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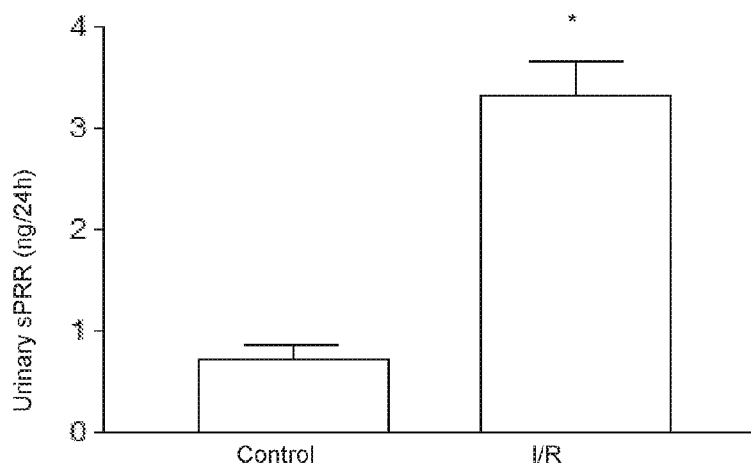


FIG. 1

(57) Abstract: Disclosed are methods of treating or diagnosing a kidney disease in a subject as well as methods of ameliorating one or more symptoms associated with a kidney disease wherein the subject has an increase of sPRR in their urine. Also disclosed are methods of determining the efficacy of a therapeutic by determining the level of sPRR in the urine of a subject and comparing the change in those levels after administration of a therapeutic.

**THE USE OF URINARY SOLUBLE (PRO)RENIN RECEPTOR AS A BIOMARKER
FOR KIDNEY DISEASE**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/095,949, filed December 23, 2014, which is hereby incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under DK094956 and DK079162 awarded by the National Institutes of Health and the Research Career Scientist award from the Department of Veterans Affairs. The government has certain rights in the invention.

BACKGROUND

[0003] Kidney diseases are a very prevalent health concern. For example, kidney disease is the most important risk factor for cardiovascular (CV) diseases which remains the number one cause of morbidity and mortality in the United States. In the United States, one in 10 adults has chronic kidney disease (CKD). CKD gradually progresses to end-stage renal disease (ESRD) that requires kidney transplantation or dialysis. Although the cause of kidney disease is multifactorial, the overactivation of renin-angiotensin system (RAS) is considered to play a major role in initiating or maintaining the disease process.

[0004] Because PRR is involved in kidney diseases, PRR could be used as system for monitoring, treating, and diagnosing subjects with kidney disease.

BRIEF SUMMARY

[0005] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject.

[0006] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor (sPRR) in a

urine sample obtained from the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA.

[0007] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor in a urine sample obtained from the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease. Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as an increase of having soluble (pro)renin receptor in a urine sample obtained from the subject, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

[0008] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject.

[0009] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA.

[0010] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

[0011] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject, wherein the therapeutic known to treat the kidney disease is an angiotensin-

converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

[0012] A method of diagnosing a kidney disease in a subject comprising detecting an increase of sPRR in a urine sample obtained from the subject; and administering a therapeutic known to treat the kidney disease to the subject.

[0013] A method of diagnosing a kidney disease in a subject comprising detecting an increase of sPRR in a urine sample obtained from the subject; and administering a therapeutic known to treat the kidney disease to the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA.

[0014] A method of diagnosing a kidney disease in a subject comprising detecting an increase of sPRR in a urine sample obtained from the subject; and administering a therapeutic known to treat the kidney disease to the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

[0015] A method of diagnosing a kidney disease in a subject comprising detecting an increase of sPRR in a urine sample obtained from the subject; and administering a therapeutic known to treat the kidney disease to the subject, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, corticosteroids, or an immunosuppressant.

[0016] Disclosed are methods of treating a kidney disease in a subject comprising determining disease progression based on the amount of increase of sPRR present in the urine of the subject, wherein the amount of increase of sPRR in the urine of the subject indicates the progression of kidney disease; and administering, to the subject, a therapeutic known to treat the kidney disease at that stage of progression.

[0017] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic.

[0018] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR

determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic, wherein an increase of the level of sPRR determined in step (c) over the level of sPRR determined in step (a) indicates a lack of efficacy of the therapeutic.

[0019] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic, wherein a decrease of the level of sPRR determined in step (c) over the level of sPRR determined in step (a) indicates good efficacy of the therapeutic.

[0020] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR.

[0021] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

[0022] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

[0023] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR, wherein the one or more symptom associated with a kidney disease is high blood pressure, fatigue, or shortness of breath.

[0024] Additional advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be

understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and compositions.

[0026] Figure 1 shows increased urinary soluble (pro)renin receptor (sPRR) in C57/BL6 mice after ischemia-reperfusion (I/R) injury. Male C57/BL6 mice were subjected to 30-min bilateral ischemia followed by reperfusion. Sham-operated C57/BL6 mice served as control. The assays for urinary sPRR were determined at 48 h after I/R. Control: n = 3; I/R: n = 6. Data are mean + SE. *, p<0.05.

[0027] Figure 2 shows the correlation of urinary sPRR with proteinuria in a mouse model of ischemia-reperfusion kidney injury. C57/BL6 mice were subjected to 30-min bilateral ischemia followed by reperfusion. The assays for urinary albumin and sPRR were determined at 48 h after I/R.

[0028] Figure 3 shows the correlation of urinary sPRR with plasma creatinine in a mouse model of I/R kidney injury. C57/BL6 mice were subjected to 30-min bilateral ischemia followed by reperfusion. T Fig. 4. Increased urinary sPRR in diabetic db/db mice. All male adult lean and db/db animals underwent unephrectomy. Urinary excretion of sPRR and albumin was determined by using ELISA. Lean: n = 4; db/db: n = 10. Data are mean + SE. *, p<0.05. The assays for plasma creatinine and sPRR were determined at 48 h of I/R.

[0029] Figure 4 shows an increased urinary sPRR in diabetic db/db mice. All male adult lean and db/db animals underwent unephrectomy. Urinary excretion of sPRR and albumin was determined by using ELISA. Lean: n = 4; db/db: n = 10. Data are mean + SE. *, p<0.05.

[0030] Figure 5 shows the correlation of urinary sPRR with albuminuria in diabetic db/db mice. All animals underwent unephrectomy to aggravate kidney injury. Urinary excretion of sPRR and albumin was determined by using ELISA.

[0031] Figure 6 shows the correlation of urinary sPRR with albuminuria in patients with type 2 diabetes. Urinary excretion of sPRR and albumin was determined by using ELISA and expressed as a ratio to creatinine.

DETAILED DESCRIPTION

[0032] The disclosed method and compositions may be understood more readily by reference to the following detailed description of particular embodiments and the Example included therein and to the Figures and their previous and following description.

[0033] It is understood that the disclosed method and compositions are not limited to the particular methodology, protocols, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

A. Definitions

[0034] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a therapeutic" includes a plurality of such therapeutics, reference to "the kidney disease" is a reference to one or more kidney diseases known to those skilled in the art, and so forth.

[0035] The term "ameliorate" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

[0036] The term "dose" refers to the quantity or amount of a composition taken, administered, or recommended to be taken or administered at or over a particular time. The time can be per administration or per day. For example, a dose of a therapeutic can be, but is not limited to, a specific amount of a composition, such as an immunosuppressant, administered 2x a day. A dose can also be a specific amount of a composition administered every day for 2 weeks.

[0037] A "kidney disease" is a disease that can be due to a variety of conditions that lead to kidney damage and deterioration of kidney function. A kidney disease can affect any part of the body as long as at some point during the disease process the kidneys are affected.

[0038] The term "efficacy" refers to the ability to produce a desired or intended result. For example, the efficacy of a therapeutic is the ability of the therapeutic to produce the intended result, such as treat a particular disease. Efficacy can be determined by evaluating laboratory tests, signs and symptoms known to be useful in evaluating the status of a kidney disease.

[0039] The phrase “increase of sPRR” refers to an increase of sPRR over normal or control levels. The increase can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or greater fold increase over normal or control levels. In some instances an increase of sPRR refers to a statistically significant increase in sPRR over normal or control levels.

[0040] “Normal levels” or “control levels” of sPRR can be levels that were previously established from a control population or can be levels that are determined from a control sample determined at the same time as the sample from the subject with kidney disease. For example, control levels can be the levels of sPRR known to exist in the urine of control individuals.

[0041] The term “control” refers to a subject or a sample taken from a subject that does not have kidney disease. In some instances, the control can be a healthy individual. In some instances, the control can be a subject having a disease other than kidney disease.

[0042] The term “therapeutic” refers to a composition that treats a disease. For example, the therapeutics disclosed herein are compositions that treat kidney diseases.

[0043] The phrase “therapeutic effect” refers to the treatment, reduction of symptoms, amelioration, prevention of disease, inhibition of disease progression, or reduced severity or incidence of disease as a result of the administration of a therapeutic.

[0044] The term “treating” refers to partially or completely alleviating, ameliorating, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0045] “Soluble (pro)renin receptor (sPRR)” is the soluble form of the (pro)renin receptor (PRR) protein. sPRR can include full-length sPRR or fragments of sPRR. The fragments of sPRR can be functional or non-functional. For example, function fragments of sPRR can retain the ability to bind (pro)renin or renin.

[0046] “Optional” or “optionally” means that the subsequently described event, circumstance, or material may or may not occur or be present, and that the description includes instances where the event, circumstance, or material occurs or is present and instances where it does not occur or is not present.

[0047] Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, also specifically contemplated and considered disclosed is the range from the one particular value and/or to the other particular value unless the context specifically indicates otherwise. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another, specifically contemplated embodiment that should be considered disclosed unless the context specifically indicates otherwise. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint unless the context specifically indicates otherwise. Finally, it should be understood that all of the individual values and sub-ranges of values contained within an explicitly disclosed range are also specifically contemplated and should be considered disclosed unless the context specifically indicates otherwise. The foregoing applies regardless of whether in particular cases some or all of these embodiments are explicitly disclosed.

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present method and compositions, the particularly useful methods, devices, and materials are as described. Publications cited herein and the material for which they are cited are hereby specifically incorporated by reference. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such disclosure by virtue of prior invention. No admission is made that any reference constitutes prior art. The discussion of references states what their authors assert, and applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of publications are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0049] Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises," means "including but not limited to," and is not intended to exclude, for example, other additives, components, integers or steps. In particular, in methods stated as comprising one or more steps or operations it is specifically contemplated that each step comprises what is listed (unless that step includes a limiting term such as "consisting of"), meaning that each step is not intended to exclude, for

example, other additives, components, integers or steps that are not listed in the step.

[0050] It is to be understood that the disclosed method and compositions are not limited to specific synthetic methods, specific analytical techniques, or to particular reagents unless otherwise specified, and, as such, may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0051] Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a PRR antagonist is disclosed and discussed and a number of modifications that can be made to a number of molecules including the PRR antagonist are discussed, each and every combination and permutation of the PRR antagonist and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

B. Methods of Treating Kidney Disease

[0052] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject. Identifying a subject as having an increase of sPRR in a urine sample obtained from the subject can occur by comparing the amount or levels of sPRR in the urine sample obtained from the subject to the amount or levels of sPRR in a control sample. For example, a control sample can be a urine sample from a healthy subject or a subject that does not have kidney disease.

[0053] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA. sPRR can also be detected by other common techniques known to detect proteins and protein fragments, such as but not limited to, western blot, dot blot, slot blot, or other known immunoassays, or mass spectrometry-based methods

[0054] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having increased or elevated soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

[0055] Therapeutics known to treat the kidney disease can be, but are not limited to, an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, corticosteroids, or an immunosuppressant. In some aspects, the therapeutic known to treat kidney disease is a non-natural compound or substance. In some instances, the therapeutic known to treat kidney disease can be an angiotensin-converting-enzyme (ACE) inhibitor or an Ang II type I (AT1) blocker. In some instances, the therapeutic can be a PRR antagonists. PRR antagonists can be polypeptides or small molecules. Examples of functional PRR antagonist polypeptides include but are not limited to IFDNIISQGVLEKDV (PR10; SEQ ID NO:1), LPTDTTTFKRIFLKRMP (PR20; SEQ ID NO:2), LPTDTTTFKRIFLKRMP (PR30; SEQ ID NO:3), and LPTRTATFERIPLKKMP (PR40; SEQ ID NO:4). Variants of the PRR antagonist polypeptides can be used. In some instances, polynucleotides that are capable of encoding the PRR antagonist polypeptides can be used.

[0056] Also disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having increased or elevated soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject, further comprising wherein the subject has been identified as not having increased or elevated sPRR in a plasma sample obtained from the subject. In some instances, determining the absence of sPRR in a plasma sample taken from the subject occurs prior to administering a therapeutic known to treat the kidney disease to the subject. In some instances, the presence of sPRR in a plasma sample taken from the subject can be indicative that no administering of a therapeutic known to treat the kidney disease is required. The same techniques used to determine the levels of sPRR in a urine sample can be used to determine the levels of sPRR in a plasma sample.

[0057] Further disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject. Detecting an increase of sPRR in a urine sample obtained from the subject can occur by comparing the amount or levels of sPRR in the urine sample obtained from the subject to the amount or levels of sPRR in a control sample. For example, a control sample can be a urine sample from a healthy subject or a subject that does not have kidney disease.

[0058] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising: detecting the presence of sPRR in a urine sample obtained from the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA. sPRR can also be detected by other techniques known to detect proteins and protein fragments, such as but not limited to, western blot, dot blot, slot blot, or other known immunoassays or mass-spectrometry-based methods.

[0059] Disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting the presence of sPRR in a urine sample obtained from the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

[0060] Therapeutics known to treat the kidney disease can be, but are not limited to, an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, corticosteroids, or an immunosuppressant. In some instances, the therapeutic can be a PRR antagonists. PRR antagonists can be polypeptides. Examples of functional PRR antagonist polypeptides include but are not limited to IFDNIISQGVLKEDVF (PR10; SEQ ID NO:1), LPTDTTTFKRIFLKRMP SI (PR20; SEQ ID NO:2), LPTDTTTFKRIFLKRMP SIRE (PR30; SEQ ID NO:3), and LPTRTATFERIPLKKMP SVRE (PR40; SEQ ID NO:4). Variants of the PRR antagonist polypeptides can be used. In some instances, polynucleotides that are capable of encoding the PRR antagonist polypeptides can be used.

[0061] Also disclosed are methods of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject, further comprising wherein the subject has been identified as not having an increase of sPRR in a plasma sample obtained from the subject. In some instances, determining the absence of sPRR in a plasma sample taken from the subject occurs prior to administering a therapeutic known to treat the kidney disease to the subject. In some instances, the presence of sPRR in a plasma sample taken from the subject can be indicative that no administering of a therapeutic known to treat the kidney disease is required. The same techniques used to determine the levels of sPRR in a urine sample can be used to determine the levels of sPRR in a plasma sample.

[0062] Further disclosed are methods of treating a kidney disease in a subject comprising determining disease progression based on the amount of sPRR present in the urine of the subject, wherein the amount of sPRR in the urine of the subject indicates the progression of kidney disease; and administering, to the subject, a therapeutic known to treat the kidney disease at that stage of progression.

[0063] Disclosed are methods of treating a kidney disease in a subject comprising determining disease progression based on the amount of sPRR present in the urine of the subject, wherein the amount of sPRR in the urine of the subject indicates the progression of kidney disease; and administering, to the subject, a therapeutic known to treat the kidney disease at that stage of progression, wherein an increased amount of sPRR in the urine indicates kidney injury. In some instances, an increased amount of sPRR in the urine can be determined by comparing the levels of sPRR in urine samples taken at different time points

from a subject. For example, a urine sample taken from an early stage of disease progression can be compared to a urine sample taken days, months, or years later. In some instances, an increased amount of sPRR in the urine can be determined by comparing the levels of sPRR in a urine sample taken from the subject with a control, wherein the control is known levels of sPRR that correspond to different stages of disease progression. For example, known levels of urinary sPRR from the different stages of chronic kidney disease can be used as the control.

C. Methods of Diagnosing a Kidney Disease

[0064] Disclosed are methods of diagnosing a kidney disease in a subject based on an increase of sPRR in a urine sample obtained from the subject.

[0065] Disclosed are methods of diagnosing a kidney disease in a subject comprising a) detecting an increase of sPRR in a urine sample obtained from the subject; and b) administering a therapeutic known to treat the kidney disease to the subject.

[0066] Disclosed are methods of diagnosing a kidney disease in a subject comprising a) detecting an increase of sPRR in a urine sample obtained from the subject; and b) administering a therapeutic known to treat the kidney disease to the subject, wherein the sPRR in the urine sample of the subject was detected by ELISA. sPRR can also be detected by other common techniques known to detect proteins and protein fragments, such as but not limited to, western blot, dot blot, slot blot, or other known immunoassays, or mass spectrometry-based methods.

[0067] Disclosed are methods of diagnosing a kidney disease in a subject comprising a) detecting an increase of sPRR in a urine sample obtained from the subject; and b) administering a therapeutic known to treat the kidney disease to the subject, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease

[0068] Therapeutics known to treat the kidney disease can be, but are not limited to, an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, corticosteroids, or an immunosuppressant. In some instances, the therapeutic can be a PRR antagonists. PRR antagonists can be polypeptides or small molecules. Examples of functional PRR antagonist polypeptides include but are not limited to IFDNIISQGVLEKEDVF (PR10; SEQ ID NO:1), LPTDTTTFKRIFLKRMPSEI (PR20; SEQ ID NO:2), LPTDTTTFKRIFLKRMPSEIRE (PR30; SEQ ID NO:3), and LPTRTATFERIPLKKMPSEVRE (PR40; SEQ ID NO:4). Variants of the PRR antagonist

polypeptides can be used. In some instances, polynucleotides that are capable of encoding the PRR antagonist polypeptides can be used.

[0069] Further disclosed are methods of diagnosing a kidney disease in a subject comprising a) detecting an increase of sPRR in a urine sample obtained from the subject; and b) administering a therapeutic known to treat the kidney disease to the subject, further comprising determining a decrease or no change in the amount of sPRR in a plasma sample taken from the subject. In some instances, determining a decrease or no change in the amount of sPRR in a plasma sample taken from the subject occurs prior to administering a therapeutic known to treat the kidney disease to the subject. In some instances, an increase of sPRR in a plasma sample taken from the subject can be indicative that no administering of a therapeutic known to treat the kidney disease is required. The same techniques used to determine the levels of sPRR in a urine sample can be used to determine the levels of sPRR in a plasma sample.

D. Methods of Determining the Efficacy of a Therapeutic

[0070] Disclosed are methods of determining the efficacy of a therapeutic based on the levels of sPRR present in a urine sample obtained from a subject that has received at least one dose of the therapeutic.

[0071] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic.

[0072] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic, wherein an increase of sPRR in the urine over control levels in the urine indicates a lack of efficacy of the therapeutic. A lack of efficacy of the therapeutic refers to the therapeutic having no therapeutic effect. No therapeutic effect can include no change in sPRR levels in the urine of a subject that was administered the therapeutic. No therapeutic effect can include an increase

over control levels in sPRR levels in the urine of a subject that was administered the therapeutic.

[0073] Disclosed are methods of determining the efficacy of a therapeutic comprising a) determining the level of sPRR in the urine of a subject; b) administering at least one dose of the therapeutic to the subject; c) determining the level of sPRR in the urine of a subject after step (b); and d) comparing the level of sPRR determined in step (c) to the level of sPRR determined in step (a), wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic, wherein a decrease of sPRR over control levels indicates good efficacy of the therapeutic. Good efficacy of the therapeutic refers to the therapeutic having a therapeutic effect. A therapeutic effect can include a reduction of disease symptoms, amelioration of symptoms, prevention of disease, inhibition of disease progression, or reduced severity or incidence of disease as a result of the administration of the therapeutic.

[0074] In some instances only one dose of a therapeutic is administered to the subject. In some instances, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 doses of a therapeutic can be administered to the subject. A dose of a therapeutic can be administered 1x, 2x, 3x, 4x, 5x, 6x, 7x, or 8x a day. A dose can be a single administration or several administrations over a particular period of time.

E. Methods of Ameliorating Symptoms Associated with Kidney Disease

[0075] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease.

[0076] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR. A subject as having an increase of sPRR in a urine sample obtained from the subject can be determined by comparing the amount or levels of sPRR in the urine sample obtained from the subject to the amount or levels of sPRR in a control sample. For example, a control sample can be a urine sample from a healthy subject or a subject that does not have kidney disease.

[0077] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease

[0078] Therapeutics known to treat kidney diseases can be, but are not limited to, an

angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant. In some instances, the therapeutic can be a PRR antagonists. PRR antagonists can be polypeptides or small molecules. Examples of functional PRR antagonist polypeptides include but are not limited to IFDNIISQGVLKEDVF (PR10; SEQ ID NO:1), LPTDTTTFKRIFLKRMP SI (PR20; SEQ ID NO:2), LPTDTTTFKRIFLKRMP SIRE (PR30; SEQ ID NO:3), and LPTRTATFERIPLKKMP SVRE (PR40; SEQ ID NO:4). Variants of the PRR antagonist polypeptides can be used. In some instances, polynucleotides that are capable of encoding the PRR antagonist polypeptides can be used.

[0079] Disclosed are methods of ameliorating one or more symptom associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR, wherein the one or more symptom associated with a kidney disease can be, but is not limited to, high blood pressure, fatigue, shortness of breath, edema, poor appetite.

F. Administration

[0080] In the methods described herein, administration or delivery of the therapeutics to cells can be via a variety of mechanisms. For example, the therapeutic can be formulated as a pharmaceutical composition.

[0081] Pharmaceutical compositions can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

[0082] Preparations of parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[0083] Formulations for optical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[0084] Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids, or binders may be desirable. Some of the compositions can be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mon-, di-, trialkyl and aryl amines and substituted ethanolamines.

Examples

[0085] A soluble form of PRR (sPRR) is generated by intracellular cleavage by furin and secreted in plasma¹. sPRR binds renin and prorenin and has been reported to activate prorenin². sPRR can be measured by ELISA³, providing a new tool for assessment of sPRR in the body fluid. By ELISA, serum levels of sPRR are increased during pregnancy^{4,5} and in patients with heart failure⁶. Serum sPRR is positively associated with serum creatinine, BUN, and urine protein, and inversely with estimated glomerular filtration rate (eGFR) in patients with CKD due to hypertension and type 2 diabetes⁷. It is puzzling however that in this study serum sPRR levels were lower in patients with primary hypertension or diabetes than in those without these conditions⁷. More recently, Nguyen et al. found no correlation of plasma sPRR with plasma renin, prorenin, and aldosterone in health subjects and patients with diabetes and hypertension⁸. So far there are no prior animal or human studies tested whether urinary sPRR is correlated with indices of kidney injury. Based on our preliminary results, urinary but not plasma renin truly reflects the activity of intrarenal RAS and proteinuria. Therefore, it will be more informative to measure urinary sPRR to indicate intrarenal RAS activity and kidney injury. To evaluate the value of urinary sPRR in predicting kidney injury, the relationship between urinary sPRR and proteinuria was examined in different types of kidney injury mouse models induced by ischemia-reperfusion (I/R) and type 2 diabetes as well as in patients with type 2 diabetes. C57/Bl6 mice were subjected to bilateral I/R. Urinary sPRR, measured at 48 h following I/R/, was significantly elevated in I/R mice as compared with sham control (Fig. 1). In I/R mice, a close correlation of urinary sPRR with proteinuria (Fig. 2) and plasma creatinine (Fig. 3) was observed. Similarly, the urinary excretion of sPRR in db/db mice, a widely used mouse model of human

type 2 diabetes, was determined and its correlation with albuminuria was studied. To aggravate kidney injury, all db/db mice and their lean controls underwent uninephrectomy. Following the recovery from the surgery, all animals were placed in metabolic cages for 24-h urine collection. Db/db mice exhibited a more than 20-fold increase in urinary sPRR excretion as compared with their lean controls (Fig. 4). In db/db mice, the urinary sPRR excretion was correlated with albuminuria (Fig. 5). To make this observation clinically relevant, the correlation of urinary sPRR and albuminuria in patients with type 2 diabetes was determined. The correlation was demonstrated in the diabetic patients (Fig. 6) as in db/db mice. The results from animal and human studies indicate the use of urinary sPRR as a reliable biomarker for both acute and chronic kidney injury of diverse etiologies.

[0086] Sample size determination was performed according to the formulae

$$n = 1 + 2C\left(\frac{s}{d}\right)^2$$
 (C, constant factor of 7.85 for power of 80%; s, standard deviation; d, the magnitude of difference). Based on the standard deviation of the data set from past experience and estimated difference between experimental groups, the minimal number of animals per experimental point can be six so that 80% power can be yielded to detect significance at 0.05 level.

[0087] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the method and compositions described herein. Such equivalents are intended to be encompassed by the claims provided below.

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CLAIMS

We claim:

1. A method of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been identified as having an increase of soluble (pro)renin receptor (sPRR) in a urine sample obtained from the subject.

2. The method of claim 1, wherein the sPRR in the urine sample of the subject was detected by ELISA.

3. The method of claim 1, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

4. The method of claim 1, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

5. A method of treating a kidney disease in a subject comprising administering a therapeutic known to treat the kidney disease in the subject, wherein the subject has been diagnosed as having a kidney disease, wherein the subject was diagnosed by a method comprising detecting an increase of sPRR in a urine sample obtained from the subject.

6. The method of claim 5, wherein the sPRR in the urine sample of the subject was detected by ELISA.

7. The method of claim 5, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

8. The method of claim 5, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

9. A method of diagnosing a kidney disease in a subject comprising:

- a) detecting an increase of sPRR in a urine sample obtained from the subject; and
- b) administering a therapeutic known to treat the kidney disease to the subject.

10. The method of claim 9, wherein the sPRR in the urine sample of the subject was detected by ELISA.

11. The method of claim 9, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

12. The method of claim 9, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, corticosteroids, or an immunosuppressant.

13. A method of treating a kidney disease in a subject comprising

a) determining disease progression based on the amount of increase of sPRR in the urine of the subject, wherein the amount of increase of sPRR in the urine of the subject indicates the progression of kidney disease; and

b) administering, to the subject, a therapeutic known to treat the kidney disease at that stage of progression.

14. A method of determining the efficacy of a therapeutic comprising:

(a) determining the level of sPRR in the urine of a subject;

(b) administering at least one dose of the therapeutic to the subject;

(c) determining the level of sPRR in the urine of a subject after step (b); and

(d) comparing the level of sPRR determined in step (c) to the level of sPRR

determined in step (a),

wherein the level of sPRR determined in step (c) compared to the level of sPRR determined in step (a) is indicative of the efficacy of the therapeutic.

15. The method of claim 14, wherein an increase of the level of sPRR determined in step (c) over the level of sPRR determined in step (a) indicates a lack of efficacy of the therapeutic.

16. The method of claim 14, wherein a decrease of the level of sPRR determined in step (c) over the level of sPRR determined in step (a) indicates good efficacy of the therapeutic.

17. A method of ameliorating one or more symptoms associated with a kidney disease comprising administering a therapeutic to a subject, wherein the subject has an increase in urinary sPRR.

18. The method of claim 17, wherein the kidney disease is acute kidney injury, chronic kidney disease, or end-stage kidney disease.

19. The method of claim 17, wherein the therapeutic known to treat the kidney disease is an angiotensin-converting-enzyme (ACE) inhibitor, an Ang II type I (AT1) blocker, a corticosteroid, or an immunosuppressant.

20. The method of claim 17, wherein the one or more symptoms associated with a kidney disease is high blood pressure, fatigue, or shortness of breath.

21. The method of any of the preceding claims, wherein the sPRR is detected with a labeled probe.

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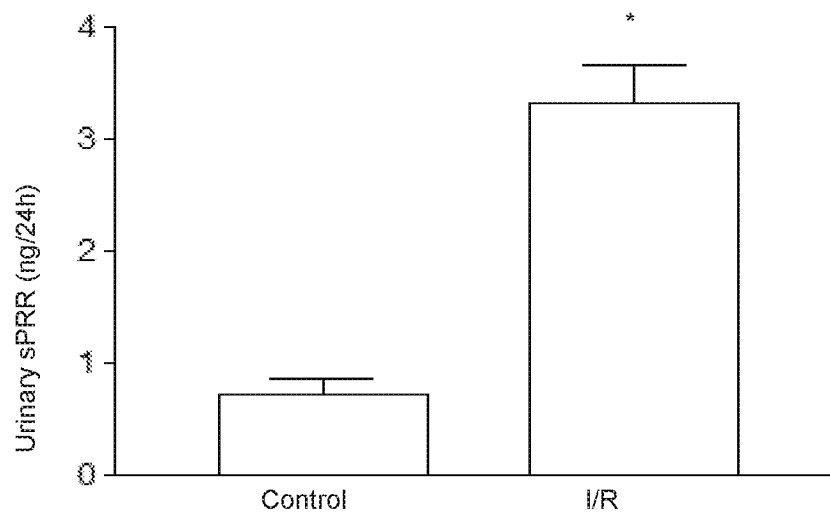


FIG. 1

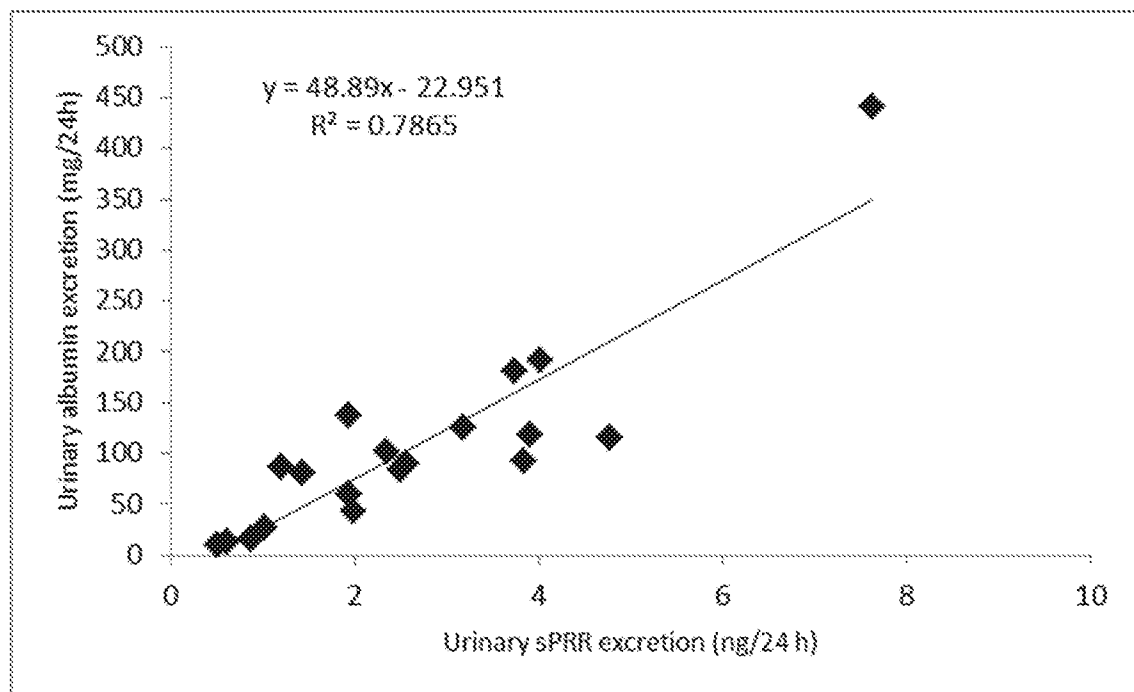


FIG. 2

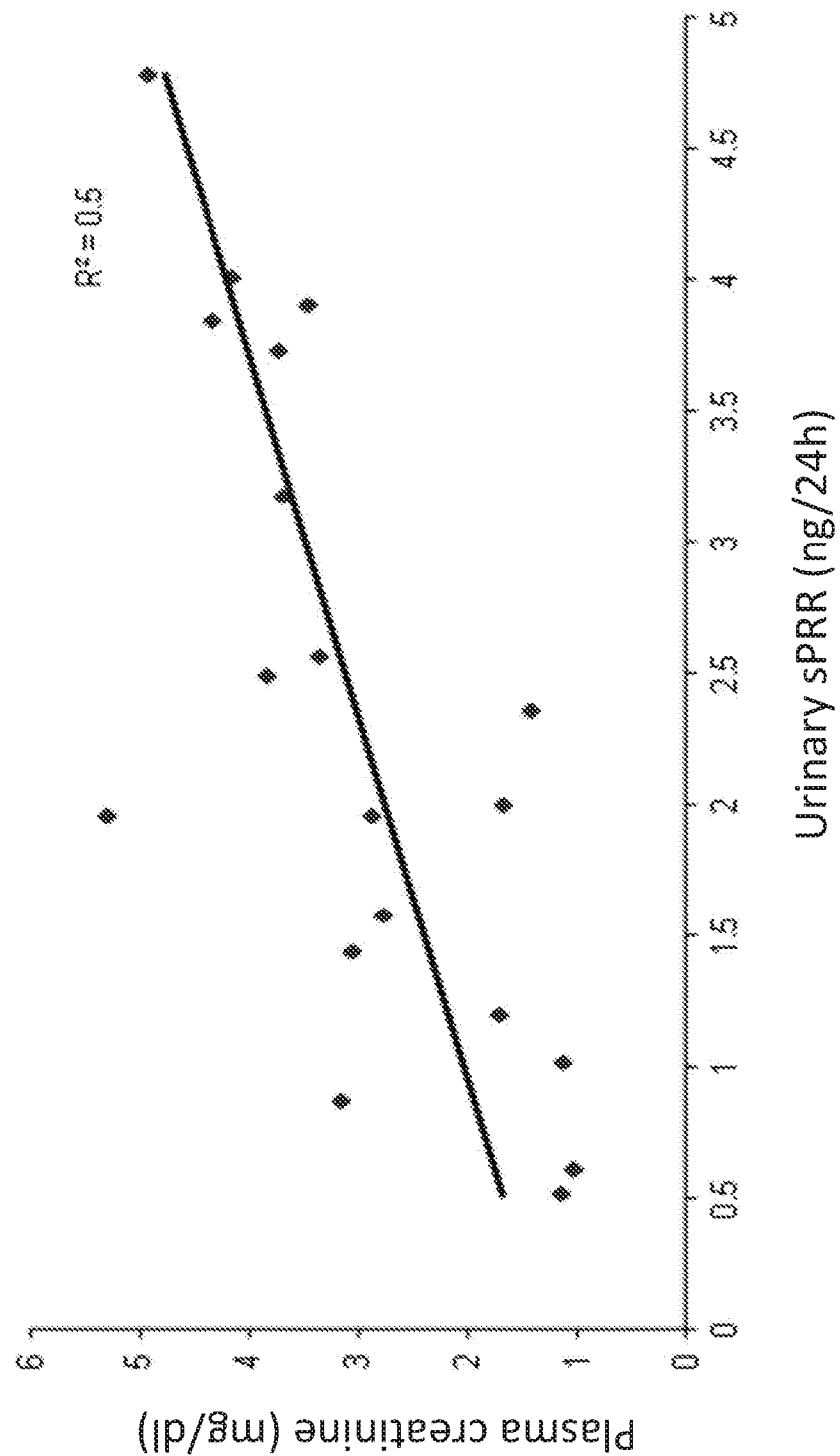


FIG. 3

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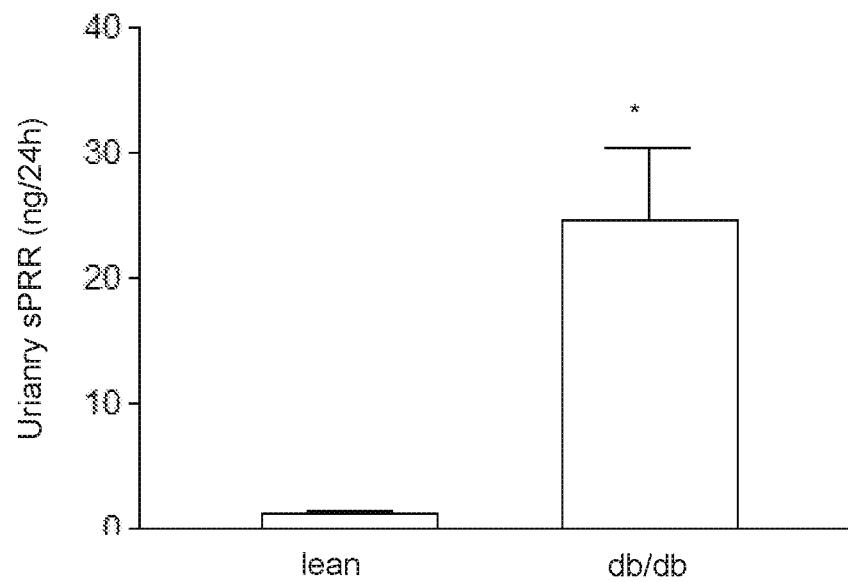


FIG. 4

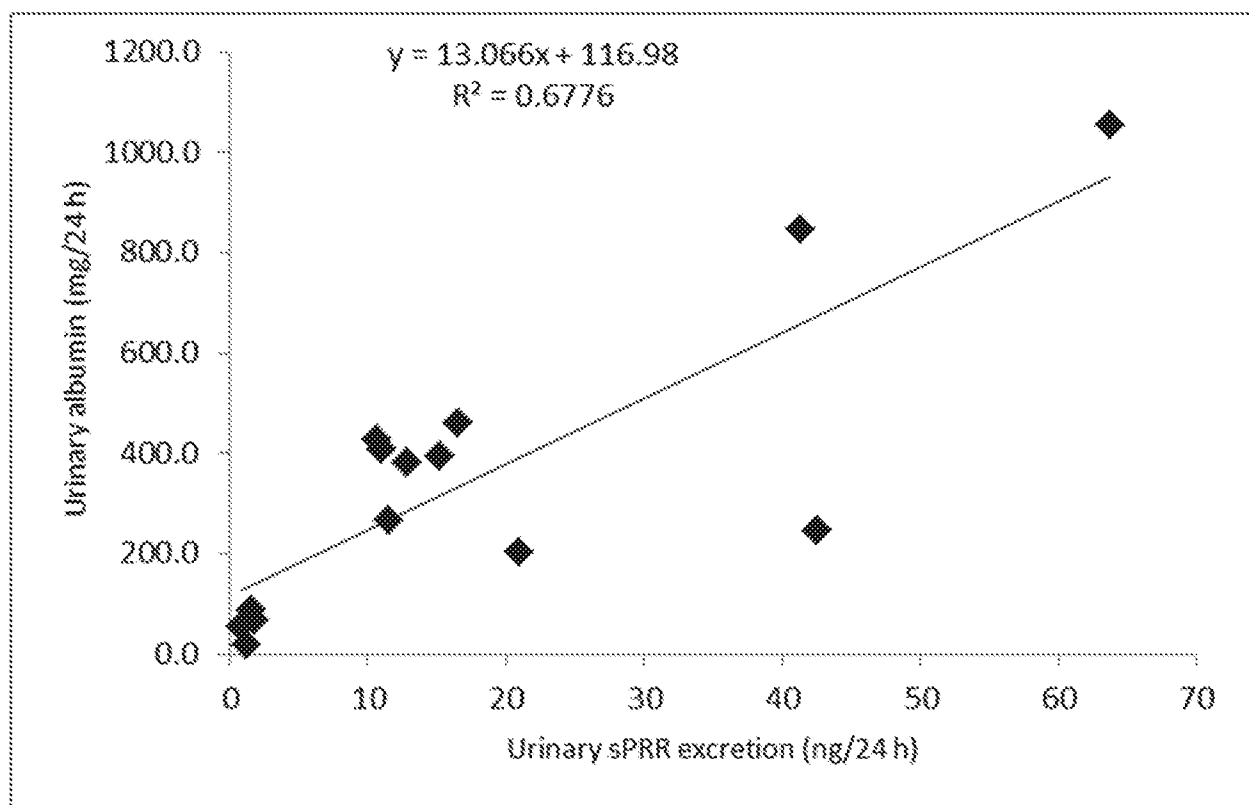


FIG. 5

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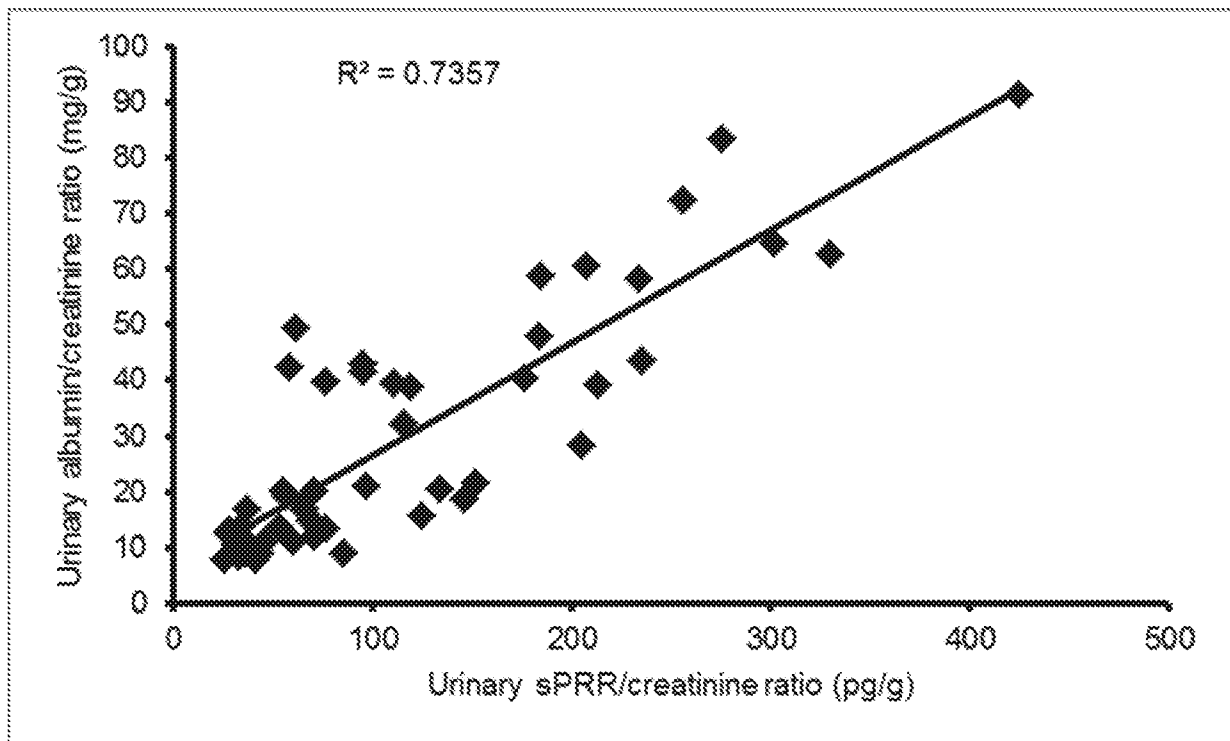


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/066367

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/50 (2016.01)

CPC - G01N 2800/347 (2016.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 38/556; A61P 13/10, 13/12; G01N 33/50, 33/53 (2016.01)

CPC - A61K 38/556; G01N 33/6827, 33/6893; G01N 2333/705; G01N 2800/34, 2800/347 (2016.02)

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

USPC - 435/7.92; 514/15.4, 16.3 (keyword delimited)

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

PatBase, Google Patents, Google Scholar, Google, ProQuest, PubMed

Search terms used: sPRR (soluble (pro)renin receptor); urine; ELISA; kidney/renal disease/failure; ACE inhibitor, AT1 inhibitor, corticosteroid, immunosuppressant; disease progression; efficacy; labeled

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/045587 A1 (CELLTREND GMBH et al) 12 April 2012 (12.04.2012) entire document	1-3, 5-7, 9-11, 13, 17-21
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Y		4, 8, 12, 14-16
Y	US 2009/0148446 A1 (SKURKOVICH et al) 11 June 2009 (11.06.2009) entire document	4, 8, 12
Y	US 2011/0287964 A1 (BONVENTRE et al) 24 November 2011 (24.11.2011) entire document	14-16
A	GONZALEZ et al. "Soluble form of the (pro)renin receptor is augmented in the collecting duct and urine of chronic angiotensin II-dependent hypertensive rats." Hypertension. Vol 57, Iss 4, Pg. 859-64. Epub 14 Feb 2011(14.02.2011) Retrieved from the internet. <URL: http://hyper.ahajournals.org/content/57/4/859.full.pdf+html > entire document	1-21
P,X	US 2015/0025013 A1 (UNIVERSITY OF UTAH) 22 January 2015 (22.01.2015) entire document	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

02 February 2016

Date of mailing of the international search report

03 MAR 2016

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