KI REPORTS
KIReports.org
CLINICAL RESEARCH

Genetic Predisposition for Renal Dysfunction and Incidence of CKD in the Malmö Diet and Cancer Study

Christina-Alexandra Schulz[1], Gunnar Engström[1], Anders Christensson[2], Peter M. Nilsson[1], Olle Melander[1] and Marju Orho-Melander[1]

[1]Department of Clinical Sciences, Lund University, Malmö, Sweden; and [2]Department of Nephrology, Lund University, Malmo, Sweden

Background: Genome-wide association studies (GWAS) have identified >50 single nucleotide polymorphisms (SNP) in association with estimated glomerular filtration rate (eGFR) and chronic kidney disease (CKD) but little is known about whether the combination of these SNPs may aid in prediction of future incidence of CKD in the population.

Methods: We included 2301 participants with baseline eGFR ≥60 mL/min per 1.73 m^2 from the Malmö Diet and Cancer Study–Cardiovascular Cohort. The eGFR was estimated during baseline (1991–1996) and after a mean follow-up of 16.6 years using the CKD–Epidemiology Collaboration 2009 creatinine equation. We combined 53 SNPs into a genetic risk score weighted by the effect size ($wGRS_{CKD}$), and examined its association with incidence of CKD stage 3A (eGFR ≤60 mL/min per 1.73 m^2).

Results: At follow-up, 453 study participants were defined as having CKD stage 3A. We observed a strong association between $wGRS_{CKD}$ and eGFR at baseline ($P = 6.5 \times 10^{-8}$) and at the follow-up reexamination ($P = 5.0 \times 10^{-10}$). The odds ratio (OR) for incidence of CKD stage 3A was 1.25 per 1 SD increment in the $wGRS_{CKD}$ (95% confidence interval [CI]: 1.12–1.39) adjusting for potential confounders (sex, age, body mass index [BMI], baseline eGFR, fasting glucose, systolic blood pressure (SBP), antihypertensive treatment, smoking, follow-up time). Adding $wGRS_{CKD}$ on the top of traditional risk factors did not improve the C-statistics ($P = 0.12$), but the Net Reclassification-Improvement-Index was significantly improved (cNRI = 21.3%; 95% CI: 21.2–21.4; $P < 0.0001$).

Conclusion: $wGRS_{CKD}$ was associated with a 25% increased incidence of CKD per 1 SD increment. Although the $wGRS_{CKD}$ did not improve the prediction model beyond clinical risk factors *per se*, the information of genetic predisposition may aid in reclassification of individuals into correct risk direction.

Kidney Int Rep (2019) **4,** 1143–1151; https://doi.org/10.1016/j.ekir.2019.05.003
KEYWORDS: CKD; eGFR; GRS; renal function
© 2019 Published by Elsevier, Inc., on behalf of the International Society of Nephrology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

With an estimated prevalence of 8% to 16% worldwide, CKD has become a global public health issue. CKD is staged based on eGFR and other markers of kidney damage, such as albuminuria.[1,2] More than 2 decades ago, genome-wide linkage analyses provided evidence that eGFR, serum creatinine, and creatinine clearance are heritable traits, with heritability estimates reaching from 19% to 46% after consideration of multiple risk factors for kidney function.[3] During the past decade, at least 53 common loci were identified in GWAS for kidney function.[4–6] Early identification of participants at high risk for future deterioration in kidney function is of importance, as it could enable early interventions to reduce progression to kidney failure or cardiovascular risk.[7] However, the question of whether genetic markers may aid to improve prediction of future kidney function remains open.[8] So far, one study of 26,000 participants from 8 population-based cohorts of European ancestry showed that most of the 16 SNPs tested associated or showed a tendency for association with incidence of CKD during a median follow-up time of 7 years.[9] In addition, 2 studies created genetic risk scores (GRS) of either 16 or 53 SNPs identified in GWAS for creatinine-based eGFR, and used them to predict incidence of stage 3 CKD, but reported no significant improvements of the prediction models beyond the traditional clinical risk factors.[10,11] On the contrary, recent results from the PREVEND study reported strong associations of a

Correspondence: Marju Orho-Melander, Department of Clinical Sciences, Malmö, CRC, Skåne University Hospital, Jan Waldenströms gata 35, SE 205 02 Malmö, Sweden. E-mail: marju.orho-melander@med.lu.se

Received 24 May 2018; revised 23 April 2019; accepted 7 May 2019; published online 16 May 2019

similar GRS with cross-sectional kidney outcomes (baseline eGFR and prevalent CKD), yet the association with CKD incidence diminished when the multivariate model was adjusted for baseline eGFR.[12] Thus, the evidence if SNPs associated with cross-sectional kidney function may aid in predicting long-term kidney outcomes remains inconclusive. Therefore, in this study we aimed to investigate the joint effect of the 53 in GWAS identified genetic markers,[4] combined into a genetic risk score (GRS_{CKD}), on longitudinal kidney function in the prospective Malmö Diet and Cancer Study–Cardiovascular Cohort (MDCS-CC).

METHODS

Study Participants

For this study, we included participants from the Malmö Diet and Cancer Study (MDCS), a Swedish population-based cohort that has been described in detail elsewhere.[13] In brief, during the baseline examination between 1991 and 1996, men and women born between 1923 and 1945, and 1923 and 1950, respectively, were invited to participate. The total participation rate was 40.8%. MDCS was approved by the ethics committee at Lund University (LU 51-90) and written informed consent was given by all the participants.

This study included individuals from the MDCS–Cardiovascular Cohort (MDCS-CC), which randomly selected 6103 participants of MDCS who underwent additional phenotyping, designed to study epidemiology of carotid artery disease, between 1991 and 1994. Between 2007 and 2012, this random sample was re-invited to a follow-up reexamination as described previously,[14] and of the total of 4924 individuals who were invited (i.e., those who were alive and had not emigrated from Sweden), 3734 attended the follow-up reexamination.

When participants with missing data on baseline or follow-up eGFR or on any covariates, lack of DNA, or an eGFR at baseline of less than 60 mL/min per 1.73 m^2 were excluded, 2301 participants were left for the analyses (Supplementary Figure S1).

Measurements

During the baseline examination, anthropometric measurements were taken by trained personnel, and all participants underwent a physical examination. BMI was calculated as weight/height^2 (kg/m^2) and SBP and diastolic blood pressure (DBP) were measured (mm Hg). Questions concerning social economic status, lifestyle factors, and medical history were assessed by a self-administrated questionnaire.[13] Fasting blood samples were drawn and immediately frozen to −80°C and stored in a biological bank.[15]

Plasma creatinine (μmol/l) was measured and analyzed with the Jaffé method, and traceable to the International Standardization with isotope dilution mass spectometry. Cystatin C was measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin; Dade Behring, Deerfield, IL). The values of cystatin C were not standardized because they were analyzed before the introduction of the world calibrator in 2010. The reference value for the method was 0.53 to 0.95 mg/l. eGFR was calculated based on the previously reported CKD–Epidemiology Collaboration 2009 creatinine-based equation.[16] A factor of 0.0113 was included to convert creatinine levels measured in μmol/l into mg/dl.

Outcome

Incidence of CKD was defined as having an eGFR <60 mL/min per 1.73 m^2 at the follow-up reexamination.

Genotyping and Creation of the GRS

For this study, we included 53 SNPs that were previously identified to be associated with kidney function in GWAS.[4] Genotyping was performed using the Illumina (San Diego, CA) Human OmniExpress BeadChip v1, at the Broad Institute, Cambridge, MA. During the quality control procedure, individuals were filtered out if the call rate was less than 0.95, an inbreeding coefficient of >3 SD away from mean was observed, discordance between inferred and reported gender occurred, duplicate samples were identified, unexpected high proportion of identity by descent sharing was observed, and if first- and second-degree relatives or deviation from the common population structure in the MDCS-CC (exceeding 8 sigma on the first 2 principal components) was observed. In addition, SNPs were filtered out if they were monomorphic or had a call rate of <0.95, had an extreme deviation from the Hardy–Weinberg equilibrium ($P < 1 \times 10^{-07}$), were missing in either cases or controls ($P < 1 \times 10^{-07}$ and minor allele frequency > 0.01) and if an error in the plate assignment occurred ($P < 1 \times 10^{-08}$ and minor allele frequency > 0.01).

A weighted genetic risk score ($wGRS_{CKD}$) was constructed by summing the number of risk alleles (0, 1, or 2) of each of the 53 SNPs per participant weighted for their published regression coefficients.[4] The allele frequencies of the 53 SNPs in MDCS-CC and further details are presented in supplementary material (Supplementary Table S1A). In addition, we updated the GRS (GRS_{CKD63}) with 10 additional SNPs recently identified by Gorski *et al.*[17] Both a weighted and unweighted GRS_{CKD63} were constructed. This updated score included in total 63 SNPs and the details of the additional 10 SNPs are provided in the supplementary material (Supplementary Table S1B). In addition, the

study participants were categorized according to the $wGRS_{CKD}$ into quartiles.

Statistics

SPSS (version 21; IBM Corporation, Armonk, NY) and STATA version 13 (StataCorp LP, College Station, TX) were used for statistical analyses.

General linear regression was used to test the association between the $wGRS_{CKD}$ and eGFR at baseline adjusted for age and sex, and eGFR at follow-up reexamination adjusted for age, sex, baseline eGFR, and follow-up time.

The relationship between the $wGRS_{CKD}$ and CKD at the follow-up reexamination was tested using logistic regression adjusting for age, sex, baseline eGFR, and follow-up time (years), and for known risk factors for CKD at baseline, including BMI, SBP, fasting glucose, use of antihypertensive treatment (AHT), and smoking status.

P for trend across genotypes was calculated assuming an additive model (i.e., genotypes coded as 0, 1, or 2 risk alleles) using $wGRS_{CKD}$ as a continuous variable in the regression models.

The Net Reclassification Improvement (NRI)[18] was calculated using *nri* STATA command for the package idi from http://personalpages.manchester.ac.uk/staff/mark.lunt. The model discrimination was tested by calculating C-statistic using *roccomp* command in STATA for models using risk factors with and without the $wGRS_{CKD}$.

All the presented *P* values are 2-sided, and $P < 0.05$ was considered as significant.

RESULTS

Associations With Clinical Characteristics at Baseline

Most of the 2301 participants were women (58.2%) and were on average 56.0 (SD 5.6) years old at baseline. The mean baseline eGFR was 78.9 (range 60.2–129.2) mL/min per 1.73 m^2 (Table 1). The maximum number of risk alleles in our population was 72 and the minimum was 40, and 71% of the population had 50 to 60 risk alleles. $wGRS_{CKD}$ was strongly associated with a lower baseline eGFR ($P = 6.5 \times 10^{-8}$). Also the $wGRS_{CKD}$ was significantly associated with baseline eGFR after Bonferroni correction for 53 tests ($P < 9.4 \times 10^{-4}$); only a total 6 SNPs reached nominal significance ($P < 0.05$) (Table 2). In addition, the $wGRS_{CKD}$ associated with baseline creatinine ($P = 9.6 \times 10^{-8}$) and cystatin C ($P = 0.001$) levels, and albeit much weaker, also with height ($P = 0.018$) and diastolic blood pressure (DBP; $P = 0.033$) but not with further baseline characteristics (Table 1).

Longitudinal Changes in Kidney Function From Baseline to Follow-up Reexamination and Incidence of CKD at the Follow-up Reexamination

The mean eGFR at follow-up was 71.7 (range 6.2–114.8) mL/min per 1.73 m^2 and the $wGRS_{CKD}$ was significantly associated with eGFR at follow-up reexamination after adjusting for age, sex, baseline eGFR, and follow-up

Table 1. Baseline characteristics of 2301 participants from the Malmö Diet and Cancer Study–Cardiovascular Cohort stratified by genetic risk score for CKD ($wGRS_{CKD}$)

		All	Quartiles of the $wGRS_{CKD}$				
	n	Mean (SD)	Q1 (*n* = 575)	Q2 (*n* = 575)	Q3 (*n* = 575)	Q4 (*n* = 575)	*P*-trend[a]
Alleles, mean (range)	2301	56 (40–72)	50 (39–53)	55 (53–56)	58 (56–59)	62 (59–72)	–
Male sex,[b] *n* (%)	2301	963 (41.8)	246 (42.8)	242 (42.1)	230 (40.0)	245 (42.5)	0.783
Age (yr)	2301	56.0 (5.6)	56.3 (5.7)	55.8 (5.5)	55.9 (5.6)	55.9 (5.6)	0.199
Height (cm)	2301	169.5 (8.8)	169.0 (8.5)	169.5 (9.0)	169.5 (9.0)	169.6 (8.7)	0.018
Weight (kg)	2301	73.1 (13.0)	73.0 (12.4)	73.1 (13.4)	73.2 (12.6)	73.0 (13.6)	0.779
BMI (kg/m^2)	2301	25.4 (3.6)	25.5 (3.7)	25.4 (3.8)	25.4 (3.5)	25.2 (3.6)	0.245
SBP (mm Hg)	2301	138.4 (17.8)	139.6 (18.2)	137.5 (17.8)	138.5 (17.3)	138.0 (17.6)	0.389
DBP (mm Hg)	2301	86.0 (9.0)	86.8 (9.0)	85.7 (9.0)	85.9 (8.7)	85.5 (9.4)	0.033
Fasting glucose (mmol/l)	2301	5.0 (1.1)	5.1 (1.1)	5.1 (1.0)	5.00 (1.1)	5.0 (1.0)	0.398
Cystatin C (mg/dl)	2170	0.75 (0.12)	0.74 (0.11)	0.74 (0.12)	0.76 (0.1)	0.76 (0.1)	0.001
Creatinine (μmol/l)	2301	82.3 (11.9)	81.2 (11.9)	81.6 (11.8)	82.5 (11.8)	83.9 (11.7)	9.6×10^{-8}
eGFR at baseline (mL/min per 1.73 m^2)	2301	78.9 (11.4)	80.1 (11.4)	79.7 (11.4)	78.4 (11.5)	77.3 (10.9)	6.5×10^{-8}
eGFR at follow-up[c] (mL/min per 1.73 m^2)	2301	71.71 (14.6)	74.2 (13.7)	72.1 (14.5)	71.0 (15.4)	69.6 (14.4)	5.0×10^{-10}
AHT,[d] *n* (%)	2301	319 (13.9)	91 (15.8)	76 (13.2)	88 (15.3)	64 (11.2)	0.077
Current smoking,[d] *n* (%)	2301	533 (23.2)	138 (24.0)	136 (23.7)	142 (24.7)	117 (20.3)	0.161

AHT, antihypertensive treatment; BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.
[a] *P* value from a sex- and age-adjusted linear regression model.
[b] *P* value for the categorical variable from an age-adjusted logistic regression.
[c] *P* value from a sex-, age-, baseline eGFR–, and follow-up time–adjusted linear regression model. eGFR was calculated according to CKD–Epidemiology Collaboration 2009 creatinine equation[16] and all 2301 study participants had baseline eGFR of ≥60 mL/min per 1.73 m^2.
[d] *P* value for the categorical variables from a sex- and age-adjusted logistic regression.
Data are shown as mean (SD) for continuous variables or *n* (%) for categorical variables.

Table 2. Kidney function based on eGFR at baseline and at the follow-up reexamination in relation to $wGRS_{CKD}$ and the individual SNPs included in the genetic risk score for CKD (GRS_{CKD}) in 2301 participants from the Malmö Diet and Cancer Study–Cardiovascular Cohort

				eGFR at baseline examination			eGFR at follow-up reexamination			Risk for incidence of CKD at follow-up reexamination		
SNP-ID	CHR	Locus	Risk allele (RAF)	Beta (SE)	*P* value[a]	*P* value[a,b]	Beta (SE)	*P* value[c]	*P* value[b,c]	OR (95% CI)	*P* value[d]	*P* value[b,d]
$wGRS_{CKD}$ per 1-SD increment				−1.26 (0.22)	5.3 e-09[e]	2.65 e09[e]	−1.27 (0.27)	2.6 e-06[e]	1.3 e06[e]	1.25 (1.12–1.39)	0.00009736	0.00004868[e]
rs1800615	1	*CASP9*	T (0.32)	−0.62 (0.33)	0.063	0.0315	−0.50 (0.41)	0.226	0.113	1.16 (0.99–1.37)	0.074	0.037
rs267734	1	*LASS2*	T (0.80)	−0.22 (0.38)	0.561	0.2805	−0.14 (0.47)	0.761	0.3805	0.98 (0.81–1.18)	0.802	0.599
rs12136063	1	*SYPL2*	G (0.33)	−0.49 (0.33)	0.131	0.0655	−0.23 (0.40)	0.556	0.278	1.07 (0.91–1.26)	0.405	0.2025
rs3850625	1	*CACNA1S*	G (0.87)	−0.08 (0.46)	0.860	0.43	−0.45 (0.57)	0.435	0.2175	1.07 (0.84–1.34)	0.592	0.296
rs2636319	1	*SDCCAG8*	C (0.45)	0.20 (0.30)	0.518	0.741	0.00 (0.38)	0.999	0.5015	0.99 (0.85–1.15)	0.897	0.552
rs807624	2	*DDX1*	G (0.65)	0.15 (0.32)	0.640	0.68	−0.23 (0.40)	0.557	0.2785	1.08 (0.92–1.27)	0.350	0.175
rs1260326	2	*GCKR*	C (0.64)	−0.27 (0.32)	0.394	0.197	−0.01 (0.39)	0.985	0.4925	0.92 (0.79–1.08)	0.309	0.846
rs6546838	2	*ALMS*	A (0.76)	−0.67 (0.36)	0.064	0.032	−0.21 (0.45)	0.636	0.318	1.00 (0.83–1.20)	0.995	0.4975
rs1047891	2	*CPS1*	A (0.33)	−0.57 (0.34)	0.097	0.0485	−0.50 (0.43)	0.246	0.123	1.07 (0.90–1.27)	0.437	0.2185
rs2712184	2	*IGFBP5*	A (0.58)	−0.52 (0.31)	0.095	0.0475	0.31 (0.39)	0.425	0.788	1.02 (0.87–1.19)	0.842	0.421
rs6759013	2	*LRP2*	A (0.51)	0.54 (0.30)	0.076	0.62	0.03 (0.38)	0.937	0.532	1.00 (0.86–1.17)	0.977	0.4885
rs7644383	3	*TFDP2*	T (0.72)	−0.70 (0.34)	0.039	0.0195	0.55 (0.42)	0.186	0.907	0.93 (0.79–1.10)	0.384	0.808
rs9682041	3	*SKIL*	T (0.88)	−0.11 (0.48)	0.827	0.4135	0.03 (0.60)	0.956	0.522	1.09 (0.85–1.40)	0.482	0.241
rs10513801	3	*ETV5*	G (0.11)	−0.49 (0.50)	0.322	0.161	−0.38 (0.62)	0.542	0.271	0.89 (0.69–1.14)	0.358	0.821
rs9864031	3	*WNT7A*	T (0.82)	0.20 (0.40)	0.624	0.688	−0.49 (0.50)	0.327	0.1635	1.08 (0.88–1.33)	0.458	0.229
rs17319721	4	*SHROOM3*	A (0.45)	−0.32 (0.31)	0.298	0.149	−0.23 (0.38)	0.545	0.2725	0.96 (0.82–1.11)	0.566	0.717
rs2866413	4	*NFKB1*	G (0.50)	0.40 (0.30)	0.186	0.907	−0.33 (0.38)	0.386	0.193	0.90 (0.77–1.05)	0.181	0.910
rs11959928	5	*DAB2*	A (0.43)	0.24 (0.31)	0.436	0.782	−0.56 (0.38)	0.140	0.07	1.00 (0.86–1.17)	0.974	0.487
rs6420094	5	*SLC34A1*	G (0.31)	−0.51 (0.33)	0.117	0.0585	−0.54 (0.40)	0.182	0.091	0.98 (0.83–1.15)	0.778	0.611
rs881858	6	*VEGFA*	A (0.71)	−0.77 (0.34)	0.021	0.0105	0.23 (0.42)	0.587	0.707	0.98 (0.83–1.17)	0.851	0.5745
rs316009	6	*SLC22A2*	C (0.90)	−0.94 (0.50)	0.063	0.0315	−0.91 (0.63)	0.146	0.073	1.21 (0.92–1.58)	0.174	0.087
rs7759001	6	*ZNF204*	A (0.77)	0.31 (0.36)	0.390	0.805	−0.08 (0.45)	0.858	0.429	1.07 (0.89–1.29)	0.441	0.2205
rs11765986	7	*TMEM60*	T (0.26)	−0.55 (0.35)	0.111	0.0555	−0.39 (0.43)	0.364	0.182	1.19 (1.00–1.41)	0.044	0.022
rs7805747	7	*PRKAG2*	A (0.26)	−0.30 (0.35)	0.401	0.2005	−0.81 (0.44)	0.066	0.033	1.10 (0.92–1.30)	0.306	0.153
rs10277115	7	*UNCX*	A (0.24)	−0.46 (0.36)	0.207	0.1035	−0.36 (0.45)	0.420	0.21	1.08 (0.90–1.30)	0.383	0.1915
rs7785065	7	*KBTBD2*	C (0.61)	0.24 (0.32)	0.449	0.776	0.15 (0.39)	0.711	0.645	0.94 (0.80–1.11)	0.473	0.764
rs6459680	7	*RNF32*	T (0.75)	−0.24 (0.36)	0.499	0.2495	0.04 (0.44)	0.932	0.534	0.95 (0.79–1.13)	0.549	0.720
rs6999484	8	*STC1*	A (0.46)	−0.35 (0.31)	0.255	0.1275	0.14 (0.38)	0.707	0.647	1.04 (0.89–1.22)	0.591	0.2955
rs1556751	9	*PIP5K1B*	G (0.39)	−0.25 (0.32)	0.425	0.2125	−0.51 (0.39)	0.195	0.0975	1.03 (0.88–1.20)	0.732	0.366
rs1044261	10	*WDR37*	T (0.07)	0.47 (0.60)	0.435	0.783	−0.27 (0.75)	0.716	0.358	1.08 (0.79–1.46)	0.637	0.3185
rs10994860	10	*A1CF*	C (0.81)	−0.17 (0.39)	0.662	0.331	−0.26 (0.49)	0.596	0.298	1.37 (1.11–1.70)	0.003	0.0015
rs3925584	11	*MPPED2*	T (0.55)	−0.21 (0.31)	0.501	0.2505	0.23 (0.39)	0.559	0.721	0.97 (0.83–1.13)	0.698	0.651
rs163158	11	*KCNQ1*	A (0.17)	0.32 (0.40)	0.418	0.791	0.09 (0.50)	0.852	0.547	1.03 (0.84–1.25)	0.807	0.4035
rs4014195	11	*AP5B1*	G (0.36)	−0.38 (0.32)	0.238	0.119	−0.42 (0.40)	0.294	0.147	1.03 (0.87–1.21)	0.739	0.3695
rs10774021	12	*SLC6A13*	T (0.67)	−1.13 (0.33)	0.00058[e]	0.00029[e]	0.18 (0.41)	0.657	0.672	0.95 (0.80–1.12)	0.544	0.728
rs10491967	12	*TSPAN9*	A (0.09)	−0.16 (0.52)	0.756	0.378	−0.56 (0.65)	0.383	0.1915	1.03 (0.80–1.33)	0.802	0.401
rs7956773	12	*PTPRO*	T (0.82)	−0.43 (0.40)	0.278	0.139	0.19 (0.49)	0.697	0.652	1.06 (0.86–1.29)	0.592	0.296

(Continued on next page)

Table 2. (Continued) Kidney function based on eGFR at baseline and at the follow-up reexamination in relation to $wGRS_{CKD}$ and the individual SNPs included in the genetic risk score for CKD (GRS_{CKD}) in 2301 participants from the Malmö Diet and Cancer Study–Cardiovascular Cohort

				eGFR at baseline examination			eGFR at follow-up reexamination			Risk for incidence of CKD at follow-up reexamination		
SNP-ID	CHR	Locus	Risk allele (RAF)	Beta (SE)	*P* value[a]	*P* value[a,b]	Beta (SE)	*P* value[c]	*P* value[b,c]	OR (95% CI)	*P* value[d]	*P* value[b,d]
rs1106766	12	*INHBC*	C (0.73)	−0.22 (0.34)	0.515	0.2575	0.02 (0.42)	0.968	0.516	1.08 (0.91–1.28)	0.381	0.1905
rs626277	13	*DACH1*	A (0.60)	−0.26 (0.31)	0.412	0.206	0.53 (0.39)	0.175	0.913	0.98 (0.84–1.15)	0.802	0.599
rs8032195	15	*INO80*	A (0.39)	−0.01 (0.32)	0.755	0.3775	−0.73 (0.39)	0.063	0.0315	1.15 (0.98–1.35)	0.077	0.0385
rs2467853	15	*GATM*	G (0.39)	−0.64 (0.31)	0.041	0.0205	−0.16 (0.39)	0.685	0.3425	1.12 (0.96–1.31)	0.155	0.0775
rs491567	15	*WDR72*	A (0.76)	−0.30 (0.36)	0.392	0.196	−1.15 (0.44)	0.009	0.0045	1.24 (1.03–1.49)	0.024	0.012
rs1394125	15	*UBE2Q2*	A (0.33)	−0.50 (0.33)	0.131	0.0655	−0.07 (0.41)	0.869	0.4345	0.97 (0.82–1.14)	0.698	0.651
rs12917707	16	*UMOD*	G (0.82)	−0.91 (0.41)	0.029	0.0145	−2.17 (0.51)	0.000019[e]	0.0000095[e]	1.59 (1.27–1.98)	0.000047[e]	0.0000235[e]
rs164749	16	*DPEP1*	G (0.42)	−0.58 (0.31)	0.065	0.0325	0.22 (0.39)	0.562	0.719	0.96 (0.82–1.13)	0.633	0.684
rs894680	17	*SLC47A1*	A (0.39)	−0.31 (0.31)	0.323	0.1615	0.31 (0.39)	0.421	0.790	1.03 (0.88–1.20)	0.716	0.358
rs7221875	17	*CDK12/ FBXL20*	G (0.76)	0.12 (0.35)	0.721	0.640	−1.11 (0.43)	0.010	0.005	1.22 (1.02–1.46)	0.032	0.016
rs9905274	17	*BCAS3*	T (0.16)	0.39 (0.44)	0.371	0.815	−0.35 (0.54)	0.517	0.2585	1.07 (0.86–1.33)	0.561	0.2805
rs8091180	18	*NFATC1*	A (0.56)	−0.63 (0.31)	0.043	0.0215	−0.16 (0.38)	0.681	0.3405	0.98 (0.84–1.14)	0.757	0.622
rs12460876	19	*SLC7A9*	T (0.66)	−0.26 (0.33)	0.428	0.214	−0.25 (0.40)	0.535	0.2675	1.00 (0.85–1.18)	0.972	0.486
rs1807157	19	*SIPA1L3*	T (0.16)	−0.05 (0.43)	0.903	0.4515	0.60 (0.53)	0.258	0.871	0.80 (0.64–1.00)	0.050	0.975
rs2273684	20	*TP53INP2*	G (0.49)	−0.57 (0.31)	0.064	0.032	−0.78 (0.38)	0.042	0.021	1.12 (0.96–1.31)	0.160	0.08
rs17216707	20	*BCAS1*	T (0.80)	0.23 (0.38)	0.548	0.726	−0.76 (0.48)	0.110	0.055	1.33 (1.09–1.63)	0.0056	0.0028

AHT, antihypertensive treatment; BMI, body mass index; CHR, chromosome; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; RAF, risk allele frequency; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; $wGRS_{CKD}$, genetic risk score weighted by the effect size.
[a]Adjusted for age and sex.
[b]One-sided *P* value.
[c]Adjusted for age, sex, baseline eGFR, and follow-up time.
[d]Adjusted for age, sex, baseline eGFR, fasting glucose, BMI, SBP, AHT, smoking status, and follow-up time.
[e]$P < 9.4 \times 10^{-4}$ (Bonferroni corrected for 53 SNPs).
eGFR calculated according to CKD–Epidemiology Collaboration 2009 creatinine equation[16] and all 2301 study participants had baseline eGFR of ≥60 mL/min per 1.73 m^2.

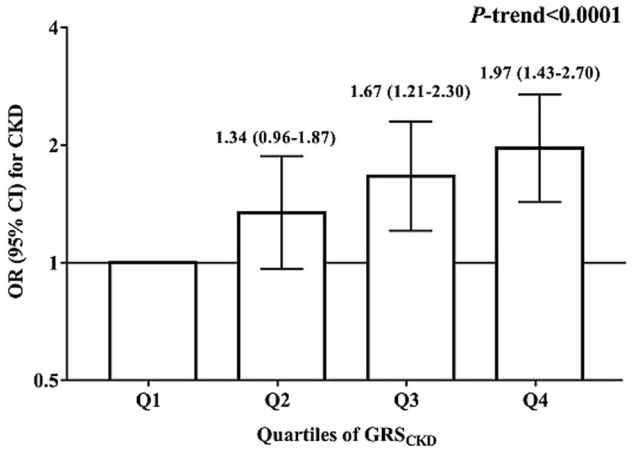

Figure 1. Incidence of chronic kidney disease (CKD) according to quartiles of the genetic risk score for CKD ($wGRS_{CKD}$) in 2301 participants of the Malmö Diet and Cancer Study–Cardiovascular Cohort after an average follow-up time of 16 years. The figure shows odds ratios (ORs) and 95% confidence intervals (CIs) for having an estimated glomerular filtration rate (eGFR) of <60 mL/min per 1.73 m^2 obtained from logistic regression analysis adjusted for age, sex, baseline levels of eGFR, fasting glucose, body mass index, systolic blood pressure, smoking status (current, former, or never smokers), use of antihypertensive drugs (yes/no), and follow-up time. The eGFR was calculated according to CKD–Epidemiology Collaboration 2009 creatinine equation,[16] and all 2301 study participants had a baseline eGFR of ≥60 mL/min per 1.73 m^2. Q1 = lowest quartile as reference was set to 1. The OR and respective 95% CI for each quartile are displayed above each column. Compared with the reference quartile Q1, the ORs for Q2, Q3, and Q4 were as follows: Q2 OR: 1.34, 95% CI: 0.96–1.87, $P = 0.081$; Q3 OR: 1.67, 95% CI: 1.21–2.30, $P = 0.001$; Q4 OR: 1.97, 95% CI: 1.43–2.70, $P = 0.00003$ in the multivariate adjusted model.

time ($P = 5.0 \times 10^{-10}$) (Table 1). During a mean follow-up time of 16.6 (13.3–20.2) years there were in total 453 (19.7%) participants whose eGFR decreased to below 60 mL/min per 1.73 m^2 by the follow-up reexamination. Baseline characteristics of participants with and without incident CKD are shown in Supplementary Table S3. We observed a significantly increased CKD incidence at follow-up reexamination with increasing $wGRS_{CKD}$ ($P = 0.00029$ per 1 SD increment in the $wGRS_{CKD}$). There was a 22% increased risk for incident CKD (OR: 1.22; 95% CI: 1.10–1.37) after adjusting for age, sex, baseline, eGFR, and follow-up time. When adjusting for further baseline risk factors, BMI, fasting glucose, SBP, antihypertensive treatment (AHT), and smoking status, the $wGRS_{CKD}$ remained significantly associated with CKD at follow-up (OR: 1.25; 95% CI: 1.12–1.39 per 1 SD increment in the $wGRS_{CKD}$) (Table 2). We found no evidence that including the covariates would markedly affect the associations between the $wGRS_{CKD}$ and kidney function (Supplementary Table S4). Participants within the highest quartile of risk alleles had a 97% increased risk for incident CKD compared with those in the lowest quartile (Q1 vs. Q4, OR: 1.97; 95 % CI: 1.43–2.70; Figure 1). The highest risk increase, 34%, was observed for participants in Q2 compared with those with the lowest number of risk alleles (Q1). Between the intermediate quartiles there was a higher risk of 33% for participants in Q3 compared with those in Q2. Participants with the highest number of risk alleles (Q4) had an additional 30% higher risk compared with those in Q3 (Figure 1); however, adding the $wGRS_{CKD}$ to the model with clinical risk factors did not improve the discrimination between participants with and without incident CKD at follow-up reexamination (area under the curve $[AUC]_{clinicalRiskfactors}$ vs. $AUC_{clinicalRiskfactors+GRS}$ 0.726 vs. 0.731; $P = 0.12$) (Supplementary Figure S3). The fit for both models was adequate (Hosmer-Lemenshow for both $P > 0.05$). However, the NRI index was significantly improved, as adding the $wGRS_{CKD}$ to the risk model reclassified 21.3% of the participants into the correct risk direction (95% CI: 21.24–21.44; $P < 0.0001$) (Supplementary Figure S4). When the $wGRS_{CKD}$ was added to the model including clinical risk factors, most of both the cases (56.07%) as well as noncases (54.60%) were reclassified into the correct risk direction.

Similar results were observed when we used the unweighted GRS_{CKD} (Supplementary Figure S2 and Supplementary Table S4).

An Updated Score Including 63 SNPs Cross-sectionally Associated With eGFR

Recently, Gorski *et al.*[17] discovered 10 additional loci that associated with eGFRcrea at $P < 5 \times 10^{-8}$. In MDCS-CC, the mean number of risk alleles for GRS_{CKD63} was 65 (SD 5; range 51–80). The associations between GRS_{CKD63} and baseline eGFR, eGFR at follow-up, and incidence of CKD were comparable to those with GRS_{CKD53}, for both weighted and unweighted GRSs (Supplementary Table S3 and Supplementary Table S4).

DISCUSSION

After the mean follow-up time of 16.6 years, we observed a direct relationship between higher $wGRS_{CKD}$ and increased CKD incidence in our Swedish population-based cohort, whereby the increase per 1-SD increment associated with a 27% increased risk, taking into account established risk factors for CKD. In our population, the highest number of risk alleles was 72 (of 106 possible) and lowest 40 (of 0 possible), and most of the participants carried 50 to 60 risk alleles; however, adding the $wGRS_{CKD}$ to a risk model with clinical risk factors did not improve the discriminatory effect of the prediction model to differentiate CKD cases from non-CKD cases. Yet, including the $wGRS_{CKD}$ in the risk model led to a significantly improved NRI index of 21.3%.

The genetic markers included in the GRS_{CDK} have previously been discovered in GWAS,[4] and earlier, Ma *et al*.[10] reported association between the same GRS of 53 SNPs and incidence of CKD stage 3 in the Framingham Heart Study. In fact, they observed similar results compared with our study, with a 37% increased CKD incidence per 10 risk alleles.[10] Compared with our study, they had a somewhat higher number of study participants (2698 vs. 2301) but fewer incident cases (292 vs. 453) and a shorter follow-up time (11 vs. 16 years). In addition, the participants were of similar age (57.6 vs. 56.0 years), had somewhat higher BMI (27.5 vs. 25.4 kg/m^2), but higher eGFR (92.3 vs. 78.9 mL/min per 1.73 m^2) at baseline, which may explain the slightly higher risk increase in our study. The C-statistics were not improved in either study after adding the GRS to the model with the traditional risk factors. Yet, it must be kept in mind that the effect on the change in the AUC in the receiver operating characteristics analysis depends not only on the predictive ability of the "traditional risk model" and the strength of the new predictor (here the GRS_{CKD}), but also on the potential correlation between them, and thus C-statistics often may be a rather insensitive measure.[19,20] Nonetheless, by NRI analysis we observed that adding the GRS_{CKD} to the risk model led to a significant improvement, indicating potential value of the $wGRS_{CKD}$ in risk classification, whereas such analyses were not reported in the Framingham Heart Study.[10] In contrast to MDCS-CC, Thio *et al*.[12] reported that association between the GRS and CKD incidence diminished when the multivariate model was adjusted for baseline eGFR. We think that this could at least partly be explained by the younger age, shorter follow-up time, higher eGFR, and lower number of cases, and thus lower statistical power in the PREVEND study as compared with our study (mean age 49 vs. 56 years, follow-up 11 vs. 16 years, baseline eGFR 96 vs. 79 mL/min per 1.73 m^2, and number of incident cases 154 vs. 453, respectively).[12]

Obviously, more studies are needed to estimate the value of genetic prediction of future kidney function, both in other population-based studies as well as among individuals at high risk for CKD, such as patients with hypertension or type 2 diabetes.

The genetic variants included in our GRS_{CKD} were initially identified in cross-sectional analyses of >130,000 individuals from 49 studies and replicated in up to 42,000 additional individuals.[4] Before our study, the question of which genetic variants associate with longitudinal decline of kidney function was recently raised in a GWAS including 63,558 individuals.[21] However, only 1 locus, the rs12917707 in the gene encoding uromodulin (*UMOD*), was identified genome-wide significantly associated with a change in eGFR.[21] The *UMOD* variant was already previously found to be associated with eGFR in the cross-sectional GWAS[4] and was thus included in our GRS_{CKD}. Indeed, of all the individual SNPs in our study, the *UMOD* SNP clearly provided the strongest association with CKD incidence, with 59% increased risk per risk allele (1.59 [1.27–1.98], $P = 0.000047$). Uromodulin is the most abundant protein excreted in the normal urine and is exclusively expressed in the thick ascending limb of the loop of Henle. The importance of *UMOD* in renal diseases was first fully appreciated when rare mutations of *UMOD* were discovered in a group of very rare autosomal-dominant tubulointerstitial renal diseases approximately 15 years ago (reviewed in Scolari *et al*.[22]). The rs12917707 was recently reported in association with urinary uromodulin levels,[23] and serum uromodulin levels were inversely associated with the development of CKD.[24] Thus, both monogenic mutations and common variants in *UMOD* seem to have causal implications in the development of kidney diseases.

Even if there was no benefit in distinguishing CKD cases from non-CKD cases when the $wGRS_{CKD}$ was added to a model including clinical risk factors (*P* value Δ AUC $P = 0.12$), it seems noteworthy that the results from the cNRI analysis show an improvement of 6.07% for CKD cases being correctly classified and likewise most participants without CKD at follow-up (54.60%) are correctly reclassified into lower risk. This shows that knowing the individual genetic risk may be of importance for those at increased risk, as it could allow them to act on this already earlier by primary regimens, such as lifestyle changes.

The heritability of eGFR has been estimated to be between 36% and 75%[25,26] and the 53 SNP variants included in our GRS_{CKD} have been estimated to explain approximately 3.2% of the variance of eGFR.[4] All these SNPs are common, with minor allele frequencies above 5% and discovery of less frequent variants with higher effect sizes could potentially explain a greater variance in eGFR[10] and improve prediction of future kidney function. Toward this direction, a very recent study identified 10 novel genome-wide significant loci in a meta-analysis of GWAS cohorts including more than 110,000 adults using 1000 Genome imputed genotypes, which enhanced the coverage of the genomic variation.[17] Nonetheless, all but 1 of the identified 10 novel variants were common and the variance of eGFR that was explained when added together with the earlier 53 variants was only slightly increased, yet remained less than 4%.[17]

Our study has some limitations that deserve clarification. The outcome in our study was incidence of CKD, defined as an eGFR <60 mL/min per 1.73 m^2 at the follow-up reexamination, which does not fulfill the

current Kidney Disease Improving Global Outcomes 2012 CKD guidelines[27] that require an eGFR <60 mL/min per 1.73 m^2 for a duration of >3 months for CKD diagnosis. We regret that, both at the baseline and the follow-up reexamination of our study, only one measurement of creatinine was performed. It is inarguable that more time points of creatinine measurement had been preferred, yet the long mean follow-up time of more than 16 years may increase the confidence in assessing the progression to CKD.[27] Further, we did not have information on albuminuria at baseline, which would have been desirable, as it is a key biomarker in CKD risk assessment. However, we adjusted our analyses for many potential risk factors, including baseline eGFR, and this did not majorly influence the results.

Our study also has some strengths. First, it was conducted in an apparently healthy middle-aged Swedish population, making the findings generalizable to the general population of European ancestry. Second, our study had a long follow-up and therefore a reasonable number of incident CKD cases. Third, the risk for reverse causation was minimized given the prospective design and the fact that the exposure was the genetic make-up of the participants.

CONCLUSION

In the prospective MDCS-CC, we observed that the $wGRS_{CDK}$ of 53 genetic markers, previously associated with creatinine-based eGFR, associated with a significantly increased incidence of CKD. Although the $wGRS_{CKD}$ did not improve the C-statistics beyond the traditional clinical risk factors, it aided in reclassification of individuals into the correct risk direction.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

We thank all participants of the Malmö Diet and Cancer Study and Ms. Malin Svensson for excellent technical assistance.

This study was supported by the European Research Council (Consolidator grant 649021, Marju Orho-Melander), the Swedish Research Council, the Swedish Heart and Lung Foundation, the Novo Nordic Foundation, the Swedish Diabetes Foundation, and the Påhlsson Foundation, and by equipment grants from the Knut and Alice Wallenberg Foundation, the Region Skåne, Skåne University Hospital, the Linneus Foundation for the Lund University Diabetes Center, and Swedish Foundation for Strategic Research for IRC15-0067. Peter M. Nilsson received grants from the Swedish Research Council (K2008-65X-20752-01-3, K2011-65X-20752-04-6). The funding organizations had no role in the design and conduct of the study; the collection, management, analyses, and interpretation of the data; or the preparation or approval of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Genetic variants included in the GRS_{CKD}.

Table S2. Kidney function based on eGFR at baseline and at the follow-up reexamination in relation to the GRS_{CKD} and the individual SNPs included in the genetic risk score for CKD (GRS_{CKD}), including 63 SNPs in 2298 participants from the Malmö Diet and Cancer Study–Cardiovascular Cohort.

Table S3. Baseline characteristics among incident CKD and non-incident CKD cases in the MDCS-CC.

Table S4. Associations between the 4 GRSs and kidney function in the MDCS-CC.

Figure S1. Participants of the Malmö Diet and Cancer Study included in the longitudinal association analyses for the GRS_{CKD} and kidney function.

Figure S2. Genetic risk score and baseline eGFR in the Malmö Diet and Cancer Study. (A) The histogram represents the frequency among 4241 participants through the number of CKD risk alleles. Further, the predicted values of the baseline eGFR resulting from a crude unadjusted linear regression plotted across the genetic risk score are shown. $n = 4241$ refers to all participants in the dataset with data on eGFR at baseline and GRS available. The mean number of SNPs per participant was 56.5 (range: 42–71) and the genetic risk score (GRS) had r^2 of 1.42%. (B) The histogram represents the frequency among 2301 participants through the number of CKD risk alleles. Further, the predicted values of the baseline eGFR resulting from a crude nonadjusted linear regression plotted across the genetic risk score are shown. $n = 2301$ refers to all participants in the dataset with data on eGFR at baseline, GRS, eGFR at follow-up reexamination, and covariates available. The mean number of SNPs per participant was 56.4 (range: 42–71) and the GRS had r^2 of 0.97%.

Figure S3. Receiver operating characteristics (ROC) curve. ROC shown in blue resulting from predicted values from logistic regression adjusted for age, sex, FU-time, eGFR at baseline, BMI, SBP, AHT, fasting glucose, and smoking. ROC shown in red resulting from predicted values from logistic regression adjusted for age, sex, FU-time, eGFR at baseline, BMI, SBP, AHT, fasting glucose, smoking, and $wGRS_{CKD}$.

Figure S4. Continuous NRI scatter plots for (A) CKD cases and (B) non-CKD cases at follow-up. The predicted probabilities from the clinical model + wGRS is given on the *y*-axis and the predicted probabilities from the clinical model on the *x*-axis. Predicted probabilities have been calculated for logistic regression models in (A) CKD cases and (B) non-CKD cases adjusted for age, sex, FU-time, eGFR at

baseline, BMI, SBP, AHT, fasting glucose, and smoking (clinical model), and age, sex, FU-time, eGFR at baseline, BMI, SBP, AHT, fasting glucose, smoking, and $wGRS_{CKD}$ (clinical model+wGRS), respectively.

REFERENCES

1. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives. *Lancet*. 2013;382:260–272.
2. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*. 2013;3:1–150.
3. Fox CS, Yang Q, Cupples LA, et al. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population:the Framingham Heart Study. *J Am Soc Nephrol*. 2004;15:2457–2461.
4. Pattaro C, Teumer A, Gorski M, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nat Commun*. 2016;7:10023.
5. Kottgen A, Glazer NL, Dehghan A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet*. 2009;41:712–717.
6. Kottgen A, Pattaro C, Boger CA, et al. New loci associated with kidney function and chronic kidney disease. *Nat Genet*. 2010;42:376–384.
7. James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. *Lancet*. 2010;375:1296–1309.
8. Echouffo-Tcheugui JB, Kengne AP. Risk models to predict chronic kidney disease and its progression:a systematic review. *PLoS Med*. 2012;9:e1001344.
9. Boger CA, Gorski M, Li M, et al. Association of eGFR-Related Loci Identified by GWAS with Incident CKD and ESRD. *PLoS Genet*. 2011;7:e1002292.
10. Ma J, Yang Q, Hwang SJ, et al. Genetic risk score and risk of stage 3 chronic kidney disease. *BMC Nephrol*. 2017;18:32.
11. O'Seaghdha CM, Yang Q, Wu H, et al. Performance of a genetic risk score for CKD stage 3 in the general population. *Am J Kidney Dis*. 2012;59:19–24.
12. Thio CHL, van der Most PJ, Nolte IM, et al. Evaluation of a genetic risk score based on creatinine-estimated glomerular filtration rate and its association with kidney outcomes. *Nephrol Dial Transplant*. 2018;33:1757–1764.
13. Berglund G, Elmstahl S, Janzon L, et al. The Malmo Diet and Cancer Study. Design and feasibility. *J Intern Med*. 1993;233:45–51.
14. Rosvall M, Persson M, Ostling G, et al. Risk factors for the progression of carotid intima-media thickness over a 16-year follow-up period: the Malmo Diet and Cancer Study. *Atherosclerosis*. 2015;239:615–621.
15. Pero RW, Olsson A, Berglund G, et al. The Malmo biological bank. *J Intern Med*. 1993;233:63–67.
16. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
17. Gorski M, van der Most PJ, Teumer A, et al. 1000 Genomes-based meta-analysis identifies 10 novel loci for kidney function. *Sci Rep*. 2017;7:45040.
18. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, et al. Evaluating the added predictive ability of a new marker:from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157–172; discussion 207–112.
19. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation*. 2007;115:928–935.
20. Cook NR. Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. *Clin Chem*. 2008;54:17–23.
21. Gorski M, Tin A, Garnaas M, et al. Genome-wide association study of kidney function decline in individuals of European descent. *Kidney Int*. 2015;87:1017–1029.
22. Scolari F, Izzi C, Ghiggeri GM. Uromodulin: from monogenic to multifactorial diseases. *Nephrol Dial Transplant*. 2015;30:1250–1256.
23. Olden M, Corre T, Hayward C, et al. Common variants in UMOD associate with urinary uromodulin levels:a meta-analysis. *J Am Soc Nephrol*. 2014;25:1869–1882.
24. Leiherer A, Muendlein A, Saely CH, et al. The value of uromodulin as a new serum marker to predict decline in renal function. *J Hypertens*. 2018;36:110–118.
25. Boger CA, Heid IM. Chronic kidney disease: novel insights from genome-wide association studies. *Kidney Blood Press Res*. 2011;34:225–234.
26. Pattaro C, Aulchenko YS, Isaacs A, et al. Genome-wide linkage analysis of serum creatinine in three isolated European populations. *Kidney Int*. 2009;76:297–306.
27. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int*. 2014;85:49–61.