microangiopathy, tubulointerstitial diseases, e.g. infections, stones, obstruction, drug toxicity as well as cycstic and congenital diseases); structural abnormalities detected by imaging (ultrasound, computed tomography and magnetic resonance with or without isotope scans, angiography) such as polycystic kidneys, dysplastic kidneys, hydronephrosis due to obstruction, cortical scarring due to infarcts, pyelonephritis or associated with vesicoureteral reflux, renal masses or enlarged kidneys due to infiltrative diseases, renal artery stenosis, small and hyperechoic kidneys; and a history of kidney transplantation. Further, AKD is characterized by structural kidney damage for <3 months and by functional criteria that are also found in AKI, or a GFR of <60ml/min per 1.73 m^2 for <3 months, or a decrease in GFR by $\geq$ 35%, or an increase in SCr by >50% for <3 months (Kidney International Supplements, Vol. 2, Issue 1, March 2012, pp. 19-36). A proper distinction between these different disease entities is important. The present invention surprisingly provides a new marker suitable for predicting the risk of incidence of CKD in healthy subjects, apparently healthy subjects, subjects with autoimmune and/or cardiovascular disease and/or hypertension and/or diabetes and/or metabolic syndrome. It is clear that an early detection of a potential risk in these patient groups would be of substantial benefit, in particular in measures that may be suitable to prevent the development of or further progression to CKD.

In one aspect, the present disclosure provides a method for predicting the risk of incidence of chronic kidney disease (CKD) in a subject comprising:

a) determining the level of Pro-Enkephalin or fragments thereof in a blood (whole blood), serum, or plasma sample obtained from said subject; and

b) correlating said level of Pro-Enkephalin or fragments thereof with a risk of incidence of chronic kidney disease, wherein the blood (whole blood), serum or plasma sample of a subject with a Pro-Enkephalin level above a certain threshold level, is classified as having an elevated risk for development of CKD, wherein the threshold associated with an elevated risk for development of CKD is between 30 and 80 pmol/L of Pro-Enkephalin,

wherein the subject is selected from the group comprising

(i) healthy subjects,

(ii) diseased subjects not having CKD,

wherein said level is determined by using at least one binder wherein said at least one binder binds to a region within the sequences selected from the group comprising SEQ ID No. 1, 2, 5, 6, 7, 8, 9 and 10.

In another aspect, the present disclosure provides a method for predicting a worsening or improvement of kidney function or dysfunction in

(i) healthy subjects, or

(ii) diseased subjects with or without kidney dysfunction,

wherein said method comprises performing the steps

(a) determining the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids in a blood (whole blood), serum, or plasma sample obtained from said subject; and

(b) correlating said level of Pro-Enkephalin or fragments thereof with a risk of incidence of chronic kidney disease, wherein the blood (whole blood), serum or plasma sample of a subject with a Pro-Enkephalin level above a certain threshold level, is classified as having an elevated risk for development of CKD, wherein the threshold associated with an elevated risk for development of CKD is between 30 and 80 pmol/L of Pro-Enkephalin, and wherein a change in the level from below the threshold as defined herein, to above the threshold is predictive for an enhanced risk of worsening of kidney function or dysfunction, and wherein a change in the level from above the threshold to below the threshold is predictive for a lowered risk or improvement of kidney function or dysfunction, optionally comprising steps as disclosed herein.

Subject matter of the present invention is a method for predicting the risk of incidence of chronic kidney disease (CKD) in a subject comprising:

a) determining the level of Pro-Enkephalin or fragments thereof in a bodily fluid obtained from said subject; and

b) correlating said level of Pro-Enkephalin or fragments thereof with a risk of incidence of CKD, wherein a level above a threshold is predictive for an enhanced risk of incidence of CKD,

wherein the subject is selected from the group comprising

(i) healthy subjects,

(ii) diseased subjects not having CKD.

The subjects referred to in (i) and (ii) and in the methods for predicting the risk of incidence of CKD referred to herein below preferably have an estimated glomerular filtration rate (eGFR) of greater than 60 ml/min/1.73 m^2 for > 3 months.

Claims

1. A method for predicting the risk of incidence of chronic kidney disease (CKD) in a subject comprising:

a) determining the level of Pro-Enkephalin or fragments thereof in a blood (whole blood), serum, or plasma sample obtained from said subject; and

b) correlating said level of Pro-Enkephalin or fragments thereof with a risk of incidence of chronic kidney disease, wherein the blood (whole blood), serum or plasma sample of the subject with a Pro-Enkephalin level above a certain threshold level, is classified as having an elevated risk for development of CKD, wherein the threshold associated with an elevated risk for development of CKD is between 30 and 80 pmol/L of Pro-Enkephalin,

wherein the subject is selected from the group comprising

(i) healthy subjects,

(ii) diseased subjects not having CKD,

wherein said level is determined by using at least one binder wherein said at least one binder binds to a region within the sequences selected from the group comprising SEQ ID No. 1, 2, 5, 6, 8, 9 and 10.

2. The method according to claim 1, wherein diseased subjects not having CKD comprises the group of subjects with metabolic syndrome, diabetes, obesity, cardiovascular disease, cardiovascular events, hypertension, and/or autoimmune disease, at the time the sample of blood (whole blood), serum or plasma sample is obtained.

3. The method according to claim 1 or claim 2, wherein the subject had a history of a cardiovascular event or cardiovascular disease at the time the sample of blood (whole blood), serum or plasma sample is taken, wherein said cardiovascular event or cardiovascular disease is selected from the group comprising myocardial infarction, stroke, acute heart failure, coronary artery disease, cardiac insufficiency, chronic heart failure, and cardiac disease associated with chronic or acute cerebrovascular disease.

4. The method according to claim 1 or claim 2, wherein the subject is diagnosed as suffering from an autoimmune disease at the time the sample of blood (whole blood), serum or plasma sample is taken from said subject, wherein the autoimmune disease is selected from the group comprising Sjögren's disease, systemic lupus erythematodes (SLE), psoriasis, polymyositis, dermatomyositis, inclusion body myositis, Myasthenia

gravis, autoimmune degenerative diseases of joints, particularly rheumatoid arthritis, or autoimmune diseases of the central nervous system, wherein said subject does not suffer from kidney disease.

5. The method according to any one of claims 1 to 4, wherein said subject has an estimated glomerular filtration rate (eGFR) of greater than 60 ml/min/1.73 m^2 for > 3 months and kidney damage for > 3 months.

6. The method according to any one of claims 1 to 5, wherein at least one additional parameter is determined, said parameter being selected from the group comprising: age, gender, systolic blood pressure and/or diastolic blood pressure (SBP and/or DBP), antihypertensive treatment (AHT), body mass index, body fat mass, body lean mass, waist circumference, waist-hip-ratio, current smoker, diabetes heredity, serum creatinine level, cystatin C level, cardiovascular disease (CVD), total cholesterol, triglyceride, low-density-lipocholesterol (LDL-C), high-density-lipocholesterol (HDL-C), whole blood or plasma glucose, plasma insulin, HOMA (Insulin (µU/ml) × glucose (mmol/l) / 22.5), and/or HbA_{1c} (%).

7. The method according to any one of claims 1 to 6, further comprising determining the status of genetic markers.

8. The method for predicting the risk of incidence of chronic kidney disease according to any one of claims 1 to 5, wherein a group of parameters is determined, said group comprising: fasting glucose, systolic blood pressure, anti-hypertensive medication and body mass index (BMI).

9. The method according to any of the preceding claims, wherein the Pro-Enkephalin concentration and/or the concentration of other substances found in the blood (whole blood), serum or plasma sample is measured in non-fasting or fasting subjects.

10. The method according to any of the preceding claims, wherein the level of Pro-Enkephalin or fragments thereof is determined by an immunoassay.

11. The method according to any of the preceding claims, wherein the Pro-Enkephalin fragment comprises Pro-Enkephalin A 119-159 (SEQ ID NO: 6) or a fragment thereof.

12. The method for predicting the risk of incidence of chronic kidney disease as defined in any of the preceding claims, wherein said method is performed at least once at time-point t0 and optionally at least one or more subsequent time-point(s) (t1…tn) to monitor the risk development of incidence of chronic kidney disease.

13. The method for predicting the risk of incidence of chronic kidney disease as defined in any of the preceding claims, wherein the threshold associated with an elevated risk for development of CKD is between 35 and 60 pmol/L, or between 40 and 50 pmol/L, or 41 and 49 pmol/L.

14. The method according to any of the preceding claims, wherein the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids, or at least of 6 amino acids, or at least of 7 amino acids, or at least of 8 amino acids, or at least of 9 amino acids, or at least of 10 amino acids, or at least of 12 amino acids is determined by using a binder to Pro-Enkephalin or fragments thereof of at least 5 amino acids, or of at least 6 amino acids, or of at least 7 amino acids, or of at least 8 amino acids, or of at least 9 amino acids, or of at least 10 amino acids, or of at least 12 amino acids.

15. The method according to any of the preceding claims, wherein a binder is used that specifically binds to Pro-Enkephalin or a fragment thereof, the binder being selected from the group comprising an antibody, an antibody fragment or a non-Ig-Scaffold binding to Pro- Enkephalin or fragments thereof.

16. The method according to any of the preceding claims, wherein said threshold is <41 pmol/L and indicates a low risk of incidence of chronic kidney disease, a range of 41-49 indicates a medium risk of incidence of chronic kidney disease, and a threshold of >49 pmol/L indicates a high risk of incidence of chronic kidney disease.

17. The method according to any of the preceding claims, wherein an assay is used comprising two binders that bind to two different regions within the region of Pro-Enkephalin that is amino acid 133-140 (SEQ ID NO. 13) and amino acid 152-159 (SEQ ID No. 14) wherein each of said regions comprises at least 4 or 5 amino acids.

18. The method according to any of the preceding claims, wherein the assay sensitivity is < 15pmol/L.

19. The method according to any of the preceding claims, further comprising performing a step of selecting suitable therapeutic or preventive measures, when the level Pro-Enkephalin or fragments thereof is above a threshold, said threshold is between 35 and 60 pmol/L, or between 40 and 50 pmol/L, or between 41 and 49 pmol/L.

20. The method according to any of the preceding claims, wherein said determination of Pro-Enkephalin or fragments thereof is performed more than once in order to monitor the risk of development of the incidence of chronic kidney disease.

21. The method according to any of the preceding claims, wherein said method is performed to stratify said subjects into risk groups.

22. A method for predicting a worsening or improvement of kidney function or dysfunction in

(i) healthy subjects, or

(ii) diseased subjects with or without kidney dysfunction,

wherein said method comprises performing the steps

(a) determining the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids in a blood (whole blood), serum, or plasma sample obtained from said subject; and

(b) correlating said level of Pro-Enkephalin or fragments thereof of at least 5 amino acids with a risk of incidence of CKD, wherein the blood (whole blood), serum or plasma sample of the subject with a Pro-Enkephalin level above a certain threshold level, is classified as having an elevated risk for development of CKD, wherein the threshold associated with an elevated risk for development of CKD is between 30 and 80 pmol/L of Pro-Enkephalin, and wherein a change in the level from below the threshold, as defined in the preceding claims, to above the threshold is predictive for an enhanced risk or worsening of kidney function or dysfunction, and wherein a change in the level from above the threshold to below the threshold is predictive for a lowered risk or improvement of kidney function or dysfunction, optionally comprising steps depicted in any one of the preceding claims.