



(11)

EP 3 358 347 A1

(12)

EUROPEAN PATENT APPLICATION
published in accordance with Art. 153(4) EPC

(43) Date of publication:

08.08.2018 Bulletin 2018/32

(51) Int Cl.:

G01N 33/49 (2006.01) **G01N 27/62** (2006.01)
G01N 33/53 (2006.01)

(21) Application number: **16850676.4**

(86) International application number:

PCT/JP2016/004394

(22) Date of filing: **29.09.2016**

(87) International publication number:

WO 2017/056498 (06.04.2017 Gazette 2017/14)

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

Designated Extension States:

BA ME

Designated Validation States:

MA MD

(72) Inventors:

- **ABE, Takaaki**
Sendai-shi
Miyagi 980-8577 (JP)
- **OGAWA, Susumu**
Sendai-shi
Miyagi 980-8577 (JP)
- **TOMIOKA, Yoshihisa**
Sendai-shi
Miyagi 980-8577 (JP)

(30) Priority: **30.09.2015 JP 2015193638**

(71) Applicant: **Tohoku University**

Sendai-shi, Miyagi 980-8577 (JP)

(74) Representative: **Wills, Andrew Jonathan et al**

Mewburn Ellis LLP
City Tower
40 Basinghall Street
London EC2V 5DE (GB)

(54) **MARKER FOR DETERMINING DIABETIC NEPHROPATHY**

(57) An object of the present invention is to provide a biomarker capable of determining the onset risk or the stage of diabetic nephropathy (early-stage diabetic nephropathy in particular). Diabetic nephropathy is determined by measuring or quantitatively determining a concentration of phenyl sulfate or a salt thereof (a phenyl sulfate) contained in a biological sample, preferably plas-

ma or urine, collected from a test subject to detect a phenyl sulfate. The present invention exhibits excellent effects particularly for stage determination for stage 1 (pre-nephropathy stage) to stage 3 (overt nephropathy stage), or for determination of onset risk of diabetic nephropathy (diabetic nephropathy at pre-nephropathy stage).

EP 3 358 347 A1

Description**Technical Field**

5 **[0001]** The present invention relates to a method for determining diabetic nephropathy, and a biomarker for determining diabetic nephropathy to be used in the method for determining diabetic nephropathy.

Background Art

10 **[0002]** It is known that diabetes causes various chronic vascular complications, and the prognosis of the disease is varied by the complications. Among the complications, diabetic nephropathy is significant for life prognosis, and it is desired to diagnose the onset and the onset risk at an early stage for inhibiting progression.

[0003] Conventionally, the stage of diabetic nephropathy has been diagnosed on the basis of not only history of diabetes but also change in urinary findings (detection of proteinuria/microalbuminuria), degradation in estimated glomerular filtration rate (eGFR), and ophthalmological findings (observation of fundus blood vessel lesion). It has been regarded, however, that such diagnoses are disadvantageously insufficient for distinction from other kidney diseases except for diabetic nephropathy.

[0004] On the other hand, novel urinary or blood biomarkers have been searched for and studied in recent years. For example, a urinary protein useful for identifying a diabetic nephropathy patient among type 2 diabetes patients by using an SELDI (Surface Enhanced Laser Desorption/Ionization) time-of-flight mass spectrometer has been reported (non-patent document 1). Besides, a pattern that can be used for distinguishing a type 2 diabetes patient from a healthy individual by profiling a urinary polypeptide using a capillary electrophoresis-mass spectrometer (CE-MS) has been found (non-patent document 2). The inventions described in these documents do not, however, relate to a biomarker capable of determining the stage of diabetic nephropathy or the onset risk of diabetic nephropathy. Furthermore, it is reported that a biomarker capable of early recognition of diabetic nephropathy using CE-TOFMS has been identified (patent document 1). Although it is described that there is a statistically significant difference in the concentration of the identified biomarker (compound) between different stages, the difference is so small that it is unknown whether or not it can be practically used. Besides, this biomarker cannot determine the onset risk of diabetic nephropathy.

Prior Art Documents**Patent Documents**

35 **[0005]** Patent Document 1: Japanese unexamined Patent Application Publication No. 2014-32046

Non-patent Documents**[0006]**

40 Non-patent Document 1: Dihazi, H. et al., Clin Chemi 2007, 53: 1636-1645

Non-patent Document 2: Mischak, H. et al., Clin Sci (Lond) 2004, 107: 485-495

Summary of the Invention**Object to be Solved by the Invention**

[0007] An object of the present invention is to provide a biomarker capable of determining the onset risk or the stage of diabetic nephropathy (early-stage diabetic nephropathy in particular).

Means to Solve the Object

[0008] In order to solve the above-described object, the present inventors have analyzed the relationship between a phenyl sulfate salt (PS) concentration and ACR, that is, an index of diabetic nephropathy, and have found that the onset risk and the stage of diabetic nephropathy (early-stage diabetic nephropathy in particular) can be determined by using increase of the PS concentration as an indicator, resulting in accomplishing the present invention.

[0009] Specifically, the present invention provides:

[1] A method for determining diabetic nephropathy, comprising detecting phenyl sulfate or salt thereof contained in

a biological sample collected from a test subject.

[2] The method for determining diabetic nephropathy according to [1], wherein the biological sample is blood or urine.

[3] The method for determining diabetic nephropathy according to [2], wherein the test subject is a test subject who has not developed diabetic nephropathy, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject is not less than a prescribed threshold value, it is indicative that the test subject has a high possibility of developing diabetic nephropathy.

[4] The method for determining diabetic nephropathy according to [3], wherein the prescribed threshold value is 5.5 (nmol/mL).

[5] The method for determining diabetic nephropathy according to [2], wherein the test subject is a test subject who is unknown to have diabetic nephropathy or not, or a test subject whose stage of diabetic nephropathy is unknown, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject is less than a prescribed threshold value, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at pre-nephropathy stage, when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject falls in a range of prescribed values A, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at early nephropathy stage or overt nephropathy stage, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject falls in a range of prescribed values B, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at overt nephropathy stage.

[6] The method for determining diabetic nephropathy according to [5], wherein the prescribed threshold value is 0.51 (nmol/mL), the prescribed values A are over 54.4 (nmol/mL) to 159 (nmol/mL), and the prescribed values B are over 74.1 (nmol/mL) to 159 (nmol/mL).

[7] A biomarker for determining diabetic nephropathy, comprising phenyl sulfate or salt thereof.

[8] The biomarker according to [7], wherein determination of diabetic nephropathy is determination of onset risk of diabetic nephropathy.

[9] The biomarker according to [7], wherein determination of diabetic nephropathy is determination of stage of diabetic nephropathy.

[0010] The determination method of the present invention is a method for assisting diagnosis of diabetic nephropathy made by a doctor, and does not include a diagnostic action carried out by the doctor. Besides, as another aspect of the determination method, a method for collecting data to be used for diagnosis of diabetic nephropathy can be provided.

[0011] Furthermore, another embodiment of the present invention provides a method for diagnosing diabetic nephropathy including detecting phenyl sulfate or salt thereof contained in a biological sample collected from a test subject. The diagnosis method preferably further includes a step of treating the test subject (patient) diagnosed with diabetic nephropathy for improving the renal function of the diagnosed test subject (patient).

[0012] Besides, as another embodiment of the present invention, phenyl sulfate or salt thereof to be used as a biomarker in a method for diagnosing (determining) diabetic nephropathy can be provided.

Effect of the Invention

[0013] According to the present invention, the onset risk or the onset of diabetic nephropathy at a comparatively early stage can be accurately determined, and therefore, the number of medical examinees for diabetic nephropathy in periodic medical examination necessary for early detection of diabetic nephropathy can be expected to increase, and in addition, it is possible to prevent the onset of diabetic nephropathy or to effectively treat diabetic nephropathy at an early stage, and thus, effects to improve QOL (Quality of Life) and to reduce medical expense can be expected by preventing progression to renal dysfunction or renal failure.

Mode of Carrying Out the Invention

[0014] A method for determining diabetic nephropathy of the present invention (hereinafter simply referred to as the "present determination method") is not especially limited as long as it is a method for determining diabetic nephropathy comprising measuring or quantitatively determining a concentration of phenyl sulfate or salt thereof (hereinafter sometimes generically referred to as a phenyl sulfate) contained in a biological sample collected from a test subject (a donor) for detecting a phenyl sulfate (excluding a diagnostic action carried out by a doctor), and a biomarker for determining diabetic nephropathy of the present invention (hereinafter simply referred to as the "present biomarker") is not especially limited as long as it is a biomarker, containing a phenyl sulfate, for determining (diagnosing) diabetic nephropathy in a test subject, and here, the "determination of diabetic nephropathy" includes determination of the onset risk of diabetic nephropathy and determination of the stage of diabetic nephropathy.

[0015] The stage of diabetic nephropathy can be classified on the basis of a urinary albumin/creatinine ratio (ACR)

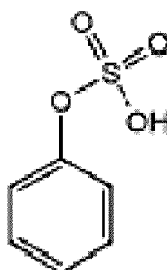
corresponding to an index of early diabetic nephropathy, or an estimated glomerular filtration rate (eGFR). For example, in employing classification based on diabetic nephropathy stage classification revised by Joint Committee on Diabetic Nephropathy in December 2013, a diabetes patient showing ACR less than 30 (mg/g) corresponding to normal albuminuria and eGFR of not less than 30 (mL/min/1.73 m²) is classified as stage 1 (pre-nephropathy stage), a diabetes patient showing ACR of 30 to 299 (mg/g) corresponding to microalbuminuria and eGFR of not less than 30 (mL/min/1.73 m²) is classified as stage 2 (early nephropathy stage), a diabetes patient showing ACR of not less than 300 (mg/g) corresponding to overt albuminuria or persistent proteinuria of not less than 0.5, and eGFR of not less than 30 (mL/min/1.73 m²) is classified as stage 3 (overt nephropathy stage), a diabetes patient showing eGFR of less than 30 (mL/min/1.73 m²) (preferably, a diabetes patient showing ACR of not less than 300 [mg/g] corresponding to overt albuminuria, or persistent proteinuria of not less than 0.5) is classified as stage 4 (renal failure stage), and a diabetes patient on dialysis treatment is classified as stage 4 (dialysis therapy stage). The present determination method and the present biomarker exhibit excellent effects particularly for making stage determination for stage 1 (pre-nephropathy stage) to stage 3 (overt nephropathy stage).

[0016] In the present invention, the term "onset of diabetic nephropathy" means a state where diabetic nephropathy at stage 2 (early nephropathy stage) or later where microalbuminuria is detected has developed. In other words, stage 1 (pre-nephropathy stage) corresponds to a preclinical state of diabetic nephropathy, and stage 2 (early nephropathy stage) corresponds to a state after the onset of diabetic nephropathy (a state where diabetic nephropathy has developed). The present determination method and the present biomarker exhibit excellent effects particularly for determination of the onset risk of diabetic nephropathy (diabetic nephropathy at early nephropathy stage) at stage 1 (pre-nephropathy stage).

[0017] Examples of the test subject include a test subject who has not developed diabetic nephropathy, a test subject who is unknown to have diabetic nephropathy or not, and a test subject (a diabetic nephropathy patient) whose stage (progression stage) of diabetic nephropathy is unknown. The test subject who is unknown to have diabetic nephropathy or not includes a test subject who has been previously affected by diabetic nephropathy but is unknown to have diabetic nephropathy or not at the time of examination, and the diabetic nephropathy patient whose stage (progression stage) of diabetic nephropathy is unknown includes a diabetic nephropathy patient that has been previously affected by diabetic nephropathy but whose stage (progression stage) of diabetic nephropathy at the time of examination is unknown.

[0018] Examples of the biological sample include a non-liquid sample such as a tissue, a cell or an organ, and a liquid sample such as blood, urine or saliva, and among these, blood (plasma or serum) or urine is preferred.

[0019] In the present determination method, phenyl sulfate to be detected or phenyl sulfate contained in the present biomarker is the following compound (molecular weight 174.17).



[0020] In the present determination method, a phenyl sulfate salt to be detected or a phenyl sulfate salt contained in the present biomarker includes a metal salt generated from aluminum, calcium, lithium, magnesium, potassium, sodium or zinc, or an organic salt generated from N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, lysine or procaine.

[0021] In the present determination method, when a concentration of a phenyl sulfate contained in a biological sample collected from a test subject who has not developed diabetic nephropathy is higher than that in a biological sample derived from a healthy individual, it is indicative that the test subject has a high possibility (risk) of developing diabetic nephropathy, and when a concentration of a phenyl sulfate contained in a biological sample collected from a test subject who has not developed diabetic nephropathy is not higher than that in a biological sample derived from a healthy individual, it is indicative that the test subject has a low possibility (risk) of developing diabetic nephropathy. Incidentally, the biological sample derived from the healthy individual is preferably subjected, after collection, to a similar treatment as the biological sample derived from the test subject.

[0022] Besides, in the present determination method, when a concentration of a phenyl sulfate contained in a biological sample collected from a test subject who is unknown to have diabetic nephropathy or not or a test subject whose stage of diabetic nephropathy is unknown is higher than that in a biological sample derived from a control, it is indicative that the test subject has a high possibility of having diabetic nephropathy at advanced stage as compared with the control,

and when a concentration of a phenyl sulfate contained in a biological sample collected from a test subject who is unknown to have diabetic nephropathy or not or a test subject whose stage of diabetic nephropathy is unknown is not higher than that in a biological sample derived from a control, it is indicative that the test subject has a low possibility of having diabetic nephropathy at advanced stage as compared with the control. Incidentally, the biological sample derived from the control is preferably subjected, after collection, to a similar treatment as the biological sample derived from the subject.

[0023] Besides, in the present determination method, when a concentration of a phenyl sulfate contained in blood collected from a test subject who has not developed diabetic nephropathy is not less than (over) a prescribed threshold value, for example, not less than 5.2 (nmol/mL), preferably not less than 5.5 (nmol/mL), more preferably not less than 6.5 (nmol/mL), and further preferably not less than 7.5 (nmol/mL), it is indicative that the test subject has a high possibility of developing diabetic nephropathy, and when a concentration of a phenyl sulfate contained in blood collected from a test subject who has not developed diabetic nephropathy is less than (not more than) a prescribed threshold value, for example, less than 5.2 (nmol/mL), preferably less than 5.5 (nmol/mL), more preferably less than 6.5 (nmol/mL), and further preferably less than 7.5 (nmol/mL), it is indicative that the test subject has a low possibility of developing diabetic nephropathy.

[0024] Furthermore, in the present determination method, when a concentration of a phenyl sulfate contained in blood collected from a test subject who is unknown to have diabetic nephropathy or not or a test subject whose stage of diabetic nephropathy is unknown is less than (not more than) a prescribed threshold value, for example, less than 0.51 (nmol/mL), preferably not more than 0.50 (nmol/mL), more preferably not more than 0.49 (nmol/mL), further preferably not more than 0.48 (nmol/mL), and still more preferably not more than 0.47 (nmol/mL), it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at stage 1 (pre-nephropathy stage), and when it is not less than (over) a prescribed threshold value, for example, not less than 0.51 (nmol/mL), preferably over 0.50 (nmol/mL), more preferably over 0.49 (nmol/mL), further preferably over 0.48 (nmol/mL), and still more preferably over 0.47 (nmol/mL), it is indicative that the test subject has a low possibility of being classified as diabetic nephropathy at stage 1 (pre-nephropathy stage).

[0025] Besides, in the present determination method, when a concentration of a phenyl sulfate contained in blood collected from a test subject who is unknown to have diabetic nephropathy or not, or from a test subject whose stage of diabetic nephropathy is unknown is over (not less than) a prescribed threshold value, for example, over 54.4 (nmol/mL), preferably not less than 54.5 (nmol/mL), more preferably not less than 54.6 (nmol/mL), and further preferably not less than 54.7 (nmol/mL), still more preferably in a range of prescribed values, for example, a range of over 54.4 (nmol/mL) to 159 (nmol/mL), particularly preferably a range of 54.5 (nmol/mL) to 159 (nmol/mL), and most preferably a range of 54.6 (nmol/mL) to 159 (nmol/mL), it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at stage 2 (early nephropathy stage) or stage 3 (overt nephropathy stage). Further, when a concentration of a phenyl sulfate contained in blood collected from a test subject who is unknown to have diabetic nephropathy or not, or from a test subject whose stage of diabetic nephropathy is unknown is not more than (less than) or not less than (over) a prescribed threshold value, for example, not more than 54.4 (nmol/mL) or not less than 159 (nmol/mL), preferably less than 54.5 (nmol/mL) or not less than 159 (nmol/mL), more preferably less than 54.6 (nmol/mL) or not less than 159 (nmol/mL), and further preferably less than 54.7 (nmol/mL) or not less than 159 (nmol/mL), it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at stage 2 (early nephropathy stage) or stage 3 (overt nephropathy stage).

[0026] Besides, in the present determination method, when a concentration of a phenyl sulfate contained in blood collected from a test subject who is unknown to have diabetic nephropathy or not, or from a test subject whose stage of diabetic nephropathy is unknown is over (not less than) a prescribed threshold value, for example, over 74.1 (nmol/mL), preferably not less than 74.2 (nmol/mL), more preferably not less than 74.3 (nmol/mL), further preferably not less than 74.4 (nmol/mL), and still more preferably in a range of prescribed values, for example, a range of over 74.1 (nmol/mL) to 159 (nmol/mL), particularly preferably a range of 74.2 (nmol/mL) to 159 (nmol/mL), and most preferably a range of 74.3 (nmol/mL) to 159 (nmol/mL), it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at stage 3 (overt nephropathy stage). Further, when a concentration of a phenyl sulfate contained in blood collected from a test subject who is unknown to have diabetic nephropathy or not, or from a test subject whose stage of diabetic nephropathy is unknown is not more than (less than) or over (not less than) a prescribed threshold value, for example, not more than 74.1 (nmol/mL) or over 159 (nmol/mL), preferably not more than 74.2 (nmol/mL) or over 159 (nmol/mL), more preferably not more than 74.3 (nmol/mL) or over 159 (nmol/mL), and further preferably not more than 74.4 (nmol/mL) or over 159 (nmol/mL), it is regarded that the test subject has a low possibility of being classified as diabetic nephropathy at stage 3 (overt nephropathy stage).

[0027] The prescribed threshold values (cut-off values) or the prescribed values can be calculated by a usual method based on data of concentrations of a phenyl sulfate in biological samples collected from diabetic nephropathy patients, healthy individuals and/or controls.

[0028] In the present determination method, a method for detecting a phenyl sulfate can be any method as long as a

concentration of a phenyl sulfate can be measured or quantitatively determined, and specific examples include mass spectrometry using a mass spectrometer (MS) and immunohistochemical staining, among which mass spectrometry is preferred.

[0029] Examples of the mass spectrometer (MS) include a liquid chromatograph mass spectrometer (LC/MS), a gas chromatograph mass spectrometer (GC/MS), a capillary electrophoresis-mass spectrometer (CE-MS), and an ELDI (Surface Enhanced Laser Desorption/Ionization) time-of-flight mass spectrometer (TOFMS).

[0030] An example of the immunohistochemical staining includes a method in which an antibody specifically bonding to a phenyl sulfate is used for performing analysis by latex agglutination immunoassay, fluorescent antibody technique, radioimmunoassay, immunoprecipitation, immune tissue staining, or Western blotting.

[0031] Now, the present invention will be more specifically described with reference to the Examples, and it is noted that the technical scope of the present invention is not limited to the following exemplified description.

Example 1

[0032] Plasma samples and urine samples were collected from 316 test subjects (including 143 males and 173 females) and the test subjects were tested for gender and eleven items (a phenyl sulfate salt [PS] concentration, a heart rate, a plasma glucose concentration, a hemoglobin A1c [HbA1c] value, a urine pH value, an estimated glomerular filtration rate [eGFR], a urinary albumin/creatinine ratio [ACR], a triglyceride [TG] level, a uric acid [UA] level, a blood urea nitrogen [BUN] level, and a pulse wave velocity [PWV] value). Multiple regression analysis was performed with the PS concentration used as a dependent function and with twelve values, that is, test values of the other ten items excluding the PS concentration and values corresponding to the age and the gender, used as independent variables. As a result, the PS concentration was more highly correlated with ACR corresponding to an index of early diabetic nephropathy than a plasma glucose concentration ($p = 0.343$) and the HbA1c value ($p = 0.351$) corresponding to indexes of diabetes, the eGFR ($p = 0.512$) and the BUN level ($p = 0.046$) corresponding to indexes of renal function, the TG level ($p = 0.368$) corresponding to an index of liver function, the UA level ($p = 0.524$) corresponding to an index of gout, or the PWV value (0.213) corresponding to an index of arteriosclerosis.

[0033] Joint Committee on Diabetic Nephropathy prescribed, in December 2013, a novel early diagnostic criterion that "a patient showing ACR of 30 to 299 mg/g is diagnosed with early diabetic nephropathy". Therefore, the test subjects were classified into groups respectively showing ACR of 0 to less than 30 (mg/g) (normal albuminuria), of 30 to 299 (mg/g) (microalbuminuria), and of not less than 300 (mg/g) (overt albuminuria) (respectively designated as a pre-nephropathy group, an early nephropathy group, and an overt nephropathy group), and the correlation with the PS concentration was checked (Table 1).

[Table 1]

ACR (mg/g)	0 to Less than 30	30 to 299	Not less than 300
Number of Test Subjects	182	89	44
Blood PS Concentration (nmol/mL)	3.12 (0.41 to 54.4)	4.88* (0.51 to 74.1)	14.7** (1.42 to 159)
Urine PS concentration (nmol/mL)	53.3 (3.47 to 843)	40.1 (1.22 to 795)	91.0*** (6.76 to 763)
eGFR (mL/min/1.73 m ²)	68.9±20.6	62.7±23.6	38.8±25.2

In this table, "*" indicates that there is a statistically significant difference ($p = 0.035$) from a blood PS concentration (nmol/mL) of "ACR of 0 to less than 30 (mg/g)".

[0034] Besides, "***" indicates that there are statistically significant differences ($p = 0.000192$ and $p = 0.00111$ respectively) respectively from blood PS concentrations (nmol/mL) of "ACR of 0 to less than 30 (mg/g)" and "ACR of 30 to 300 (mg/g)".

[0035] Furthermore, "****" indicates that there are statistically significant differences ($p = 0.000167$ and $p = 0.00392$ respectively) respectively from urine PS concentrations (nmol/mL) of "ACR of 0 to less than 30 (mg/g)" and "ACR of 30 to 300 (mg/g)".

[0036] As a result, the blood PS concentration was higher in the early nephropathy group than in the pre-nephropathy group, and in the overt nephropathy group than in the early nephropathy group. This result reveals that the blood PS concentration increases as the stage of diabetic nephropathy defined based on a urinary albumin level proceeds (Table 1).

[0037] Besides, although there was no difference in the urine PS concentration between the pre-nephropathy group and the early nephropathy group, the urine PS concentration was higher in the overt nephropathy group than in the pre-nephropathy group and the early nephropathy group.

[0038] It was revealed, based on these results, that the stage (the early stage in particular) of diabetic nephropathy can be determined by using, as an index, the increase of the blood or urine PS concentration.

[0039] On the other hand, it is known that a blood PS concentration of a healthy individual is 0.07 ± 0.02 (mg/dL) ($\approx 4.0 \pm 1.1$ [nmol/mL]) (document "Itoh, Y. et al., Anal. Bioanal. Chem. 2012, 403: 1841-1850"). In other words, assuming that the blood PS concentration derived from a healthy individual is normally distributed, healthy individuals showing a blood PS concentration of 4.0 ± 1.1 (nmol/mL) occupy about 68% of the whole, healthy individuals showing a blood PS concentration of 4.0 ± 2.2 (nmol/mL) occupy about 95% of the whole, and healthy individuals showing a blood PS concentration of 4.0 ± 3.3 (nmol/mL) occupy about 99% of the whole. Therefore, the threshold value of the PS concentration was set to 5.5 (nmol/mL), 6.5 (nmol/mL) or 7.5 (nmol/mL) to check whether or not the PS concentration had increased before the onset of diabetic nephropathy (early nephropathy stage) (Table 2).

[Table 2]

ACR (mg/g)	0 to Less than 30 (Pre-nephropathy Stage)	30 to 299 (Early Nephropathy Stage)	Not less than 300 (Overt Nephropathy Stage)
Number of Test Subjects	182	89	44
Less than 5.5	118 (65%)	46 (52%)	10 (23%)
Less than 6.5	125 (69%)	54 (61%)	12 (27%)
Less than 7.5	131 (72%)	58 (65%)	14 (32%)
Not less than 5.5	64 (35%)	43 (48%)	34 (77%)
Not less than 6.5	57 (31%)	35 (39%)	32 (73%)
Not less than 7.5	51 (28%)	31 (35%)	30 (68%)

[0040] In this table, "Less than 5.5", "Less than 6.5", "Less than 7.5", "Not less than 5.5", "Not less than 6.5" and "Not less than 7.5" indicate blood PS concentrations (nmol/mL). Each parenthesized value (%) indicates a proportion in all the test subjects.

[0041] As a result, when the threshold values of the PS concentration was set to any of 5.5 (nmol/mL), 6.5 (nmol/mL) and 7.5 (nmol/mL), the PS concentration was increased in some test subjects in the pre-nephropathy stage before the onset of diabetic nephropathy (diabetic nephropathy at the early nephropathy stage) ("Not less than 5.5", "Not less than 6.5" and "Not less than 7.5" of Table 2).

[0042] Also in consideration of the results of Table 1 revealing that the stage of diabetic nephropathy can be determined by using the increase of the PS concentration as an index, it is presumed that a test subject showing the increase of the PS concentration before the onset of diabetic nephropathy has high risk of developing diabetic nephropathy. Besides, assuming that the blood PS concentration is normally distributed, the following can be said: In a test subject showing a blood PS concentration not less than 5.5 (nmol/mL), the blood PS concentration is higher than that of a healthy individual with at least a 84% probability, in a test subject showing a blood PS concentration not less than 6.5 (nmol/mL), the blood PS concentration is higher than that of a healthy individual with at least a 97.5% probability, and in a test subject showing a blood PS concentration not less than 7.5 (nmol/mL), the blood PS concentration is higher than that of a healthy individual with at least a 99.5% probability.

Industrial Applicability

[0043] The present invention contributes to early detection and early treatment of diabetic nephropathy and prevention of onset of diabetic nephropathy.

Claims

1. A method for determining diabetic nephropathy, comprising detecting phenyl sulfate or salt thereof contained in a biological sample collected from a test subject.
2. The method for determining diabetic nephropathy according to claim 1, wherein the biological sample is blood or urine.

3. The method for determining diabetic nephropathy according to claim 2, wherein the test subject is a test subject who has not developed diabetic nephropathy, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject is not less than a prescribed threshold value, it is indicative that the test subject has a high possibility of developing diabetic nephropathy.
4. The method for determining diabetic nephropathy according to claim 3, wherein the prescribed threshold value is 5.5 (nmol/mL).
5. The method for determining diabetic nephropathy according to claim 2, wherein the test subject is a test subject who is unknown to have diabetic nephropathy or not, or a test subject whose stage of diabetic nephropathy is unknown, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject is less than a prescribed threshold value, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at pre-nephropathy stage, when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject falls in a range of prescribed values A, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at early nephropathy stage or overt nephropathy stage, and when a concentration of the phenyl sulfate or salt thereof contained in blood collected from the test subject falls in a range of prescribed values B, it is indicative that the test subject has a high possibility of being classified as diabetic nephropathy at overt nephropathy stage.
6. The method for determining diabetic nephropathy according to claim 5, wherein the prescribed threshold value is 0.51 (nmol/mL), the prescribed values A are over 54.4 (nmol/mL) to 159 (nmol/mL), and the prescribed values B are over 74.1 (nmol/mL) to 159 (nmol/mL).
7. A biomarker for determining diabetic nephropathy, comprising phenyl sulfate or salt thereof.
8. The biomarker according to claim 7, wherein determination of diabetic nephropathy is determination of onset risk of diabetic nephropathy.
9. The biomarker according to claim 7, wherein determination of diabetic nephropathy is determination of stage of diabetic nephropathy.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2016/004394

A. CLASSIFICATION OF SUBJECT MATTER

G01N33/49(2006.01)i, G01N27/62(2006.01)n, G01N33/53(2006.01)n

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)

G01N33/49, G01N27/62, G01N33/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2016

Kokai Jitsuyo Shinan Koho 1971-2016 Toroku Jitsuyo Shinan Koho 1994-2016

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus/JMEDPlus/JST7580(JDREAMIII),

CAplus/REGISTRY/MEDLINE/BIOSIS(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NG D. P. K., A metabolomic study of low estimated GFR in non-proteinuric type 2 diabetes mellitus, Diabetologia, 2012, Vol.55 No.2, Page.499-508, Abstract, Methods (Metabolomic data preprocessing), page 503, right column, Table.1, Table.3	1-2, 7-9
X	Jun'ya HIRATA, "Indoxyl Sulfate Oyobi sonota no Nyodokusho Busshitsu no Hinketsu Yuhatsu Sayo ni Kansuru Hikaku Kento", The Japanese Journal of Nephrology, 2013, vol.55, no.3, page 428, P-490	1-9

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* 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

09 December 2016 (09.12.16)

Date of mailing of the international search report

20 December 2016 (20.12.16)

Name and mailing address of the ISA/

Japan Patent Office

3-4-3, Kasumigaseki, Chiyoda-ku,

Tokyo 100-8915, Japan

Authorized officer

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2016/004394

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOBAYASHI Toshihiro, Exploration of novel predictive markers in rat plasma of the early stages of chronic renal failure, Anal Bioanal Chem, 2014, Vol.406 No.5, Page.1365-1376, Abstract, Fig. 3(F)	1-9
X	Toshimitsu NIWA, "Analysis of Phenols in Uremic Serum by Gas Chromatography-mass Spectrometry", The Japanese Journal of Nephrology, 1981, vol.23, no.6, pages 777 to 788, page 782	1-9
X	KIKUCHI Kaori, Metabolomic search for uremic toxins as indicators of the effect of an oral sorbent AST-120 by liquid chromatography/tandem mass spectrometry, J Chromatogr B, 2010, Vol.878 No.29, Page.2997-3002, Abstract, Table.2	1-9
A	Susumu OGAWA, "Tonyobyosei Jinsho no Shinko ni Okeru Kecchu Oyobi Nyochu osteopontin to Igi", Folia endocrinologica Japonica, 01 April 2015 (01.04.2015), vol.91, no.1, page 320, P1-10-1	1-9
A	Takuya SAKAMOTO, "Tonyobyosei Jinsho ni Okeru Kecchu CyclophilinA no Shinki Biomarker to shite no Yuyosei ni Tsuite", The Journal of the Japan Diabetic Society, 2013, vol.56, no.Supplement 1, page S.365, III-8-20	1-9
A	Susumu OGAWA, "Tonyobyosei Jinsho no Byoki Shinko to Ensho Marker no Zodai Oyobi Shinkekkan Shogai Shinko no Kento", Diabetes Front, 2006, vol.17, no.5, page 671	1-9
A	JP 2012-237623 A (Kureha Corp.), 06 December 2012 (06.12.2012), entire text; all drawings (Family: none)	1-9
A	WO 2013/188333 A1 (METABOLON, INC.), 19 December 2013 (19.12.2013), entire text; all drawings (Family: none)	1-9

Form PCT/ISA/210 (continuation of second sheet) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- JP 2014032046 A [0005]

Non-patent literature cited in the description

- **DIHAZI, H. et al.** *Clin Chemi*, 2007, vol. 53, 1636-1645 [0006]
- **MISCHAK, H. et al.** *Clin Sci (Lond)*, 2004, vol. 107, 485-495 [0006]
- **ITOH, Y. et al.** *Anal. Bioanal. Chem.*, 2012, vol. 403, 1841-1850 [0039]

专利名称(译)	确定糖尿病肾病的标志物		
公开(公告)号	EP3358347A4	公开(公告)日	2019-05-29
申请号	EP2016850676	申请日	2016-09-29
申请(专利权)人(译)	东北大学		
当前申请(专利权)人(译)	东北大学		
[标]发明人	ABE TAKAAKI OGAWA SUSUMU TOMIOKA YOSHIHISA		
发明人	ABE, TAKAAKI OGAWA, SUSUMU TOMIOKA, YOSHIHISA		
IPC分类号	G01N33/49 G01N27/62 G01N33/53		
CPC分类号	G01N33/6893 G01N33/53 G01N33/5308 G01N33/84 G01N2800/347 G01N2800/56		
优先权	2015193638 2015-09-30 JP		
其他公开文献	EP3358347A1		
外部链接	Espacenet		

摘要(译)

本发明的一个目的是提供一种能够确定糖尿病肾病（特别是早期糖尿病肾病）的发病风险或阶段的生物标志物。糖尿病肾病通过测量或定量测定从受试者收集的生物样品，优选血浆或尿液中含有的苯基硫酸盐或其盐（苯基硫酸盐）的浓度来确定，以检测苯基硫酸盐。本发明显示出优异的效果，特别是对于阶段1（肾病前阶段）至阶段3（明显肾病阶段）的阶段确定，或用于确定糖尿病肾病（肾病前阶段的糖尿病肾病）的发病风险。