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(54) **Title**: PHENOTYPIC RATIO OF SERUM AMYLOID IN PRE- AND TYPE 2 DIABETES

(57) **Abstract**: The present invention is directed to diagnosing, determining, and/or monitoring type 2 diabetes, pre-diabetes, insulin resistance, and their related conditions by detecting levels and modulations of Serum amyloid A protein (SAA), SAA variants and/or the phenotypic ratio of SAA. The present invention is also directed to methods for identifying and evaluating therapeutic treatments for type 2 diabetes, pre-diabetes, insulin resistance, and their related conditions by monitoring SAA, SAA variants, and/or the phenotypic ration of SAA.

# PHENOTYPIC RATIO OF SERUM AMYLOID IN PRE- AND TYPE 2 DIABETES

## FIELD OF THE INVENTION

This invention relates to methods of determining, diagnosing, and/or assessing pre-diabetes, type 2 diabetes, insulin resistance and their elated conditions by detecting levels and modulations of the phenotypic ratio of Serum amyloid A protein (SAA). This invention also relates to methods for screening molecules that modulate this ratio and their use in the treatment and/or amelioration of symptoms that are related to pre-diabetes, type 2 diabetes, insulin resistance and their related conditions.

## BACKGROUND OF THE INVENTION

Type 2 diabetes (also known as diabetes mellitus type 2, non-insulin-dependent diabetes mellitus or adult on-set diabetes) is a metabolic disorder that is characterized by high blood glucose. This condition is a result of either a lack of insulin produced by the body or the developed tolerance to insulin by the cells of the body (Insulin Resistance). Since insulin is essential for the absorption of glucose from the blood, perturbations in insulin homeostasis eventually result in loss of glycemic control and the development of the diabetic condition. If left uncontrolled, excessive blood glucose will eventually lead to a multitude of complications, including but not limited to: blindness, skin ulcerations, amputations, heart disease and kidney disease. Many proposed theories exist regarding the association of multiple complications that are linked to the loss of glycemic control, however, the mechanisms in which these multiple pathways interact is still unknown.

As of 2007, the Centers of Disease Control determined that 7.8% of the US population (23.6 million people) have type 2 diabetes and it is estimated that >20% of Americans are pre-diabetic. Due to the debilitating nature of these conditions and their associated cost of treatment, more effective and efficient methods for earlier detection are paramount to the health of the US populace and western civilization. The "gold standard" for evaluating the level of glycemic control is the Oral Glucose Tolerance Test (OGTT). This test involves measuring

the fasting blood glucose of an individual or patient and then obtaining a blood glucose measurement 2 hours post administration of an oral glucose solution. Current guidelines define normal glucose tolerance (NGT) as a 2 hour glucose level ≤ 140 mg/dl. The criteria for type 2 diabetes (DM) is a 2 hour glucose of ≥ 200 mg/dl and the definition of impaired glucose tolerance (IGT or pre-diabetes) is between 140 and 200 mg/dl. Even though these criteria are sanctioned by both the American Diabetes Association and the World Health Organization, these values are not without clinical error or subject to interpretation.

A recent development in diabetes care included the adoption of guidelines for the use of the protein biomarker hemoglobin A1C (HbA1C) in the diagnosis of diabetes and pre-diabetes. Due to the longevity of hemoglobin in the blood, it serves as a long term measure of glycemic control. Although long used as a biomarker of whether or not a patient's diabetes treatment is sufficient, conflicting data previously prevented the use of HbA1C in diabetes determination. Even with the acceptance of these new guidelines, and their use in conjunction with the standard OGTT criteria and fasting blood glucose levels, the diagnosis of pre- or type 2 diabetes still ultimately relies on the interpretation and the judgment of the diagnosing physician.

Due to the pandemic nature of type 2 diabetes, pre-diabetes, insulin resistance and the development of associated complications, there is a world wide need for additional detection/diagnostic/assessment methods to allow for the earliest possible intervention and provide a means to evaluate the effectiveness of treatment through life-style changes and/or medication. A need also exists for additional metabolic or endocrine targets for the development of treatments that alleviate or ameliorate the problems and symptoms associated with these related conditions.

## SUMMARY OF THE INVENTION

As shown here for the first time, modulations in the phenotypic ratio of SAA correlate with the 2 hour glucose values obtained after the application of an OGTT and the clinical diagnosis of certain metabolic disease states. These findings identify the phenotypic ratio of SAA as a biomarker for the clinical assessment/diagnosis of type 2 diabetes, pre- diabetes, insulin resistance and

their related conditions. It also stands to reason that SAA may also mechanistically participate in the initiation/development and pathology of these disease states and therefore the phenotypic ratio of SAA may also have utility as a therapeutic target for the amelioration and/or treatment of the disease symptoms or condition.

Another objective of the present invention is to use SAA and the calculation of the phenotypic ratio of SAA as new targets and screening methods for the in-vitro and in-vivo detection and/or diagnosis of type 2 diabetes, pre-diabetes, insulin resistance and their related conditions.

A further objective of the present invention is to use the phenotypic ratio of SAA as a new target for molecules that modulate SAA and/or in the screening of molecules aimed at the in-vitro and/or in-vivo modulation of its expression, activity and/or clearance in the treatment of Type 2 diabetes, pre-diabetes, insulin resistance and their related conditions.

Some novel features that are considered characteristic of the invention are set forth with particularity in the claims. The invention itself, however, both as to its structure and its operation together with the additional objectives and advantages thereof will best be understood from the following description of the exemplary embodiments of the present invention when read in conjunction with the accompanying drawings. Unless specifically noted, it is intended that the words and phrases in the specification and claims be given the ordinary and accustomed meaning for those of ordinary skill in the application of the art or arts. If any other meaning is intended, the specification will specifically state that special meaning is being applied to the word or phrase. Likewise, the use of the words "function" or "means" in the Description of Preferred Embodiments is not intended to indicate a desire to invoke the special provisions of 35 U.S.C. § 112, paragraph 6 to define the invention. To the contrary, if the provisions of 35 U.S.C. § 112, paragraph 6, are sought to be invoked to define the invention(s), the claims will specifically state the phrases "means for" or "step for" and a function, without also reciting in such phrases any structure, material, or act in support of the function. Even when the claims recite a "means for" or "step for" performing a function, if they also recite any structure, material or acts in support of that means or step, then the intention is not to invoke the provisions of 35 U.S.C. § 112, paragraph 6. Moreover, even if the provisions of 35 U.S.C. § 112, paragraph 6,

are invoked to define the inventions, it is intended that the inventions not be limited only to the specific structure, material or acts that are described in the preferred embodiments, but in addition, include all structures, materials or acts that perform the claimed function, along with any known or later-developed equivalent structures, materials or acts for performing the claimed function.

## BRIEF DESCRIPTION OF THE DRAWINGS

The object and features of the present invention will be better understood with reference to the following detailed description and drawings.

FIG. 1 illustrates mass spectral results of the analysis of SAA and the phenotypic ratio of SAA from human plasma samples using mass spectrometric immunoassay.

FIG. 2 is a dot histogram of the observed phenotypic ratio of SAA from the human plasma samples from patients with normal glucose tolerance (NGT) and patients with impaired glucose tolerance (IGT, pre-diabetes) that were analyzed and shown in FIG. 1.

FIG. 3 is a dot histogram of the observed phenotypic ratio of SAA from the human plasma samples from patients with normal glucose tolerance (NGT) and patients with type 2 diabetes (DM) that were analyzed and shown in FIG. 1.

FIG. 4 shows the resultant ROC curves established from the mass spectral phenotypic ratio of SAA data obtained from each sample population, namely the human plasma samples from patients with normal glucose tolerance (NGT), the human plasma samples from patients with impaired glucose tolerance (IGT, pre-diabetes), and the human plasma samples from patients with type 2 diabetes (DM), that were analyzed and shown in FIG. 1.

## DETAILED DESCRIPTION

It is demonstrated for the first time in the present invention that the phenotypic ratio of SAA correlates with the clinical diagnosis of both the pre-diabetes and type 2 diabetes disease states. It is also demonstrated for the first time that the modulations in the phenotypic ratio of SAA compare to the results of the OGTT. This observed phenomenon directly displays the utility of this novel

marker as it relates to type 2 diabetes, pre-diabetes, insulin resistance and/or their related conditions or complications.

The present invention encompasses the use of the phenotypic ratio of SAA as a biomarker for the in-vitro and/or in-vivo determination/diagnosis/assessment of type 2 diabetes, pre-diabetes, insulin resistance and/or their related conditions or complications.

In addition, the present invention includes the use of the phenotypic ratio of SAA as a marker for the in-vitro and/or in-vivo screening of compounds, aimed at modulating this ratio by altering the expression, modification, activity and/or clearance of SAA for the purpose of treating, or reducing symptoms that are associated with, pre-diabetes, type 2 diabetes, insulin resistance and/or their related conditions or complications.

The present invention also includes methodologies for the measurement of the forms of SAA for the calculation of the phenotypic ratio of SAA from biological samples for the determination/diagnosis/assessment of type 2 diabetes, pre-diabetes, insulin resistance and/or their related conditions or complications.

The present invention also extends these methodologies for the determination of the phenotypic ratio of SAA in order to screen compounds aimed at modulating this ratio by altering the expression, activity and/or clearance of SAA for the purpose of treating, or reducing symptoms that are associated with, pre-diabetes, type 2 diabetes, insulin resistance and/or their related conditions or complications.

Definitions

All the technical and scientific terms used herein, unless defined otherwise, have the meaning commonly understood by a person skilled in art to which this invention pertains. As used herein, the following terms have the meaning ascribed to them unless specified otherwise.

As used herein, "SAA" is a general term describing the serum amyloid A acute phase family of proteins. There are two isoforms of SAA, which are expressed by the genes SAA1 and SAA2, with multiple allelic variations of each. These are collectively known as SAA. SAA serves as an apolipoprotein and is expressed in both hepatocytes and adipocytes.

As used herein, "Parent SAA" refers to the most common intact endogenous form of SAA observed within the sample. This is normally SAA1 (MW = 11,683) but may also include an allelic variant or a point mutated forms.

As used herein, "SAA-Arg" refers to the specific truncated variant of SAA which is missing its N-terminal Arginine residue.

As used herein, "Analysis" refers to the determination of the phenotypic ratio of SAA from a biological sample.

As used herein, "Phenotypic ratio of SAA" refers to the quotient calculated by dividing the measured amount of parent SAA by the measured amount of SAA-Arg from a given biological sample.

As used herein, "Biological sample" refers to a fluid or extract having a plurality of components. Complex media may include, but is not limited to, tissue, cell extracts, nuclear extracts, cell lysates and excretions, blood, sera, plasma, saliva, urine, sputum, synovial fluid, cerebral-spinal fluid, tears, feces, saliva, membrane extracts, industrial fluids and the like.

As used herein, "type 2 diabetes (DM)" is a clinical condition defined by excess glucose in the blood. Reference limits that aid in the clinical determination are a Fasting glucose value ≥126 mg/dl and/or a 2 hour oral glucose tolerance test value ≥ 200 mg/dl.

As used herein, "pre-diabetes, impaired glucose tolerance (IGT)" is a clinical condition defined by above normal amounts of glucose present in the blood. Reference limits that aid in the clinical determination are Fasting glucose values that are between 110 and 125 mg/dl and/or 2 hour oral glucose tolerance test values that are between 140 and 199 mg/dl.

As used herein, "normal glucose tolerance (NGT)" is a clinical determination when blood glucose levels are within normal concentration ranges. Reference limits that aid in the clinical determination are a Fasting glucose value < 110 mg/dl and/or a 2 hour oral glucose tolerance test value < 140 mg/dl.

As used herein, "insulin resistance" is a physical condition in which the hormone insulin becomes less effective in reducing blood glucose levels. The gold standard for evaluating insulin resistance is the administration of a hyperinsulinemic euglycemic clamp.

As used herein, "related conditions or complications" is defined as a variety of ailments that are commonly associated with the development and progression

of type 2 diabetes. Some of these ailments include but are not limited to: heart attacks, skin ulcerations, blindness, amputations and kidney failure.

As used herein, "mass spectrometry" refers to the ability to volatilize/ionize analytes to form vapor-phase ions and determine their absolute or relative molecular masses. Suitable forms of volatilization/ionization are laser/light, thermal, electrical, atomized/sprayed and the like or combinations thereof. Suitable forms of mass spectrometry include, but are not limited to, Matrix Assisted Laser Desorption/Time of Flight Mass Spectrometry (MALDI-TOF MS), electrospray (or nanospray) ionization (ESI) mass spectrometry, or the like or combinations thereof.

As used herein, "subject" refers to any human, animal, or other living organism which possesses measurable levels of SAA and/or SAA variants in a biological sample taken therefrom.

In the present invention, the method for the establishment of the phenotypic ratio of SAA is performed using a biological sample (biological matrix). Possible biological matrices include, but are not limited to; tissue, whole blood, serum, plasma, saliva, cerebral spinal fluid or urine. The sample may also be free or immobilized onto a solid support. Examples of solid supports include, but are not limited to, filter paper, beads, arrays, etc. Any solid support material known in the art for immobilizing samples may be used. The sample may also be native in form or have undergone processing including, but not limited to, denaturation, reduction, proteolytic digestion and the like.

In order to measure the phenotypic ratio of SAA, the present invention may utilize a separation or purification step to first isolate or remove SAA from the biological sample. This separation can be performed by several means, including, but not limited to, chromatography, centrifugation, affinity interactions, chemical methodologies, staining methodologies, and gel electrophoresis. Chromatography techniques may include but are not limited to: high performance liquid chromatography (HPLC) thin layer chromatography (TLC), paper chromatography (PC), affinity chromatography, size exclusion, ion-exchange, reverse phase, etc. Affinity interactions employ the ability of a molecule to bind with another molecule having proper fitting conformation. Affinity methods employed may include, but are not limited to, affinity interactions utilizing antibodies (Ab), antibody fragments (FAb), aptamers, proteins, peptides, lectins,

etc. For sandwich assays, combinations of primary and secondary affinity ligands may be used. Gel electrophoresis methods may include, but are not limited to, acrylamide, agarose, etc. Chemical methodologies may include, but are not limited to, amino acid analysis, sequencing, etc. Staining methods may include, but are not limited to, the use of any dye, and the like, that directly binds to the SAA protein and its variants, or to molecules that bind to SAA and its variants.

The method for detecting the forms of SAA used to calculate the phenotypic ratio of SAA will utilize some form of detection technology for the isolated protein (i.e. isolated SAA and/or SAA variants). Detection methods may be direct or indirect in nature. Detection technologies include but are not limited to: staining, spectroscopy, spectrometroscopy, spectrophotometry, colorimetry, fluorescence, luminescence, magnetism, radioisotopes, nuclear magnetic resonance spectroscopy, x-ray crystallography, surface plasmon resonance, mass spectrometry, etc. Depending on the state of the biological sample, the detected forms of SAA measured and used to calculate the phenotypic ratio of SAA may be intact or a fragment thereof.

Analyses Demonstrating Biomarker Utility

EXAMPLE

**ANALYSIS OF THE PHENOTYPIC RATIO OF SERUM AMYLOID A FROM HUMAN PLASMA**

One exemplary embodiment of the invention is the measurement of the phenotypic ratio of SAA for use as a biomarker(s) to differentiate between clinically defined populations of samples. The analysis of this exemplary embodiment was performed using mass spectrometric immunoassay (MSIA) technology.

In this example, the analyses were performed on human citrate plasma samples obtained from patients that were newly diagnosed and clinically defined as having NGT, IGT or DM. There were 237 plasma samples obtained from NGT individuals, 98 plasma samples obtained from patients with IGT, and 25 plasma samples from patients with DM which were all analyzed in the same manner.

The MSIA was performed with the aid of affinity pipette tips. The affinity pipette tips are made of small, porous microcolumns that are fitted at the entrance

of a pipettor tip. The microcolumns are derivatized with an affinity ligand. The affinity ligand used to derivatize the affinity pipettes was anti-SAA antibody. The sample preparation utilized the addition of an internal reference standard (IRS) for the semi-quantitative measurement of the parent SAA and SAA-Arg. Each sample mixture consisted of 10 μL of human plasma, 20 μL of ten-fold diluted horse serum (horse SAA is the IRS) and 170 μL of HEPES buffered saline. SAA was extracted from the samples by interrogation with the anti-SAA affinity pipette tips by repetitive drawing of the samples through the affinity pipettes. The purification process used HEPES buffered saline and water rinses following the SAA affinity retrieval. Enriched and purified proteins were then eluted from the affinity pipettes with sinapic acid MALDI matrix and were deposited directly on a MALDI mass spectrometer target for subsequent mass spectral detection.

Examination of the resultant mass spectra showed signals from multiple forms of SAA that are present in human plasma, as well as the IRS. Each spectrum was normalized to the integral of the SAA signal obtained from the IRS. Within each mass spectrum, multiple forms of SAA are clearly resolved, but are dominated by the parent SAA and SAA-Arg as shown in FIG. 1. FIG. 1 also illustrates that the phenotypic ratio of SAA is different in human plasma samples from patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (DM). The integral of the parent SAA and SAA-Arg signals were collected and the phenotypic ratio of SAA was calculated. The phenotypic ratio of SAA was established by taking the integral for the parent SAA and dividing it by the integral of the SAA-Arg. The ratio of SAA-Arg to parent SAA is clearly skewed in the IGT and DM spectra.

The resulting mass spectrometry data showed the ability to discriminate between samples that originate from NGT patients and IGT/DM patients. FIG. 2 is a dot histogram of the observed phenotypic ratio of SAA from the samples of patients having NGT and IGT. Using the phenotypic ratio of SAA as a biomarker for IGT, a cut-off value of 1.3760 was established for IGT determination. The mean value and one standard deviation of each population are also denoted in FIG. 2. FIG. 3 is a dot histogram of the observed phenotypic ratio of SAA from the samples of patients having NGT and DM. Using the phenotypic ratio of SAA as a biomarker for DM, a cut-off value of 1.4780 was established for DM determination.

The mean value and one standard deviation of each population are also denoted in FIG. 3.

The clinical sensitivity and specificity for the phenotypic ratio of SAA were also determined. These values are presented below in **Table 1**.

| Biomarker | Sensitivity | Specificity | Cut-off Value |
|---|---|---|---|
| **IGT** | | | |
| SAA ratio | 80.4% | 80.3% | 1.3760 |
| **DM** | | | |
| SAA ratio | 84.0% | 87.0% | 1.4780 |

The accuracy of a test to discriminate diseased cases from normal cases is elevated using Receiver Operating Characteristic (ROC) curve analysis. For comparative purposes the receiver operator characteristic (ROC) curves of phenotypic ratio of SAA for IGT and DM were also plotted. These plots are shown in **FIG. 4**. The displayed plots illustrate the clinical utility of the SAA phenotypic ratio biomarker in determining/diagnosing both IGT and DM. The calculated areas under the curve for each plot are 0.8360 and 0.8731, respectively.

Finally, an algorithm-assisted bio-statistical evaluation of the SAA phenotypic ratio dataset was performed, resulting in improved clinical sensitivity and specificity for determining/diagnosing both IGT and DM. This data is presented below in **Table 2**.

| Biomarker | Sensitivity | Specificity |
|---|---|---|
| **IGT** | | |
| SAA ratio | 87.0% | 79.0% |
| **DM** | | |
| SAA ratio | 100% | 91.1% |

All of the analyses set forth and/or described above can also be performed on other SAA variants and/or applied to combinations of various observed SAA variants to determine additional biomarkers or groupings of biomarkers for determining IGT, DM and their related conditions.

The preferred embodiment of the invention is described above in the Drawings and Description of Preferred Embodiments. While these descriptions directly describe the above embodiments, it is understood that those skilled in the

art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s). The foregoing description of an exemplary embodiment and best mode of the invention known to the applicant at the time of filing the application has been presented and is intended for the purposes of illustration, and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed, and many modifications and variations are possible in the light of the above teachings. The embodiment was chosen and described in order to best explain the principles of the invention and its practical application and to enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated.

## CLAIMS

1. A method for at least one of determining, diagnosing, and monitoring at least one of type 2 diabetes, pre-diabetes, insulin resistance, and their related conditions in one or more subjects which includes the step of analyzing at least one of SAA, an SAA variant, and a phenotypic ratio of SAA.

2. The method of claim 1 wherein the step of analyzing at least one of SAA, an SAA variant, and a phenotypic ration of SAA includes the step of analyzing at least one of Parent SAA and SAA-Arg.

3. The method of claim 1 wherein the step of analyzing at least one of SAA, an SAA variant, and a phenotypic ratio of SAA comprises the step of performing at least one of mass spectroscopy, chromatography, an affinity reaction, gel electrophoresis, centrifugation, chemical methodology, and staining methodology.

4. The method of claim 1 wherein the step of analyzing at least one of SAA, an SAA variant, and a phenotypic ratio of SAA comprises the step of performing a single assay.

5. The method of claim 4 further comprising the step of performing single mass spectrometry.

6. A method for diagnosing pre-diabetes in a subject comprising the steps of detecting a level of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA in a biological sample of the subject and diagnosing said subject as having pre-diabetes when the level of said at least one of SAA, an SAA variant, and a phenotypic ratio of SAA is modulated by a statistically significant amount from a level present in one or more subjects with normal glucose tolerance.

7. The method of claim 6 wherein the step of diagnosing said subject as having pre-diabetes comprises the step of diagnosing said subject as having pre-diabetes when the level of said at least one of SAA, an SAA

variant, and a phenotypic ratio of SAA is increased by a statistically significant amount over a level present in one or more subjects with normal glucose tolerance.

8. The method of claim 6 wherein the step of detecting a level of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA comprise the step of detecting the levels of Parent SAA and SAA-Arg and the step of diagnosing said subject as having pre-diabetes comprises the step of diagnosing said subject as having pre-diabetes when the phenotypic ratio of SAA is modulated by a statistically significant amount from a phenotypic ratio of SAA present in one or more subjects with normal glucose tolerance.

9. The method of claim 8 wherein the step of diagnosing said subject as having pre-diabetes comprises the step of diagnosing said subject as having pre-diabetes when the phenotypic ratio of SAA is increased by a statistically significant amount over a phenotypic ratio of SAA present in one or more subjects with normal glucose tolerance.

10. A method for diagnosing type 2 diabetes in a subject comprising the steps of detecting a level of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA in a biological sample of the subject and diagnosing said subject as having type 2 diabetes when the level of said at least one of SAA, an SAA variant, and a phenotypic ratio of SAA is modulated by a statistically significant amount from a level present in one or more subjects with normal glucose tolerance.

11. The method of claim 10 wherein the step of diagnosing said subject as having type 2 diabetes comprises the step of diagnosing said subject as having type 2 diabetes when the level of said at least one of SAA, an SAA variant, and a phenotypic ratio of SAA is increased by a statistically significant amount over a level present in one or more subjects with normal glucose tolerance.

12. The method of claim 10 wherein the step of detecting a level of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA comprise the step of detecting the levels of Parent-SAA and SAA-Arg and the step of

diagnosing said subject as having type 2 diabetes comprises the step of diagnosing said subject as having type 2 diabetes when the phenotypic ratio of SAA is modulated by a statistically significant amount from a phenotypic ratio of SAA present in one or more subjects with normal glucose tolerance.

13. The method of claim 12 wherein the step of diagnosing said subject as having type 2 diabetes comprises the step of diagnosing said subject as having type 2 diabetes when the phenotypic ratio of SAA is increased by a statistically significant amount over a phenotypic ratio of SAA present in one or more subjects with normal glucose tolerance.

14. A method for evaluating a therapeutic treatment for at least one of type 2 diabetes, pre-diabetes, insulin resistance and their related conditions in one or more subjects which includes the step of monitoring at least one of SAA, an SAA variant, and a phenotypic ratio of SAA in a biological sample from said one or more subjects.

15. The method of claim 14 wherein the step of monitoring at least one of SAA, an SAA variant, and a phenotypic ratio of SAA includes the step of monitoring at least one of Parent-SAA and SAA-Arg.

16. A method for indentifying a therapeutic treatment for at least one of type 2 diabetes, pre-diabetes, insulin resistance and their related conditions which includes the step of determining whether the therapeutic treatment can modulate activity or concentration of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA within a subject.

17. The method of claim 16 wherein the step of determining whether the therapeutic treatment can modulate activity or concentration of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA includes the step of determining whether the therapeutic treatment can modulate activity or concentration of at least one of SAA, an SAA variant, and a phenotypic ratio of SAA.

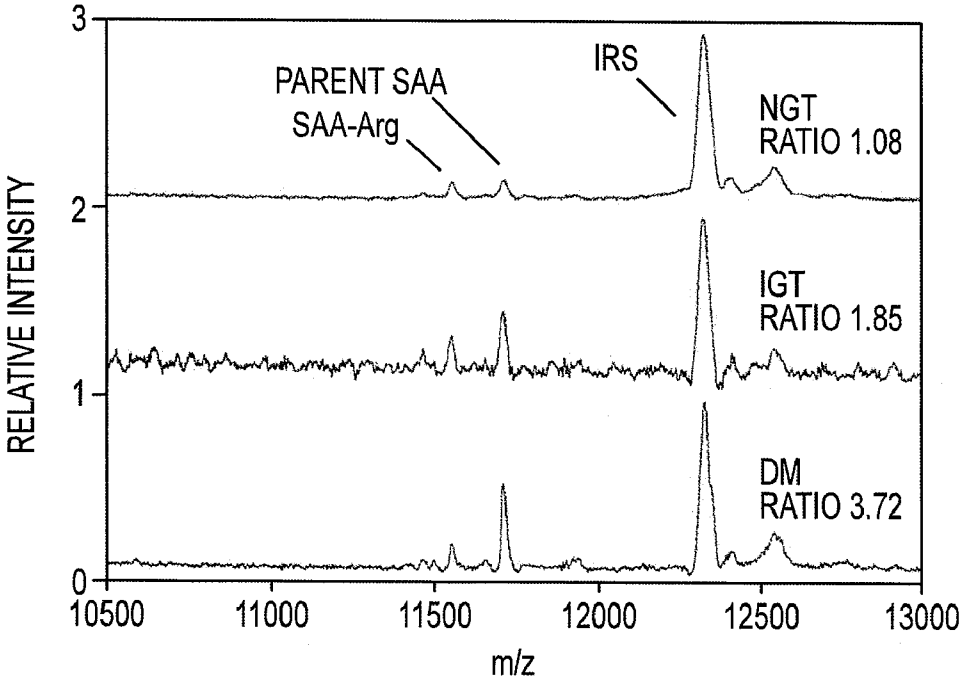
RELATIVE INTENSITY
3
2
1
0
PARENT SAA
SAA-Arg
IRS
NGT
RATIO 1.08
IGT
RATIO 1.85
DM
RATIO 3.72
10500
11000
11500
12000
12500
13000
m/z

FIG.1

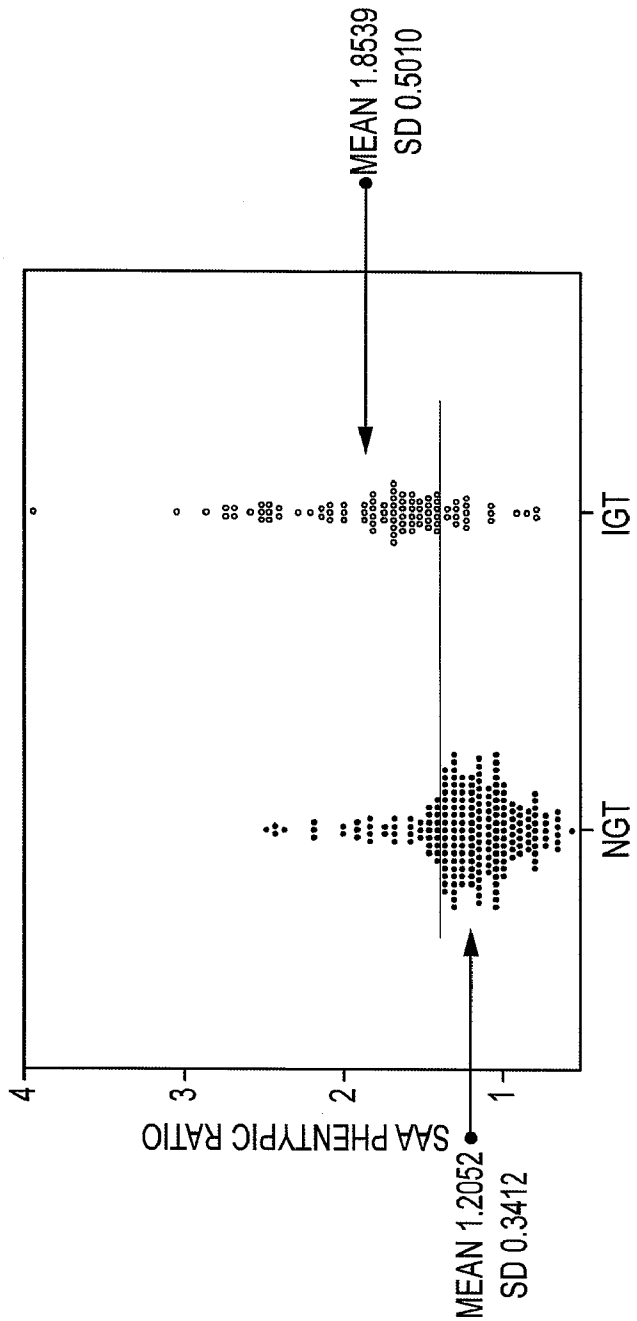
MEAN 1.8539
SD 0.5010
SAA PHENTYPIC RATIO
4
3
2
1
IGT
NGT
MEAN 1.2052
SD 0.3412

FIG.2

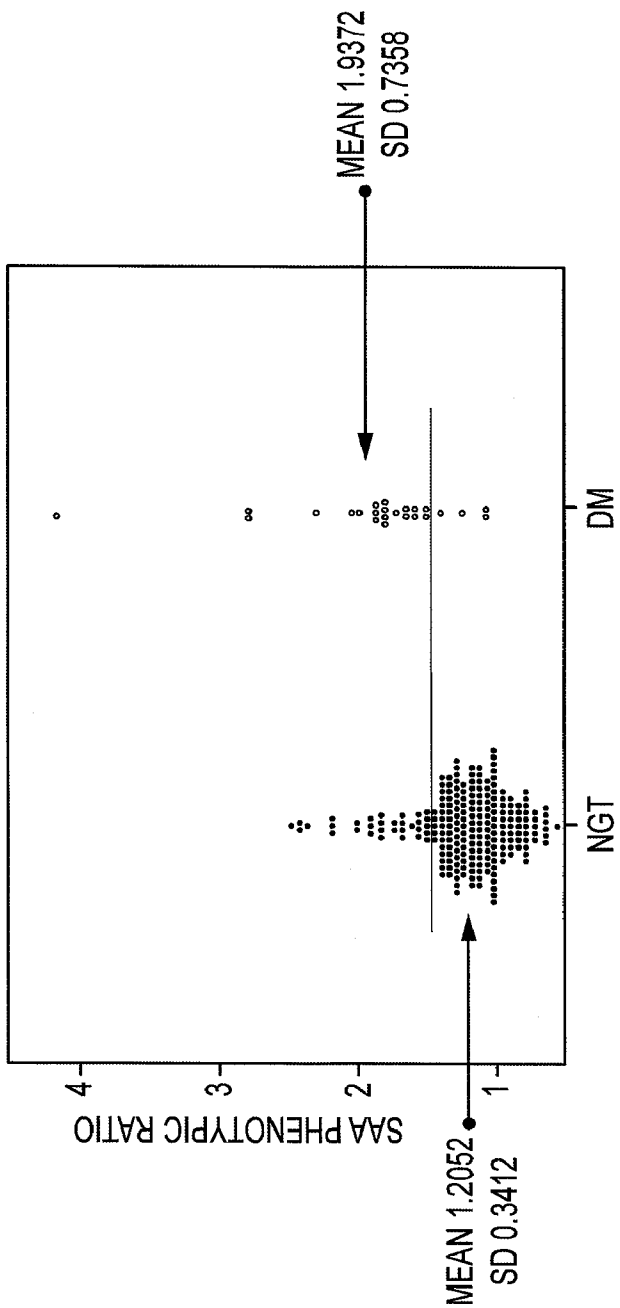
MEAN 1.9372
SD 0.7358
DM
NGT
SAA PHENOTYPIC RATIO
4
3
2
1
MEAN 1.2052
SD 0.3412

FIG.3

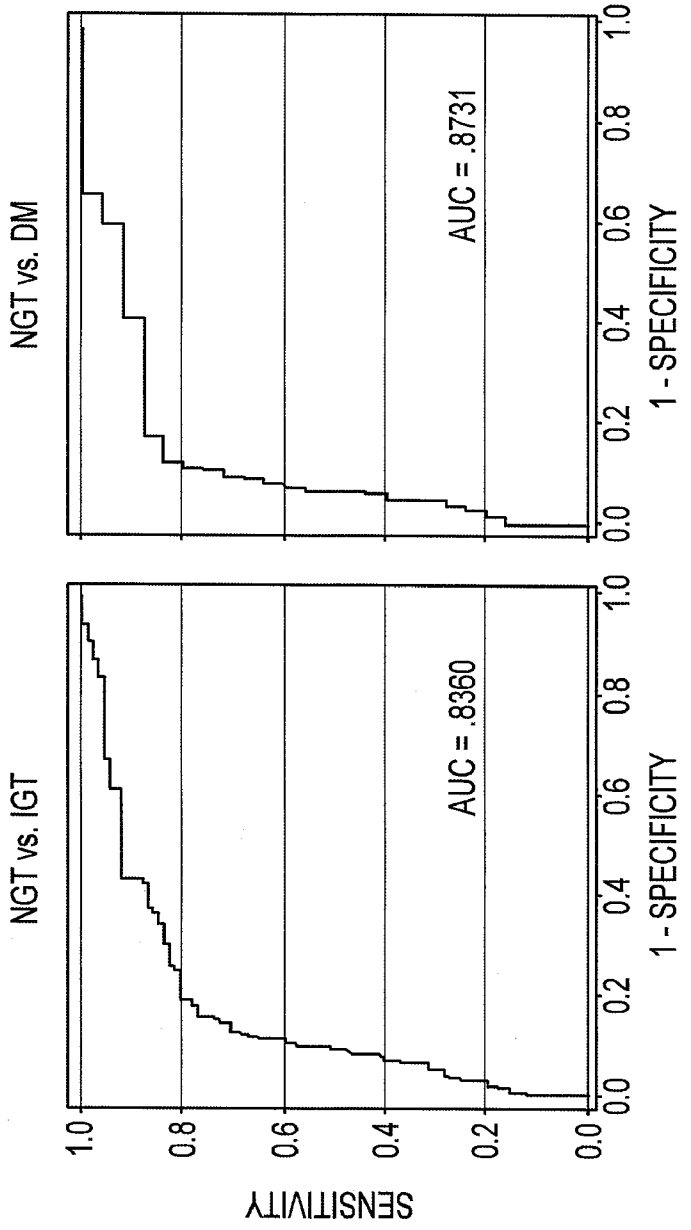
NGT vs. DM
AUC = .8731
1 - SPECIFICITY
0.0
0.2
0.4
0.6
0.8
1.0
NGT vs. IGT
AUC = .8360
1 - SPECIFICITY
SENSITIVITY
1.0
0.8
0.6
0.4
0.2
0.0

FIG.4

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/40997

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/16 (2010.01)
USPC - 530/380
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 530/380

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 530/350 (text search, see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,EPAB,JPAB); Google/Scholar (text search, see terms below)
Search Terms: SAA, SAA-Arg, serum amyloid A, type 2, NIDDM, T2DM, diabetes, insulin resistance, N-terminal, N-terminus, NH2-terminus, NH2-terminal, amino-terminal, amino-terminus, arginine, Arg, apoC1, apoCI, apolipoprotein C1, apolipoprotein CI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>----<br>Y | US 2007/0087448 A1 (NELSESTUEN) 19 April 2007 (19.04.2007); Figure 12, Table 1, Claim 1, Claim 5, Claim 6, paras [0006], [0009], [0012], [0062], [0112], [0113], [0139], [0165], [0202], [0236], [0259] | 1-6, 8, 14, 15<br>----------<br>7, 9, 12, 13 |
| X<br>----<br>Y | US 2008/0039364 A1 (COLLIER et al.) 14 February 2008 (14.02.2008); paras [0005] - [0007], [0078], [0111] | 16, 17<br>----------<br>9, 13 |
| X<br>-----<br>Y | Hatanaka et al. Interaction between serum amyloid A and leukocytes - A possible role in the progression of vascular complications in diabetes. Immunology Letters, 2007, vol 108, pp 160-166; esp: Table 1, (page 161, col 1, para 5), (page 161, col 2, para 2), (page 163, col 2, para 2) | 10, 11<br>----------<br>7, 12, 13 |
| Y | Kiernan et al. Detection of novel truncated forms of human serum amyloid A protein in human plasma. FEBS Letters, 2003, vol 537, pp 166-170; esp: page 169, col 2, para 1 | 9, 13 |

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

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

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 Augsut 2010 (17.08.2010) | 30 AUG 2010 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

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