

The adaptation of all actions according to the absolute risk (the probability that a person develops a coronary disease in a certain period of time) is important, because it allows reach a suitable balance between efficacy, safety and therapy costs. The estimation of the absolute risk requires adding up the contribution of each risk factor and the result is "the determination of the global risk". The *Framingham Heart Study*, as mentioned above, performed a quantitative estimation of the global risk based on the contribution of each risk.

Although traditional risk factors are important predictors of cardiac events in individuals with CKD, the Framingham equations do not accurately weight these risk factors in predicting coronary heart disease events in CKD (J Am Coll Cardiol 2007;50:217-224). Much of this failure is driven by competing events with death as well as the significantly higher cardiac event rate in CKD patients. While it is possible to somewhat improve coronary heart disease prediction in individuals with CKD, particularly in women, both by recalibrating the equations and by assigning different importance to traditional Framingham risk factors, it is likely that future predictive equations with adequate sample size for development and validation of a predictive model in CKD will need to examine both cardiac events and mortality in this high risk population with the goal of identifying shared modifiable risk factors for adverse outcomes.

Accordingly, there is a need for novel markers, including new genetic markers and combinations thereof that could successfully and advantageously define the cardiovascular risk of a subject suffering from chronic kidney disease and predict who is at high risk of developing cardiovascular disease and/or cardiovascular disease complications such as –but not limited to– fatal or non-fatal myocardial infarction or angina pectoris or stroke or transient ischemic attack or peripheral arteriopathy, in a way that preventive and/or therapeutic measures could be implemented to keep that risk at the lowest possible level.

SUMMARY OF THE INVENTION

In a first embodiment, the invention disclosure provides a method which is suitable to solve the limitations of the scales/methods nowadays in use to calculate the cardiovascular risk in patients suffering from chronic kidney disease.

The method provided according to the present invention disclosure solves the above mentioned limitations by determining the cardiovascular risk of a subject suffering from chronic kidney disease and predicts his/her risk of developing cardiovascular disease and/or cardiovascular disease complications comprising the steps of determining in a sample isolated from said subject suffering from chronic kidney disease the presence of a polymorphism, wherein said polymorphism is at positions 27 within the nucleic acid sequences of SEQ ID NO:1 to 8, or said polymorphism is in a sequence that is in strong linkage disequilibrium with the sequence of any of SEQ ID NO:1 to 8, wherein the presence at position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, and/or G in SEQ ID NO:8, which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned is indicative of a risk of having a cardiovascular event.

For example, rs1746048, which is represented herein by SEQ ID NO:48, is in strong linkage disequilibrium with rs501120; or e.g. rs2133189, which is represented herein by SEQ ID NO:49, is in strong linkage disequilibrium

with rs17465637. These sequences may thus also be used for the purposes of the present ~~invention~~disclosure, as the rs that is in strong linkage disequilibrium with any of those rs mentioned, as described herein.

In any of said rs sequences that are in strong linkage disequilibrium with an rs sequence mentioned herein, the polymorphism may, for example, be at position 27. The skilled person may access rs sequences on the NCBI SNP database ("dbSNP", <http://www.ncbi.nlm.nih.gov/snp>).

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

In one aspect, there is provided a method for a cardiovascular risk assessment in a subject comprising the steps of determining in a sample isolated from said subject suffering from chronic kidney disease the presence of a polymorphism, wherein said polymorphism is at positions 27 within the nucleic acid sequences of SEQ ID NO:1 to 8, or said polymorphism is in a sequence that is in strong linkage disequilibrium with the sequence of any of SEQ ID NO:1 to 8, wherein the presence at position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, and/or G in SEQ ID NO:8, which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned, is indicative of a risk of having a cardiovascular event.

In another aspectone embodiment, the ~~invention~~disclosure relates to a method of determining the cardiovascular risk of a subject suffering from chronic kidney disease and who has suffered one or more cardiovascular event to predict the risk of developing cardiovascular disease and/or cardiovascular disease complications comprising the steps of determining in a sample isolated from said subject the presence of a polymorphism, wherein said polymorphism is at positions 27 in the nucleotide sequences of SEQ ID NO:1 to 11, or said polymorphism is in a sequence that is in strong linkage disequilibrium with the sequence of any of SEQ ID NO:1 to 11, wherein said polymorphism at said position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, G in SEQ ID NO:8, A in SEQ ID NO:9, A in SEQ ID NO:10 and/or G in SEQ ID NO:11, which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, rs10507391, rs9315051, and/or rs17222842, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned is indicative of a risk of having an adverse cardiovascular disease or disorder.

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

The ~~invention~~disclosure further provides a method for identifying a subject suffering from chronic kidney disease in need of early and/or a more aggressive cardiovascular therapy or in need of prophylactic cardiovascular therapy comprising the steps of determining in a sample isolated from said subject the presence in at least one allele of a polymorphism, wherein said polymorphism is at positions 27 within the nucleic acid sequenceof any of SEQ ID NO:1 to 11, , or said polymorphism is in a sequence that is in strong linkage disequilibrium with any of SEQ ID NO:1 to 11, wherein the presence at position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID

NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, G in SEQ ID NO:8, A in SEQ ID NO:9, A in SEQ ID NO:10 and/or G in SEQ ID NO:11, which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, rs10507391, rs9315051, and/or rs17222842, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned is indicative of being in need of early and/or a more aggressive cardiovascular therapy or in need of prophylactic cardiovascular treatment.

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

A “prophylactic” therapy means a therapy which is started before symptomatic onset of a disease.

An “early or a more aggressive” therapy means a therapy which is started at an early stage of a disease or with higher amounts or higher frequency than on average due, for instance, to be at a higher risk of developing CHD when using this inventiondisclosure and therefore therapeutic objectives have to be adapted according to guidelines. These new objectives would require a more aggressive therapy.

In another ~~aspect~~one embodiment, the inventiondisclosure relates to a method of treatment of a patient suffering from chronic kidney disease and suffering from a cardiovascular disease with a cardiovascular therapy wherein the patient is selected for said therapy based on the presence in a sample isolated from said subject of a polymorphism, wherein said polymorphism is at position 27 in the nucleotide sequences of SEQ ID NO:1 to 11, or said polymorphism is in a sequence that is in strong linkage disequilibrium with the sequence of any of SEQ ID NO:1 to 11, wherein the presence at said position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, G in SEQ ID NO:8, A in SEQ ID NO:9, A in SEQ ID NO:10 and/or G in SEQ ID NO:11, which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, rs10507391, rs9315051, and/or rs17222842, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned indicates that the patient is selected for said therapy.

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

In another ~~aspect~~embodiment, the inventiondisclosure relates to a method of determining the cardiovascular risk of a subject suffering from chronic kidney disease at any of the stages 1 to 5 of chronic kidney disease and/or under replacement therapy with dialysis (hemodialysis or peritoneal dialysis) and/or with a kidney transplant to predict who is at high risk of developing cardiovascular disease and/or cardiovascular disease complications and/or of determining the response to a cardiovascular therapy comprising the steps of determining in a sample isolated from said subject the presence of a polymorphism, wherein said polymorphism is at positions 27 within the nucleic acid sequences of SEQ ID NO:1 to 11, , or said polymorphism is in a sequence that is in strong linkage disequilibrium with the sequence of any of SEQ ID NO:1 to 11, wherein the presence at position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, G in SEQ ID NO:8, A in SEQ ID NO:9, A in SEQ ID NO:10 and/or G in SEQ ID NO:11 which correspond to db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, rs10507391, rs9315051, and/or rs17222842, respectively, or the presence of a polymorphism within the sequence of any other rs that is in strong linkage disequilibrium with any of those rs mentioned is indicative of an increased risk of having an adverse cardiovascular disease or disorder or of a low response to a cardiovascular therapy.

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

In further ~~aspect~~embodiments, the inventiondisclosure relates to methods for the determination of the probability of an individual suffering from CKD of presenting a fatal or non-fatal myocardial infarction or angina in a 10 year period based on the presence of one or more of the polymorphisms mentioned above in combination with one or more clinical/biochemical risk factors, wherein the relative contribution of each polymorphism or risk factor to the

probability is corrected using the hazard ratios. The hazard ratio is a measure of how often a coronary event happens in the group with each of the polymorphisms or risk factors compared to how often it happens in the group without each of the polymorphisms or risk factors, over time).

In an advantageous embodiment, it is possible to use the combination of SEQ ID NO: 1 – 7, or 1- 8, or 1 – 11, for the above method.

"Improved cardiovascular risk assessment" in the context of this application should be understood as a prediction of the probability to develop a cardiovascular event that fits better than the risk assessment done by scales/methods nowadays in use, such as but not limited to Framingham risk score, adapted Framingham risk score (such as but not limited to Regicor), Score, HeartScore, Procam, Reynolds, QRisk, with the number of events that a particular patient has suffered (within the context of a retrospective study) or will suffer, and the MACE and all-cause mortality calculator construction using the ALERT extension trial data that includes as variable of interest age, previous coronary heart disease, smoking, serum creatinine, diabetes mellitus, LDL-cholesterol (for MACE only), total time on renal replacement therapy (for MACE only), and number of transplants (for mortality only), calculator that can be accessed at <http://www.anst.uu.se/insov254/calculator/> (Transplantation 2013;95:142-147). The improvement can be measured as an increase in the area under the ROC curve, measures as a higher c statistic value calculated according to Acad Radiol 1997;4:49-58., the net reclassification improvement and/or integrated discrimination improvement calculated according to Statist Med 2008;27:157-172.

The validation of the markers included in the present ~~invention~~disclosure have been done following the recommendations of the AHA (Circulation 2009;119:2408-2416).

allele of a polymorphism, may also be determined in any other rs that is in strong linkage disequilibrium with any of those rs or SEQ ID NOs mentioned. For example, presence of a polymorphism, or an allele of a polymorphism, may be determined at position 27 of any such other rs that is in strong linkage disequilibrium with any rs or SEQ ID NOs mentioned herein.

In a preferred embodiment the presence of the following alleles of polymorphisms is determined: polymorphisms at positions 27 within specific nucleic acid sequences in particular the presence at position 27 of a C in SEQ ID NO:1, C in SEQ ID NO:2, T in SEQ ID NO:3, C in SEQ ID NO:4, C in SEQ ID NO:5, A in SEQ ID NO:6, T in SEQ ID NO:7, G in SEQ ID NO:8, A in SEQ ID NO:9, A in SEQ ID NO:10 and G in SEQ ID NO:11 with db SNP accession number rs17465637, rs6725887, rs9818870, rs12526453, rs1333049, rs501120, rs9982601, rs10455872, rs10507391, rs9315051, and/or rs17222842, respectively. The presence of a polymorphism, or an allele of a polymorphism, may also be determined (e.g. at position 27) in any other rs in strong linkage disequilibrium with any of those rs or SEQ ID NOs mentioned. The A in SEQ ID NO:9, A in SEQ ID NO:10 and G in SEQ ID NO:11 with db SNP accession number rs10507391, rs9315051, and rs17222842, respectively (or the polymorphism in any other rs in strong linkage disequilibrium with any of those rs mentioned), are together forming the haplotype B ALOX5AP and being considered as one risk genetic component in addition to the other 8 sequences.

These embodiments, i.e. specific combinations of SNPs, in particular the combination of SEQ ID NO 1-7, or 1 -8, or 1 – 11, are preferred embodiments of ~~all aspects of this invention~~disclosure described below.

In another ~~aspect~~embodiment, the ~~invention~~disclosure relates to methods for the estimation of the probability (known as cardiovascular risk) of an individual suffering from chronic kidney disease of presenting a fatal or non-fatal myocardial infarction, or angina, or stroke, or transient ischemic attack or peripheral arteriopathy in a ten year period and/or long-life period based on the presence of one or more of the polymorphisms mentioned above in combination with one or more clinical and/or biochemical risk factors, wherein the relative contribution of the polymorphisms is given as a genetic score risk.

In another ~~aspect~~embodiment, the ~~invention~~disclosure relates to methods for the estimation of the probability (known as cardiovascular risk) of an individual suffering from chronic kidney disease of presenting a fatal or non-fatal myocardial infarction, or angina, or stroke, or transient ischemic attack or peripheral arteriopathy in a ten year period and/or long-life period based on the presence of one or more of the polymorphisms mentioned above, preferably in combination with one or more clinical and/or biochemical risk factors, wherein the relative contribution of the polymorphisms is given as a genetic score risk. The use of the present ~~invention~~disclosure may imply a reclassification of the cardiovascular risk in comparison to the classical way of estimating the risk in patients suffering from chronic kidney disease (e.g. Framingham, Regicor, Score, MACE or total mortality risk calculators among others) where the risk is estimated without the consideration of the genetic and/or clinic/biochemical data considered in the present ~~invention~~disclosure. Reclassification means that by considering the present ~~invention~~disclosure, the new risk can be lower or higher than that calculated by classic means.

"Cardiovascular event" in the context of this application is used interchangeably with "cardiovascular complication".

"AHA" in the context of this application should be understood as American Heart Association.

"GWAS" in the context of this application should be understood as genome-wide association studies.

The term "disease" and "disorder" shall be interpreted in the context of this application interchangeably.

The term "chronic kidney disease" is meant to define a Kidney damage for ≥ 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased Glomerular Filtration Rate (GFR), that can lead to decreased GFR, manifest by either pathological abnormalities or markers of kidney damage, including abnormalities in imaging test or a GFR < 60 mL/min/1.73 m² for ≥ 3 months, with or without kidney damage (Am J Kidney Dis 39:S1-S266, 2002 suppl 1, incorporated herein by reference).

In another ~~aspect~~embodiment, the ~~invention~~disclosure relates to methods for the reclassification of the probability (known as cardiovascular risk) of an individual suffering from chronic kidney disease classified as having a moderate risk to suffer a cardiovascular event (fatal or non-fatal myocardial infarction, or angina, or stroke, or transient ischemic attack or peripheral arteriopathy) in a ten year period and/or long-life period according to the methods nowadays in use based on the presence of one or more of the polymorphisms mentioned above in combination with one or more conventional risk factors, wherein the relative contribution of the polymorphisms is given as a genetic score risk.

In further ~~aspect~~embodiments, the ~~invention~~disclosure relates to methods for the determination of the probability of an individual suffering from chronic kidney disease of presenting a fatal or non-fatal myocardial infarction or angina pectoris or stroke or transient ischemic attack or peripheral arteriopathy in a ten year period or in a long-life period based on the presence of one or more of the polymorphisms mentioned above in combination with one or more conventional risk factors, wherein the relative contribution of the polymorphisms is given as a genetic score risk.

In further ~~aspect~~embodiments, the ~~invention~~disclosure relates to methods for the determination of the probability of an individual suffering from chronic kidney disease, and having suffered from one or more non-fatal myocardial infarction or angina pectoris or stroke or transient ischemic attack or peripheral arteriopathy of presenting a fatal or non-fatal myocardial infarction or angina pectoris or stroke or transient ischemic attack or peripheral arteriopathy in a ten year period or in a long-life period based on the presence of one or more of the polymorphisms mentioned above, preferably in combination with one or more conventional risk factors, wherein the relative contribution of the polymorphisms is given as a genetic score risk.

~~In a further aspect, the invention relates to~~ In one aspect, there is provided a computer program or a computer-readable media containing means for carrying out ~~any of the a methods of the invention as defined herein.~~

~~In yet a further aspect, the invention relates to~~ In one aspect, there is provided a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO:1 to 11.

~~In yet a further aspect~~ one embodiment, the ~~invention~~disclosure relates to a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO:1 to 8, and the following SEQ ID NO: 9, 10, and/or 11 being considered as haplotype B ALOX5AP and the alleles AAG in those SEQ being considered as a single risk allele.

In one aspect, there is provided a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO: 1 to 8.

In yet a further aspect one embodiment, the inventiondisclosure relates to a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, and/or 8.

In one aspect, there is provided a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO: 1 to 7.

In yet a further aspect one embodiment, the inventiondisclosure relates to a kit comprising reagents for detecting the identity of the nucleotide at position 27 within a nucleic acid sequence selected from the group of SEQ ID NO:1, 2, 3, 4, 5, 6, and/or 7.

In a further aspect one embodiment, the inventiondisclosure relates to a computer program or a computer-readable media containing means for carrying out any of the methods of the inventiondisclosure.

The inventiondisclosure is also further defined by the figures, wherein Figure 1 shows an overview of preferred genes and SNPs and Figure 2 shows how CKD population had a higher cardiovascular and coronary risk than a non-CKD general Spanish population (Rev Esp Cardiol 2003;56(3):253).

In one aspect, there is provided a set of probes/primers, selected from the following group:

SEQ ID NO:12 and 13 (rs1333049), and

SEQ ID NO:14 and 15 (rs1333049);

SEQ ID NO:16 and 17 (rs10455872) and

SEQ ID NO:18 and 19 (rs10455872);

SEQ ID NO:20 and 21 (rs6725887) and

SEQ ID NO:22 and 23 (rs6725887);

SEQ ID NO:24 and 25 (rs9818870) and

SEQ ID NO:26 and 27 (rs9818870);

SEQ ID NO:28 and 29 (rs10507391) and

SEQ ID NO:30 and 31 (rs10507391);

SEQ ID NO:32 and 33 (rs9982601) and

SEQ ID NO:34 and 35 (rs9982601);

SEQ ID NO:36 and 37 (rs9315051) and

SEQ ID NO:38 and 39 (rs9315051);

SEQ ID NO:40 and 41 (rs12526453) and

SEQ ID NO:42 and 43 (rs12526453);

SEQ ID NO:44 and 45 (rs17222842) and

SEQ ID NO:46 and 47 (rs17222842);

SEQ ID NO: 50 and 51 (rs17465637) and

SEQ ID NO: 52 and 53 (rs17465637);

SEQ ID NO: 54 and 55 (rs501120) and

SEQ ID NO: 56 and 57 (rs501120);

SEQ ID NO: 58 and 59 (rs2133189) and

SEQ ID NO: 60 and 61 (rs2133189);

SEQ ID NO: 62 and 63 (rs1746048) and

SEQ ID NO: 64 and 65 (rs1746048);

SEQ ID NO: 66 and 67 (rs2133189) and

SEQ ID NO: 68 and 69 (rs2133189).

DETAILED DESCRIPTION OF THE INVENTION

The authors of the present invention have identified a series of single nucleotide polymorphism (SNP) markers which, when used in combination, are associated with a risk of CVD and/or with a risk of CVD complications including, but not limited to, myocardial infarction, stroke, angina pectoris, and peripheral arteriopathy in patients suffering from chronic kidney disease. These polymorphic markers are superior to the polymorphisms or combination of polymorphisms currently in use.

The authors of the present invention have solved the problem identified above in the scales/methods/equations in use nowadays for the calculation of the risk of a subject suffering from chronic kidney disease to develop cardiovascular disease, cardiovascular events and/or cardiovascular complications including, but not limited to, fatal- and non-fatal myocardial infarction, stroke, angina pectoris, transient ischemic attacks, and peripheral arteriopathy.

The present application thus also pertains to a method to overcome the limitations of the existent scales/methods/equations, which do not accurately predict a cardiovascular disease in patients suffering from chronic kidney disease.

The present application thus also pertains to a method to overcome the limitations of the existent scales/methods/equations, which still allow a significant number of cardiovascular events to occur in subjects suffering from chronic kidney disease with only a calculated intermediate risk, using the tools nowadays in use for cardiovascular risk.

The present application thus also pertains to a method to overcome the limitations of the scales/methods/equations, which do not accurately predict a cardiovascular disease in patients suffering from chronic kidney disease in particular those who already have suffered one or more cardiovascular diseases.

The present application overcomes the above-described limitations of the scales/methods/equations used nowadays to calculate the cardiovascular risk by providing a method to accurately calculate the cardiovascular risk in patients suffering from chronic kidney disease. A particular combination (as described above) of genetic markers is used. The particularly advantageous combination is a combination of SNPs in SEQ ID NO: 1-7, or 1-8, namely rs 17465637, rs 6725887, rs 9818870, rs 12526453, rs 1333049, rs 501120, rs 9982601 and rs 10455872. Even more preferred and advantageous is the combination as listed in table 1 (see Figure 1), selected and evaluated by the inventors after a complex and genuine analysis of thousands of possible markers. Of the different possibilities to construct a genetic risk score (GRS), the inventors have been successful to identify a