP1	rabaptin, RAB GTPase binding effector protein 1
200905_x_at	HLA-E	major histocompatibility complex, class I, E
201010_s_at	TXNIP	thioredoxin interacting protein
221607_x_at	ACTG1	actin, gamma 1
201085_s_at	SON	SON DNA binding protein
214723_x_at	KIAA1641	KIAA1641
201565_s_at	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
201861_s_at	LRRFIP1	leucine rich repeat (in FLII) interacting protein 1
207785_s_at	RBPSUH	recombining binding protein suppressor of hairless (*Drosophila*)
230415_at	—	—
236620_at	RIF1	RAP1 interacting factor homolog (yeast)
206363_at	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
1558748_at	NAPE-PLD	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D
223101_s_at	ARPC5L	actin related protein 2/3 complex, subunit 5-like
236370_at	SMURF1	SMAD specific E3 ubiquitin protein ligase 1
200702_s_at	DDX24	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24
1557227_s_at	TPR	translocated promoter region (to activated MET oncogene)
220934_s_at	MGC3196	hypothetical protein MGC3196
233333_x_at	AVIL	advillin
231798_at	NOG	Noggin
228986_at	OSBPL8	oxysterol binding protein-like 8
241786_at	PPP3R1	Protein phosphatase 3 (formerly 2B), regulatory subunit B, 19 kDa, alpha isoform (calcineurin B, type I)
212227_x_at	EIF1	eukaryotic translation initiation factor 1
222471_s_at	KCMF1	potassium channel modulatory factor 1
203580_s_at	SLC7A6	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6
208900_s_at	TOP1	topoisomerase (DNA) I
240070_at	FLJ39873	hypothetical protein FLJ39873
213305_s_at	PPP2R5C	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
229470_at	—	CDNA FLJ27196 fis, clone SYN02831
204048_s_at	PHACTR2	phosphatase and actin regulator 2
1561690_at	—	CDNA clone IMAGE: 5303966
1556728_at	—	CDNA FLJ43665 fis, clone SYNOV4006327
212027_at	RBM25	RNA binding motif protein 25
210218_s_at	SP100	nuclear antigen Sp100
232356_at	—	CDNA FLJ13539 fis, clone PLACE1006640
241891_at	DOCK8	Dedicator of cytokinesis 8
235925_at	LOC440282	Hypothetical protein LOC145783
211745_x_at	HBA1	hemoglobin, alpha 1 /// hemoglobin, alpha 1
240452_at	GSPT1	G1 to S phase transition 1
212669_at	CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma
209791_at	PADI2	peptidyl arginine deiminase, type II
221952_x_at	TRMT5	TRM5 tRNA methyltransferase 5 homolog (*S. cerevisiae*)

TABLE 1-continued

Probe	Gene Symbol	Gene Title
226942_at	PHF20L1	PHD finger protein 20-like 1
203939_at	NT5E	5'-nucleotidase, ecto (CD73)
208705_s_at	EIF5	eukaryotic translation initiation factor 5
1557718_at	PPP2R5C	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
212251_at	MTDH	metadherin
226384_at	PPAPDC1B	phosphatidic acid phosphatase type 2 domain containing 1B
212487_at	KIAA0553	KIAA0553 protein
227402_s_at	C8orf53	chromosome 8 open reading frame 53
221875_x_at	HLA-F	major histocompatibility complex, class I, F
225506_at	KIAA1468	KIAA1468
201730_s_at	TPR	translocated promoter region (to activated MET oncogene)
235645_at	ESCO1	establishment of cohesion 1 homolog 1 (*S. cerevisiae*)
208993_s_at	PPIG	peptidyl-prolyl isomerase G (cyclophilin G)
233690_at	C21orf96	Chromosome 21 open reading frame 96
221798_x_at	RPS2	Ribosomal protein S2
1569898_a_at	—	CDNA FLJ32047 fis, clone NTONG2001137
202368_at	TRAM2	translocation associated membrane protein 2
215128_at	—	—
230761_at	USP7	Unknown protein
243_g_at	MAP4	microtubule-associated protein 4
223081_at	PHF23	PHD finger protein 23
224736_at	CCAR1	cell division cycle and apoptosis regulator 1
236962_at	PTBP2	Polypyrimidine tract binding protein 2
225893_at	—	MRNA; cDNA DKFZp686D04119 (from clone DKFZp686D04119)
244414_at	MAML2	Mastermind-like 2 (*Drosophila*)
221234_s_at	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2 /// BTB and CNC homology 1, basic leucine zipper transcription factor 2
218135_at	PTX1	PTX1 protein
229353_s_at	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1
228408_s_at	SDAD1	SDA1 domain containing 1
234723_x_at	—	—
212130_x_at	EIF1	eukaryotic translation initiation factor 1
232565_at	RAB6IP2	RAB6 interacting protein 2
210479_s_at	RORA	RAR-related orphan receptor A
226320_at	THOC4	THO complex 4
208859_s_at	ATRX	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, *S. cerevisiae*)
238645_at	VIL2	Villin 2 (ezrin)
243578_at	—	Transcribed locus
202868_s_at	POP4	processing of precursor 4, ribonuclease P/MRP subunit (*S. cerevisiae*)
224585_x_at	ACTG1	actin, gamma 1
221768_at	SFPQ	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)
1557459_at	SNF1LK2	SNF1-like kinase 2
225583_at	UXS1	UDP-glucuronate decarboxylase 1
225125_at	TMEM32	transmembrane protein 32
202408_s_at	PRPF31	PRP31 pre-mRNA processing factor 31 homolog (yeast)
236355_s_at	LOC439993	LOC439993
209458_x_at	HBA1 /// HBA2	hemoglobin, alpha 1 /// hemoglobin, alpha 1 /// hemoglobin, alpha 2 /// hemoglobin, alpha 2
211948_x_at	BAT2D1	BAT2 domain containing 1
203682_s_at	IVD	isovaleryl Coenzyme A dehydrogenase
203184_at	FBN2	fibrillin 2 (congenital contractural arachnodactyly)
1560082_at	NOL10	Nucleolar protein 10
212794_s_at	KIAA1033	KIAA1033
226159_at	LOC285636	hypothetical protein LOC285636
225276_at	GSPT1	G1 to S phase transition 1
205859_at	LY86	lymphocyte antigen 86
200977_s_at	TAX1BP1	Tax1 (human T-cell leukemia virus type I) binding protein 1
239418_x_at	ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1
208638_at	PDIA6	protein disulfide isomerase family A, member 6
203228_at	PAFAH1B3	platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit 29 kDa
208812_x_at	HLA-C	major histocompatibility complex, class I, C
220924_s_at	SLC38A2	solute carrier family 38, member 2
235705_at	—	—
208974_x_at	KPNB1	karyopherin (importin) beta 1
201854_s_at	ASCIZ	ATM/ATR-Substrate Chk2-Interacting Zn2+-finger protein
209116_x_at	HBB	hemoglobin, beta /// hemoglobin, beta
218150_at	ARL5	ADP-ribosylation factor-like 5
208042_at	AGGF1	angiogenic factor with G patch and FHA domains 1
226718_at	AMIGO1	adhesion molecule with Ig-like domain 1
235328_at	CCDC41	Coiled-coil domain containing 41
225609_at	GSR	glutathione reductase
242972_at	—	CDNA FLJ46556 fis, clone THYMU3039807
239811_at	MLL5	Myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, *Drosophila*)
201027_s_at	EIF5B	eukaryotic translation initiation factor 5B
233742_at	MGC2654	LP8272

TABLE 1-continued

Probe	Gene Symbol	Gene Title
1556323_at	CUGBP2	CUG triplet repeat, RNA binding protein 2
202926_at	NAG	neuroblastoma-amplified protein
220966_x_at	ARPC5L	actin related protein 2/3 complex, subunit 5-like /// actin related protein 2/3 complex, subunit 5-like
1552302_at	MGC20235	hypothetical protein MGC20235
238787_at	—	Transcribed locus
213505_s_at	SFRS14	splicing factor, arginine/serine-rich 14
1555920_at	CBX3	Chromobox homolog 3 (HP1 gamma homolog, *Drosophila*)
207186_s_at	FALZ	fetal Alzheimer antigen
210426_x_at	RORA	RAR-related orphan receptor A
1559993_at	—	—
201602_s_at	PPP1R12A	protein phosphatase 1, regulatory (inhibitor) subunit 12A
216088_s_at	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7
236254_at	VPS13B	vacuolar protein sorting 13B (yeast)
204731_at	TGFBR3	transforming growth factor, beta receptor III (betaglycan, 300 kDa)
202269_x_at	GBP1	guanylate binding protein 1, interferon-inducible, 67 kDa /// guanylate binding protein 1, interferon-inducible, 67 kDa
216981_x_at	SPN	sialophorin (gpL115, leukosialin, CD43)
212007_at	UBXD2	UBX domain containing 2
217755_at	HN1	hematological and neurological expressed 1
213940_s_at	FNBP1	formin binding protein 1
201831_s_at	VDP	vesicle docking protein p115
225041_at	HSMPP8	M-phase phosphoprotein, mpp8
1552584_at	IL12RB1	interleukin 12 receptor, beta 1
206133_at	BIRC4BP	XIAP associated factor-1
229625_at	GBP5	Guanylate binding protein 5
206500_s_at	C14orf106	chromosome 14 open reading frame 106
201881_s_at	ARIH1	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (*Drosophila*)
202323_s_at	ACBD3	acyl-Coenzyme A binding domain containing 3
204021_s_at	PURA	purine-rich element binding protein A
215313_x_at	HLA-A	major histocompatibility complex, class I, A
207966_s_at	GLG1	golgi apparatus protein 1
235461_at	FLJ20032	hypothetical protein FLJ20032
223983_s_at	C19orf12	chromosome 19 open reading frame 12
202021_x_at	EIF1	eukaryotic translation initiation factor 1
231577_s_at	GBP1	guanylate binding protein 1, interferon-inducible, 67 kDa
218927_s_at	CHST12	carbohydrate (chondroitin 4) sulfotransferase 12

TABLE 11

Gene	Description (functions, domains)
	SELECTED GENES
TCF7L2	The TCL7L2 gene product is a high mobility group (HMG) box-containing transcription factor implicated in blood glucose homeostasis. High mobility group (HMG or HMGB) proteins are a family of relatively low molecular weight non-histone components in chromatin. HMG1 (also called HMG-T in fish) and HMG2 are two highly related proteins that bind single-stranded DNA preferentially and unwind double-stranded DNA. Although they have no sequence specificity, they have a high affinity for bent or distorted DNA, and bend linear DNA. HMG1 and HMG2 contain two DNA-binding HMG-box domains (A and B) that show structural and functional differences, and have a long acidic C-terminal domain rich in aspartic and glutamic acid residues. The acidic tail modulates the affinity of the tandem HMG boxes in HMG1 and 2 for a variety of DNA targets. HMG1 and 2 appear to play important architectural roles in the assembly of nucleoprotein complexes in a variety of biological processes, for example V(D)J recombination, the initiation of transcription, and DNA repair.
CLC	The protein encoded by this gene is a lysophospholipase expressed in eosinophils and basophils. It hydrolyzes lysophosphatidylcholine to glycerophosphocholine and a free fatty acid. This protein may possess carbohydrate or IgE-binding activities. It is both structurally and functionally related to the galectin family of beta-galactoside binding proteins. It may be associated with inflammation and some myeloid leukemias. Galectins (previously S-lectins) bind exclusively beta-galactosides like lactose. They do not require metal ions for activity. Galectins are found predominantly, but not exclusively in mammals. Their function is unclear. They are developmentally regulated and may be involved in differentiation, cellular regulation and tissue construction.
CDKN1C	protein encoded by this gene is a tight-binding, strong inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation. Mutations in this gene are implicated in sporadic cancers and Beckwith-Wiedemann syndorome, suggesting that this gene is a tumor suppressor candidate. Three transcript variants encoding two different isoforms have been found for this gene.
	3 GENE SIGNATURE
TOP1	This gene encodes a DNA topoisomerase, an enzyme that controls and alters the topologic states of DNA during transcription. DNA topoisomerases regulate the number of topological links between two DNA strands (i.e. change the number of superhelical turns) by catalysing transient single- or double-strand breaks, crossing the strands through one another, then resealing the breaks. These enzymes have several functions: to remove DNA supercolls during transcription and DNA replication; for strand breakage during recombination; for chromosome condensation; and to disentangle intertwined DNA during mitosis. DNA topoisomerases are divided into two classes: type I enzymes break single-strand DNA, and type II enzymes break double-strand DNA. Type I topoisomerases are ATP-independent enzymes (except for reverse gyrase), and can be subdivided according to their

TABLE 11-continued

Gene	Description (functions, domains)
	structure and reaction mechanisms: type IA (bacterial and archaeal topoisomerase I, topoisomerase III and reverse gyrase) and type IB (eukaryotic topoisomerase I and topoisomerase V). These enzymes are primarily responsible for relaxing positively and/or negatively supercolled DNA, except for reverse gyrase, which can introduce positive supercoils into DNA. The crystal structures of human topoisomerase I comprising the core and carboxyl-terminal domains in covalent and noncovalent complexes with 22-base pair DNA duplexes reveal an enzyme that "clamps" around essentially B-form DNA. The core domain and the first eight residues of the carboxyl-terminal domain of the enzyme, including the active-site nucleophile tyrosine-723, share significant structural similarity with the bacteriophage family of DNA integrases. A binding mode for the anticancer drug camptothecin has been proposed on the basis of chemical and biochemical information combined with the three-dimensional structures of topoisomerase I-DNA complexes.
CD24	This gene encodes a sialoglycoprotein that is expressed on mature granulocytes and in many B cells. The encoded protein is anchored via a glycosyl phosphatidylinositol (GPI) link to the cell surface.
STAP1	The protein encoded by this gene functions as a docking protein acting downstream of Tec tyrosine kinase in B cell antigen receptor signaling. The protein is directly phosphorylated by Tec in vitro where it participates in a positive feedback loop, increasing Tec activity.
	10 GENE SIGNATURE
TULP4	Tubby like protein 4 contains WD40 and SOCS domains. WD-40 repeats (also known as WD or beta-transducin repeats) are short ~40 amino acid motifs, often terminating in a Trp-Asp (W-D) dipeptide. WD-containing proteins have 4 to 16 repeating units, all of which are thought to form a circularised beta-propeller structure. WD-repeat proteins are a large family found in all eukaryotes and are implicated in a variety of functions ranging from signal transduction and transcription regulation to cell cycle control and apoptosis. The underlying common function of all WD-repeat proteins is coordinating multi-protein complex assemblies, where the repeating units serve as a rigid scaffold for protein interactions. The specificity of the proteins is determined by the sequences outside the repeats themselves. Examples of such complexes are G proteins (beta subunit is a beta-propeller), TAFII transcription factor, and E3 ubiquitin ligase. The SOCS box was first identified in SH2-domain-containing proteins of the suppressor of cytokines signaling (SOCS) family but was later also found in: the WSB (WD-40-repeat-containing proteins with a SOCS box) family, the SSB (SPRY domain-containing proteins with a SOCS box) family, the ASB (ankyrin-repeat-containing proteins with a SOCS box) family, and ras and ras-like GTPases. The SOCS box found in these proteins is an about 50 amino acid carboxy-terminal domain composed of two blocks of well-conserved residues separated by between 2 and 10 nonconserved residues. The C-terminal conserved region is an L/P-rich sequence of unknown function, whereas the N-terminal conserved region is a consensus BC box, which binds to the Elongin BC complex. It has been proposed that this association could couple bound proteins to the ubiquitination or proteasomal compartments.
AA741300	Unknown protein (New protein)
ESCO1	establishment of cohesion 1 homolog 1 (ESCO1) belongs to a conserved family of acetyltransferases involved in sister chromatid cohesion.
EIF5B	Accurate initiation of translation in eukaryotes is complex and requires many factors, some of which are composed of multiple subunits. The process is simpler in prokaryotes which have only three initiation factors (IF1, IF2, IF3). Two of these factors are conserved in eukaryotes: the homolog of IF1 is eIF1A and the homolog of IF2 is eIF5B. This gene encodes eIF5B. Factors eIF1A and eIF5B interact on the ribosome along with other initiation factors and GTP to position the initiation methionine tRNA on the start codon of the mRNA so that translation initiates accurately.
ACTR2	ARP2 actin-related protein 2 homolog (ACTR2) is known to be a major constituent of the ARP2/3 complex. This complex is located at the cell surface and is essential to cell shape and motility through lamellipodial actin assembly and protrusion. Two transcript variants encoding different isoforms have been found for this gene.
WNK1	The WNK1 gene encodes a cytoplasmic serine-threonine kinase expressed in distal nephron. Protein kinases are a group of enzymes that possess a catalytic subunit which transfers the gamma phosphate from nucleotide triphosphates (often ATP) to one or more amino acid residues in a protein substrate side chain, resulting in a conformational change affecting protein function. The enzymes fall into two broad classes, characterised with respect to substrate specificity: serine/threonine specific and tyrosine specific. Protein kinase function has been evolutionarily conserved from *Escherichia coli* to *Homo sapiens*. Protein kinases play a role in a mulitude of cellular processes, including division, proliferation, apoptosis, and differentiation. Phosphorylation usually results in a functional change of the target protein by changing enzyme activity, cellular location, or association with other proteins. The catalytic subunits of protein kinases are highly conserved, and several structures have been solved, leading to large screens to develop kinase-specific inhibitors for the treatments of a number of diseases. Eukaryotic protein kinases are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common with both serine/threonine and tyrosine protein kinases. There are a number of conserved regions in the catalytic domain of protein kinases. In the N-terminal extremity of the catalytic domain there is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. In the central part of the catalytic domain there is a conserved aspartic acid residue which is important for the catalytic activity of the enzyme.
COCH	The protein encoded by this gene is highly conserved in human, mouse, and chicken, showing 94% and 79% amino acid identity of human to mouse and chicken sequences, respectively. Hybridization to this gene was detected in spindle-shaped cells located along nerve fibers between the auditory ganglion and sensory epithelium. These cells accompany neurites at the habenula perforate, the opening through which neurites extend to innervate hair cells. This and the pattern of expression of this gene in chicken inner ear paralleled the histologic findings of acidophilic deposits, consistent with mucopolysaccharide ground substance, in temporal bones from DFNA9 (autosomal dominant nonsyndromic sensorineural deafness 9) patients. Mutations that cause DFNA9 have been reported in this gene. Alternative splicing results in multiple transcript variants encoding the same protein. Additional splice variants encoding distinct isoforms have been described but their biological validities have not been demonstrated. The protein contains a VWA domains in extracellular eukaryotic proteins mediate adhesion via metal ion-dependent adhesion sites (MIDAS). Intracellular VWA domains and homologues in prokaryotes have recently been identified. The proposed VWA domains in integrin beta subunits have recently been substantiated using sequence-based methods.
SON	The protein encoded by this gene binds to a specific DNA sequence upstream of the upstream regulatory sequence of the core promoter and second enhancer of human hepatitis B virus (HBV). Through this binding, it represses HBV core promoter activity, transcription of HBV genes, and production of HBV virions. The protein shows sequence similarities with other DNA-binding structural proteins such as gallin, oncoproteins of the MYC family, and the oncoprotein MOS. It may also be involved in protecting cells from apoptosis and in pre-mRNA splicing. Several transcript variants encoding different isoforms have been described for this gene, but the full-length nature of only two of them has been determined. Members of this family belong to the collagen

TABLE 11-continued

Gene	Description (functions, domains)
	superfamily. Collagens are generally extracellular structural proteins involved in formation of connective tissue structure. The sequence is predominantly repeats of the G-X-Y and the polypeptide chains form a triple helix. The first position of the repeat is glycine, the second and third positions can be any residue but are frequently proline and hydroxyproline. Collagens are post-translationally modified by proline hydroxylase to form the hydroxyproline residues. Defective hydroxylation is the cause of scurvy. Some members of the collagen superfamily are not involved in connective tissue structure but share the same triple helical structure.
TPR	This gene encodes a large coiled-coil protein that forms intranuclear filaments attached to the inner surface of nuclear pore complexes (NPCs). The protein directly interacts with several components of the NPC. It is required for the nuclear export of mRNAs and some proteins. Oncogenic fusions of the 5' end of this gene with several different kinase genes occur in some neoplasias. Intermediate filaments (IF) are proteins which are primordial components of the cytoskeleton and the nuclear envelope. They generally form filamentous structures 8 to 14 nm wide. IF proteins are members of a very large multigene family of proteins which has been subdivided in five major subgroups: Type I: Acidic cytokeratins. Type II: Basic cytokeratins. Type III: Vimentin, desmin, glial fibrillary acidic protein (GFAP), peripherin, and plasticin. Type IV: Neurofilaments L, H and M, alpha-internexin and nestin. Type V: Nuclear lamins A, B1, B2 and C. All IF proteins are structurally similar in that they consist of: a central rod domain comprising some 300 to 350 residues which is arranged in coiled-coiled alpha-helices, with at least two short characteristic interruptions; a N-terminal non-helical domain (head) of variable length; and a C-terminal domain (tall) which is also non-helical, and which shows extreme length variation between different IF proteins. While IF proteins are evolutionary and structurally related, they have limited sequence homologies except in several regions of the rod domain. This entry represents the central rod domain found in IF proteins.
NOG	The secreted polypeptide, encoded by this gene, binds and inactivates members of the transforming growth factor-beta (TGF-beta) superfamily signaling proteins, such as bone morphogenetic protein-4 (BMP4). By diffusing through extracellular matrices more efficiently than members of the TGF-beta superfamily, this protein may have a principal role in creating morphogenic gradients. The protein appears to have pleiotropic effect, both early in development as well as in later stages. It was originally isolated from *Xenopus* based on its ability to restore normal dorsal-ventral body axis in embryos that had been artificially ventralized by UV treatment. The results of the mouse knockout of the ortholog suggest that it is involved in numerous developmental processes, such as neural tube fusion and joint formation. Recently, several dominant human NOG mutations in unrelated families with proximal symphalangism (SYM1) and multiple synostoses syndrome (SYNS1) were identified; both SYM1 and SYNS1 have multiple joint fusion as their principal feature, and map to the same region (17q22) as this gene. All of these mutations altered evolutionarily conserved amino acid residues. The amino acid sequence of this human gene is highly homologous to that of *Xenopus*, rat and mouse. This family consists of the eukaryotic Noggin proteins. Noggin is a glycoprotein that binds bone morphogenetic proteins (BMPs) selectively and, when added to osteoblasts, it opposes the effects of BMPs. It has been found that noggin arrests the differentiation of stromal cells, preventing cellular maturation.

TABLE 12A

Gene symbol	GenBank Accession Number
CDKN1C	gi|169790897|ref|NM_000076.2|
TCF7L2	gi|170014695|ref|NM_030756.3
CLC	gi|20357558|ref|NM_001828.4|
WFS1	NM_006005
TSPAN8	NM_004616
THADA	NM_022065
TCF7L2	NM_030756
SLC30A8	NM_173851
PPARG	NM_138712
NOTCH2	NM_024408
LGR5	NM_003667
KCNJ11	NM_000525
JAZF1	NM_175061
IGF2BP2	NM_001007225
HHEX-IDE	NM_002729, NM_004969
FTO	NM_001080432
CDKN2B	NM_078487
CDKN2A	NM_058195
CDC123	NM_006023
CAMK1D	NM_153498

TABLE 12A-continued

Gene symbol	GenBank Accession Number
ADAMTS9	NM_182920
	3-gene signature
TOP1	NM_003286
CD24	NM_013230
STAP1	NM_012108
	10-gene signature
TULP4	NM_020245
AA741300	AA741300
ESCO1	NM_052911
EIF5B	NM_015904
ACTR2	NM_001005386
WNK1	NM_018979
COCH	NM_004086
SON	NM_138927
TPR	NM_003292
NOG	NM_005450

TABLE 12 B

3-gene signature Gene Symbol	Accession	Upper primer Sequence	Probe Sequence	Lower primer Sequence
TOP1	NM_003286	CCCTGTACTTCATCGACAAGC	AGCAGCAGCCCACAGTGT	AGAGCAGGCAATGAAAAGGAGGAAG
CD24	NM_013230	GCCAGGGCAATGATGAATG	CTCAATATGGATAATCAAGAGTTGCT	TCTACCCCCAGATCCAAGCAGCCT

TABLE 12 B-continued

STAP1	NM_012108	TGAAAAGAACTGTGCGAAATTC CACTTTCTGTGTTCTCTGTCTTCAG CCTTGTTTTGCCGAAAGAGGAAGTACA	
	STAP1-331F22	TGAAAAGAACTGTGCGAAATTC	
	STAP1-407R25	CACTTTCTGTGTTCTCTGTCTTCAG	
	STAP1-355P27	CCTTGTTTTGCCGAAAGAGGAAGTACA	
	CD24_996_U19	GCCAGGGCAATGATGAATG	
	CD24_1069_L26	CTCAATATGGATAATCAAGAGTTGCT	
	CD24_1019_P24	TCTACCCCCAGATCCAAGCAGCCT	
	TOP1_1679_U22	CCCTGTACTTCATCGACAAGC	
	TOP1_1762_118	AGCAGCAGCCCACAGTGT	
	TOP1_1708_P26	AGAGCAGGCAATGAAAAGGAGGAAG	

TABLE 12 C

10-gene signature

Gene Symbol	Upper primer Sequence	Probe Sequence	Lower primer Sequence
TULP4	GAAGAGTGTGTGTCTATGTGCATTTAAA	CAAGTTGCTCCATCTGATTCTTAAATT	CACATTCACACGGGAAGACAGGCTCA
AA741300	Not available		
ESCO1	CTAAACGGCAGCACAAAAGGA	CATGTCTTATGGCTAACACGTTTCTT	TGCAAACCAACAGACTCAGCAAACAAGG
EIF5B	CAGCCAAGGCATCAAGATCA	GAGCGCCATTGACAAGCAAT	TCATCCTTGGTGCTGTCTTCGCTCTTGTT
ACTR2	CATTCAACTCCAGGACATGGAA	TCCCCAAGACACCAGAATAAAACT	AGGCCTCTCTCTGCCCTTTGACTGGA
WNK1	GCATGCTTGAGATGGCTACATC	TGGTCACGCGACGGTAGAT	TCCTTACTCGGAGTGCCAAAATGCTGC
COCH	CCATTTAGGCAAATAAGCACTCCTT	GCCTCAGCAGTGTTTTTAACAAAG	AAGCCGCTGCCTTCTGGTTACAATTTACA
SON	GCTCTGCTCAGCCCTAAAGAAA	TCCTCAATATTGGCAGAAAATCCT	CCTCCCCCTCCTAAAGAGACACTGCCTG
TPR	CTGCCCAAGTCTGTCCAGAAC	CCTGACTGTGGGACAACCTCTT	ATCAGCAATCCGAGATCGATGGCCT
NOG	CACCCGGACACTTGATCGAT	GTTCATTGAAAACCCTCGCTAGA	ACCGCCTCCAACCAGTTCCACCAC

Gene Symbol	Primer sequences	Gene Symbol	Primer sequences
TULP4-F1	GAAGAGTGTGTGTCTATGTGCATTTAAA	COCH-F1	CCATTTAGGCAAATAAGCACTCCTT
TULP4-R1	CAAGTTGCTCCATCTGATTCTTAAATT	COCH-R1	GCCTCAGCAGTGTTTTTAACAAAG
TULP4-Pro1	CACATTCACACGGGAAGACAGGCTCA	COCH-Pro1	AAGCCGCTGCCTTCTGGTTACAATTTACA
ESCO1-F1	CTAAACGGCAGCACAAAAGGA	SON-F1	GCTCTGCTCAGCCCTAAAGAAA
ESCO1-R1	CATGTCTTATGGCTAACACGTTTCTT	SON-R1	TCCTCAATATTGGCAGAAAATCCT
ESCO1-Pro2	TGCAAACCAACAGACTCAGCAAACAAGG	SON-Pro1	CCTCCCCCTCCTAAAGAGACACTGCCTG
NOG-F1	CACCCGGACACTTGATCGAT	EIF5B-F1	CAGCCAAGGCATCAAGATCA
NOG-R1	GTTCATTGAAAACCCTCGCTAGA	EIF5B-R1	GAGCGCCATTGACAAGCAAT
NOG-Pro1	ACCGCCTCCAACCAGTTCCACCAC	EIF5B-Prot1	TCATCCTTGGTGCTGTCTTCGCTCTTGTT
WNK1-F1	GCATGCTTGAGATGGCTACATC	ACTR2-F1	CATTCAACTCCAGGACATGGAA
WNK1-R1	TGGTCACGCGACGGTAGAT	ACTR2-R1	TCCCCAAGACACCAGAATAAAACT
WNK1-Pro1	TCCTTACTCGGAGTGCCAAAATGCTGC	ACTR2-Pro1	AGGCCTCTCTCTGCCCTTTGACTGGA
		TPR-F1	CTGCCCAAGTCTGTCCAGAAC
		TPR-R1	CCTGACTGTGGGACAACCTCTT
		TPR-Prot	ATCAGCAATCCGAGATCGATGGCCT

SEQUENCE LISTING

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1. A method of diagnosing Diabetes Mellitus in a patient comprising the steps of:
a. providing a test sample taken from a patient;
b. measuring the gene expression profile of a gene signature comprising two or more genes selected from the group consisting of the TOP1, CD24 and STAP1 genes;
c. comparing said gene expression profile with a diagnostic gene expression profile of said gene signature;
d. determining a diabetic disease state in said patient based at least in part upon a substantial match between said gene expression profile and said diagnostic gene expression profile;
e. displaying said determination to a medical professional.

2. The method of claim 1, wherein said determining step is executed by a computer system, said computer system running one or more algorithms selected from the group consisting of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM).

3. The method of claim 2, wherein said determining step further comprises an analysis of the patient's metabolic disease profile.

4. The method of claim 1, wherein said gene signature further comprises one or more genes selected from the genes listed in TABLES 1 or 6.

5. The method of claim 1, wherein said diabetic disease state is a pre-diabetic disease state or a Type 2 Diabetes disease state.

6. The method of claim 1, wherein said test sample is a blood sample.

7. The method of claim 1, wherein said test sample comprises PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$ or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $CD14^+$ monocytes.

8. The method of claim 1, wherein said measuring step involves real-time PCR or an immunochemical assay or specific oligonucleotide hybridization.

9. A method of diagnosing Diabetes Mellitus in a patient comprising the steps of:
a. providing a test sample taken from a patient;
b. measuring the gene expression profile of a gene signature comprising two or more genes selected from the group consisting of the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes in said test sample; and
c. comparing said gene expression profile with a diagnostic gene expression profile, of said gene signature;
d. determining a diabetic disease state in said patient based at least in part upon a substantial match between said gene expression profile and said diagnostic gene expression profile; and
e. displaying said determination to a medical professional.

10. The method of claim 9, wherein said gene expression profile of a gene signature comprises the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes.

11. The method of claim 10, wherein said determining step is executed by a computer system, said computer system running one or more algorithms selected from the group consisting of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM).

12. The method of claim 9, wherein said determining step further comprises an analysis of the patient's metabolic disease profile.

13. The method of claim 9, wherein said diabetic disease state is a pre-diabetic disease state or a Type 2 Diabetes disease state.

14. The method of claim 9, wherein said test sample is a blood sample.

15. The method of claim 9, wherein said test sample comprises PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$] or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $CD14^+$ monocytes.

16. The method of claim 9, wherein said measuring step involves real-time PCR, an immunochemical assay or specific oligonucleotide hybridization.

17. A method of diagnosing Diabetes Mellitus in a patient comprising the steps of:
a. providing a test sample taken from a patient;
b. measuring the gene expression profile of a gene signature comprising the TCF7L2 and CLC genes in said test sample; and
c. comparing said gene expression profile with a diagnostic gene expression profile of said gene signature;
d. determining a diabetic disease state in said patient based at least in part upon a substantial match between said gene expression profile and said diagnostic gene expression profile; and
e. displaying said determination to a medical professional.

18. The method of claim 17, wherein said determining step is executed by a computer system, said computer system running one or more algorithms selected from the group consisting of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM).

19. The method of claim 18, wherein said determining step further comprises an analysis of the patient's metabolic disease profile.

20. The method of claim 17, wherein said gene signature further comprises one or more genes selected from the genes listed in TABLES 1 or 6.

21. The method of claim 17, wherein said diabetic disease state is a pre-diabetic disease state or a Type 2 Diabetes disease state.

22. The method of claim **17**, wherein said test sample is a blood sample.

23. The method of claim **17**, wherein said test sample comprises PBMCs or $CD11c^+$ or $CD11b^+$ or Emr^+ or [$CD11b^+$ $CD11c^+$ or [Emr^+ $CD11b^+$] or [Emr^+ $CD11c^+$] or [Emr^+ $CD11b^+$ $CD11c^+$] cells or $CD14^+$ monocytes.

24. The method of claim **17**, wherein said measuring step involves real-time PCR, an immunochemical assay or specific oligonucleotide hybridization.

25. A method of diagnosing a change in the diabetic disease state of a patient comprising the steps of:

a. providing a first test sample taken from a patient at a first time point;

b. measuring a first expression profile of a gene signature in said first test sample, said gene signature being selected from a first group of genes comprising the TOP1, CD24 and STAP1 genes or from a second group of genes comprising the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes;

c. providing a second test sample taken from said patient at a second time point;

d. measuring a second expression profile of said gene signature in said second test sample;

e. comparing said first expression profile with said second expression profile;

f. determining a change in the diabetic disease state in said patient based at least in part upon a substantial difference between said first gene expression profile and said second gene expression profile; and

g. displaying said determination to a medical professional.

26. The method of claim **25**, wherein said determining step is executed by a computer system, said computer system running one or more algorithms selected from the group consisting of Linear combination of gene expression signals, Linear regression model, Logistic regression model, Linear discrimination analysis (LDA) model, The nearest neighbor model and the Prediction Analysis of Microarrays (PAM).

27. The method of claim **25**, wherein said determining step further comprises an analysis of the patient's metabolic disease profile.

28. The method of claim **25**, wherein the time period between said first time point and said second time point is from 0 to 2 years or from ¼ to 2 years or from ½ to 2 years or from 2 to 5 years, or from 5 to 10 years or more.

29. The method of claim **25**, wherein said diabetic disease state is a pre-diabetic disease state or a Type 2 Diabetes disease state.

30. The method of claim **25**, wherein said first and second test samples are blood samples.

31. The method of claim **25**, wherein said measuring step involves real-time PCR, an immunochemical assay or specific oligonucleotide hybridization.

32. A kit for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus, the kit comprising:

a. reagents for collecting a test sample from a patient; and

b. reagents for measuring the expression profile of a gene signature comprising the TCF7L2 and CLC genes or variants thereof in a patient's test sample and packaging therefor.

33. The kit of claim **32**, wherein said reagents for collecting a test sample are reagents for collecting a blood sample.

34. The kit of claim **32**, said reagents for measuring the expression profile of a gene signature are reagents for real-time PCR or an immunochemical assay or specific oligonucleotide hybridization.

35. A kit for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus, the kit comprising:

a. reagents for collecting a test sample from a patient; and

b. reagents for measuring the gene expression profile of a gene signature comprising the TOP1, CD24 and STAP1 genes in a patient's test sample; and packaging therefor.

36. The kit of claim **35**, wherein said reagents for collecting a test sample are reagents for collecting a blood sample.

37. The kit of claim **35**, said reagents for measuring the expression profile of a gene signature are reagents for real-time PCR or an immunochemical assay or specific oligonucleotide hybridization.

38. A kit for assessing a patient's susceptibility to Diabetes in which the assessment is made with a test apparatus, the kit comprising:

a. reagents for collecting a test sample from a patient; and

b. reagents for measuring the gene expression profile of a gene signature comprising the TULP4, AA741300, ESCO1, EIF5B, ACTR2, WNK1, COCH, SON, TPR and NOG genes in a patient's test sample and packaging therefor.

39. The kit of claim **38**, wherein said reagents for collecting a test sample are reagents for collecting a blood sample.

40. The kit of claim **38**, said reagents for measuring the expression profile of a gene signature are reagents for real-time PCR or an immunochemical assay or specific oligonucleotide hybridization.

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摘要(译)

公开了用于鉴定在诊断患有前糖尿病疾病状态和明显的II型糖尿病的患者的PBMC中差异表达的基因组的方法。 3基因和10个基因标记，以准确地预测患者的糖尿病疾病状态。该申请还描述了用于在护理点的患者中快速诊断糖尿病疾病状态的试剂盒。