CLAIMS

We claim:

1. A method for the treatment of chronic kidney disease in a subject in need thereof, comprising administering to a subject having or at risk for a chronic kidney disease a therapeutically effective amount of a composition comprising a ROBO2 inhibitor.

2. A method for the reduction of proteinuria in a subject in need thereof, comprising administering to a subject having or at risk for proteinuria a therapeutically effective amount of a composition comprising a ROBO2 inhibitor.

3. The method of any one of claims 1or 2, wherein the ROBO2 inhibitor is a blocking antibody or antigen-binding fragment thereof specific for ROBO2, an antisense molecule specific for ROBO2, a short interfering RNA (siRNA) specific for ROBO2, a small molecule inhibitor of ROBO2, a ROBO2 inhibitory polypeptide, or a ROBO2 structural analog.

4. The method of any one of claims 1-3, wherein the ROBO2 inhibitor blocks or reduces binding of ROBO2 to SLIT, to Nck, or to both.

5. The method of any one of claims 1-4, wherein the ROBO2 inhibitor is specific for the Ig1 SLIT binding domain, the Ig1 and Ig2 SLIT binding domains, the Nck intracellular binding domain, or any combination thereof.

6. The method of claim 3, wherein the ROBO2 inhibitory polypeptide is a dominant negative ROBO2 fusion protein, a polypeptide comprising a ROBO2 extracellular domain without the intracellular domain, or a polypeptide comprising a ROBO2 intracellular domain without the extracellular domain.

7. The method of any one of claims 1-6, wherein the subject having or at risk for a chronic kidney disease has diabetic nephropathy or high blood pressure.

8. The method of any one of claims 1-7, further comprising administering to the subject an additional therapeutic agent.

9. The method of claim 8, wherein the additional therapeutic agent is an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin II receptor blocker (ARB).

10. A method comprising:
    a. assaying a biological test sample from a subject to determine an expression level of ROBO2 polypeptide or an RNA encoding a ROBO2 polypeptide;
    b. determining whether the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide in the biological test sample is above a reference threshold level; and

c. diagnosing the subject as in need of treatment or therapy for chronic kidney disease.

11. The method of claim 10, wherein assaying the expression level of ROBO2 polypeptide is performed using an antibody or antigen-binding fragment thereof specific for the ROBO2 polypeptide.

12. The method of claim 10, wherein assaying the expression level of the RNA encoding a ROBO2 polypeptide is performed using PCR or a hybridization assay.

13. The method of any one of claims 10-12, wherein the biological test sample is a kidney biopsy, urine, blood, serum sample, or cells pelleted from a urine sample.

14. The method of any one of claims 10-13, wherein the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide is at least 20% above the reference threshold level.

15. The method of any one of claims 10-13, wherein the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide is at least two standard deviations above the reference threshold level.

16. An assay comprising:

a. contacting a biological test sample isolated from a subject with a reagent that detects ROBO2 polypeptide or an RNA encoding a ROBO2 polypeptide; and

b. measuring the level of ROBO2 polypeptide or an RNA encoding a ROBO2 polypeptide,

wherein an increased level of said ROBO2 polypeptide or said RNA encoding a ROBO2 polypeptide, relative to a normal biological sample, identifies a subject having chronic kidney disease and/or progression of chronic kidney disease or proteinuria.

17. The assay of claim 16, wherein detecting the expression level of ROBO2 polypeptide is performed using an antibody or antigen-binding fragment thereof specific for the ROBO2 polypeptide.

18. The assay of claim 16, wherein detecting the expression level of the RNA encoding a ROBO2 polypeptide is performed using PCR or a hybridization assay.

19. The assay of any one of claims 16-18, wherein the biological test sample is a kidney biopsy, urine, blood, serum sample, or cells pelleted from a urine sample.

20. The assay of any one of claims 16-19, wherein the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide is at least 20% above the reference threshold level.

21. The assay of any one of claims 16-19, wherein the expression level of ROBO2 polypeptide or

the expression level of the RNA encoding a ROBO2 polypeptide is at least two standard deviations above the reference threshold level.

22. The assay of any one of claims 16-21, wherein the subject has been diagnosed with diabetes or high blood pressure.

23. A system for determining if a subject is at risk for chronic kidney disease or proteinuria, or has chronic kidney disease comprising:
    a. a measuring module configured to determine the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide in a biological sample obtained from a subject;
    b. a comparison module configured to receive said expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide determined by the measuring module and perform at least one analysis to determine whether the expression level of ROBO2 polypeptide or the expression level of the RNA encoding a ROBO2 polypeptide is greater than a pre-determined reference level or ratio, and to provide a retrieved content; and
    c. a display module for displaying a content based the data output from said comparison module, wherein the content comprises a signal indicative that the expression level or ratio of ROBO2 polypeptide or RNA is greater than the pre-determined reference level or ratio, or a signal indicative that the level or expression ratio of ROBO2 is not greater than the reference level or pre-determined ratio.

24. The system of claim 23, wherein the content displayed on said display module further comprises a signal indicative of the subject being recommended to receive a particular treatment regimen.

25. A system for determining if a subject is at risk for chronic kidney disease or proteinuria, or has chronic kidney disease comprising:
    a. a determination module configured to receive at least one test sample obtained from a subject and perform at least one analysis on said at least one test sample to determine the presence or absence of either of the following conditions:
        i. an expression ratio of ROBO2 greater than a pre-determined ratio, or
        ii. an expression level of ROBO2 greater than a pre-determined level
    b. a storage device configured to store data output from said determination module; and
    c. a display module for displaying a content based on the data output from said determination module, wherein the content comprises a signal indicative that the expression ratio of ROBO2 is greater than the pre-determined ratio or level of ROBO2 greater than a pre-determined level, or a signal indicative that the expression ratio of

ROBO2 is not greater than the pre-determined ratio or not greater than a pre-determined level.

26. The system of claim 25, wherein the content displayed on said display module further comprises a signal indicative of the subject being recommended to receive a particular treatment regimen.

27. A method for treating a human subject with a risk of chronic kidney disease or proteinuria, comprising administering a treatment or therapy to prevent the occurrence of chronic kidney disease or proteinuria to a human subject who is determined to have a level of ROBO2 protein above a reference threshold level.

28. The method of claim 27, wherein the level of ROBO2 protein is at least 20% above the reference level.

29. The method of claim 27, wherein the level of ROBO2 protein is at least two standard deviations above the reference level.

30. The method of any one of claims 27-29, wherein the treatment or therapy to prevent the occurrence of chronic kidney disease or proteinuria comprises a ROBO2 inhibitor.

31. The method of claim 30, wherein the ROBO2 inhibitor is a blocking antibody or antigen-binding fragment thereof specific for ROBO2, an antisense molecule specific for ROBO2, a short interfering RNA (siRNA) specific for ROBO2, a small molecule inhibitor of ROBO2, a ROBO2 inhibitory polypeptide, or a ROBO2 structural analog.

32. The method of any one of claims 30-31, wherein the ROBO2 inhibitor blocks or reduces binding of ROBO2 to SLIT, to Nck, or to both.

33. The method of any one of claims 30-32, wherein the ROBO2 inhibitor is specific for the Ig1 SLIT binding domain, the Ig1 and Ig2 SLIT binding domains, the Nck intracellular binding domain, or any combination thereof.

34. The method of claim 31, wherein the ROBO2 inhibitory polypeptide is a dominant negative ROBO2 fusion protein, a polypeptide comprising a ROBO2 extracellular domain without the intracellular domain, or a polypeptide comprising a ROBO2 intracellular domain without the extracellular domain.

35. A ROBO2 inhibitor for use in treating a chronic kidney disease.

36. A ROBO2 inhibitor for use in treating proteinuria.

37. The use of any one of claims 35 or 36, wherein the ROBO2 inhibitor is a blocking antibody or antigen-binding fragment thereof specific for ROBO2, an antisense molecule specific for ROBO2, a short interfering RNA (siRNA) specific for ROBO2, a small molecule inhibitor of

ROBO2, a ROBO2 inhibitory polypeptide, or a ROBO2 structural analog.

38. The use of any one of claims 35-37, wherein the ROBO2 inhibitor blocks or reduces binding of ROBO2 to SLIT, to Nck, or to both.

39. The use of any one of claims 35-38, wherein the ROBO2 inhibitor is specific for the Ig1 SLIT binding domain, the Ig1 and Ig2 SLIT binding domains, the Nck intracellular binding domain, or any combination thereof.

40. The use of claim 37, wherein the ROBO2 inhibitory polypeptide is a dominant negative ROBO2 fusion protein, a polypeptide comprising a ROBO2 extracellular domain without the intracellular domain, or a polypeptide comprising a ROBO2 intracellular domain without the extracellular domain.

41. The use of any one of claims 35-40, wherein the chronic kidney disease or proteinuria is caused by diabetic nephropathy or high blood pressure.

CLAIMS

1. A pharmaceutical composition comprising a ROBO2 inhibitor that inhibits ROBO2 biological activity, in which the ROBO2 inhibitor is selected from the group consisting of an anti-ROBO2 antibody or an antigen-binding fragment thereof, that specifically binds ROBO2, an antisense molecule directed to a nucleic acid encoding ROBO2, a siRNA molecule directed to a nucleic acid encoding ROBO2, a RNA or DNA aptamer that binds to ROBO2, a ROBO2 structural analogue, a soluble ROBO2 protein and a ROBO2 inhibitory polypeptide; and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, in which the ROBO2 inhibitor prevents or reduces the binding of the ROBO2 polypeptide to SLIT2, to Nck, or to both.

3. A pharmaceutical composition according to Claim 1 or 2, in which the ROBO2 inhibitor is a structural analogue.

4. A pharmaceutical composition according to any of Claims 1 to 3, in which the ROBO2 inhibitor is a dominant negative ROBO2 polypeptide.

5. A pharmaceutical composition according to any of Claims 1 to 4, in which the ROBO2 inhibitor is a dominant negative fusion ROBO2 polypeptide.

6. A pharmaceutical composition according to any of Claims 1 to 5, in which the soluble ROBO2 protein inhibitor comprises

a fusion polypeptide containing at least one Ig SLIT binding domain selected from the group consisting of: amino acid residues 46-145 of SEQ ID NO: 1, amino acid residues 151-237 of SEQ ID NO: 1, amino acid residues 30-129 of SEQ ID NO: 3 or amino acid residues 135-221 of SEQ ID NO:3; or

a fusion polypeptide containing amino acid residues 881-1378 of SEQ ID NO: 3.

7. A pharmaceutical composition according to any of Claims 1 to 5, in which the anti-ROBO2 antibody or antigen-binding fragment thereof specifically binds to (i) at least one Ig SLIT binding domain of ROBO2 corresponding to amino acid residues 46-145 of SEQ ID NO: 1, amino acid residues 151-237 of SEQ ID NO: 1, amino acid residues 30-129 of SEQ ID NO: 3, amino acid residues 135-221 of SEQ ID NO:3, or (ii) specifically binds to amino acids

881-1378 of SEQ ID NO:3, in which the anti-ROBO2 antibody or antigen-binding fragment thereof prevents binding of SLIT2 or Nck to the ROBO2 polypeptide.

8. A pharmaceutical composition according to Claim 7, in which the anti-ROBO2 antibody or antigen binding fragment thereof is selected from the group consisting of; a monoclonal antibody, a chimeric antibody, a human antibody and a humanized antibody.

9. A pharmaceutical composition according to any of Claims 1 to 8, in which the ROBO2 inhibitor is specific for the Ig1 SLIT binding domain, the Ig1 and the Ig2 SLIT binding domains, the Nck intracellular binding domain, or any combination thereof.

10. A pharmaceutical composition according to any of Claims 1 to 9, in which the ROBO2 inhibitory polypeptide comprises a ROBO2 extracellular domain without the intracellular domain, or comprises a ROBO2 intracellular domain without the extracellular domain.

11. A pharmaceutical composition according to any of Claims 1 to 10, in which the anti-ROBO2 antibody or antigen-binding fragment thereof specifically binds to the ROBO2 polypeptide to (i) prevent or reduce SLIT2 binding to the ROBO2 polypeptide at amino acid residues selected from the group consisting of amino acid residues 46-145 of SEQ ID NO: 1, amino acid residues 151-237 of SEQ ID NO: 1, amino acid residues 30-129 of SEQ ID NO: 3, amino acid residues 135-221 of SEQ ID NO: 3, or (ii) prevent or reduce Nck binding to the ROBO2 polypeptide at amino acid residues 881-1378 of SEQ ID NO:3.

12. A pharmaceutical composition according to any of Claims 1 to 11, in which the anti-ROBO2 antibody or antigen-binding fragment thereof is selected from the group consisting of; an antibody fragment, a monoclonal antibody, a chimeric antibody, a human antibody, a humanized antibody.

13. A pharmaceutical composition according to any of Claims 1 to 12 for use in treating a subject having or at risk for a chronic kidney disease or proteinuria.

14. A pharmaceutical composition according to Claim 13, in which the chronic kidney disease or proteinuria is caused by diabetic nephropathy or high blood pressure.

15. A pharmaceutical composition according to any of Claims 1 to 12 for use in treating a subject having a dysfunctional SLIT2 protein or ROBO2 protein or both.