



LUND UNIVERSITY

The role of circulating biomarkers, genetics, and diet in kidney function

Schulz, Christina-Alexandra

2017

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Schulz, C.-A. (2017). *The role of circulating biomarkers, genetics, and diet in kidney function*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Malmö]. Lund University: Faculty of Medicine.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

The role of circulating biomarkers, genetics, and diet in kidney function

CHRISTINA-ALEXANDRA SCHULZ

DEPARTMENT OF CLINICAL SCIENCES IN MALMÖ | LUND UNIVERSITY



The role of circulating biomarkers, genetics, and diet in kidney function

The role of circulating biomarkers, genetics, and diet in kidney function

Christina-Alexandra Schulz



LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty Medicine, Lund University, Sweden.

To be defended at Jubileumsaulan, Skånes universitetssjukhus,

Jan Waldenströms gata 5, SE-20502 Malmö,

Sweden on Friday December 1st 2017 at 13:00.

Faculty opponent

Professor Peter Rossing, MD, PhD

Steno Diabetes Center Copenhagen, DK

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION	
	Date of issue: December, 01 2017	
Author(s) Christina-Alexandra Schulz	Sponsoring organization	
Title and subtitle: The role of circulating biomarkers, genetics, and diet in kidney function		
<p>Abstract</p> <p>Today, between 8% and 16% of all individuals worldwide suffer from chronic kidney disease (CKD), which is becoming a growing public health issue. Creatinine and cystatin C are commonly used markers for kidney function, and estimated glomerular filtration rate (eGFR) and albuminuria are used to stage CKD. However, creatinine and eGFR are rather insensitive in identifying individuals at high risk of future CKD. Several biomarkers have been proposed to play a role, yet the clinical evidence, particularly from longitudinal studies, is limited. During the recent years, several genetic markers associated with kidney function have been identified by genome-wide association studies. However, the question of whether genetic markers may aid in improving prediction on the top of the commonly used clinical risk factors remains open. Environmental factors, such as diet, have been suggested to be of importance for kidney function, but current knowledge from individuals free of CKD is limited.</p> <p>Therefore, this thesis aimed to investigate if 1) circulating biomarkers (studies I-III) or 2) information on genetic predisposition (study IV) may show advantages in the prediction of future kidney function deterioration after a long-term follow-up. Furthermore, we studied if 3) dietary intake at baseline associates with future kidney function (study V). The work in this thesis was conducted within the setting of the Malmö Diet and Cancer Study, a population-based cohort of middle-aged individuals from Southern Sweden.</p> <p>In studies I-III we observed that in participants with an eGFR > 60 mL/min/1.73m² elevated plasma levels of pro-enkephalin (study I), soluble urokinase receptor (study II), or kidney injury molecule-1 (study III), were each associated with an ~20% increased risk of incident CKD stage 3A per increase in one SD of the respective biomarker, independently of known risk factors (OR 1.17 95 %CI=1.05-1.30; 1.25 95 %CI=1.10-1.41, and 1.21 95 %CI=1.08-1.34, respectively).</p> <p>In study IV, we report that adding a genetic risk score composed of 53 SNPs, previously identified by GWAS to associate with kidney function, to a risk model including clinical risk factors, may be helpful in evaluating the risk of incident CKD (OR per risk allele 1.04, 95%CI 1.01-1.07; NRI 17.7.% P=0.0007).</p> <p>In study V, we investigated the association between dietary intakes and occurrence of CKD in participants with a preserved kidney function at baseline. We investigated reported intakes of 15 food groups focusing on different sources of protein, and intakes of macronutrients and three beverages. We observed that lower sucrose intake as well as higher fiber and coffee intake associated with favorable long-term kidney function.</p> <p>In conclusion, the studies included in this PhD-thesis demonstrate that novel circulating and genetic biomarkers may be clinically useful in estimating future kidney function, and emphasize genetic susceptibility and diet as important determinants of longitudinal kidney function.</p>		
Key words: biomarker, chronic kidney disease, CKD, diet , eGFR, genetic, genetic, risk score, glomerular filtration rate, GRS, kidney function, kidney injury molecule 1, KIM-1, pro-enkephalin, proENK, soluble urokinase receptor, suPAR		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language: English
ISSN and key title 1652-8220		ISBN 978-91-7619-561-1
Recipient's notes	Number of pages 100	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date



The role of circulating biomarkers, genetics, and diet in kidney function

Christina-Alexandra Schulz



LUND
UNIVERSITY

Coverphoto: Ribersborgsstranden, Malmö Sverige

© Andrew Clelland

Copyright Christina-Alexandra Schulz

Faculty of Medicine
Department of Clinical Sciences in Malmö
Medicine Doctoral Dissertation Series 2017:179

ISBN 978-91-7619-561-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2017



To my beloved family

“Declare the past, diagnose the present, foretell the future.”

(Hippocrates)

Content

List of papers included in this thesis	11
List of papers not included in this thesis	12
List of figures	14
Table legend	15
Abbreviations	16
Introduction	19
Anatomy and physiology of the kidneys	20
The conceptual model of CKD	24
Definition and classification of CKD	25
Prevalence of CKD	26
Quantification of kidney function	26
Creatinine and cystatin C	26
Cockcroft-Gault (CG)-equation	27
Modification of Diet in Renal Disease (MDRD) Study equation	27
Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations	27
Limitations of estimating GFR	28
Etiology of CKD	29
Risk factors for CKD	31
Comorbidities and Mortality of CKD	31
Prevention and management of CKD	32
Awareness of CKD	32
Genetic susceptibility and kidney function	33
Human Genome	33
Heritability of kidney function	33
Monogenic kidney disease	33
Multifactorial kidney diseases	34
Prediction of CKD utilizing genetic markers	35
Diet and kidney disease	35
Biomarkers	36
Definition and applications	36
Biomarkers for kidney disease	37
Pro-enkephalin A (pro-ENK)	37
Soluble urokinase-type plasminogen activator receptor (suPAR)	38

Kidney injury molecule -1 (KIM-1)	39
Aims	41
Methods	43
The Malmö Diet and Cancer Study (MDCS) cohort.....	43
The MDCS-Cardiovascular cohort.....	43
Exclusion criteria in studies I-V	44
Clinical examination and assays.....	45
Measurements.....	45
Genotyping	45
Dietary assessment	46
Renal endpoints	46
Primary Endpoint.....	46
Secondary Endpoint.....	47
Predictors.....	47
Pro-enkephalin (study I)	47
suPAR (study II).....	47
KIM-1 (study III).....	48
GWAS and Mendelian Randomization (MR)-analysis (study I and study III)	48
Genetic risk score (study IV).....	48
Dietary intakes (study V).....	49
Statistics	50
Covariates	50
Results	53
Study I	53
Study II.....	58
Study III.....	61
Study IV	63
Study V.....	67
Discussion.....	71
Summary and conclusions.....	81
References	83
Copyright references.....	95
Popular Science summary	97
Populärvetenskaplig sammanfattning.....	99
Acknowledgement.....	101

List of papers included in this thesis

- I. **Schulz, CA**, Christensson, A, Ericson, U, Almgren, P, Hindy, G, Nilsson, PM, Struck, J, Bergmann, A, Melander, O, Orho-Melander, M: High Level of Fasting Plasma Proenkephalin-A Predicts Deterioration of Kidney Function and Incidence of CKD. *Journal of the American Society of Nephrology : JASN*, 28: 291-303, 2017.*
- II. **Schulz CA**, PM, Christensson A, Hindy G, Almgren P, Nilsson PM, Engström G, Orho-Melander M.: Soluble Urokinase-type Plasminogen Activator Receptor (suPAR) and Impaired Kidney Function in the Population-based Malmö Diet and Cancer Study. *KI Reports*, 2017.*
- III. **Schulz CA**, Engström G, Nilsson J, Almgren P, Petkovic M, Christensson A, Nilsson PM, Melander O, Orho-Melander M: Plasma kidney injury molecule 1 (p-KIM-1) levels predict deterioration of kidney function over 16 years.
- IV. **Schulz CA**, Ericson U, Engström G, Nilsson PM, Melander O, Orho-Melander M: Genetic predisposition for decline in kidney function: results from the Malmö Diet and Cancer Study.
- V. **Schulz CA**, Engström G, Christensson A, Nilsson PM, Ericson U ,Orho-Melander M: Dietary intake and longitudinal kidney function: results from the Malmö Diet and Cancer Study.

Published papers were reproduced with the permission of the publishers*

Detailed information can be found on page 96

List of papers not included in this thesis

- I. Li, M, Li, Y, Weeks, O, Mijatovic, V, Teumer, A, Huffman, JE, Tromp, G, Fuchsberger, C, Gorski, M, Lyttikainen, LP, Nobile, T, Sedaghat, S, Sorice, R, Tin, A, Yang, Q, Ahluwalia, TS, Arking, DE, Bihlmeyer, NA, Boger, CA, Carroll, RJ, Chasman, DI, Cornelis, MC, Dehghan, A, Faul, JD, Feitosa, MF, Gambaro, G, Gasparini, P, Giulianini, F, Heid, I, Huang, J, Imboden, M, Jackson, AU, Jeff, J, Jhun, MA, Katz, R, Kifley, A, Kilpelainen, TO, Kumar, A, Laakso, M, Li-Gao, R, Lohman, K, Lu, Y, Magi, R, Malerba, G, Mihailov, E, Mohlke, KL, Mook-Kanamori, DO, Robino, A, Ruderfer, D, Salvi, E, Schick, UM, **Schulz, CA**, Smith, AV, Smith, JA, Traglia, M, Yerges-Armstrong, LM, Zhao, W, Goodarzi, MO, Kraja, AT, Liu, C, Wessel, J, Group, CG-TDW, Group, CBPW, Boerwinkle, E, Borecki, IB, Bork-Jensen, J, Bottinger, EP, Braga, D, Brandslund, I, Brody, JA, Campbell, A, Carey, DJ, Christensen, C, Coresh, J, Crook, E, Curhan, GC, Cusi, D, de Boer, IH, de Vries, AP, Denny, JC, Devuyst, O, Dreisbach, AW, Endlich, K, Esko, T, Franco, OH, Fulop, T, Gerhard, GS, Glumer, C, Gottesman, O, Grarup, N, Gudnason, V, Hansen, T, Harris, TB, Hayward, C, Hocking, L, Hofman, A, Hu, FB, Husemoen, LL, Jackson, RD, Jorgensen, T, Jorgensen, ME, Kahonen, M, Kardia, SL, Konig, W, Kooperberg, C, Kriebel, J, Launer, LJ, Lauritzen, T, Lehtimaki, T, Levy, D, Linksted, P, Linneberg, A, Liu, Y, Loos, RJ, Lupo, A, Meisinger, C, Melander, O, Metspalu, A, Mitchell, P, Nauck, M, Nurnberg, P, Orho-Melander, M, Parsa, A, Pedersen, O, Peters, A, Peters, U, Polasek, O, Porteous, D, Probst-Hensch, NM, Psaty, BM, Qi, L, Raitakari, OT, Reiner, AP, Rettig, R, Ridker, PM, Rivadeneira, F, Rossouw, JE, Schmidt, F, Siscovick, D, Soranzo, N, Strauch, K, Toniolo, D, Turner, ST, Uitterlinden, AG, Ulivi, S, Velayutham, D, Volker, U, Volzke, H, Waldenberger, M, Wang, JJ, Weir, DR, Witte, D, Kuivaniemi, H, Fox, CS, Franceschini, N, Goessling, W, Kottgen, A, Chu, AY: SOS2 and ACP1 Loci Identified through Large-Scale Exome Chip Analysis Regulate Kidney Development and Function. *Journal of the American Society of Nephrology* : JASN, 28: 981-994, 2017.
- II. Varga, TV, Kurbasic, A, Aine, M, Eriksson, P, Ali, A, Hindy, G, Gustafsson, S, Luan, J, Shungin, D, Chen, Y, **Schulz, CA**, Nilsson, PM, Hallmans, G, Barroso, I, Deloukas, P, Langenberg, C, Scott, RA, Wareham, NJ, Lind, L, Ingelsson, E, Melander, O, Orho-Melander, M, Renstrom, F, Franks, PW: Novel genetic loci associated with long-term deterioration in blood lipid concentrations and coronary artery disease in European adults. *International journal of epidemiology*, 2016.
- III. Sonestedt, E, Hellstrand, S, Drake, I, **Schulz, CA**, Ericson, U, Hlebowicz, J, Persson, MM, Gullberg, B, Hedblad, B, Engstrom, G, Orho-Melander, M: Diet Quality and Change in Blood Lipids during 16 Years of Follow-up and Their Interaction with Genetic Risk for Dyslipidemia. *Nutrients*, 8, 2016.

- IV. Norby, FL, Eryd, SA, Niemeijer, MN, Rose, LM, Smith, AV, Yin, X, Agarwal, SK, Arking, DE, Chasman, DL, Chen, LY, Eijgelsheim, M, Engstrom, G, Franco, OH, Heeringa, J, Hindy, G, Hofman, A, Lutsey, PL, Magnani, JW, McManus, DD, Orho-Melander, M, Pankow, JS, Rukh, G, **Schulz, CA**, Uitterlinden, AG, Albert, CM, Benjamin, EJ, Gudnason, V, Smith, JG, Stricker, BH, Alonso, A: Association of Lipid-Related Genetic Variants with the Incidence of Atrial Fibrillation: The AFGen Consortium. *PLoS one*, 11: e0151932, 2016.
- V. Hellstrand, S, Ericson, U, **Schulz, CA**, Drake, I, Gullberg, B, Hedblad, B, Engstrom, G, Orho-Melander, M, Sonestedt, E: Genetic susceptibility to dyslipidemia and incidence of cardiovascular disease depending on a diet quality index in the Malmo Diet and Cancer cohort. *Genes Nutr*, 11: 20, 2016.
- VI. Grotenfelt, NE, Wasenius, NS, Rono, K, Laivuori, H, Stach-Lempinen, B, Orho-Melander, M, **Schulz, CA**, Kautiainen, H, Koivusalo, SB, Eriksson, JG: Interaction between rs10830963 polymorphism in MTNR1B and lifestyle intervention on occurrence of gestational diabetes. *Diabetologia*, 59: 1655-1658, 2016.
- VII. Ali, A, Varga, TV, Stojkovic, IA, **Schulz, CA**, Hallmans, G, Barroso, I, Poveda, A, Renstrom, F, Orho-Melander, M, Franks, PW: Do Genetic Factors Modify the Relationship Between Obesity and Hypertriglyceridemia? Findings From the GLACIER and the MDC Studies. *Circulation Cardiovascular genetics*, 9: 162-171, 2016.
- VIII. Sonestedt, E, Hellstrand, S, **Schulz, CA**, Wallstrom, P, Drake, I, Ericson, U, Gullberg, B, Hedblad, B, Orho-Melander, M: The association between carbohydrate-rich foods and risk of cardiovascular disease is not modified by genetic susceptibility to dyslipidemia as determined by 80 validated variants. *PLoS one*, 10: e0126104, 2015.
- IX. Ericson, U, Hellstrand, S, Brunkwall, L, **Schulz, CA**, Sonestedt, E, Wallstrom, P, Gullberg, B, Wirfalt, E, Orho-Melander, M: Food sources of fat may clarify the inconsistent role of dietary fat intake for incidence of type 2 diabetes. *The American journal of clinical nutrition*, 101: 1065-1080, 2015.
- X. Smith, JG, Luk, K, **Schulz, CA**, Engert, JC, Do, R, Hindy, G, Rukh, G, Dufresne, L, Almgren, P, Owens, DS, Harris, TB, Peloso, GM, Kerr, KF, Wong, Q, Smith, AV, Budoff, MJ, Rotter, JI, Cupples, LA, Rich, S, Kathiresan, S, Orho-Melander, M, Gudnason, V, O'Donnell, CJ, Post, WS, Thanassoulis, G, Cohorts for, H, Aging Research in Genetic Epidemiology Extracoronary Calcium Working, G: Association of low-density lipoprotein cholesterol-related genetic variants with aortic valve calcium and incident aortic stenosis. *JAMA : the journal of the American Medical Association*, 312: 1764-1771, 2014.

List of figures

Figure 1 The structure of the nephron	22
Figure 2 Effect of kidney function on essential homoeostatic processes	23
Figure 3 The conceptual model of CKD.....	24
Figure 4 Progression of CKD by GFR and albuminuria categories according to KDIGO2012	25
Figure 5 Non-GFR determinants that affect estimated GFR	29
Figure 6 Classification and selected examples of causes of chronic kidney disease.....	30
Figure 7 SNPs associated with different diseases and traits with a P-value $\leq 5.0 \times 10^{-8}$ in the GWAS Catalog.....	34
Figure 8 Flowchart of the recruitment process and participants included in the Malmö Diet and Cancer Study and the random subsample MDCS-CC at baseline and follow-up re-examination.....	44
Figure 9 Association between baseline levels of pro-ENK and longitudinal kidney function, and risk of incident CKD in MDCS-CC	54
Figure 10 Genome-wide association analysis for fasting plasma concentration of pro-ENK in the 4,150 participants of MDCS-CC	56
Figure 11 Association of rs1012178 genotypes with longitudinal kidney function and risk of incident CKD in the MDCS-CC.....	57
Figure 12 Cumulative incidence of hospitalization due to impaired renal function as main diagnosis during a mean follow-up time of 19 years according to quartiles of baseline suPAR in 5,129 participants of MDCS-CC.....	59
Figure 13 Risk of incident CKD at follow-up re-examination according to quartiles of KIM-1 among 2,765 participants of the MDCS-CC after a mean follow-up time of 16.6 (± 1.5) years.....	62
Figure 14 Cumulative incidence of hospitalization due to impaired renal function (main diagnosis, n=90) during follow-up according to quartiles of KIM-1 in 4,406 participants of the MDCS-CC.....	63
Figure 15 Association between the GRS_{CKD} of 53 SNPs and baseline eGFR in the Malmö Diet and Cancer Study.....	66
Figure 16 Risk of incident CKD according to quartiles of GRS_{CKD} in 2,301 participants of the MDCS-CC after an average follow-up-time of 16 years	66
Figure 17 Annual change in eGFR (Δ eGFR) according to dietary intakes in 2,918 participants from the Malmö Diet and Cancer Study with an eGFR >60 mL/min/1.73m ² at baseline	69
Figure 18 Risk of incident CKD (eGFR <60 mL/min/1.73m ²) at follow-up re-examination according to dietary intakes in 2,918 participants from the Malmö Diet and Cancer Study with an eGFR >60 mL/min/1.73m ² at baseline	70

Table legend

Table 1 Estimation of GFR according to CKD-EPI creatinine cystatin C equation.....	28
Table 2 Overview of the study design characteristics.....	51
Table 3 Incidence of impaired kidney function during a mean follow-up time of 19 years, in relation to baseline concentration of suPAR in the Malmö Diet and Cancer- Cardiovascular Cohort	60
Table 4 Baseline characteristics of the 2,301 participants from the MDCS-CC according to quartiles of GRS_{CKD}	65
Table 5 Dietary intakes among 2,918 participants from the MDCS-CC	68

Abbreviations

AHT	Anti-hypertensive treatment
AUC	Area under the curve
BMI	Body-mass-index
Chr	Chromosome
CI	Confidence Interval
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
CVD	Cardiovascular disease
Da	Dalton
DALY	Disability-adjusted life-years
DASH	Dietary Approaches to Stop Hypertension
DNA	Deoxyribonucleic acids
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate
eGFR _{crea}	Estimated glomerular filtration rate based on creatinine
eGFR _{cystatin}	Estimated glomerular filtration rate based on cystatin C
EN%	Energy percentage
ESRD	End stage renal disease
GBDP	Global burden of disease project
GRS	Genetic risk score
GWAS	Genome-wide association study
HDL	High-density lipoprotein cholesterol
HWE	Hardy–Weinberg equilibrium
HR	Hazard Ratio
Kb	Kilobase
kDa	Kilo Dalton
KDIGO	Kidney Disease Improving Global Outcomes Initiative
KIM-1	Kidney injury molecule 1
LDL	Low-density lipoprotein cholesterol
MAF	Minor allele frequency
Mb	Megabase
MDCS	Malmö Diet and Cancer Study
MDCS-CC	Malmö Diet and Cancer Study - Cardiovascular Cohort
Met-enkephalin	Methionine-enkephalin
mGFR	Measured GFR
mg/dl	Milligram/deciliter
mmHG	Millimeter mercury
MR	Mendelian Randomization
ng/mL	Nanogram/milliliter
NKF-KDQOI	National Kidney Foundation’s Kidney Disease Quality Outcome Initiative

NRI-Index	Net-reclassification-improvement index
OGF	Opioid growth factor
OGFR	Opioid growth factor -receptor
OR	Odds ratio
PH	Proportional hazard
P-value	Probability value
pro-ENK	Pro-enkephalin
QC	Quality control
RCT	Randomized controlled trial
ROC-Curve	Receiver operating characteristics curve
SSB	Sugar-sweetened beverages
SBP	Systolic blood pressure
SD	Standard deviation
SNP	Single nucleotide polymorphism
suPAR	Soluble urokinase plasminogen activating receptor
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TG	Triglycerides
WHO	World Health Organization
YLD	years lived with disabilities
μmol/L	micromoles/litre

Introduction

*“Health is a state of complete physical, mental, and social well-being
and
not merely the absence of disease or infirmity.”*

*World Health Organization (WHO) in 1946*¹

It is estimated that 8 to 16% of the population suffer from Chronic Kidney Disease (CKD) worldwide.² Moreover, based on data by the global burden of disease project (GBDP), disability-adjusted life-years (DALYs) associated with CKD have been estimated to have increased by 19.6% globally from 2005 to 2015.^{3,4} In addition, systematic analysis among 301 acute and chronic diseases in the GBDP showed that years lived with disabilities (YLD) due to chronic kidney disease increased by almost 50% from 1993 to 2010.⁵ Declined kidney function associates with increased risk of cardiovascular events, hospitalization and death,⁶ and kidney disease related mortality is on the rise. Indeed, in 2010 CKD was ranked 18th in the list of total numbers of deaths worldwide, according to the GBDP, while it was listed as number 27 twenty years before in 1990.² Thus, it is evident CKD is becoming a growing public health issue.

With every heartbeat approximately 20-25% of the cardiac output is dedicated to the kidneys, thereby around 1.2 liters per minute⁷ and around 600 liters of blood daily are circulating through the kidneys. Thus, due to its central role in circulation, kidney is target of hemodynamic, vascular, inflammatory and metabolic diseases.⁸ CKD is a heterogenous disease with a complex etiology, characterized by altered endocrine, exocrine, and paracrine functions, and many of the biomedical and metabolic abnormalities have been acknowledged for a long time.⁹ Indeed, the interest in urine as an indicator of disease status was already documented in the late middle ages, when Ullrich Pinder described possibilities of colors, smells and tests in “the urine wheel”,¹⁰ and approaches to measure kidney function from urine reach back more than hundred years ago.¹¹ Even though the physiological and pathophysiological characteristics have been studied since at least a century,¹¹ the understanding of the interconnections and relationships of the metabolic interplay the kidneys are involved in remains to be fully understood.⁹

It is known that kidney function has a heritable component.¹² So far, more than 110 genes underlying rare monogenic kidney diseases,⁸ and more than 50 loci associated with estimated glomerular filtration rate (eGFR) have been described.^{13,14} However, less than 4% of the variance in eGFR can be explained by the currently identified loci,¹⁴ and

attempts to use genetic risk scores (GRS), including genetic variants associated with reduced eGFR have not substantially improved prediction of CKD stage 3 beyond the traditional clinical risk factors.^{15,16}

Onset of multifactorial diseases, such as kidney diseases, may be triggered by both genetic and environmental factors.¹⁷ Current management advices for patients with CKD recommend lowering the protein intake to $\leq 0.8\text{g/kg/d}$ with appropriate education in patients with CKD stage 4 or 5, regardless of diabetes status.¹⁸ Further, high protein intake ($>1.3\text{ g/kg/d}$) is suggested to be avoided by adults at risk for progression of CKD.¹⁸ However, today there are no dietary recommendations for prevention of CKD in individuals free of kidney disease.

Awareness of CKD in the general population is low¹⁹ and CKD typically evolves over a long time. First the disease may be undetected, then the lengthy latent period is followed by late onset of symptoms, caused by complications of decreased kidney function.²⁰ Thus, identification of individuals at elevated risk earlier in the disease process is crucial to facilitate interventions for prevention of the progression to CKD. Hence, markers of kidney damage and/or eGFR should make it possible to detect CKD prior to kidney failure.²⁰ However, as both eGFR and proteinuria are relatively insensitive measures of early kidney injury, more sensitive biomarkers are required.²¹

Therefore, this PhD-thesis undertakes the attempt to describe the clinical potential of three circulating biomarkers, ie. pro-ENKephalin (pro-ENK), soluble urokinase-type plasminogen activator receptor (suPAR), and kidney injury molecule -1 (KIM-1), previously associated with kidney function.²²⁻²⁷ Furthermore, this thesis examines the applicability of a GRS, including genetic variants prior identified in genome-wide association studies (GWAS), to associate with kidney function for prediction of future CKD stage 3. Lastly, it investigates the relationship between diet and longitudinal kidney function. All five individual studies are based on investigations within the Malmö Diet and Cancer Study, a population-based cohort from the South of Sweden.

Anatomy and physiology of the kidneys

Together with the ureters, bladder and the urethra, the kidneys form the urinary system, also known as the renal system.²⁸

The kidneys are two paired organs, which weigh approximately 150 g each, have a vertical length of 10-12 cm, a transversal width of 5-7 cm, and an anterior-posterior diameter of 3 cm. They are located in the abdominal cavity left and right of the spine. Typically, the left kidney lays slightly superior in the abdomen and reaches from the 12th thoracic to the 3rd lumbar vertebra. The silhouette of the kidney is bean-shaped, with a convex shape lateral and a concave side medial. The renal hilus, located in the concave medial side (*Sinus renalis*), is the starting point for the renal artery, renal vein, ureter, renal nerves and lymphatics to enter and exit, respectively, the kidney.²⁸⁻³⁰

The kidneys are located in a retroperitoneal position between the parietal peritoneum and the posterior abdominal wall. On the outside each kidney is surrounded by a layer of pararenal (or paranephric) fat, followed by the renal fascia, a thin layer of connective tissue, which encapsulates the kidney together with the adrenal gland (located on top of the kidney). In-between the renal fascial and the renal capsule there is a layer of perirenal (or perinephral) fat (*Capusla adiposa*). The renal capsule (*Capsula fibrosa*) is a thin layer of fibrous connective tissue which coats the kidney.^{28,30}

The renal parenchyma consists of the outer renal cortex (*Cortex renalis*) and the inner renal medulla (*Medulla renalis*), which can be divided in approximately 8-10 cone-shaped renal lobes. The renal pyramids lay in the medulla, separated by the renal columns. The renal pyramids taper into renal papillae, which are a cluster of collecting ducts transporting urine via the calyces into the renal pelvis. From there the urine will be transported to the urinary bladder by the ueter.^{28,30,31}

The functional unit of the kidney is the nephron. Each kidney contains approximately 1 million nephrons. They are built of a filtering body, i.e. the glomerulus, and a long tubule segment. The tubules are interconnected in the end and form the collecting ducts and open into the renal pelvis.⁸ (**Figure 1**)

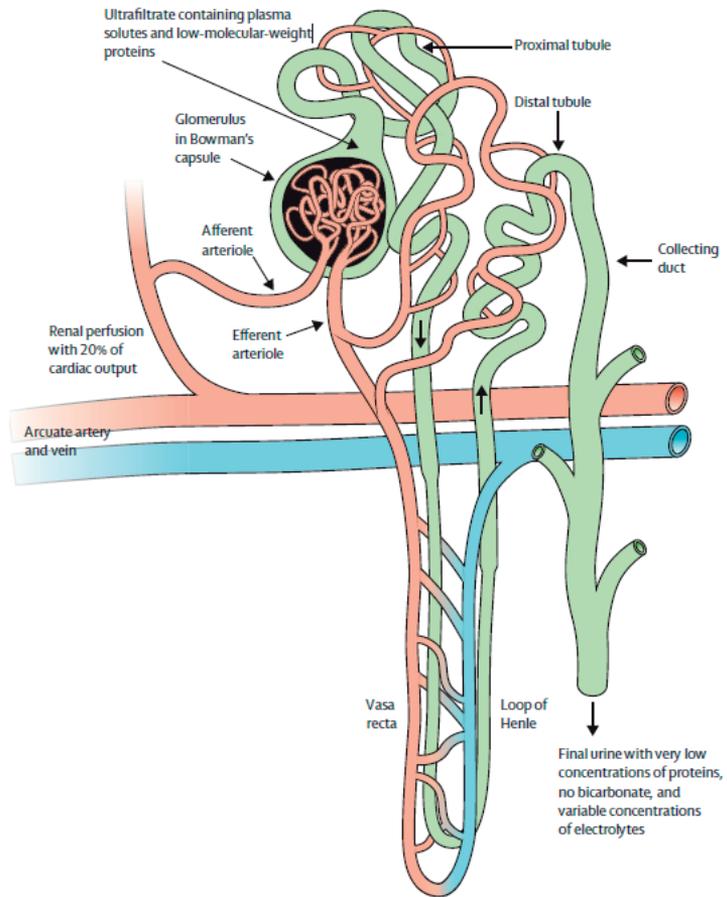


Figure 1 The structure of the nephron

From Eckardt et al. 2013⁹ with the permission of the publisher. Detailed information can be found on page 95.

The kidneys are provided with around 1.2 l of blood per minute from the heart. Thus, the renal blood flow is almost similar to those of the brain or liver (20%-25% versus 15% versus 20%). Considering the much smaller mass of kidneys, the renal perfusion rate is by far the highest of all organs.⁷ By this, each day the kidneys produce around 180 L of ultrafiltrate, which is modified during tubular passages and only about 1.5 L of urine is excreted daily.⁸

The kidneys perform several endocrine, exocrine and metabolic functions, and thus are interconnected with several organs.⁸ (Figure 2)

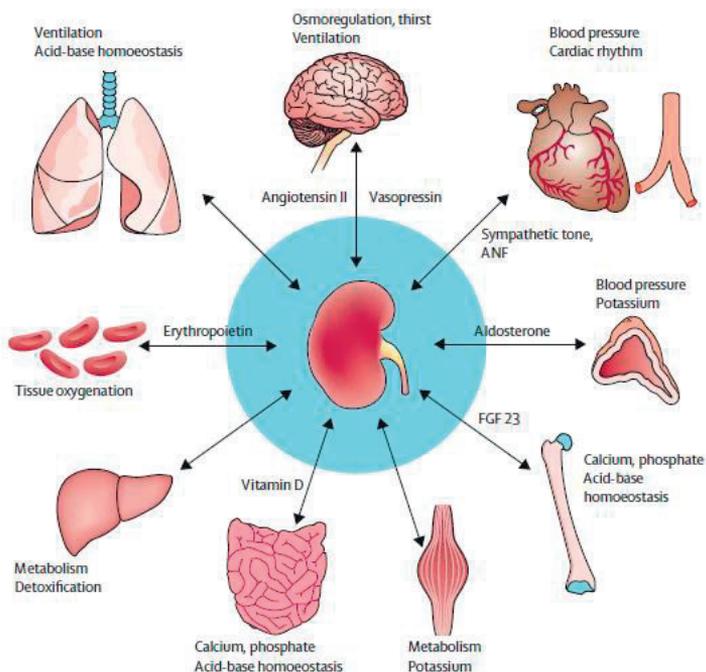


Figure 2 Effect of kidney function on essential homeostatic processes

ANF= atrial natriuretic factor FGF=fibroblast growth factor.

From Eckardt et al. Lancet 2013⁸ with the permission of the publisher.

Detailed information can be found on page 95.

The conceptual model of CKD

The current conceptual model of CKD incorporates development, progression and complications of CKD. The course of CKD is presented in **Figure 3** including the stages of CKD, potential antecedent conditions and consequences of CKD, outcomes, risk factors for adverse outcomes, as well as actions to improve outcomes.³² It was introduced in 2002 by the *National Kidney Foundation's Kidney Disease Quality Outcome Initiative* (NKF-KDQOI) and further adopted and revised in under the auspices of *Kidney Disease: Improving Global Outcomes* (KDIGO) by international consensus in 2005.²⁰

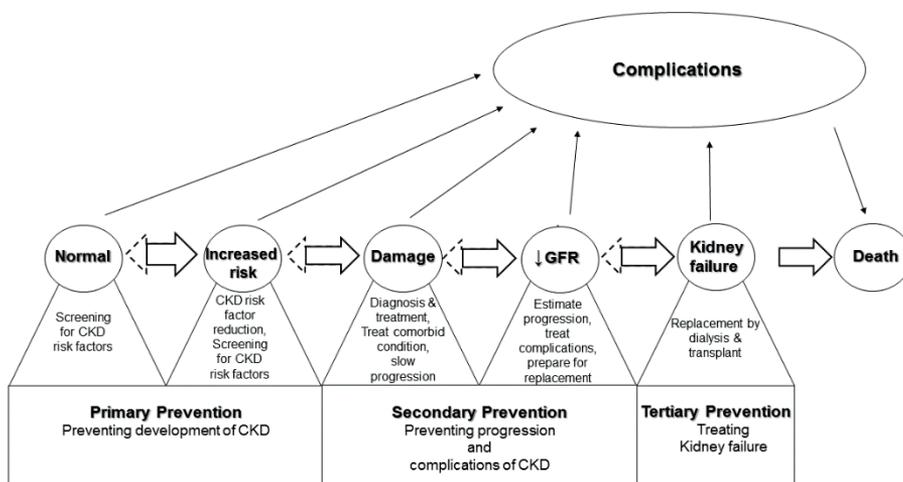


Figure 3 The conceptual model of CKD

"Complications" refer to all complications of CKD and its treatment, including complications of decreased eGFR (hypertension, anemia, malnutrition, bone diseases, neuropathy, and decreased quality of life). Remission is less frequent than progression, indicated by the dashed arrows.

Modified from Levey et al. 2005³², Levey et al. 2009²⁰, and Levey et al. 2012³³.

In general, CKD is a heterogeneous condition partly due to the related cause, the pathophysiological features of a particular kidney disease, severity, rate of progression, and presence of comorbidities. Thus, variety in the expression of CKD among the individual patient exists.^{33,34}

Definition and classification of CKD

For CKD management and prevention planning, estimation of CKD prevalence is central.³⁵ To guide identification of cases and facilitate management, the US NKF-KDQOI established a five-stage classification system, which was adopted internationally by the KDIGO Initiative^{32,36-39}

Today, CKD is defined by abnormalities of kidney structure or function occurring for ≥ 3 months.¹⁸ Usually, eGFR and albuminuria are used to diagnose CKD, and determine the severity stage.² Moreover, identification of the specific underlying disease, when possible, is recommended by the most recent KDIGO 2012 CKD guidelines. The enhanced classification framework for CKD recommends classifying CKD based on three dimensions: cause, GFR category and albuminuria (CGA).¹⁸ **Figure 4** illustrates the risk for progression of CKD in a matrix of the five GFR categories by three albuminuria categories:

				Persistent albuminuria categories		
				Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min per 1.73 m ²) Description and range	G1	Normal or high	≥ 90			
	G2	Mildly decreased	60–89			
	G3a	Mildly to moderately decreased	45–59			
	G3b	Moderately to severely decreased	30–44			
	G4	Severely decreased	15–29			
	G5	Kidney failure	<15			

Figure 4 Progression of CKD by GFR and albuminuria categories according to KDIGO2012

The color scheme refer to: low risk (if no other markers of kidney disease, no CKD) [green],

moderately increased risk [yellow], high risk [orange], and very high risk [red].

Modified from Levin et al. 2014¹⁸ with the permission of the publisher.

Detailed information can be found on page 95.

Prevalence of CKD

Kidney diseases have been recognized as a major public health burden, affecting between 8-16% of the population globally.² A recent study in different European countries has shown that there is variation in CKD prevalence across countries and regions. Among adult populations between 20 and 74 years of age, the prevalence of CKD stages 1-5 varied between 3.3% in Norway and 17.3% in Northeast Germany.^{40,41}

Quantification of kidney function

GFR is considered to be the best index for renal function,²⁰ and ideally it should be measured (mGFR).⁴² Historically, the ideal filter marker to determine GFR has been inulin. Measuring inulin clearance is yet not practical for day-to-day clinical practice, as it requires careful timing of blood sampling, multiple repeated blood and urine collections and continuous intravenous infusion of inulin.⁴³

Therefore, GFR is usually estimated, and numerous equations have been developed during the last decades.

Creatinine and cystatin C

Today, serum creatinine or cystatin C are the two most used biomarkers to estimate GFR.⁴²

Creatinine, also known as 2-amino-1-methyl- methylimidazolidin-4-on in the chemical nomenclature, is a 113 Dalton (Da) amino acid derivative⁴⁴ that is generated from the breakdown of creatine phosphate in the muscles²⁸ and excreted by the kidneys primarily by glomerular filtration.

Cystatin C, encoded by the *CST3* gene on chromosome 20⁴⁵, is a 146 amino acid long protein (in humans). It belongs to the cystatin superfamily and has a mass of 13.4 kDa. *CST3* is a housekeeping gene, expressed throughout the whole body.⁴⁵ As such, cystatin C is produced by all nucleated cells at constant rate.^{46,47}

Cockcroft-Gault (CG)-equation

In 1976, the Cockcroft-Gault (CG)-equation was the first published formula, which was used to predict creatinine clearance (Ccr) from serum creatinine (Scr) in 249 men of age 18 to 92 years. The authors concluded that both age and body weight must be included in the formula for reasonable prediction.⁴⁸

$$CCr = [((140 - \text{age}) \times \text{weight}) / (72 \times \text{SCr})] \times (0.85 \text{ if female})$$

with C_{Cr} = mL/minute, Age = years, Weight = kg, SCr = mg/dL

Since then, several equations have been developed:

Modification of Diet in Renal Disease (MDRD) Study equation

The Modification of Diet in Renal Disease (MDRD) Study equation, based on four variables, was developed in 1999 in 1,628 individuals with CKD.⁴⁹ It was re-expressed in 2006 for the use of IDMS traceable creatinine assay.⁵⁰

$$\text{eGFR (mL/min/1.73 m}^2\text{)} = 175 \times (\text{Scr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \\ \times (1.212 \text{ if African American})$$

with Scr(serum creatinine)= mg/dl, Age=years

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations

In 2009, the Chronic Kidney Disease Epidemiology Collaboration established the CKD-EPI creatinine formula (eGFR_{crea}), which modeled the relationship between GFR and serum creatinine, age, sex, and race using a 2-slope "spline" in 12,150 individuals from diverse populations (of which 8,254 were included in the development and 3,896 in the validation).⁵¹

eGFR is calculated as following:

$$\text{for males eGFR} = 141 \times (\text{S}_{\text{cr}}/0.9)^{\alpha} \times 0.993^{\text{age}},$$

where α is -0.411 if creatinine is ≤ 0.9 and -1.209 if creatinine is > 0.9

$$\text{for females eGFR} = 144 \times (\text{S}_{\text{cr}}/0.7)^{\alpha} \times 0.993^{\text{age}},$$

where α is -0.329 if creatinine is ≤ 0.7 and -1.209 if creatinine is > 0.7

with Scr(serum creatinine)= mg/dl, Age=years

Three years later in 2012, the CKD-EPI creatinine-cystatin C equation was published. The equation was developed in 5,352 participants from 13 studies and validated in 1,119

participants from 5 different studies. It included both creatinine and cystatin C and was shown to perform better than equations based on either of the markers alone.⁵² (Table 1)

In addition, an equation solely based on cystatin C was developed in the same study population.

The CKD-EPI cystatin C equation (2012)⁵² estimates GFR as following:

$$\text{cystatin C} \leq 0.8 \text{ eGFR} = 133 \times (\text{Scr}/0.8) - 0.499 \times 0.996\text{Age} \times [x 0.932 \text{ if female}]$$

$$\text{cystatin C} > 0.8 \text{ eGFR} = 133 \times (\text{Scr}/0.8) - 1.328 \times 0.996\text{Age} \times [x 0.932 \text{ if female}]$$

with Scr=serum cystatin C (mg/L), Age=years

Table 1 Estimation of GFR according to CKD-EPI creatinine cystatin C equation

Gender	Creatinine	Cystatin C	eGFR equation
Female	≤ 0.7	≤ 0.8	130 x (creatinine /0.7)-0.248 x (cystatin C / 0.8) -0.375 x 0.995Age x [1.08 if black]
Female	≤ 0.7	> 0.8	130 x (creatinine /0.7)-0.248 x (cystatin C / 0.8) -0.711 x 0.995Age x [1.08 if black]
Female	> 0.7	≤ 0.8	130 x (creatinine /0.7)-0.601 x (cystatin C / 0.8) -0.375 x 0.995Age x [1.08 if black]
Female	> 0.7	> 0.8	130 x (creatinine /0.7)-0.601 x (cystatin C / 0.8) -0.711 x 0.995Age x [1.08 if black]
Male	≤ 0.8	≤ 0.8	135 x (creatinine /0.9)-0.207 x (cystatin C / 0.8) -0.375 x 0.995Age x [1.08 if black]
Male	≤ 0.8	> 0.8	135 x (creatinine /0.9)-0.207 x (cystatin C / 0.8) -0.711 x 0.995Age x [1.08 if black]
Male	> 0.8	≤ 0.8	135 x (creatinine /0.9)-0.601x (cystatin C / 0.8) -0.375 x 0.995Age x [1.08 if black]
Male	> 0.8	> 0.8	135 x (creatinine /0.9)-0.601x (cystatin C / 0.8) -0.711 x 0.995Age x [1.08 if black]

With creatinine= mg/dl, cystatin C=mg/L, Age=years.

Modified based on Inker et al.⁵²

Limitations of estimating GFR

Directly measuring kidney function has limitation,⁴³ as described above. In the clinical setting, GFR is generally estimated on the basis of serum creatinine and including demographic characteristics such as age, sex, and ethnic origin into the equation.³⁶ Indeed, eGFR values, derived from equations based on serum creatinine or cystatin C, can be affected by GFR-independent factors as their production, and concentrations can be influenced by extra-renal determinants. Thus, the accuracy of these biomarkers in estimating GFR and/or predicting outcomes may be affected by diverse extra-renal factors such as muscle mass, i.e. the effect of muscle wasting or increased muscle mass, which may affect creatinine levels, or the possible influence of inflammation and/or obesity on cystatin C levels.⁴² (Figure 5)

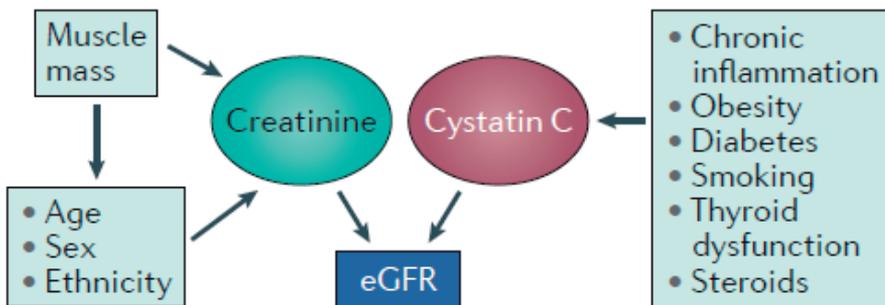


Figure 5 Non-GFR determinants that affect estimated GFR

Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Nephrology] (Glassock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nat Rev Nephrol* 2017;13:104-14.), copyright (2016)

Etiology of CKD

Ageing associates with physiological decline of kidney function. At birth the function of kidneys is relatively low and reaches adults levels at around two years of age. Until the fourth decade it is maintained at approximately 140 mL/min/1.73m².⁵³ Thereafter, it declines around 8 mL/min/1.73m² per decade according to inulin clearance studies by Davies and Shock.^{53,54}

In the majority of cases, CKD evolves over decades and during many years of follow-up some patients do not progress. However, rapid progression can lead to kidney failure within months. A decline of 4 mL/min/1.73m² per year is considered fast, and would lead to kidney failure in 12 years or less, from the onset of CKD stage 3.³³

The etiology of CKD is complex and many potential factors can contribute. Hypertension, metabolic syndrome, and diabetes may affect the function of kidneys, as well as other less common diseases. In high risk populations, more than 50% of the population may suffer from CKD.⁸ Broadly, CKD may thus be classified into Diabetic CKD and Non-diabetic CKD.³⁶

Based on the location of the pathological-anatomical findings within the kidney, and presence or absence of systemic disease, non-diabetic CKD can be classified into cystic, glomerular, tubulointerstitial or vascular.³⁶ (Figure 6)

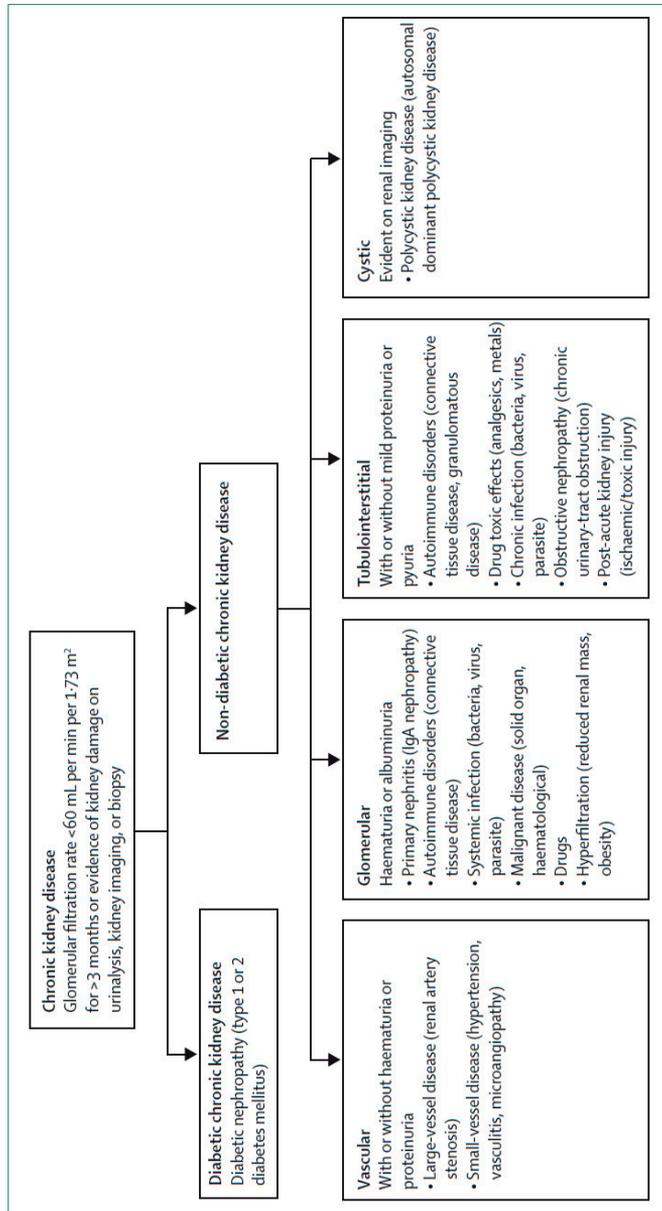


Figure 6 Classification and selected examples of causes of chronic kidney disease
From James et al.³⁶ with the permission of the publisher.
Detailed information can be found on page 95.

Risk factors for CKD

"A risk factor is any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury."

According to the WHO⁵⁵

Risk factors for CKD can affect the 1) development, 2) progression, and/or 3) complications of the disease. As such they may 1) increase the susceptibility to kidney damage and/or directly initiate kidney damage, 2) worsen kidney damage or accelerate GFR decrease, and/or 3) increase risk of complications of decreased GFR, accelerate onset or recurrence of CVD and/or increase morbidity and mortality in kidney failure.²⁰

The risk factors of CKD may be both acquired and inherited, and some of them are well known, such as hypertension and diabetes, while others remain still less understood or unknown.⁴¹ Indeed, the cumulative lifetime risk of CKD at the age of 50 has been found to be modified by the presence of known CKD risk factors, i.e. diabetes, hypertension, or obesity.⁵⁶

Diabetic nephropathy is the leading cause of end stage renal disease (ESRD).^{57,58} Between 1990 and 2013, the number of cases of CKD due to diabetes mellitus has increased by more than 50% according to the recent results of the global burden of diseases study.⁵ Given that obesity is closely linked to diabetes and hypertension, it may as well predispose individuals to CKD.⁴¹

Comorbidities and Mortality of CKD

Kidney function is complex and might affect several essential homeostatic processes. As such, kidneys can be both a cause and a target organ of diseases.⁸ Hypertension, anemia, malnutrition, bone diseases, neuropathy, and decreased quality of life are complications of decreased GFR.^{20,32} Furthermore, major outcomes of CKD are: complications of decreased GFR, kidney failure, and increased risk of cardio-vascular diseases (CVD).²⁰ Indeed, cardiovascular events occur more often in patients with CKD, than kidney failure.²⁰ Likewise, CVD seems to be an independent risk factor for CKD.³² Thus, CVD can be considered both a risk factor for and a comorbidity of CKD. Both diseases share a number of common bidirectional pathways, including cardio-vascular-disease associated mechanisms, haemodynamic mechanisms, and (neuro) hormonal mechanisms.⁵⁹

CKD is associated with an increased risk for mortality.⁶ From 1990 to 2010, the ranking of CKD in the list of total numbers of death worldwide rose from 27th to 18th, indicating the increased importance of CKD as a risk factor for mortality.²

Prevention and management of CKD

The aim of primary prevention is preventing CKD development, which is particularly important in individuals at increased risk (i.e. patients with diabetes or hypertension), and therefore preventing the development of CKD risk factors is important. Secondary prevention aims to slowing the progression, and to prevent and treat complications in patients with decreased GFR (stage 3 and 4) and with kidney damage (stage 1 and 2). Early identification of individuals at increased risk, i.e. patients with hypertension, diabetes or CVD, and individuals with a family history of CKD, are key to secondary prevention. The primary aim in tertiary prevention includes treating kidney failure and improving the care of patients with kidney failure.³⁴

The management of kidney disease includes prevention of disease development, slowing down its progression, reducing complications of decreased GFR, reducing the risk of CVD, and improving the quality of life and survival.³³ Management is oriented towards a) clinical diagnosis, which allows for specific therapies directed towards the pathophysiologic processes and causes, and b) the stage (according to albuminuria and GFR), which can be used for non-specific therapy guidance to slow down disease progression and reduce the risk for complications.³³ There are several treatment possibilities available for patients with CKD. Potential treatment options include a) specific treatment of the underlying renal disease (e.g. diabetic nephropathy, glomerulonephritis, nephrosclerosis, hereditary renal disease), b) lifestyle interventions, and/or c) pharmacological interventions.⁶⁰

The most recent clinical practice guideline for diagnosis and management was published by the KDIGO in 2012.^{18,61}

Awareness of CKD

Early identification of CKD is crucial for preventing the progression of the disease and reducing the risk of cardiovascular morbidity and mortality. In spite of the wide spread of CKD, its awareness is low among health-care providers and patients.² Although awareness increases with progressing disease stage, the results from the 2000-2004 National Health and Nutritional Examination Survey show that CKD awareness has remained considerably low in the public i.e. less than half of the persons with CKD stage 4 and less than 10% with CKD stage 3 were aware of their condition.¹⁹ Hence, CKD can be considered a ‘silent’ epidemic.⁶⁰ Due to the initially asymptomatic nature of CKD in the early stages, the detection of CKD can often be delayed until its later stages when symptoms begin to emerge. By then, opportunities for preventive approaches may already have diminished.⁶⁰

Therefore, awareness campaigns are needed, both aimed at parties and health care providers. In order to raise awareness of the global pandemic of kidney disease and

associated CVD, the International Society of Nephrology and the International Federation of Kidney Foundation jointly launched the World Kidney Day in 2006.⁶²

Genetic susceptibility and kidney function

Human Genome

The human genome consists of 23 chromosome pairs, 22 pairs of autosomal chromosomes and one pair of sex-chromosomes. Each chromosome consists of a double strand of deoxyribonucleic acids (DNA) build of four nucleotides: cytosine, guanine, adenine, and thymine.⁶³ The molecular structure of the DNA was first discovered by James Watson and Francis Crick in Cambridge, UK, in 1953.⁶⁴

In 2001, the human genome project published the first draft of the sequenced human genome.^{65,66} Shortly after in 2004, the complete sequence, covering 99% of the genome, was published. It consists of 2.85 billion nucleotides and has been estimated to include 20000-25000 protein-coding genes.⁶⁷ Variation in one nucleotide in the DNA sequence, a so called single nucleotide polymorphism (SNP), occurs every 1 in 500 to 1 in 2000 base pairs,⁶³ and is thus the most common variation in the human genome.

Heritability of kidney function

More than two decades ago kidney function measures were suggested to have an underlying genetic component.¹² In the community-based Framingham Heart Study, the heritability estimates for serum creatinine, GFR, and creatinine clearance reached from 29 to 46%, after consideration of traditional risk factors for kidney function. Heritability of eGFR has been estimated to be above 30% (h^2 0.33; 95% CI 0.19-0.47).¹² Very recently, an even higher heritability estimate for eGFR has been reported from a multicenter family-based cohort in Switzerland ($h^2=0.46$ for CKD-EPI creatinine equation).⁶⁸ Heritability (h^2), is referred to as the proportion of the phenotypic variance that is explained by a genetic component.

Monogenic kidney disease

Diseases caused by mutations in one gene, i.e. monogenic diseases, have been identified since 1980 by linkage analysis, positional cloning, homozygosity mapping or sequencing. Today, more than 110 genes underlying rare monogenic kidney diseases have been described, of which the autosomal-dominant polycystic kidney disease may be the most prominent example.⁸

Multifactorial kidney diseases

In contrast, complex diseases or traits (also referred to as multifactorial diseases) have both genetic and environmental risk factors,^{17,69} and variations in several genes and other parts of the genome as well as in the epigenome may contribute to the genetic component.⁷⁰ To identify genetic variants associated with the susceptibility of kidney diseases genome-wide association studies (GWAS) have been conducted. The aim of GWAS is to identify genetic variations, most commonly SNPs that associate with clinical measures or disease status.⁷¹ The first GWAS, based on a high-throughput SNP genotyping array, was published in 2005.^{71,72} As of July 31st 2017, the GWAS Catalog contained 3,057 publications and 39,366 unique SNP-trait associations.⁷³ (Figure 7)

So far, GWAS in nephrology have been focused on studying specific CKD etiologies, such as diabetic kidney disease, IgA nephropathy or membranous nephropathy, and CKD-defining traits, like eGFR and urinary albumin-to-creatinine ratio (UACR).⁷⁴

In 2007, the first GWAS for a wide range of kidney function traits, i.e. eGFR, urinary albumin excretion, cystatin C, was performed including 70,987 autosomal SNPs in the Framingham Heart Study.⁷⁵ Thereafter, the first two-stage GWAS was conducted among two community based prospective studies.⁷⁶ These efforts have been followed by numerous GWAS studies for renal traits, including associations with cross-sectional eGFR_{crea},^{13,77-80} eGFR_{cystatin},^{13,78,80} CKD,^{13,78,80} kidney function decline,⁸¹ and for eGFR_{crea} and CKD stratified by key CKD risk factors (age, sex, diabetes or hypertension status).⁸⁰ So far, more than 50 genetic variants associated with eGFR_{crea} have been identified.^{13,79,81} However, less than 4 % of the variance in eGFR_{crea} is explained by the genetic markers identified to date.^{13,81}



Figure 7 SNPs associated with different diseases and traits with a P-value $\leq 5.0 \times 10^{-8}$ in the GWAS Catalog⁸²

The colors represent different traits: Biological process (iris), Body weight measures (light blue), Cancer (violet), Cardiovascular disease (red), Cardiovascular measurement (cornflower), Digestive system disease (brown), Hematological measurement (jade), Immune system disease (yellow), Inflammatory marker measurement (mint), Lipid or lipoprotein measurement (light green), Liver enzyme measurement (green), Metabolic disease (orange), Nervous system disease (light yellow), Other diseases (magenta), Other measurement (navy), Other trait (apricot), Response to drugs (light pink)

Prediction of CKD utilizing genetic markers

The question of whether the information on genetic predisposition for kidney function may be useful in predicting development of renal impairment, such as incidence of CKD or ESRD, remains open. It seems likely that complex disease may be affected by many genes and variations, of which most have a small effect on the disease risk.^{69,83} Therefore, individual SNPs are usually combined to a GRS to study the combined effect in relation to a disease.

It has been attempted to study the association between the identified genetic markers in relation to incidence of CKD. So far such GRSs for eGFR_{crea}, containing 16¹⁵ or 53¹⁶ SNPs associated with the trait, have not shown to be useful in prediction of new cases of CKD stage 3 beyond commonly used risk factors.^{15,16}

Diet and kidney disease

The current knowledge on how diet may affect kidney function in the long term is narrow and today there are, to my knowledge, no recommendations for prevention of CKD in individuals free of kidney disease.

It has been suggested that healthy dietary patterns, such as the Dietary Approaches to Stop Hypertension (DASH) style diet,⁸⁴ could be beneficial in prevention of kidney disease. In addition, higher diet quality has been shown to associate with decreased risk of incident CKD stage 3A,⁸⁵ and adherence to dietary recommendations, particularly regarding meat, legumes and dairy products, have been suggested to be of importance for normal kidney function.⁸⁶

High protein intake has been discussed to influence progression of moderate renal diseases.⁸⁷ Current management advices for patients with CKD recommend lowering the protein intake to $\leq 0.8\text{g/kg/d}$ including an appropriate education of patients with CKD stages 4 or 5, regardless of diabetes status. Further, high protein intake ($>1.3\text{g/kg/d}$) is suggested to be avoided by adults at increased risk for progression of CKD.¹⁸ In addition, patients with CKD should receive expert dietary advice and information tailored to the severity of CKD, and if necessary, intervention on salt, phosphate, potassium and protein intake, according to the current management guidelines.¹⁸

Biomarkers

Definition and applications

A biomarker i.e. a biological marker is defined as ‘*A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*’ according to the Biomarkers Definitions Working Group.⁸⁸

Already in 1506, Ullrich Pinder made use of colors, smells, and taste of urine to diagnose disease.¹⁰ Biomarkers have been used for disease diagnostics for over a century, probably starting with the discovery of the ABO blood group system, which is used to detect ABO hemolytic diseases of newborn (HDN) and transfusion reaction.⁸⁹

The entire spectrum of diseases from the earliest manifestation to terminal stages can be reflected by biomarkers,⁹⁰ and biomarkers can thus serve as tools in prediction, diagnosis, progression or regression of a disease, to understand the causes of diseases, and the outcome of treatment of a disease.⁹⁰

Broadly, biomarkers can be classified into two major types:

biomarker of exposure and biomarker of disease.

The latter can be used in screening, diagnosis and monitoring of disease progression.⁹⁰ As such, biomarkers may aid in detecting and monitoring of health status, serve as diagnostic markers (to identify individuals with a pathophysiological condition or disease), and/or as a tool for classification of the disease stage, and/or staging of the disease process, as well as an indicator of disease prognosis.⁸⁸ In addition, to predict future disease course, a prognostic biomarker may also be applicable in predicting recurrence, therapy response, and/or monitoring of the efficiency of a therapy.⁸⁹

Biomarkers of exposure primarily serve in risk prediction including exposure to environmental factors, genetic susceptibility and genetic response to environmental exposures.⁸⁹ In addition, they may also serve as markers of subclinical or clinical disease, or as indicators of response to therapy,⁸⁹ similar to biomarkers of disease.

Furthermore, a biomarker may also function as a surrogate marker for a clinical endpoint in intervention studies.⁸⁸

Biomarker characteristics

Regardless if a biomarker is supposed to be a measure of risk exposure, disease progression or serves as a surrogate marker, the crucial characteristics of a clinically useful biomarker are high accuracy, high reproducibility, and being easy to measure. Additionally, it must be acceptable to the patient and physician.^{89,91}

Biomarker sources

The source of a biomarker is dependent on the characteristics of the disease, disease stage and/or the biomarker itself. Theoretically, it can be measured in a wide range of biological specimens (e.g. blood, urine, saliva, other body fluids, faeces, cells, or body tissues), but it may also result from other techniques such as imaging.

Costs and acceptability

Whether in a research or a clinical setting, costs and acceptability of a biomarker play important roles. The choice of a biomarker should consider financial aspects, such as costs per sample. Likewise, it may need to be taken into account that urine and blood sample are commonly well tolerated, whereas samples originating from biopsies are more difficult to obtain.⁹⁰

Biomarkers for kidney disease

Since creatinine was recognized to be correlated with renal function, biomarkers have been used as screening tools for kidney disease.⁹² Nonetheless, creatinine has limitations based on its physiology and the fact that it primarily reflects the capacity of renal filtering, and therefore creatinine is not a particularly sensitive biomarker of CKD. During the last decades it has therefore been recognized, that ‘by relying only on serum creatinine to screen for kidney disease, we may only be seeing the tip of the iceberg of kidney diseases’.⁹² Likewise does cystatin C have limitations as a biomarker for CKD.⁴² Currently, proteinuria, especially when combined with eGFR, are considered the most sensitive biomarkers for CKD progression in clinical practice. Yet, there are limitations also to this approach.²¹

Hence, novel biomarkers for CKD or for elevated risk of future CKD are needed.²¹

In this thesis we have examined three potential circulating biomarkers for kidney function: pro-enkephalin (pro-ENK), soluble urokinase-type plasminogen activator receptor (suPAR), and kidney injury molecule -1 (KIM-1), which all have previously been reported to be associated with kidney function. Certainly, there are several other potential biomarkers for CKD,²¹ yet this is beyond the scope of this doctoral thesis.

Pro-enkephalin A (pro-ENK)

Enkephalins were the first endogenous opioids discovered almost 40 years ago.⁹³ The 10,061 basepair long gene that encodes pro-enkephalin A (*PENK*) is located within the first band of the long arm of chromosome 8 (8p12.1).⁹⁴

Similar to other neuropeptides, the biosynthesis of the active enkephalins involves several steps including proteolytic cleavage, through which four copies of methionine-enkephalin (Met-enkephalin) and one copy of leucin-enkephalin, enkelytin, one hexa-

and one octa-peptide are processed. All these share the first 4 amino acids Tyrosin-Glycin-Glycin-Phenylalanin, but vary in their carboxy-terminal sequence.⁹⁵ Prior to the proteolytic cleavage, posttranscriptional biosynthesis continues by translation of the *PENK* mRNA into a precursor preproneuropeptide. When the amino-terminal signal peptide is enzymatically removed at the rough endoplasmic reticulum (ER), the resulting pre-enkephalin is guided to the golgi apparatus and together with processing proteases packed into secretory vesicles.⁹⁶ Subsequently, the mono- and dibasic residuals flanking the eight active neuropeptides are removed by proteolytic processing inside the secretory vesicle.^{96,97} First endoproteolytic cleaving is performed by cysteine protease cathepsin L⁹⁸ and prohormone convertases (PC) 1 and 2^{99,100}. Then cleaving by the exopeptidases follows and amino terminal residues are removed by cathepsin H and/or aminopeptidase B and the carboxy-terminal residuals by carboxypeptidase E.⁹⁷

PENK is expressed within both neuronal as well as non-neuronal tissues. Although not many studies in humans have been conducted yet, the precursor preprotein pro-ENK was found expressed in human heart, subcutaneous and epicardial adipose tissues, airway epithelial cells, hepatocytes⁹⁵ and in epidermal keratinocytes and melanocytes.¹⁰¹ Furthermore, animal studies have shown expression in additional tissues like brain, kidney, adrenal gland, testes, liver, myoblast and intestine.⁹⁵

Earlier, high plasma levels of pro-ENK have been associated with decline in eGFR and kidney function in two observational studies,^{22,23} and with a worse prognosis after acute myocardial infarction.²² Among patients with sepsis or a septic shock, elevated levels of pro-ENK associated with acute kidney injury.²³ However, this evidence resulted from studies including rather ill patients, and we were not aware of any study that had investigated the association between pro-ENK and kidney function in a general population.

Soluble urokinase-type plasminogen activator receptor (suPAR)

The soluble urokinase-type plasminogen activator receptor (suPAR), is the soluble form of the membrane bound cellular receptor uPAR, also known as urokinase plasminogen activator receptor. This serine protease is an important regulator of proteolysis in the extra cellular matrix and it regulates the activity of the plasminogen activator system.¹⁰²

Highly elevated suPAR levels have been implicated as potentially causal in the pathogenesis of focal segmental glomerulosclerosis (FSGS).¹⁰³⁻¹⁰⁶ In patients with CVD undergoing cardiac catheterization, elevated plasma levels of suPAR were shown to strongly associate with increased decline in kidney function and incidence of CKD.²⁴ Further, elevated suPAR levels have been reported in earlier studies to associate with increased risk for several adverse health conditions, such as CVD outcomes,¹⁰⁷⁻¹⁰⁹ inflammation,¹¹⁰ and cancer¹¹¹ in addition to kidney function. Hence, elevated serum suPAR levels seem to play a role in FSGS and associate with CKD in severely ill

patients, yet little is known about if circulating suPAR plays a role in kidney function of healthy individuals.

Kidney injury molecule -1 (KIM-1)

The kidney injury molecule 1 (KIM-1), also known as T-cell immunoglobulin and mucin-containing molecule (TIM-1), is one of the three members of the TIM protein family in humans. All these transmembrane glycoproteins share a similar structure, with an extracellular region consisting of an immunoglobulin V-like region which is followed by a mucin-like region. These proteins are encoded by three genes on the long arm of chromosome 5 (5q33.2). The hepatitis A virus cellular receptor 1 gene (*HAVCR1*) encodes KIM-1,¹¹² and is primarily expressed in the kidney cortex.¹¹³

However, under physiological conditions KIM-1 is undetectable, but ischemic and toxic insults of the kidney have been found to strongly induce KIM-1 expression in rodents, first detected in 1998.¹¹⁴ A few years later in 2007, KIM-1 was found to be upregulated in humans with renal diseases and to co-localize with inflammation and renal fibrosis.²⁶ Hence, KIM-1 has been suggested to be a urinary biomarker for kidney injury.²⁷ Very recently it has been reported that chronic expression of KIM-1 lead to kidney fibrosis in mice. At 12 weeks of age these transgenic mice, generated to chronically express KIM-1 in epithelial cells of the renal cortex and outer medulla, showed extensive interstitial inflammation with tubular dedifferentiation, fibrosis, microcystic tubular dilation and hyaline casts.¹¹⁵

In addition, studies in patients with T1D¹¹⁶ and T2D¹¹⁷ have reported that an increased decline of eGFR associated with higher urinary KIM-1 (u-KIM-1) levels, whereas lower levels were associated with regression of microalbuminuria in patients with T1D.¹¹⁸

However, in a prediction model with known risk factors, u-KIM-1 did not add significant additional independent prognostic information in the studies of patients with T1D and T2D^{116,117}, neither did u-KIM-1 associate with CKD progression in a recent study of the Chronic Renal Insufficiency Cohort when the analyses were adjusted for urinary albumin to creatinine ratio and eGFR.¹¹⁹ Similar results were reported in patients with T1D from the FinnDiane study, where compared to current biomarkers, u-KIM-1 did not add prognostic benefit for progression of diabetic nephropathy.¹²⁰ Fewer studies have investigated plasma KIM-1 (p-KIM-1).¹²¹⁻¹²³ In the Joslin Kidney Study, higher p-KIM-1 associated with deterioration of kidney function in T1D patients.¹²¹ Higher p-KIM-1 levels were likewise associated with greater eGFR decline independently of known clinical risk factors in two controlled trails (ACCORD and VA NEPHRON-D) for early and advanced diabetic kidney disease (DKD).¹²²

However, most of the evidence deducts either from animal studies,^{114,115} studies in patients with diabetes (T1D^{116,120,121,123} or T2D¹¹⁷), or studies that have investigated u-KIM-1,¹¹⁶⁻¹²⁰ and little is known about if p-KIM-1 serves as a biomarker for long term decline in kidney function or occurrence of CKD in a general healthy population.

Aims

The overall aim of these PhD studies was to investigate how novel circulating biomarkers (pro-ENK, suPAR and KIM-1), genetic markers and dietary intake associate with longitudinal deterioration of kidney function in a generally healthy middle-age Swedish population. Moreover, we challenged the question of whether the investigated plasma biomarkers and genetic markers aid to improve clinical prediction models.

Specific aims:

Study 1: To test if fasting plasma pro-ENK associates with changes in eGFR, creatinine and cystatin C levels per year, or with incidence of CKD in the prospective population based MDCS-CC. Further, to gain insight as to whether any such relationship may be causal, we performed a GWAS for pro-ENK levels and used the associated effect of the strongest GWAS-significant SNP as instrumental variable in Mendelian Randomization (MR) analysis.

Study 2: To test if plasma levels of suPAR associate with longitudinal decline in kidney function, incidence of CKD or hospitalization due to impairment in renal function.

Study 3: To study the association between fasting plasma levels of KIM-1 and longitudinal kidney function, incidence of CKD or hospitalization due to impairment in renal function. Furthermore, to test if KIM-1 improves clinical prediction models for incidence of CKD.

Study 4: To investigate if 53 genetic variants, previously discovered to associate with kidney function in GWAS and combined into a GRS, predict longitudinal deterioration of kidney function and incidence of CKD.

Study 5: With a primary focus on protein intake, we investigate if reported dietary intake levels of macronutrients, three beverages and 15 food groups associate with longitudinal deterioration of kidney function in participants without CKD stage 3A at baseline.

Methods

The results presented in this PhD-thesis are based on investigations within the MDCS-cohort. This chapter describes the population, measurements taken (anthropometrics, assays, biological specimens and dietary assessment), outcomes investigated and statistics applied. An overview of the study design characteristics for studies I-V is given in Table 2 in the end of this chapter.

The Malmö Diet and Cancer Study (MDCS) cohort

The MDCS is a population-based cohort from the South of Sweden. Between 1991 and 1996 all inhabitant living in the city of Malmö (approximately 230,000 inhabitants at the of the study) were the background population to this cohort. All women and men (n=74,138) born 1923-1950, and 1923-1945, respectively, were invited to participate in the baseline examination between 1991 and 1996. Mental incapacity and inadequate Swedish language skills were the only exclusion criteria. The participation rate was 40.8% and in total 28,098 men and women were included in the cohort.¹²⁴ All participants have given written informed consent. The study was approved by the local ethics committee at Lund University (LU 51-90). More information about the MDCS-cohort has been described previously.^{124,125}

The MDCS-Cardiovascular cohort

For the current study we included participants that were part of the 6,103 randomly selected individuals who underwent additional phenotyping during 1991-1994 as part of the MDCS- Cardiovascular Cohort (MDCS-CC), designed to study the epidemiology of carotid artery. Between 2007 and 2012, all participants of this random sample who were alive and had not emigrated from Sweden were invited to a follow-up re-examination, as previously described.¹²⁶ In total, 4,924 MDCS-CC participants were invited of which 3,734 attended the follow-up re-examination. **(Figure 8)**

Exclusion criteria in studies I-V

For the respective studies included in this thesis, the main exclusion criteria were lack of data on a) the measured biomarkers (study I-III), b) genetic markers (study IV), or c) information on diet intakes (study V). In addition, participants were excluded if data for estimating eGFR or any covariate data was missing at either baseline or follow-up re-examination. Details are further given in the appended manuscripts.

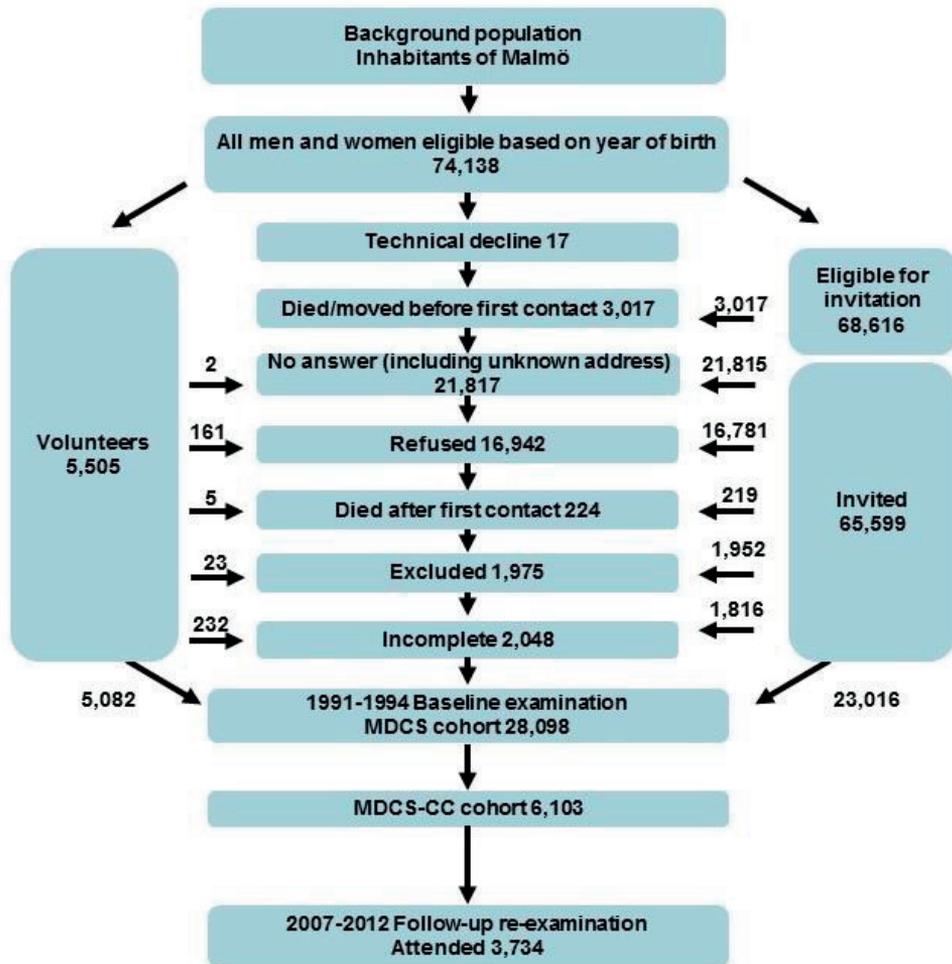


Figure 8 Flowchart of the recruitment process and participants included in the Malmö Diet and Cancer Study and the random subsample MDCS-CC at baseline and follow-up re-examination

Modified from Manjer et al. 2001¹²⁴

Clinical examination and assays

Measurements

At the baseline, the participants underwent a physical examination and trained nurses measured height (cm), weight (kg) and waist and hip circumference (cm). BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure (diastolic and systolic) was measured (mmHg) in supine position with a mercury column sphygmomanometer after 10 minutes of rests. Questions concerning socio-economic status, lifestyle factors and medical history were answered by the participants via a self-administrated questionnaire.¹²⁵ Use of anti-hypertensive treatment (AHT) at baseline was categorized as “yes” or “no”. Smoking status was defined as a categorical variable: current smokers including irregular smokers, former-smokers, and never smokers.

At baseline, fasting blood samples were drawn and immediately frozen to -80°C and stored in a biobank.¹²⁷ Creatinine ($\mu\text{mol}/\text{L}$) and cystatin C (mg/L) were quantified from fasting blood samples. Creatinine was analyzed using the Jaffé method, and traceable to the International Standardization with isotope dilution mass spectrometry (IDMS). Cystatin C was measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin; Dade Behring, Deerfield, Illinois). The values of cystatin C were not standardized because they were analyzed before the introduction of the world calibrator in 2010. The reference values for the method were 0.53–0.95 mg/L .

During the follow-up re-examination (2007-2012), following similar approaches as at baseline, anthropometric characteristics (height, weight, waist and hip circumference, SBP and DBP) were measured and plasma concentrations of glucose (mmol/L), creatinine ($\mu\text{mol}/\text{L}$) and cystatin C (mg/L) were quantified in fasting blood samples using similar analytical methods as at baseline.

Genotyping

Genotyping was performed using the Illumina HumanOmniExpress BeadChip v1, at the Broad Institute, Cambridge, MA USA. During the quality control individuals were filtered out if the call rate was less than 0.95, an inbreeding coefficient of > 3 SD away from the mean was observed, discordance between inferred and reported gender occurred, duplicate samples were identified, unexpected high proportion of IBD sharing was observed, first and second degree relatives or deviating from the common population structure in the MDCS-CC (exceeding 8 sigma on first two principal components) was observed. In addition, SNPs were filtered out if they were monomorphic or had a call rate of < 0.95 , an extreme deviation from HWE ($P < 1 \times 10^{-07}$), were missing in either cases or controls ($P < 1 \times 10^{-07}$ and $\text{MAF} > 0.01$) or an error in the plate assignment occurred ($P < 1 \times 10^{-08}$ and $\text{MAF} > 0.01$).

In addition, the dataset was imputed up to 38,028,468 genetic variants with the 1000 Genomes (1000G) Phase 1 version 3 reference panel¹²⁸ using SHAPEIT2¹²⁹ for pre-phasing and IMPUTE¹³⁰ at the Division of Translational Medicine and Human Genetics, Perelman School of Medicine, University of Pennsylvania, USA. After QC, 21,575,257 imputed variants were left for the analysis.

Dietary assessment

At the baseline diet was assessed using a modified diet history methodology,¹³¹ specifically designed for the MDCS and consisting of 1) a 7 day menu book for recording of intakes from meals that varied from day to day (usually lunches and dinner meals), cold beverages, and nutrient supplements, 2) a 168-item questionnaire for the assessment of consumption frequencies and portion sizes of regularly eaten foods from meals which were not covered by the menu book, and 3) a 45-60 minute interview. In 1984-1985, the Malmö Food study evaluated the relative validity of the MDCS diet methods, comparing them with an 18 day weighted-food record.^{132,133} Energy adjusted Pearson correlation coefficients between the reference methods and the MDCS methods were 0.54 and 0.53 for protein, 0.64 and 0.69 for total fat, 0.66 and 0.70 for carbohydrates, and 0.74 and 0.69 for fiber, for men and women, respectively.¹³²

The questionnaire and menu book were used to calculate the mean daily intake of foods based on the frequencies and portion-size estimates. The reported food intakes were converted to energy and nutrient intakes using the MDCS Food and Nutrient Database, with the nutrient information mainly originating from PC-Kost2-93 from the National Food Agency in Uppsala, Sweden.

Renal endpoints

Primary Endpoint

Kidney function, described by eGFR at the follow-up re-examination was our primary outcome for all studies. Calculation of eGFR was done according to the CKD-Epidemiology Collaboration 2012 creatinine-cystatin C equation⁵² (studies I, III and V) or the CKD-Epidemiology Collaboration 2009 creatinine equation⁵¹ (studies II and IV).

We investigated decline in eGFR calculated both as

- a) absolute [(eGFR at follow-up) – (eGFR at baseline)]
- b) and annual decline [(absolute decline) / years of follow-up], and

risk of incident CKD stage 3A (eGFR at follow-up \leq 60 mL/min/1.73m²) after excluding prevalent cases of CKD (i.e. eGFR < 60 mL/min/1.73m²) at baseline.

Secondary Endpoint

Hospitalization due to impaired renal function was used as the secondary outcome in studies II and III. Data on hospitalization due to impaired renal function were obtained from Swedish registries by linking the individual personal number to the Swedish patient registries, which cover all hospitalization in Sweden since 1987 and hospital outpatient visits from 2001 onwards. The register has been previously described and validated for outcome classification.¹³⁴ All participants were followed until occurrence of a hospital diagnosis of impaired renal function, death, emigration from Sweden, or until December 31st 2013. Admission to the hospital due to impaired renal function was defined according to ICD 9 as 585-586, and according to ICD10 as N18 and N19. We differentiated between impaired kidney function as the main diagnosis, registered as the first diagnosis in the patient record, and impaired kidney function as the main or contributing diagnosis (i.e. patients with the above mentioned ICD codes at any position). All participants with prevalent impaired kidney function were excluded from the analysis of incident hospitalization due to impaired renal function.

Predictors

The three biomarkers pro-ENK, suPAR, and KIM-1 were measured in fasting plasma of the MDCS-CC participants from the baseline examination.

Pro-enkephalin (study I)

Chemiluminometric sandwich immunoassay was used to measure PENK A 119-159, a surrogate marker for endogenous pro-ENK A, which utilizes a stable solid phase and tracer antibody detecting the endogenous pro-ENK A precursor fragments.¹³⁵

Before the analysis we transformed the positively skewed concentration of fasting plasma pro-ENK with the natural logarithm to achieve normal distribution. In addition, individuals were stratified to tertiles based on their pro-ENK concentration, defining the first tertile (T1=lowest pro-ENK concentration) as the reference. Sex-specific tertiles were used, because women had a significantly higher mean pro-ENK concentration at baseline compared to men (one-way ANOVA *P*-value < 0.000001).

suPAR (study II)

The plasma concentration of suPAR (ng/ml) was analyzed in 2012 using a commercial ELISA suPARnostic[®]-kit. The intra-assay coefficient of variation was 3% and the inter-assay coefficient variation was 5%.¹³⁶

To reach normal distribution we log-transformed the suPAR levels before the analysis and the study sample was categorized according suPAR levels into equal quartiles (Q1=lowest suPAR concentration).

KIM-1 (study III)

The Olink Proseek Multiplex 96 proximity extension assay was used to quantify concentrations of KIM-1 as normalized to protein expression (NPX).¹³⁷ The lower limit of detection (LOD) was 0.48 pg/mL, and inter-coefficient and between-coefficient of variations were 11% and 9%, respectively.¹³⁸

Baseline levels of KIM-1 per 1 standard deviation (SD) increment of the log value were analyzed and the study sample was categorized according to KIM-1 levels into sex-specific equal quartiles (Q1=lowest KIM-1 concentration).

GWAS and Mendelian Randomization (MR)-analysis (study I and study III)

In studies I and III, the GWAS approach was used to identify genetic markers that significantly associate with pro-ENK and KIM-1 levels, respectively. Thereafter, MR-analysis was conducted in study I. MR is an approach that uses genetic markers aiming to assess causal relationship between a risk factor (often a biomarker) and disease outcome.¹³⁹ The genetic marker with the strongest association with pro-ENK was used as a proxy for levels of pro-ENK. Given the random allocation of genes at conception, the genetic marker divides the study population into random groups similar to a randomized clinical trial. Thus, by random the participants who inherit the biomarker-raising allele are assigned to a group with higher levels, whereas those who do not inherit this allele are assigned to the group with lower levels.¹³⁹ Conditional analysis was performed, prior to the identification of the most suitable pro-ENK associated SNP for the instrumental variable analyses.

Genetic risk score (study IV)

In total 53 SNPs, which have been previously identified to be associated with kidney function¹³ were used to construct an unweighted genetic risk score (GRS_{CKD}) by summing up the number of risk alleles (0,1 or 2 per SNP) per participant. The allele frequencies of the 53 SNPs in the MDC-CC and further details are presented in the supplementary material of the appended manuscript. In addition, the participants were categorized according to the GRS_{CKD} into quartiles.

Dietary intakes (study V)

In the final study, macronutrients, 15 food groups (with a focus on protein intake), and three beverages were examined. These were as following: carbohydrates (E%), fat (E%), protein (E%), sucrose (E%), fiber (g/1000 kcal), fruits and vegetables (g/day), refined cereals (including pasta, rice, refined breakfast cereals and low fiber bread; portions/day), fiber-rich cereals (including fiber-rich bread and breakfast cereals; portions/day), egg (g/day), poultry (g/day), total fish (g/day), total red meat (g/day), unprocessed red meat (g/d), processed meat and meat products (g/d), total dairy (including unfermented milk, yoghurt, sour milk, cream and cheese; portions/day), yoghurt (g/day), cheese (g/day), cream (g/day), milk (g/day), legumes (g/day), and sugar-sweetened beverages (SSB) (g/day), coffee (g/day), tea (g/day). Before the analysis, all food variables were log-transformed to normalize the distribution. A very small amount was added to handle log transformation of zero-consumers. All food variables were adjusted for total energy intake using the residual method by regressing the dietary intake on total non-alcohol energy intake. Subsequently, the participants were ranked in equal intake groups (quartiles). If more than 25% of the consumers were zero-consumers (in the case of SSB, tea, yoghurt, poultry, and legumes) these were classified as zero-consumers, and the consumers, were classified into tertiles. Participants ranked in the lowest quartile of consumption or as zero-consumers, where applicable, were the reference in all analyses.

The season of the dietary data collection (spring, summer, autumn and winter) was defined as “season”. A 4-class categorical variable was used to categorize the participants according to their alcohol consumption. Zero-consumers were participants who had reported zero consumption in the menu book and indicated no consumption of any type of alcohol during the previous year. Consumers were categorized as “low” if their alcohol consumption was <15 g/d for women or < 20 g/d for men, as “medium” if 15-30 g/d for women or 20-40 g/d for men, and “high” if >30 g/d for women and >40 g/d for men. The education level of the participants was recorded by dividing the participants into 4 categories according to their highest level of education (≤ 8 , 9-10, or 11-13 years, or university degree).

Previous studies in the MDCS cohort have shown that changes in food habits related to obesity and other lifestyle and socio-economic factors and might confound observed relationships between diet and disease.¹⁴⁰ Therefore, sensitivity analyses were performed excluding participants who reported a dietary change in the past (“yes” or “no”), assessed based on the question “Have you substantially changed your eating habits because of illness or some other reason?”.

Statistics

We used the χ^2 -test to test differences in the distribution of the categorical variables (i.e. sex, smoking status, or use of AHT) across equal groups of participants (either tertiles or quartiles) of the respective biomarkers, or the GRS_{CKD} (studies I-IV).

General linear regression was used to test association between the respective predictor variable and kidney function markers at baseline and follow-up re-examination (studies I-V).

We applied logistic regression to estimate the Odds Ratios (OR) and 95 % confidence intervals (CI) for risk for incident CKD (studies I-V). Model fitting was tested using the Hosmer-Lemeshow test for logistic regression and an adequate model fit was assumed by a P -value > 0.05 .

In addition, we used Cox proportional hazard regression (study II and III) to estimate the Hazard Ratios (HR) and 95 % CI for incident hospitalization due to impaired renal function. Age was used as the underlying time-variable. The proportional hazard (PH) assumption was tested using Schoenfeld residuals (estat STATA command), and the graphical (stphplot STATA command) and hazard functions were graphically examined by plotting the Kaplan Meier failure function (sts graph STATA command).

To test the clinical applicability of the circulating biomarkers in studies I-III and the GRS_{CKD} in study IV, Net Reclassification Improvement (NRI) was calculated using the *nri* STATA command for the package *idi* from <http://personalpages.manchester.ac.uk/staff/mark.lunt>.¹⁴¹ Model discrimination was tested by calculating the C-statistics using the *roccomp* command in STATA for models using risk factors with and without the circulating biomarker or GRS_{CKD} , respectively. P -values of ≤ 0.05 were considered statistically significant.

Covariates

To take into account factors that may affect kidney function and could therefore confound the relationship between kidney function and the prediction variables in our studies (i.e. circulating biomarkers, GRS_{CKD} or dietary intakes), the analyses were adjusted for several established kidney risk factors (age, sex and baseline eGFR, BMI, fasting glucose, SBP, use of AHT and smoking status). Furthermore, the linear and logistic regression models were adjusted for the follow-up time.

Moreover, the dietary analyses were adjusted for additional covariates including season, total energy intake, alcohol intake and physical activity.

Details on the adjustments for covariates are stated in the results section.

SPSS (version 21, IBM Corporation, Armonk, NY), STATA version 13 (Stata Corp LP, College Station, TX) and PLINK version 1.07¹⁴² were used for the performed analysis.

Table 2 Overview of the study design characteristics

	Study I	Study II	Study III	Study IV	Study V
Exclusion criteria	Lack of information on biomarker levels (study I-II), genetic markers (study IV), diet intake (study V), measurements of creatinine and/or cystatin C at baseline and/or follow-up, and/or covariates				
Outcomes	Annual change in eGFR Incident CKD	Annual change in eGFR Incident CKD Hospitalization due to impaired renal function (ICD 9 585-586 and ICD 10 N19 N20)	Annual change in eGFR Incident CKD Hospitalization due to impaired renal function (ICD 9 585-586 and ICD 10 N19 N20)	Change in eGFR Incident CKD	Annual change in eGFR Incident CKD
Equation to estimate GFR	CKD-EPI crea-cystatin C ⁵²	CKD-EPI crea ⁵¹	CKD-EPI crea-cystatin C ⁵²	CKD-EPI crea ⁵¹	CKD-EPI crea-cystatin C ⁵²
Statistical analyses	Linear and Logistic regression ROC NRI GWAS MR	Linear and Logistic regression Cox-regression* ROC NRI	Linear and Logistic regression Cox-regression* ROC NRI GWAS	Linear and Logistic regression ROC NRI	Linear and Logistic regression
Exposures	Fasting plasma levels of pro-ENK	Fasting plasma levels of suPAR	Fasting plasma levels of KIM-1	GRS, including 53 SNPs previously associated with eGFR ^{crea13}	Dietary intakes including five macronutrients, 15 food groups, and three beverages
Adjustments					
Basic	Age, sex, baseline eGFR and follow-up time	Age, sex, baseline eGFR and follow-up time*	Age, sex, baseline eGFR and follow-up time*	Age, sex, baseline eGFR and follow-up time	Age, sex, baseline eGFR, follow-up time, total energy intake, and season
Full	Basic + fasting glucose, BMI, SBP and use of AHT	Basic + fasting glucose, BMI, SBP, use of AHT and smoking status	Basic + fasting glucose, BMI, SBP, use of AHT and smoking status	Basic + fasting glucose, BMI, SBP, use of AHT and smoking status	Basic + daily fiber intake, education, physical activity, alcohol intake, fasting glucose, BMI, SBP, use of AHT and smoking status

* in study II and III age was used as the underlying time scale in the Cox regression models.

Results

Study I

High Level of Fasting Plasma Proenkephalin-A Predicts Deterioration of Kidney Function and Incidence of CKD

In study I, we investigated the relationship between fasting plasma levels of pro-ENK and kidney function.

Cross-sectional association between pro-ENK and kidney function

We observed that among 1,970 men and 2,664 women included in the study, participants with high baseline levels of pro-ENK had a significantly lower eGFR (P -trend<0.001). In addition, pro-ENK associated positively with creatinine and cystatin C levels (P -trend<0.001 for both) at baseline.

Longitudinal association between pro-ENK and kidney function

In addition, we found that a greater decline in eGFR (P -trend<0.001) as well as incidence of CKD occurred more frequently in participants classified within the highest tertiles of baseline pro-ENK during a mean follow-up time of 16 years (OR:1.51; 95% CI 1.18-1.94).¹⁴³ The associations across the tertiles of pro-ENK for all participants, and separately for men and women, are shown in **Figure 9**. Adding pro-ENK to a risk model including age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, AHT, and follow-up time did only marginally improve the C-statistics (AUC without vs. with pro-ENK 0.789 vs. 0.791, $P=0.079$), but did lead to a significant NRI of 14.1% of the individuals ($P=0.001$). The results remained similar when participants with diabetes or CVD at baseline were excluded in the sensitivity analysis (OR: 1.54; 95% CI 1.19-1.97).

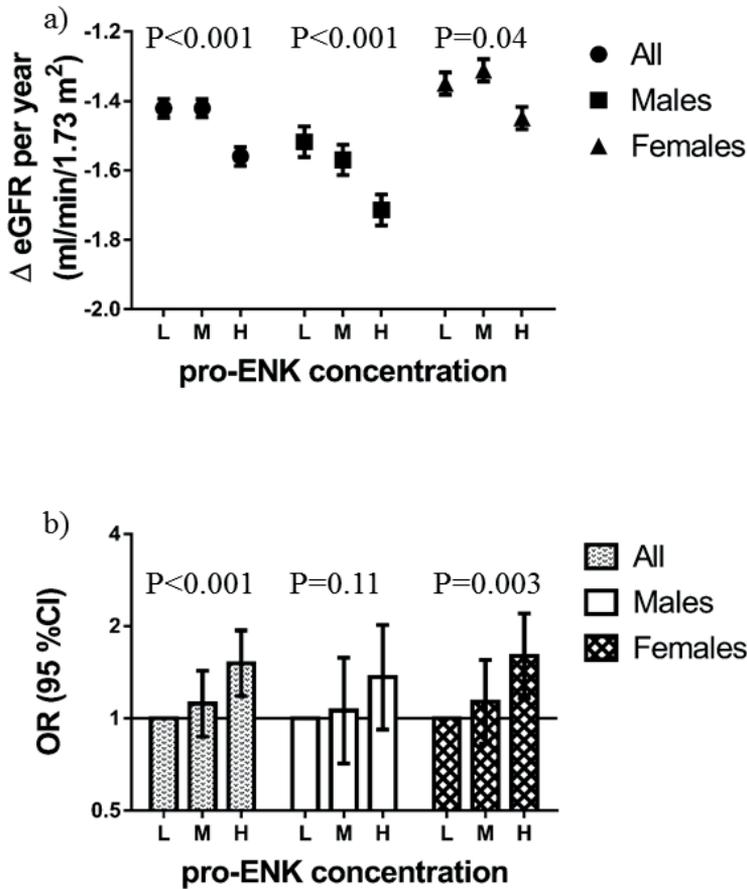


Figure 9 Association between baseline levels of pro-ENK and longitudinal kidney function, and risk of incident CKD in MDCCS-CC and stratified by gender

High levels of baseline pro-ENK associate with a greater decline in eGFR and increased risk of incident CKD at follow-up re-examination compared to low levels of baseline pro-ENK.

Figure 1a) shows the change in eGFR according to levels of pro-ENK in linear regression model adjusted for age, sex (when applicable), and baseline levels of eGFR.

Figure 1b) shows the risk of incident CKD (eGFR at follow-up re-examination <60 mL/min/1.73m²) in tertiles of pro-ENK among 2,568 participants with a baseline eGFR of >60 mL/min/1.73m². Altogether 813 individuals were classified as having CKD at the follow-up examination (293 males and 520 females). The logistic regression model was adjusted for age, sex (when applicable), baseline levels of eGFR, fasting glucose, BMI, SBP, use of AHT (yes/no), and follow-up time. eGFR according CKD-EPI creatinine-cystatin C equation.⁵²

Abbreviations: AHT=anti-hypertensive treatment, BMI= body mass index, eGFR=estimated glomerular filtration rate, MDCCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, pro-ENK=pro-enkephalin, SBP=systolic blood pressure.

Modified from Schulz et al. 2016¹⁴³ Reprinted with the permission of the publisher.

Detailed information can be found on page 96.

Genome-wide association analysis for plasma pro-ENK levels and associations between the identified SNPs and kidney function

Next we performed GWAS for pro-ENK levels in 4,150 participants of MDCS-CC. We observed that 24 of the 850,658 directly genotyped SNPs tested, all in the vicinity of the *PENK* locus, associated with pro-ENK levels at $P < 5 \times 10^{-8}$. The strongest association was found with the SNP rs1012178, which associated with a 0.057 $\mu\text{mol/L}$ higher baseline pro-ENK per minor T-allele (MAF 0.22 $P = 4.7 \times 10^{-21}$). (**Figure 10**) In conditional analysis, the SNP rs1012178 was observed to provide the strongest association with pro-ENK in the region.

We next tested association between rs1012178 and risk of incident CKD and found that the T-allele associated with increased risk of incident CKD (OR:1.19, 95% CI 1.02-1.38) after adjustment for age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, use of AHT, and follow-up time. In addition, the decline in eGFR was greater in the T-allele carriers compared to major allele carriers ($-0.083 \text{ mL/min/1.73m}^2$ per copy of the minor allele and year of follow-up; $P < 0.001$). In line with these results, the increases of the creatinine and cystatin C levels from baseline to follow-up were higher among the minor allele carriers ($+0.123 \mu\text{mol/L}$; $P = 0.002$ and $+0.001 \text{ mg/L}$; $P = 0.03$, respectively, per minor allele and year of follow-up). Moreover, in MR-analysis rs1012178 associated with a non-significant trend for increased risk of incident CKD (OR:1.95; 95% CI 0.97-3.92) after adjustment for age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, and AHT and follow-up time. (**Figure 11**) Yet, the instrumental variable associated with significant decline of eGFR ($P = 0.008$) and increase in creatinine ($P = 0.019$) from baseline to follow-up.¹⁴³

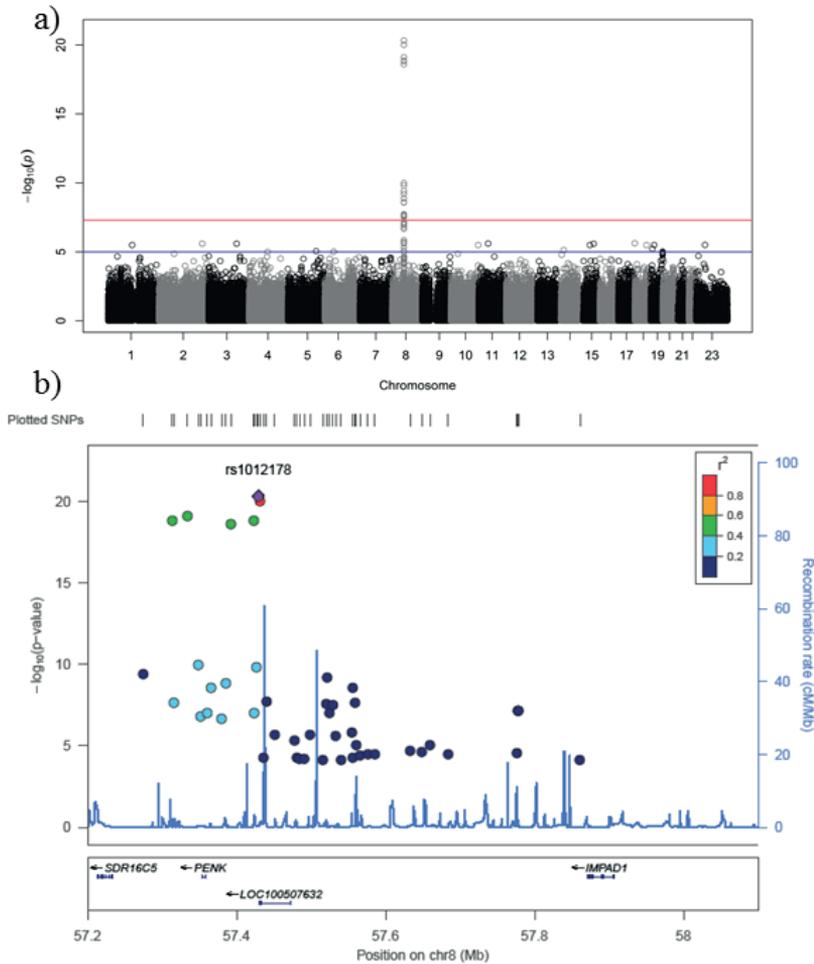


Figure 10 Genome-wide association analysis for fasting plasma concentration of pro-ENK in the 4,150 participants of MDCS-CC

Figure 2a) presents the Manhattan-plot for association between fasting plasma levels of pro-ENK and 850,658 directly genotyped SNPs after QC. The red line indicates $P < 5 \times 10^{-8}$ and the blue line indicates $P < 5 \times 10^{-5}$.

Figure 2b) illustrates the regional locus zoom plot of the chromosome 8q12.1 locus. The purple diamond indicates the lead SNP rs1012178 and the circles indicate SNPs with different degrees of LD [$r^2 \geq 0.80\%$ (red), 0.8-0.6 (orange), 0.6-0.4 (green), 0.4-0.2 (light blue) and ≤ 0.2]. Genes encoded in the region are shown in the box above the X-axis, which indicates the nucleotide position on chromosome 8.

Abbreviations: chr8= chromosome 8, GWA= Genome-wide Association, LD=Linkage-disequilibrium, MDCS-CC= Malmö Diet and Cancer Study-Cardiovascular Cohort, Mb=megabase, pro-ENK=pro-enkephalin, SNP=single nucleotide polymorphism, QC=Quality Control.

Modified from Schulz et al. 2016¹⁴³ Reprinted with the permission of the publisher. Detailed information can be found on page 96.

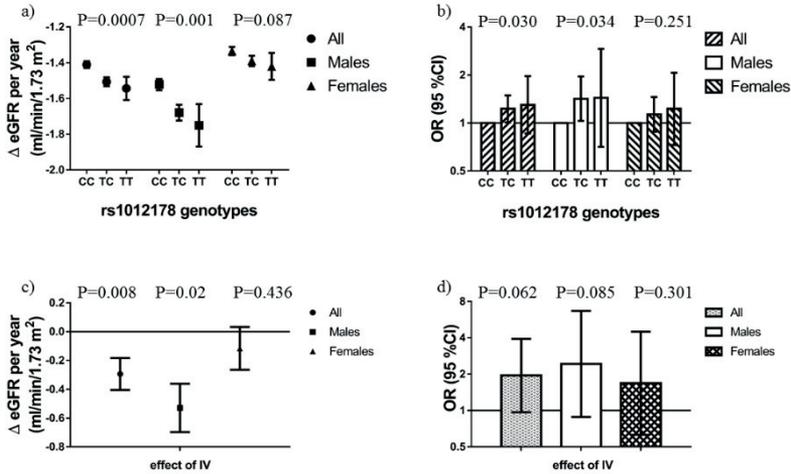


Figure 11 Association of rs1012178 genotypes with longitudinal kidney function and risk of incident CKD in the MDCS-CC

At the follow-up re-examination, a greater decline in eGFR and increased risk of incident CKD was more likely in rs1012178 minor allele carriers compared to major allele carriers. The instrumental variable associated with a decline in eGFR and a trend for increased risk of incident CKD.

Figure 3a) shows change in eGFR through genotypes of rs1012178 in a linear model adjusted for age, sex (when applicable), and baseline levels of eGFR.

Figure 3b) shows the risk of incident CKD (eGFR at follow-up re-examination < 60 mL/min/1.73m²) according to genotypes of rs1012178 in 2,766 participants from the MDCS-CC. The logistic regression model was adjusted for age, sex (when applicable), baseline levels of eGFR, SBP, BMI, fasting glucose, use of AHT (yes/no) and follow-up time.

Figure 3c) shows the IV analysis for rs1012178 genotypes and change in eGFR in a linear model adjusted for age, sex (when applicable), and baseline levels of eGFR.

Figure 3d) shows the IV analysis for rs1012178 genotypes and risk of incident CKD (eGFR at follow-up re-examination < 60 mL/min/1.73m²) in 2,308 participants from the MDCS-CC. The logistic regression model was adjusted for age, sex (when applicable), baseline levels of eGFR, fasting glucose, BMI, SBP, use of AHT (yes/no) at baseline, and follow-up time. eGFR according CKD-EPI creatinine-cystatin C equation.⁵²

Abbreviations: AHT=anti-hypertensive treatment, BMI= body mass index, eGFR=estimated glomerular filtration rate, MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, pro-ENK=pro-enkephalin, SBP=systolic blood pressure.

Modified from Schulz et al. 2016¹⁴³ Reprinted with the permission of the publisher
Detailed information can be found on page 96.

Study II

Soluble urokinase-type plasminogen activator receptor (suPAR) and impaired kidney function in the population-based Malmö Diet and Cancer Study

Hayek et al. reported recently that in patients with cardiovascular disease, undergoing cardiac catheterization, elevated plasma levels of suPAR strongly associated with increased decline in kidney function and incidence of CKD.²⁴ Thus, in study II we attempted to investigate if fasting plasma levels of suPAR associate with longitudinal kidney function in generally healthy middle-aged participants from the MDCS-CC.

Longitudinal association between baseline concentration of suPAR and kidney function

We observed that the study participants classified in the highest quartile of baseline suPAR had a significantly lower eGFR at the follow-up re-examination (P -trend= 4.3×10^{-7}). The annual decline in eGFR was higher among participants classified within the top quartile of baseline suPAR compared to participants within the lowest quartile (P -trend= 2.9×10^{-8}). At the follow-up visit, after an average follow-up time of 16.6 years, 561 participants had eGFR of $< 60 \text{ mL/min } 1.73\text{m}^2$ and were classified as having CKD. After adjusting for potential confounders, we observed that participants classified within the highest quartile of suPAR had a 69% higher risk of incident CKD compared to participants classified in the lowest quartile (OR:1.69; 95% CI 1.25-2.30).

Clinical applicability of suPAR

To investigate whether suPAR may improve the ROC-curve, we added the variable to a risk model including age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking, AHT and follow-up time and observed that it did not statistically improve the C-statistics (AUC without suPAR vs. with suPAR 0.729 vs. 0.733, $P=0.17$). Nonetheless, adding suPAR to the model significantly improved the NRI-Index (NRI=15.5%, $P=0.0010$). Model calibration was acceptable for both models (Hosmer-Lemeshow's $P>0.05$).

Hospitalization due to impaired renal function

In addition, in the Cox-regression analysis we observed that higher baseline concentrations of suPAR associated with a higher hazard of hospitalization (HR per 1SD increase in baseline suPAR 1.59; 95% CI 1.41-1.74 in the basic model and 1.49; 95% CI 1.27-1.74 in the multivariate adjusted model).¹⁴⁴ Of the 110 participants, hospitalized due to impaired renal function during the mean follow-up time of 19.0 years, 47 were classified within the highest quartile of suPAR, whereas only seven were categorized in the lowest quartile (Q1 vs. Q4 HR: 3.73; 95% CI 1.65-8.44). Similar results were observed when we included cases hospitalized with impaired renal function as a secondary diagnosis (n=97, in total 207 cases). (**Table 3** and **Figure 12**)

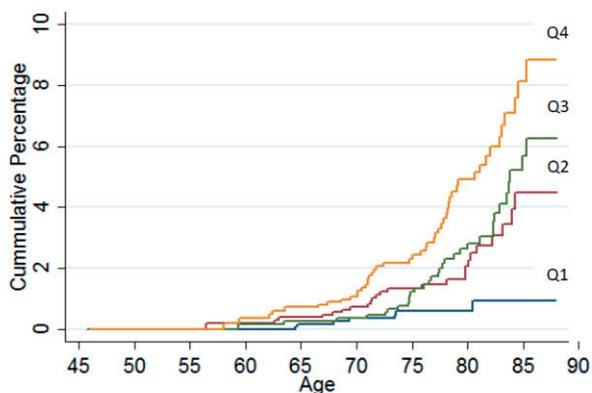


Figure 12 Cumulative incidence of hospitalization due to impaired renal function as the main diagnosis during a mean follow-up time of 19 years according to quartiles of baseline suPAR in 5,129 participants of MDCS-CC

Cumulative incidence of hospitalization due to impaired renal function as the main diagnosis during a mean follow-up time of 19 years according to quartiles of baseline suPAR in 5,129 participants of MDCS-CC

In the final model, male sex (HR:2.53; 95% CI 1.69-3.79), BMI (HR:1.10; 95% CI 1.05-1.15), baseline glucose (HR:1.21; 95% CI 1.14-1.30), AHT (HR:1.59; 95% CI 1.05-2.41), eGFR (HR:0.96; 95% CI 0.95-0.98) and current smoking (HR:2.23; 95% CI 1.34-3.73) were significantly associated with hospitalization due to impaired renal function, in addition to suPAR.

The Kaplan-Meier plot shows cumulative percentages of patients that were hospitalized due to impaired renal function as the main diagnosis during the follow-up in quartiles of baseline suPAR level [first (lowest values) to fourth (highest values)]. Median (range) concentrations of the quartiles 1 to 4 are shown in Table 3.

Cox regression adjusted for sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking status (current, former or never smokers), and use of AHT (yes/no) at baseline. Age was used as underlying time-variable. eGFR according CKD-EPI creatinine equation.⁵¹

Abbreviations: AHT=anti-hypertensive treatment, BMI= body mass index, eGFR=estimated glomerular filtration rate, MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP=systolic blood pressure, suPAR= soluble urokinase plasminogen activating receptor.

Modified from Schulz et al. 2017¹⁴⁴

Detailed information can be found on page 96.

Table 3 Incidence of impaired kidney function during a mean follow-up time of 19 years, in relation to baseline concentration of suPAR in the MDCCS-CC

	Per 1SD log suPAR				Per quartile of suPAR			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Mean (range) suPAR (ng/mL)	2.12 (0.03-2.43)	2.62 (2.43-2.83)	3.10 (2.83-3.41)	4.36 (3.41-35.86)				
Impaired kidney function as main diagnosis								
N/Cases1	5129/110	1282/24	1283/32	1282/47				
Sex adjusted HR (95 %CI)	1.59 (1.41-1.79)	1.69 (1.40-2.04)	1.0	4.18 (3.11-15.28)				
Risk factor3- adjusted HR (95 %CI)	1.49 (1.27-1.74)	1.37 (1.22-1.67)	1.0	3.11 (1.36-7.10)				
All impaired kidney function cases								
N/Cases2	5129/207	1282/23	1282/47	1283/57				
Sex adjusted HR (95 %CI)	1.46 (1.32-1.61)	1.0	1.84 (1.12-3.03)	2.20 (1.35-3.59)				
Risk factor3- adjusted HR (95 %CI)2	1.35 (1.18-1.53)	1.0	1.55 (0.94-2.56)	1.69 (1.04-2.76)				

¹Admission to the hospital due to impaired kidney function as main diagnosis. ²Admission to the hospital due to impaired kidney function as main diagnosis (n=110) and secondary diagnosis (n=97, in total n=207).

³adjusted for sex, fasting glucose levels, eGFR, BMI, SBP, smoking status (current, former or never smokers) and use of AHT (yes/no) at baseline. Age was used as underlying time-variable. Abbreviations: BMI= Body Mass Index, SBP=systolic blood pressure, DBP=diastolic blood pressure, AHT=anti-hypertensive treatment, eGFR= estimated glomerular filtration rate according CKD-EPI creatinine equation.⁵¹

Modified from Schulz et al. 2017¹⁴⁴ Detailed information can be found on page 96.

Study III

High levels of plasma kidney injury molecule-1 (KIM-1) predict deterioration of kidney function and incidence of chronic kidney disease

In paper III we investigated the relationship between plasma levels of KIM-1 and kidney function.

Cross-sectional association between KIM-1 and kidney function

The 4,412 participants (39.3% men) included in the study were 57.4 (SD 5.9) years old, had a mean BMI of 25.6 (SD 3.9) kg/m² and an eGFR of 89.1 mL/min/1.73m² (range 13.6 -153.6). Further, we observed that participants within the higher quartile of KIM-1 had a significantly lower eGFR compared to participants within the lowest quartile of KIM-1 (Q4: 85.3 vs. Q1: 91.6 mL/min/1.73m²; *P*-trend=0.0007).

Longitudinal association between baseline concentrations of KIM-1 and kidney function

At the follow-up re-examination the annual decline in eGFR was greater among participants with higher baseline levels of KIM-1 (*P*-trend<0.0001). Likewise, in comparison to participants within the lowest quartile of baseline KIM-1, participants within the highest quartile had higher levels of creatinine and cystatin C as compared to participants within the lowest quartile (*P*-trend for both < 0.0001).

When 34 participants with a baseline eGFR of < 60 mL/min/1.73m² were excluded, incidence of CKD at follow-up re-examination was observed in 882 of 2,765 participants. As illustrated in **Figure 13** the incidence of CKD was higher among participants in the highest quartile of baseline KIM-1 in the multivariate model (Q1 vs. Q4 OR: 1.45; 95% CI 1.10-1.92).

When KIM-1 was added to a risk model, including age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking, and AHT, the AUC improved marginally yet significantly (*P*=0.043) and the NRI-Index improved significantly (*P*=0.023). Both models had an adequate model calibration (Lemeshow's *P*-value> 0.05 for both models).

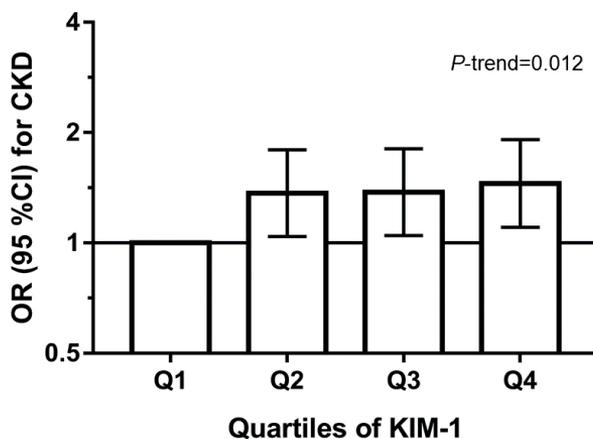


Figure 13 Risk of incident CKD at follow-up re-examination according to quartiles of KIM-1 among 2,765 participants of the MDCS-CC after a mean follow-up time of 16.6 (\pm 1.5) years

High levels of baseline KIM-1 associate with increased risk of incident CKD at follow-up re-examination compared to low levels of baseline KIM-1 (P -trend=0.012).

The logistic regression model was adjusted for age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking status (current, former or never smokers), use of AHT (yes/no) and follow-up time.

eGFR according CKD-EPI creatinine-cystatin C equation.⁵²

Abbreviations: AHT=anti-hypertensive treatment, BMI= body mass index, eGFR=estimated glomerular filtration SBP=systolic blood pressure. Q1=lowest quartile; Q4=highest quartile.

Hospitalization due to impaired renal function

In total 90 participants were admitted to the hospital due to impaired renal function during the follow-up time of 19 (range 0-22) years. Participants classified into the highest quartile of baseline KIM-1 were more likely to be admitted to the hospital. When adjusted for the known risk factors the hazard for hospitalization due to impaired renal function was increased for participants classified within the highest quartile of baseline KIM-1 compared to the lowest quartile (Q1 vs. Q4 HR: 1.84; 95% CI 0.93-3.63). (Figure 14) Likewise, when 82 additional cases with hospitalization due to impaired renal function as a secondary diagnosis were induced into the analysis, the risk was significantly increased (Q1 vs. Q4 HR: 1.78; 95% CI 1.08-2.94). PH-assumption was fulfilled for both analyses (global P -value >0.42 for both primary and all cases of hospitalization due to impaired renal function).

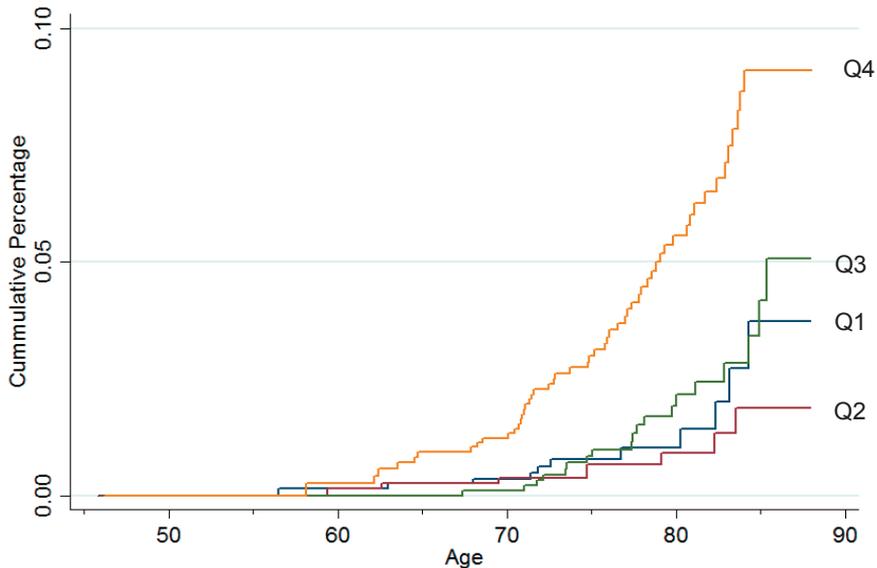


Figure 14 Cumulative incidence of hospitalization due to impaired renal function (main diagnosis, n=90) during follow-up according to quartiles of KIM-1 in 4,406 participants of the MDCS-CC

In the final model, in addition to KIM-1, male sex (HR: 2.62; 95% CI 1.69-4.07), fasting glucose (HR:1.21; 95% CI 1.12-1.31), BMI (HR:1.10; 95% CI 1.05-1.15), and eGFR (HR:0.95; 95% CI 0.94-0.96) were significantly associated with hospitalization due to impaired renal function,

The Kaplan-Meier plot shows cumulative percentages of main cases of patients (n=90) that were hospitalized due to impaired renal function during the follow-up (19.2± 4.0 years) in quartiles of baseline fasting plasma KIM-1 [first (lowest values) to fourth (highest values)].

The Cox regression was adjusted for sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking status (current, former or never smokers), and use of AHT (yes/no) at baseline. Age was used as the underlying time-variable. eGFR according CKD-EPI creatinine-cystatin C equation.⁵²

Abbreviations: AHT=anti-hypertensive treatment, BMI= body mass index, eGFR=estimated glomerular filtration rate, MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP=systolic blood pressure.

Study IV

Genetic predisposition for renal dysfunction and incidence of chronic kidney disease in the Malmö Diet and Cancer study

Early identification of persons at increased risk of CKD is of great importance³⁴ and several genetic markers associated with kidney function have been discovered in the recent years.^{13,77-81} In study IV, we therefore investigated the clinical potential of a GRS, consisting of 53 SNPs associated with eGFR_{crea},¹³ to predict risk of incident CKD at follow-up re-examination.

The average number of risk alleles carried by the 2,301 participants included in this study was 56 (range 42-71). (**Figure 15**) We observed that higher GRS_{CKD} associated strongly with lower baseline eGFR (-0.26 mL/min/1.73m² per risk allele;

$P=3.34 \times 10^{-8}$), as well as with higher levels of creatinine and cystatin C ($P<0.0001$ and $P<0.001$, respectively). (**Table 4**)

Risk of incident CKD

After a mean follow-up time of 16.6 years incidence of CKD occurred in 453 (19.7%) participants. We observed that participants classified within the highest quartile of the GRS_{CKD} had a lower eGFR at the follow-up re-examination compared to participants within the lowest quartile, independent of age, sex, baseline eGFR, and follow-up time (P -trend=0.0009). Likewise, there was a 43% increased risk of incident CKD (OR=1.43; 95% CI 1.12-1.81) per 10 risk alleles after adjusting for age, sex, baseline, eGFR and follow-up time. When adjusting for further baseline risk factors BMI, fasting glucose, SBP, AHT and smoking status, GRS_{CKD} remained significantly associated with the risk of incident CKD (OR: 1.48; 95% CI 1.16-1.88 per 10 risk alleles). Higher GRS_{CKD} associated with increased risk of incident CKD at follow-up examination in multivariate adjusted analysis (Q1 vs. Q4 OR: 1.78; 95% CI 1.29-2.46). (**Figure 16**)

Yet, the GRS_{CKD} did not significantly improve the C-statistics when compared to a risk model including solely the conventional risk factors ($P=0.22$). Nonetheless, adding the GRS_{CKD} did improve the NRI-Index (NRI=17.7%; $P=0.0007$).

Table 4 Baseline characteristics of the 2,301 participants from the MDCCS-CC according to quartiles of GRS_{KID}

	n	Mean (SD)	Q1 (n=574)	Q2 (n=576)	Q3 (n=574)	Q4 (n=577)	P-trend ¹
Alleles, mean (range)	2301	56 (43-71)	51 (43-53)	55 (53-57)	58 (57-59)	62 (59-71)	/
Male sex, n (%)	2301	963 (41.8)	237 (41.3)	241 (41.8)	236 (41.1)	249 (43.2)	0.895
Age (years)	2301	56.0 (5.6)	56.2 (5.7)	55.9 (5.5)	55.9 (5.6)	55.9 (5.6)	0.380
Height (cm)	2301	169.5 (8.8)	168.9 (8.5)	169.6 (8.9)	169.8 (8.9)	169.9 (8.9)	0.021
Weight (kg)	2301	73.1 (13.0)	72.7 (13.2)	72.8 (12.5)	73.7 (12.7)	73.1 (13.6)	0.516
BMI (kg/m ²)	2301	25.4 (3.6)	25.5 (3.8)	25.3 (3.6)	25.5 (3.49)	25.2 (3.6)	0.488
SBP (mmHG)	2301	138.4 (17.8)	138.9 (18.5)	138.4 (17.9)	138.2 (17.3)	138.2 (17.7)	0.628
DBP (mmHG)	2301	86.0 (9.0)	86.6 (9.3)	86.1 (8.6)	85.8 (9.0)	85.5 (9.3)	0.041
Glucose (mmol/L)	2301	5.0 (1.1)	5.1 (1.1)	5.0 (1.0)	5.03 (1.3)	5.0 (0.8)	0.278
Cystatin C (mg/dL)	2170	0.75 (0.12)	0.74 (0.12)	0.75 (0.12)	0.76 (0.1)	0.76 (0.1)	0.0003
Creatinine (μmol/L)	2301	82.3 (11.9)	80.8 (11.9)	81.9 (12.0)	82.7 (11.5)	83.9 (11.8)	5.5 x 10 ⁻⁸
eGFR at baseline (mL/min/1.73m ²)	2301	78.9 (11.4)	80.4 (11.6)	79.4 (11.5)	78.4 (11.4)	77.4 (10.9)	2.9 10 ⁻⁸
eGFR at follow-up (mL/min/1.73m ²)	2301	71.71 (14.6)	74.2 (14.0)	71.8 (14.8)	70.7 (14.5)	70.2 (14.7)	0.00009
AHT, n (%)	2301	319 (13.9)	84 (14.6)	78 (13.5)	89 (15.5)	68 (11.8)	0.294
Current smoking, n (%)	2301	533 (23.2)	137 (23.9)	144 (25.0)	136 (23.7)	116 (20.1)	0.224

Data shown as mean (SD) for continuous variables or n (%) for categorical variables.

¹P-value from sex and age adjusted linear regression model, or chi2-test.

eGFR calculated according to CKD-EPI 2009 creatinine equation.⁵¹

Abbreviations: AHT=anti-hypertensive treatment; BMI= body mass index; DBP= diastolic blood pressure; eGFR= estimated glomerular filtration rate; MDCCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort. SBP=systolic blood pressure.

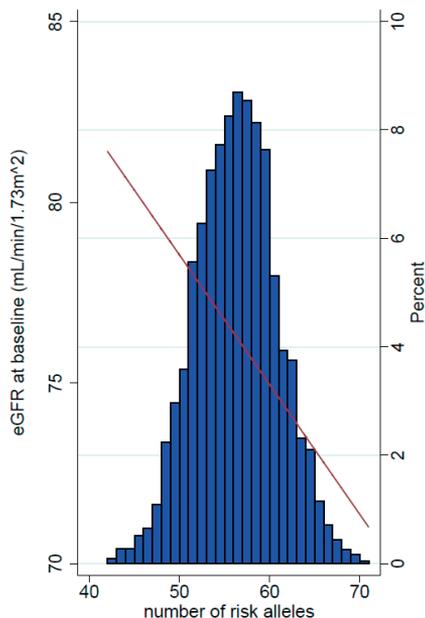


Figure 15 Association between the GRS_{CKD} of 53 SNPs and baseline eGFR in the Malmö Diet and Cancer Study

The blue histogram represents the frequency of the number of risk alleles carried by the 2,301 participants, and the predicted values of the baseline eGFR (the red line) resulting from a linear regression plotted across the GRS_{CKD} . eGFR calculated according to CKD-EPI 2009 creatinine equation.⁵¹

Abbreviations: eGFR= estimated glomerular filtration rate; MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort.

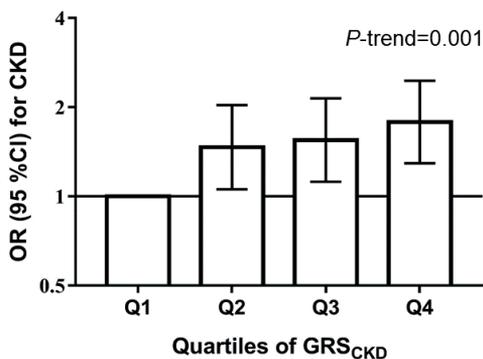


Figure 16 Risk of incident CKD according to quartiles of GRS_{CKD} in 2,301 participants of the MDCS-CC after an average follow-up-time of 16 years

A higher GRS_{CKD} was associated with increased risk of incident CKD at follow-up examination (Q1 vs. Q4 OR: 1.78; 95% CI 1.29-2.46).

OR and 95%CI were obtained from logistic regression adjusted for age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, smoking status (current, former or never smokers), use of AHT (yes/no), and follow-up time. Number of risk alleles per quartile are shown in Table 4.

eGFR calculated according to CKD-EPI 2009 creatinine equation.⁵¹

Q1=lower quartile as reference was set to 1.

Abbreviation: AHT=anti-hypertensive treatment; BMI= body mass index; DBP= diastolic blood pressure; eGFR= estimated glomerular filtration rate; MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP=systolic blood pressure.

Study V

Dietary intake and longitudinal kidney function: results from the Malmö Diet and Cancer Study

Lifestyle factors, such as diet, play a role in kidney function. However, the knowledge on how dietary intake may influence kidney function of individuals with a preserved kidney function is limited. Thus, in study V we investigate the relationship between dietary intake and longitudinal kidney function.

The 2,918 study participants were on average 56.3 (SD 5.7) years old and had a mean BMI of 25.3 (SD 3.6) kg/m² and their median dietary intakes are shown in **Table 5**.

All participants were free of CKD at baseline and had a mean eGFR of 90.4 (range 60.1-153.6) mL/min/1.73m². There were no associations between any of the dietary variables and baseline eGFR, except for poultry intake which associated with higher eGFR (details are presented in the appended manuscript). The average decline in eGFR was 1.44 ml/min/1.73m² (SD 0.82) per year during the mean follow-up time of 16.6 (13.3-20.2) years.

Longitudinal association between dietary intake and kidney function

When adjusted for all covariates (model 3), we observed that higher intake of sucrose was associated with a greater decline in eGFR. When participants were categorized to quartiles of sucrose intake, participants in the highest quartile had a significantly greater decline of eGFR compared to participants in the first quartile. In contrast, participants categorized within the highest quartiles of fiber, coffee, or total protein intake had a significantly lower decline in eGFR from baseline to follow-up re-examination. In addition, higher intake of egg, total red meat and cream associated with a lower mean decline of eGFR. Higher total dairy as well as non-fermented milk intake showed a tendency for association with lower eGFR decline. (**Figure 17**)

Incident CKD occurred in 930 (31.4%) study participants, and higher intake of fiber, coffee, and milk associated with a lower risk of incident CKD. Likewise, there was a tendency for a decreased risk of incident CKD among participants with higher intake of cream and fiber-rich cereals. In contrast, higher intake of sucrose associated with a tendency for higher risk of incident CKD. (**Figure 18**)

In the mutual adjustment analysis the positive association between total protein intake or red meat intake and change in eGFR disappeared when adjusted for sucrose intake. In contrast, higher intake of sucrose remained significantly associated with a lower decline in eGFR when adjusted for total protein intake. However, sucrose intake was no longer associated with risk of incident CKD when adjusted for total protein intake. Neither remained milk nor total dairy intake significantly associated with decline in eGFR after adjusting for protein intake (data not shown).

In sensitivity analysis we excluded participants who reported having changed their diet prior to baseline (n=703; 24%), but this did not majorly change the results. Further details can be found in the appended manuscript.

Table 5 Dietary intakes¹ among 2,918 participants from the MDCC-CC

Daily dietary intake	Mean ± SD	Daily dietary intake	Mean ± SD
Total energy (kcal/d)	2339.65±658.54	Egg (g/day)	23.14±18.21
Protein (EN %)	15.48±2.40	Poultry (g/day)	12.90±17.65
Fat (EN %)	37.92±6.32	Fish, total (g/day)	42.79±31.64
Carbohydrates (EN %)	44.48±5.68	Red meat – processed and nonprocessed (g/day)	99.12±52.24
Sucrose (E%)	8.52 (3.2)	Red meat, nonprocessed (g/day)	60.20±35.56
Fiber (g/1000 kcal)	9.74±2.85	Meat and meat products - processed (g/day)	38.91±30.66
Fruits & vegetables (g/day)	399.79±182.69	Dairy products, total (portions/day)	4.83±2.22
Alcohol (g/day)²	10.69±11.6	Yoghurt (g/day)	92.32±109.51
not during the last year	102 (3.5%)	Cheese (g/day)	47.69±33.05
low	2217 (75.9%)	Cream (g/day)	15.80±17.43
medium	501 (17.2%)	Milk, non-fermented (g/day)	270.20±231.43
high	98 (3.4%)	Cereals - refined (portions/day)	2.36±1.55
SSB (g/day)	69.75±131.70	Cereals - fiber rich (portions/day)	1.13±1.09
Coffee (g/day)	532.47±385.56	Legumes (g/day)	8.97±18.07
Tea (g/day)	146.83±244.52		

Data shown as mean±SD.

¹assessed by self-reported assessed modified dietary history method.¹³¹

² low=<15 g alcohol/d for women or < 20 g/d for men; medium= 15-30g/d for women or 20-40 g/d for men; high=>30 g/d for women and >40 g/d for men

Abbreviation: EN %=Energy percentage; MDCC-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort; SSB=Sugar sweetened beverages.

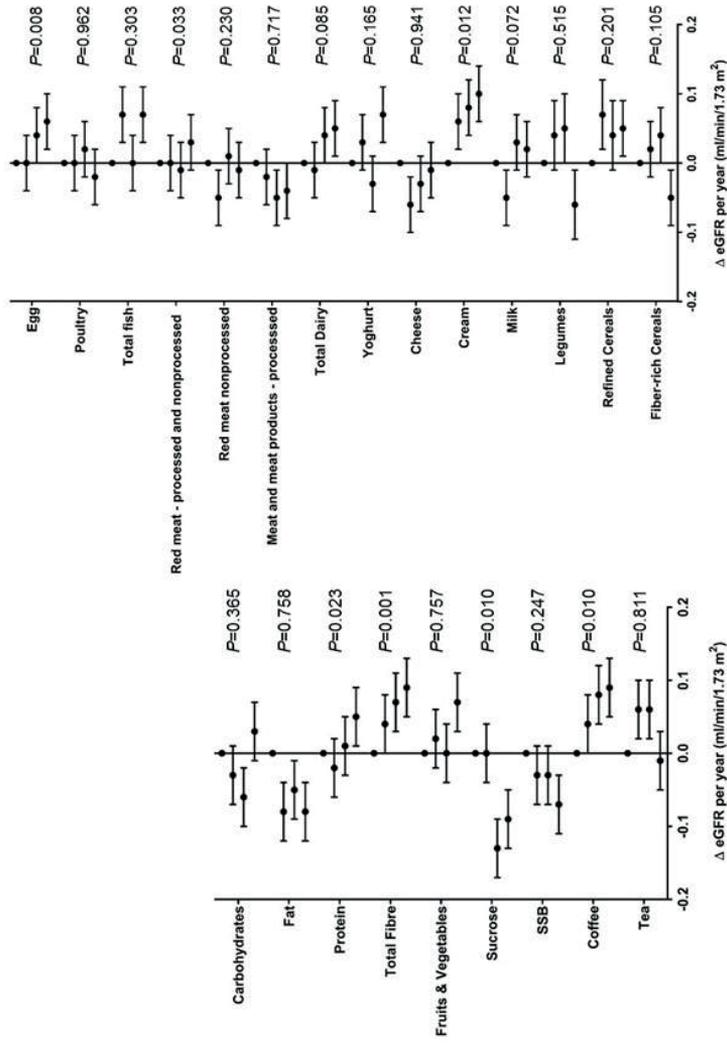


Figure 17 Annual change in eGFR (Δ eGFR) according to dietary intakes in 2,918 participants from the Malmö Diet and Cancer Study

with an eGFR >60 mL/min/1.73m² at baseline

Mean Δ eGFR ±SE was obtained from linear regression and P=trend through quartile 1(lowest=reference) to 4 (highest) adjusted for: age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, total energy intake, season, daily fiber intake, education, physical activity, alcohol intake, smoking status (current, former or never smokers), use of AHT (yes/no), and follow-up time.

eGFR according CKD-EPI creatinine-cystatin C equation.⁵²

Abbreviation: AHT=anti-hypertensive treatment; BMI= body mass index; DBP= diastolic blood pressure; eGFR= estimated glomerular filtration rate; MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP=systolic blood pressure.

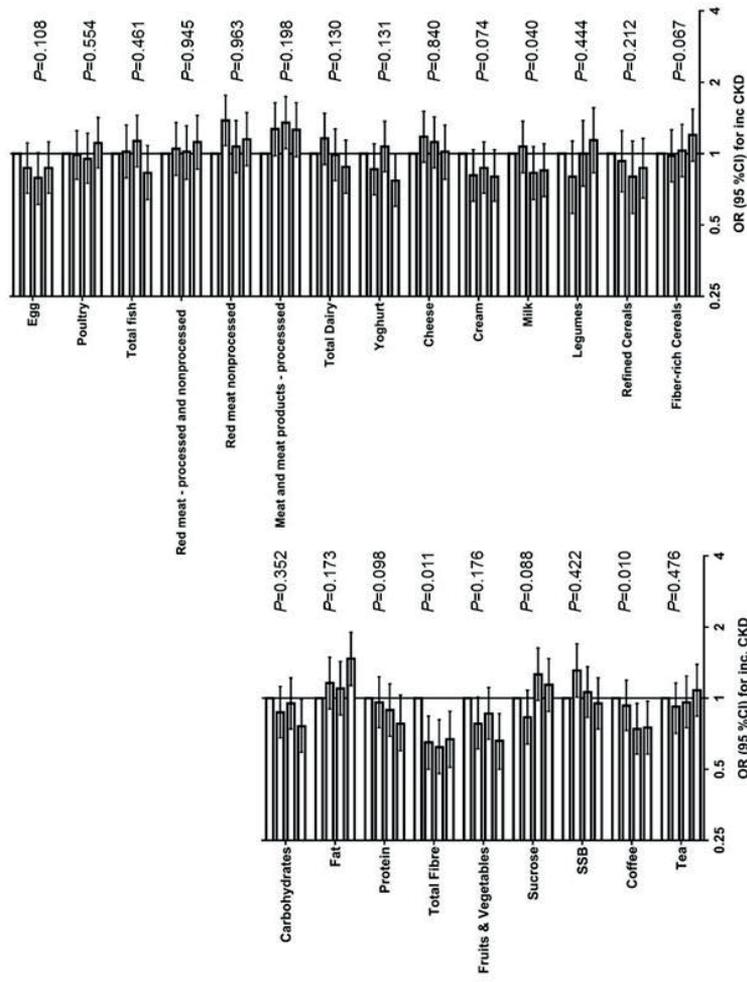


Figure 18 Risk of incident CKD (eGFR<60 mL/min/1.73m²) at follow-up re-examination according to dietary intakes in 2,918 participants from the Malmö Diet and Cancer Study with an eGFR >60 mL/min/1.73m² at baseline

OR and 95CI were obtained from logistic regression and $P=P$ -trend through quartile 1 (lowest=reference) to 4 (highest) adjusted for age, sex, baseline levels of eGFR, fasting glucose, BMI, SBP, total energy intake, season, daily fiber intake, education, physical activity, alcohol intake, smoking status (current, former or never smokers), use of AHT (yes/no), and follow-up time.

eGFR according CKD-EPI creatinine-cystatin C equation.⁵²
 Abbreviation: AHT=anti-hypertensive treatment; BMI= body mass index; DBP= diastolic blood pressure; eGFR= estimated glomerular filtration rate; MDCS-CC=Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP=systolic blood pressure.

Discussion

Overall, this thesis provides evidence for that the three investigated circulating biomarkers (pro-ENK, suPAR and KIM-1) may be clinically useful in estimating long-term kidney function, based on that they associated with longitudinal eGFR decline and have shown benefits in predicting risk of incident CKD. Furthermore, we observed that participants who were carrying a higher number of risk alleles had an increased risk of incident CKD at follow-up re-examination, independent of conventional risk factors. Thus, the results suggest that incorporating information on genetic predisposition in risk prediction models may be helpful in prediction of future kidney function. Lastly, we observed that of the investigated macronutrients, three beverages and 15 food groups, higher intake of sucrose associated with a greater long term kidney function decline, whereas higher intake of fiber associated with a lower decline in kidney function throughout follow-up. In addition, higher coffee intake associated with a lower decline of eGFR.

The three investigated circulating biomarkers – in relation to current literature and potential mechanisms

pro-ENK (study I)

In line with our study, previous observational studies reported an association between pro-ENK and kidney function, and were in fact the motivation for us to initiate and design study I.^{22,23} However, the previous findings were observed in patients with acute myocardial infarction²², or sepsis.²³ Very recently, plasma levels of pro-ENK were strongly correlated with eGFR in renal transplant recipients and with mGFR in healthy donors.¹⁴⁵ To our knowledge, we were the first to report that high levels of pro-ENK associate with longitudinal decline in kidney function and risk of incident CKD in a population-based cohort of middle-aged generally healthy participants.¹⁴³

PENK is widely expressed throughout the body within both neuronal as well as non-neuronal tissues, including the kidney epithelium.⁹⁵ Hitherto, the physiological role of enkephalins still remains to be fully understood, even though they were first discovered almost 40 years ago.⁹³ Already in 1987, a four times higher plasma concentration of met-enkephalin was reported in uraemic patients compared to healthy individuals.¹⁴⁶ Met-enkephalin was observed to directly correlate with plasma levels of creatinine and urea in the uremic patients, and the study suggested that the retention of met-enkephalin in blood could be involved in the pathogenesis of uraemic syndrome.¹⁴⁶ In 1981, met-enkephalin was observed to be elevated in plasma of patients with renal failure, and it

was proposed that this could potentially be a consequence of either impaired clearance of met-enkephalin or of its precursor, or because of increased production.¹⁴⁷ Met-enkephalin, also known as the opioid growth factor (OGF), is involved in cell proliferation.^{148,149} Met-enkephalin has been reported to modulate the cyclin-dependent kinase inhibitory pathway by interacting with the OGF-receptor (OGFR) on the outer nuclear envelope.¹⁴⁸ Further, it has been suggested that the OGF-OGFR axis might play a major role in the regulation of cell proliferation of human cancer cells.¹⁵⁰ It is yet unknown if the nuclear pro-ENK reflects or affects the plasma levels of pro-ENK. To investigate if and how nuclear pro-ENK and/or an unbalanced OGF-OGFR axis may affect cell proliferation and apoptosis in the cells of renal tissues, and which long-term consequences such actions would entail, needs to be investigated further.

suPAR (study II)

Emerging evidence from observational studies suggest a pathogenic role for suPAR in kidney disease.^{14-17,24} However, we were the first to report that high plasma levels of suPAR associate with deterioration of kidney function and risk of incident CKD in a general population of middle-aged Swedish participants.¹⁴⁴ Indeed, patients undergoing cardiac catheterization were observed to have a greater decline of eGFR and increased incident CKD at follow-up if they presented increased levels of suPAR at baseline.²⁴ Moreover, suPAR has been implicated in the pathogenesis of focal segmental glomerulosclerosis (FSGS).^{24,103-106} FSGS can be referred to as a morphological/histological pattern of injury rather than a specific glomerular disease.¹⁵¹ FSGS is recognized via kidney biopsy and is characterized by sclerotic (fibrotic) lesions in the glomeruli that are focal (less than 50% of all glomeruli affected on light microscopy) and segmental (less than 50% of the glomerular tuft affected).¹⁵¹ Interestingly, increased levels of suPAR occurred in two-thirds of the patients with FSGS, but not in patients with other glomerular diseases with podocyte involvement (including minimal change disease and membranous nephropathy) or preeclampsia.¹⁰⁵ Likewise, elevated levels of suPAR were observed in patients with biopsy proven primary FSGS from two cohorts.¹⁰⁶ It has been postulated that suPAR may act via interference with podocyte migration and apoptosis.^{24,103-106} Indeed, both suPAR and uPAR have been suggested to be directly involved in podocyte migration through binding of integrins and regulation of cell adherence.^{24,152} Experimental studies have shown that uPAR is required for activating the $\alpha v \beta_3$ integrin in the podocytes and a pathophysiology similar to primary FSGS was observed when uPAR was overexpressed in mice, whilst neither proteinuria nor podocyte pathology was observed in knock-out mice without the gene encoding uPAR (*Plaur*^{-/-}).¹⁰³

KIM-1 (study III)

Several studies have previously suggested an association between KIM-1 and adverse kidney function outcomes. However, adding u-KIM-1 to prediction models has not shown additional prognostic benefits beyond the common clinical risk factors,^{116,117,120} and only a few studies have investigated p-KIM-1.¹²¹⁻¹²³ Interestingly, one study in

patients with T1D reported that the association of u-KIM-1 with deterioration of kidney function diminished and was not significant when adjusted for p-KIM-1, whilst the association remained significant for p-KIM-1 when adjusted for u-KIM-1.¹²¹ These findings lead to the discussion that u-KIM-1 may reflect the acute production, which may vary over time, whereas p-KIM-1 might represent the synthesis over time, and may be less affected by variation over time and thus time of collection.¹²¹ *HAVCR1* (the gene encoding for KIM-1) is primarily expressed in the kidney cortex,¹¹³ and a recent paper reported that the levels of urinary and plasma KIM-1 are correlated ($r=0.43$).¹²³ The movement of KIM-1 into the circulation may be facilitated by loss of tubular cell polarity with injury, when KIM-1 may be released directly to the interstitium, but also by increased transepithelial permeability and disruption of the actin cytoskeletal architecture¹⁵³ in renal microvascular endothelial cells.^{123,153-155}

There are a few aspects, which deserve some further clarification when interpreting the results from studies I-III. The biomarkers investigated were only measured once at the baseline examination and therefore all our longitudinal analyses were either adjusted for the follow-up time, or the reported change in kidney function was expressed per year of follow-up. Further, it could be argued that this may not be much of an issue at least for study III. As mentioned above, p-KIM-1 measured in plasma has been discussed to probably reflect long-term synthesis, which may be less affected by variation over time. Of note, we have investigated circulating KIM-1 measured in plasma, whereas the majorities of the studies have been investigating u-KIM-1.¹¹⁶⁻¹²⁰ Given that there was no urine available from the baseline examination it was not possible to investigate u-KIM-1 in the MDCS-CC.

In addition, in study I, we performed MR-analysis to investigate the causal link between pro-ENK and kidney function. Instead of plasma levels of pro-ENK we used an instrumental variable for the strongest genetic marker associated with pro-ENK that we identified in GWAS. Considering that alleles are randomly allocated during conception, and that the analysis using the instrumental variable showed similar results (i.e. which suggested that pro-ENK may be causally linked to kidney function), it can be argued that using a one-time measurement at baseline in study I might not be of major concern. Lastly, the biomarkers pro-ENK, suPAR and KIM-1^{135,136,138} have been evaluated prior in regards to their stability, and intra- as well as inter-assay variation.

We observed that the circulating biomarkers associated with age at baseline, i.e. older individuals had higher levels, thus all analyses were age adjusted. Given the particular relationship between age and kidney function (i.e. the decline in eGFR starting approximately in the fourth decade),^{53,54} we used age as the underlying time variable in the Cox-regression models in studies II and III, and the PH-assumption was fulfilled and the Schoenfeld residuals were acceptable (global P-value of > 0.05).¹⁴⁴

Genetic predisposition and potential pathophysiological consequences (study IV)

The variants included in the GRS_{CKD} in study IV were initially discovered in the to date largest meta-analysis of GWAS for $eGFR_{crea}$, which identified 24 novel loci and replicated 29 earlier identified loci in the combined data set of more than 175,000 individuals.¹³ In addition, a variety of extensive locus characterization and bioinformatics approaches were performed by Pattaro et al. to gain further insight into the potential mechanisms behind the associations of the 53 loci with kidney function. The key findings of that study were that: 1) Many of the genetic variants associated with $eGFR_{crea}$ seemed to affect processes within the kidney. The genes identified at the loci associated with $eGFR_{crea}$ were enriched for expression in kidney tissues and could be assigned to gene regulatory functions in the epithelial cells, but not in the general vasculature or the glomerular endothelial cells; 2) Of the identified SNPs, in total 19 were shown to associate with $eGFR_{crea}$ in individuals with diabetes. Moreover, in pathway analysis the identified gene sets associated with abnormal glucose homeostasis and glucose transport activity 3) Developmental processes related to the kidneys and urogenital tract such as placenta morphology, embryo size and kidney weight were observed in pathway analyses.¹³

A crucial factor in manifestation of many diseases is genetic predisposition.⁶⁹ In contrast to monogenic forms of kidney disease where mutation of a single gene cause the disease, most individuals with kidney disease may suffer from a multifactorial disease with several genetic and environmental factors affecting the risk and disease manifestation.

For multifactorial kidney diseases the development and progression of the disease may result from where, when and how the genetic risk and environmental factors act together. However, the main challenge has been and still remains for the future, to reveal the functional mechanisms between the identified genetic risk factors and the disease phenotype.⁶⁹ Today, GWAS is the widely used tool to identify genetic variants that associate with multifactorial diseases,⁶⁹ and these studies have immensely contributed to understanding of these diseases, including kidney diseases.⁷⁴ GWAS is a powerful tool to systematically characterize the genetic predisposition of a disease and to set light on the complex biology of fundamental disease processes.¹⁵⁶

However, the GWAS approach has some limitations. Commonly there are around one million SNPs tested (instead of the close to 20 million genetic variations), because these one million SNPs resemble the genetic variation of larger region the so called haplotype- or LD blocks. However, this makes it challenging to identify the functionally relevant SNP or SNPs driving the associations.¹⁵⁷ Moreover, little is known about how the associated SNPs translate to pathophysiological mechanisms, considering that more than 80% of the identified variants are located in the noncoding regions of the genome.^{157,158}

Considerations about the predictive ability of biomarkers

Overall, all the biomarkers investigated in studies I-IV showed to be beneficial in risk prediction models. However, to be exact, for both pro-ENK and suPAR, the C-statistics did not show statistical significance, and purely based on the *P*-values when adding the pro-ENK or suPAR to the risk model with conventional risk markers did not majorly seem to improve the discrimination. Of note, for pro-ENK the *P*-value was marginally significant (*P*=0.08). Nevertheless, in all studies (I-IV) the continuous NRI analyses indicated that adding the respective biomarker helped to correctly reclassify participants into the correct risk direction.

Therefore, it needs to be pointed out that there are methodological particularities in ROC-curve analysis and NRI-Index, which need to be kept in mind when interpreting the results. ROC-curve is commonly used to evaluate how well a test or a model can distinguish between a diseased and a non-diseased status (CKD in our study). This may bear some limitations. The effect on the change in AUC depends on both the predictive ability of the “traditional risk model”, the strengths of the new marker and the correlations between these two. Thus, assessing the additional impact of adding a new predictor in comparison to an existing model is often insensitive.^{159,160} The purpose of reclassification is different. The NRI-Index has the ability to determine how many individuals would be classified into the clinically relevant risk strata, which therefore directly compares the clinical impact of two models.¹⁶⁰

Similar to study I-III. we have tested if adding a GRS, including 53 SNP previously associated with eGFR_{crea} in GWAS,¹³ improved the prediction model for incidence of CKD in study IV. However, adding the GRS_{CKD} to a logistic regression model including commonly used risk factors did not lead to a significantly improved C-statistics.

The heritability of eGFR has been estimated to range between 36–75%,^{161,162} yet today the phenotypic variance explained by the 53 identified SNPs only is around 3.2%.¹³ Considering that all these SNPs are common, with minor allele frequencies above 5%, the discovery of less frequent variants with higher effect sizes could potentially explain a greater variance in eGFR,¹⁶ and improve prediction of future kidney function. A very recent study including over 110,000 adult individuals identified 10 novel genome-wide significant loci in a meta-analysis of GWAS using the 1000Genome imputed genotypes, which enhanced the coverage of the genomic variation.¹⁴ Nonetheless, of the identified 10 novel variants all but one were common and the variance of eGFR explained when added together with the earlier 53 variants was only slightly increased yet remained under 4%.¹⁴

It is interesting that investigation in the Framing Heart Study have shown that a GRS similar to the ones we used, including 53 SNPs, did significantly associate with incidence of CKD stage 3.¹⁶ However, previous investigations using a GRS consisting of 16 SNPs showed a borderline significant association with incidence of CKD stage 3 in the same cohort.¹⁵

However, if adding these newly identified variants¹⁴ may improve prediction remains to be investigated.

Dietary intake and kidney function in relation to the current literature (study V)

Increased protein intake has been shown to associate with adverse kidney outcomes in rats.¹⁶³ Yet, the results from the so far largest clinical trial The Modification of Diet in Renal Disease (MDRD) Study have been less conclusive.¹⁶⁴ Therefore, our observations that higher protein intake was associated with a less decline in eGFR and a tendency for a lower risk of incident CKD, were rather surprising. Our observations deserve some further clarification as this relationship seems complex. Cautious interpretation is requested, given that the association between higher intake of red meat and less decline of eGFR was only significant in the final fully adjusted model but not in the less adjusted models. In addition, for intakes of both total protein and red meat, the association vanished when we adjusted for the intake of sucrose, and no associations were observed with non-processed red meat nor processed meat or meat products. Our results differ from some earlier reports. Earlier studies in the ARIC cohort of 14,882 individuals reported an association between higher risk for kidney diseases and higher intake of red- and processed meat.⁸⁴ Additionally, an increased risk for end stage renal disease (ESRD, 951 cases) associated with increased consumption of total protein and red meat in a recent study including 63,257 participants from the Singapore Chinese Health Study.¹⁶⁵ More studies on how protein intake may impact kidney function in individuals with normal kidney function are warranted.

Last but not least, it needs to be highlighted that the KDIGO 2012 CKD Guideline recommends expert dietary advice tailored to the severity of CKD for individuals with CKD and if indicated, to intervene on salt, phosphate, potassium, and protein intakes.¹⁸ In addition, for adults with CKD stages 4 and 5, regardless of diabetes status, lowering the protein intake to $\leq 0.8\text{g/kg/d}$ including an appropriate education is suggested.¹⁸ Further, high protein intake ($>1.3\text{ g/kg/d}$) is suggested to be avoided by adults at risk for progression of CKD.¹⁸

Today, no direct recommendations in terms of carbohydrate intake *per se* are included in the current KDIGO 2012 CKD Guideline.¹⁸ No association between total carbohydrate intake and kidney function was observed in our study. In line with this, the Australian Blue Mountain Eye Study did not observe any association between carbohydrate intake and prevalence of CKD, nor did the dietary glycemic index at baseline influence the occurrence of CKD after 5 years.¹⁶⁶ Yet, there was a trend for higher incidence of CKD with increasing intake of sugar.¹⁶⁶ Likewise, we observed that higher sucrose intake associated with a greater decline in eGFR and a tendency for increased risk of incident CKD in the MDCS-CC.

Our prospective study provides novel evidence for that higher fiber intake associates with a better longitudinal kidney function, which is in line with the recently reported findings that high fiber intake associates with a better kidney function and decreased prevalence of CKD in cross-sectional analyses.^{167,168} In line with this, the DASH dietary

pattern, which is rich in fiber, has been shown to associate with a lower risk for kidney disease⁸⁴.

Moreover, we observed a lesser decline of eGFR over time and decreased risk of incident CKD with increasing coffee intake. Similar to our results, a couple of studies have reported cross-sectional associations between higher coffee intake and higher eGFR.¹⁶⁹⁻¹⁷² However, our study is the first to report a longitudinal relationship between coffee consumption and kidney function.

The results from MDSCS-CC with detailed information on dietary intake underlines the importance of dietary factors for longitudinal kidney function. The current knowledge on of the relationship between diet intakes and long-term kidney function is limited and more studies in individuals with a preserved kidney function are needed.

Strengths and Limitations

Some aspects regarding the endpoints investigated in this PhD thesis deserve further discussion. The primary outcomes of the studies in this thesis were decline in eGFR and occurrence of CKD stage 3A at follow-up re-examination. We used two different equations to estimate eGFR in our studies. In studies I, II and V we used the CKD-EPI 2012 equation based on both creatinine and cystatin C,⁵² while in studies II and IV we used the CKD-EPI equation based on creatinine alone.⁵¹ The rationale of using the creatinine-based equation instead of the most recent equation for estimating GFR⁵² was that we intended to use the same equation as Hayek et al.²⁴ (study II) and because the SNPs included in the GRS_{CKD} in study IV were originally identified to be associated with eGFR_{crea}.¹³

It can be argued that measuring GFR would have been ideal, yet given the sample size of the MDSCS cohort this had not been feasible. Moreover, GFR is not trivial to measure, as discussed in chapter one.⁴³ Therefore, creatinine as well as cystatin C have been utilized as proxies to describe kidney function and are included in equations to estimate GFR. So far, they are the most commonly used biomarkers, yet, both carry some limitations as already discussed in the introduction.⁴³ Thus, new biomarkers to predict kidney function particularly in the long-term, would be desired.²¹ Therefore, this thesis was focused on investigating novel circulating biomarkers and their clinical potential. However, there are some limitations which warrant some further clarification when studying the association between potential biomarkers with kidney function. As discussed in the first chapter, CKD is classified by at least two factors: GFR and albuminuria.² Yet, in all five studies included in this thesis solely eGFR was used for the definition of CKD. Lack of assessing albuminuria certainly is a key limitation in the assessment of CKD stages 1 and 2, yet the primary outcome of the studies was CKD stage 3A (eGFR < 60 mL/min/1.73m²). Unfortunately, no urinary samples from the MDSCS baseline examination were available. In addition, the current KDIGO 2012 guidelines require an eGFR < 60 mL/min/1.73m² for > 3 months to confirm CKD.¹⁸ In the MDSCS-CC, creatinine and cystatin C were measured at two time points, and more measurements would have been desired, but due to only one follow-up visit, this was

not possible to obtain. However, this study has a relatively long follow-up time of on average 16 years, which thus may increase the confidence in assessing CKD progression.¹⁸

In addition, in studies II and III our secondary outcome was incidence of hospitalization due to impaired kidney function. This outcome was obtained from the Swedish registry data and was independent of the CKD definition at the follow-up re-examination, but nonetheless supported the results in studies II¹⁴⁴ and III. The Swedish patient register covers all hospitalizations in Sweden since 1987 in addition to hospital outpatient visits from 2001 onwards and has been described and validated for outcome classification previously.¹³⁴

It might be arguable that classifying CKD as an eGFR $<60\text{mL}/\text{min}/1.73\text{m}^2$ to define incident CKD may explain the fairly high incidence rate of around 30% in the MDCS-CC. It has been questioned earlier if defining incidence of CKD as an eGFR $<60\text{mL}/\text{min}/1.73\text{m}^2$ may be clinically significant in the elderly.¹⁷³ Yet, as mentioned, we used outcomes from several sources and it needs to be underlined that the associations between the biomarkers and hospitalization due to impaired kidney function were comparably strong in studies II¹⁴⁴ and III. So, even if an overestimation of the incidence rate may have occurred this does not seem to have affected the conclusions of our studies.

We adjusted our analyses for commonly known risk factors for kidney function, such as sex, BMI, systolic blood pressure, AHT, SBP and smoking. Although we have presented rather convincing evidence for that the three biomarkers pro-ENK, suPAR and KIM-1, associate with kidney function, residual confounding cannot be entirely excluded. However, we thoughtfully investigated the relationships between the corresponding biomarkers and kidney function. We acknowledge that several associations between the investigated biomarkers and baseline characteristics were observed, others than those with eGFR. Therefore, we approached with mutual adjustment of these analyses, yet this did not appear to majorly influence the results of the cross-sectional analyses in studies I-III (data shown in the supplementary of the appended manuscripts).

In study V, dietary intake is assessed via a modified diet history, which besides an interview of 1h, included a self-administrated 7-day menu book and an 168-item questionnaire.¹³¹ It deserves to be pointed out that the diet-history, which has been especially developed for the MDCS, showed adequate relative validity in the Malmö Food study, which was prior to MDCS baseline compared with a 18-d weighted-food record.^{132,133} However, no data on salt intake is available in MDCS, which made it impossible to account for this key variable.

Many factors may need to be considered when studying diet as an exposure for a disease. Therefore, we have adjusted our analysis for known factors that could influence the results: such as total energy intake, season, physical activity, education and alcohol intake, in addition to known kidney function risk markers. Furthermore, in MDCS it has

previously been observed that changes in food habits relate to obesity and other lifestyle and socio-economic factors and thus might influence the relationship between diet and diseases outcome.¹⁴⁰ Therefore, we conducted a sensitivity analyses excluding the participants (24%) that had reported to have changed their diet prior to MDCS-baseline examination. However, this did not majorly influence the results.

Summary and conclusions

Today, CKD has become a major public health issue,² with a high burden of comorbidities and increased risk for mortality.⁶ Given that early identification of individuals at increased risk could allow to intervene earlier in the disease process,³⁴ and that the commonly used biomarkers (i.e. creatinine, eGFR and albuminuria) are rather insensitive,²¹ three papers of this thesis focused primarily on investigating potential novel biomarkers for kidney function.

The results of papers I to III can be summarized as follows:

To the best of our knowledge, we were the first to provide evidence for that the investigated biomarkers associate with decline in kidney function and risk of incident CKD in a general population of apparently healthy middle-aged participants.

In paper I we have shown that high plasma levels of pro-ENK may be an early marker for longitudinal deterioration of kidney function, and provided evidence for that it may be causally linked to kidney function using a MR-approach.

In paper II we have shown that high plasma suPAR levels predict longitudinal decline in kidney function and associate with hospitalization due to impaired renal function. Moreover, we were able to show that suPAR may be useful in clinical prediction in addition to conventional risk factors.

In paper III we observed that higher plasma KIM-1 levels may serve as a biomarker for longitudinal kidney function. Moreover, NRI-Index analysis suggested that it may add useful information to a prediction model in addition to commonly used risk factors.

In paper IV we aimed to investigate if genetic predisposition for decreased kidney function, captured by a GRS consisting of 53 SNPs previously associated with eGFR_{crea} in GWAS,¹³ may be useful in prediction of risk of incident CKD. We observed that an increased number of risk-alleles associated with a higher risk of incident CKD, and that adding the GRS_{CKD} to a risk model aided in re-classification of study participants into the correct risk direction.

In paper V we investigated association between dietary intakes and long-term kidney function, with a focus on protein intake. We observed that reported higher intake of sucrose associated with decreased kidney function while reported higher intakes of fiber and coffee associated with favorable kidney function. No evidence could be found for that protein intake or intake of meat would associate with deterioration of kidney function by time.

The results of this thesis point out the need to investigate further the studied biomarkers, in particular concerning the underlying mechanisms by which they may affect kidney function. Moreover, the current knowledge on the genetic underpinning of CKD is still limited, as upon today less than 4% of the phenotypic variance in eGFR_{crea} can be explained by the 53 genetic variants that were investigated. Last but not least, the current KDIGO 2012 CKD guideline includes dietary recommendations for patients with CKD. Yet, not much is known about how dietary intakes may effect long-term kidney function in individuals with a preserved kidney function, calling for more studies in this topic in the future.

Thus, given the background of this PhD-thesis as well as the data presented, it can be finally concluded that the circulating biomarkers (pro-ENK, suPAR, and KIM-1) as well as genetic predisposition may be useful in identification of individuals with an increased risk for decline of kidney function in the future. In addition, the importance of dietary intakes in kidney function of healthy individuals has been highlighted.

References

1. Constitution of the World Health Organization. *Am J Public Health Nations Health* 1946;36:1315-23.
2. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY, Yang CW. Chronic kidney disease: global dimension and perspectives. *Lancet* 2013;382:260-72.
3. Coresh J. Update on the Burden of CKD. *Journal of the American Society of Nephrology : JASN* 2017;28:1020-2.
4. DALYs GBD, Collaborators H. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388:1603-58.
5. Global Burden of Disease Study C. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015;386:743-800.
6. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *The New England journal of medicine* 2004;351:1296-305.
7. Calzia E, IZ, Radermacher P. (2005) Determinants of Blood Flow and Organ Perfusion. In: Pinsky M.R., Payen D. (eds) *Functional Hemodynamic Monitoring. Update in Intensive Care and Emergency Medicine*, vol 42. Springer, Berlin, Heidelberg.
8. Eckardt KU, Coresh J, Devuyst O, Johnson RJ, Kottgen A, Levey AS, Levin A. Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet* 2013;382:158-69.
9. Levin A, Lancashire W, Fassett RG. Targets, trends, excesses, and deficiencies: refocusing clinical investigation to improve patient outcomes. *Kidney international* 2013;83:1001-9.
10. Nicholson JK, Lindon JC. Systems biology: Metabonomics. *Nature* 2008;455:1054-6.
11. Rehberg PB. Studies on Kidney Function: The Rate of Filtration and Reabsorption in the Human Kidney. *The Biochemical journal* 1926;20:447-60.
12. Fox CS, Yang Q, Cupples LA, Guo CY, Larson MG, Leip EP, Wilson PW, Levy D. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: the Framingham Heart Study. *Journal of the American Society of Nephrology : JASN* 2004;15:2457-61.
13. Pattaro C, Teumer A, Gorski M, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nature communications* 2016;7:10023.

14. Gorski M, van der Most PJ, Teumer A, et al. 1000 Genomes-based meta-analysis identifies 10 novel loci for kidney function. *Scientific reports* 2017;7:45040.
15. O'Seaghdha CM, Yang Q, Wu H, Hwang SJ, Fox CS. Performance of a genetic risk score for CKD stage 3 in the general population. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2012;59:19-24.
16. Ma J, Yang Q, Hwang SJ, Fox CS, Chu AY. Genetic risk score and risk of stage 3 chronic kidney disease. *BMC nephrology* 2017;18:32.
17. Tiret L. Gene-environment interaction: a central concept in multifactorial diseases. *The Proceedings of the Nutrition Society* 2002;61:457-63.
18. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney international* 2014;85:49-61.
19. Plantinga LC, Boulware LE, Coresh J, Stevens LA, Miller ER, 3rd, Saran R, Messer KL, Levey AS, Powe NR. Patient awareness of chronic kidney disease: trends and predictors. *Arch Intern Med* 2008;168:2268-75.
20. Levey AS, Stevens LA, Coresh J. Conceptual model of CKD: applications and implications. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009;53:S4-16.
21. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. *Kidney international* 2011;80:806-21.
22. Ng LL, Sandhu JK, Narayan H, Quinn PA, Squire IB, Davies JE, Bergmann A, Maisel A, Jones DJ. Proenkephalin and prognosis after acute myocardial infarction. *Journal of the American College of Cardiology* 2014;63:280-9.
23. Marino R, Struck J, Hartmann O, Maisel AS, Rehfeldt M, Magrini L, Melander O, Bergmann A, Di Somma S. Diagnostic and short-term prognostic utility of plasma pro-enkephalin (pro-ENK) for acute kidney injury in patients admitted with sepsis in the emergency department. *Journal of nephrology* 2014.
24. Hayek SS, Sever S, Ko YA, Trachtman H, Awad M, Wadhvani S, Altintas MM, Wei C, Hotton AL, French AL, Sperling LS, Lerakis S, Quyyumi AA, Reiser J. Soluble Urokinase Receptor and Chronic Kidney Disease. *The New England journal of medicine* 2015;373:1916-25.
25. Hayek SS, Koh KH, Grams ME, et al. A tripartite complex of suPAR, APOL1 risk variants and alphavbeta3 integrin on podocytes mediates chronic kidney disease. *Nature medicine* 2017.
26. van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. *The Journal of pathology* 2007;212:209-17.
27. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney international* 2002;62:237-44.
28. Marieb E N, Hoehn K N. *Human Anatomy & Physiology, Global Edition*. Harlow, United Kingdom: Pearson Education Limited, 2015.
29. Boron W BEMP, 2e Updated Edition. Philadelphia, PA. Saunders Elsevier, 2012.
30. Drake R, Vogl A W, Mitchell A W M. *Gray's Anatomy for Students 3rd Edition*. Philadelphia, PA: Elsevier Churchill Livingstone , 2015.

31. Rhoades A, Bell D R. Medical physiology : principles for clinical medicine. Baltimore, MD: Lippincott Williams & Wilkins; 2009.
32. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney international* 2005;67:2089-100.
33. Levey AS, Coresh J. Chronic kidney disease. *Lancet* 2012;379:165-80.
34. Levey AS, Schoolwerth AC, Burrows NR, Williams DE, Stith KR, McClellan W, Centers for Disease C, Prevention Expert P. Comprehensive public health strategies for preventing the development, progression, and complications of CKD: report of an expert panel convened by the Centers for Disease Control and Prevention. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009;53:522-35.
35. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA : the journal of the American Medical Association* 2007;298:2038-47.
36. James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. *Lancet* 2010;375:1296-309.
37. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G, National Kidney F. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Annals of internal medicine* 2003;139:137-47.
38. Levin A, Hemmelgarn B, Culeton B, Tobe S, McFarlane P, Ruzicka M, Burns K, Manns B, White C, Madore F, Moist L, Klarenbach S, Barrett B, Foley R, Jindal K, Senior P, Pannu N, Shurraw S, Akbari A, Cohn A, Reslerova M, Deved V, Mendelssohn D, Nesrallah G, Kappel J, Tonelli M, Canadian Society of N. Guidelines for the management of chronic kidney disease. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2008;179:1154-62.
39. Crowe E, Halpin D, Stevens P, Guideline Development G. Early identification and management of chronic kidney disease: summary of NICE guidance. *Bmj* 2008;337:a1530.
40. Bruck K, Stel VS, Gambaro G, et al. CKD Prevalence Varies across the European General Population. *Journal of the American Society of Nephrology : JASN* 2016;27:2135-47.
41. Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, Fox CS, Gansevoort RT, Heerspink HJL, Jardine M, Kasiske B, Kottgen A, Kretzler M, Levey AS, Luyckx VA, Mehta R, Moe O, Obrador G, Pannu N, Parikh CR, Perkovic V, Pollock C, Stenvinkel P, Tuttle KR, Wheeler DC, Eckardt KU, participants ISNGKHS. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. *Lancet* 2017.
42. Glasscock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nat Rev Nephrol* 2017;13:104-14.
43. Hsu CY, Bansal N. Measured GFR as "gold standard"--all that glitters is not gold? *Clinical journal of the American Society of Nephrology : CJASN* 2011;6:1813-4.
44. Georg Thieme Verlag KG. Kreatinin [Internet]. 2017 [Accessed 20th October 2017]. Available from <https://roempp.thieme.de/roempp4.0/do/data/RD-11-02075>

45. Abrahamson M, Olafsson I, Palsdottir A, Ulvsback M, Lundwall A, Jensson O, Grubb A. Structure and expression of the human cystatin C gene. *The Biochemical journal* 1990;268:287-94.
46. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clinical chemistry* 1994;40:1921-6.
47. Randers E, Erlandsen EJ. Serum cystatin C as an endogenous marker of the renal function--a review. *Clinical chemistry and laboratory medicine : CCLM / FESCC* 1999;37:389-95.
48. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31-41.
49. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of internal medicine* 1999;130:461-70.
50. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F, Chronic Kidney Disease Epidemiology C. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Annals of internal medicine* 2006;145:247-54.
51. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, Ckd EPI. A new equation to estimate glomerular filtration rate. *Annals of internal medicine* 2009;150:604-12.
52. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS, Investigators C-E. Estimating glomerular filtration rate from serum creatinine and cystatin C. *The New England journal of medicine* 2012;367:20-9.
53. Weinstein JR, Anderson S. The aging kidney: physiological changes. *Advances in chronic kidney disease* 2010;17:302-7.
54. Davies DF, Shock NW. Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. *The Journal of clinical investigation* 1950;29:496-507.
55. World Health Organization. Riskfactors [Internet]. 2017 [Accessed 25th June 2017]. Available from: http://www.who.int/topics/risk_factors/en/
56. McMahon GM, Hwang SJ, Fox CS. Residual lifetime risk of chronic kidney disease. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2016.
57. Collins AJ, Foley RN, Chavers B, et al. 'United States Renal Data System 2011 Annual Data Report: Atlas of chronic kidney disease & end-stage renal disease in the United States. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2012;59:A7, e1-420.
58. Sandholm N, Van Zuydam N, Ahlqvist E, et al. The Genetic Landscape of Renal Complications in Type 1 Diabetes. *Journal of the American Society of Nephrology : JASN* 2017;28:557-74.

59. Schefold JC, Filippatos G, Hasenfuss G, Anker SD, von Haehling S. Heart failure and kidney dysfunction: epidemiology, mechanisms and management. *Nat Rev Nephrol* 2016;12:610-23.
60. Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *Journal of internal medicine* 2010;268:456-67.
61. 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.
62. Collins AJ, Couser WG, Dirks JH, Kopple JD, Reiser T, Riella MC, Robinson S, Shah SV, Wilson A. World Kidney Day: an idea whose time has come. *Journal of the American Society of Nephrology : JASN* 2006;17:600-1.
63. Turnpenny P D, Ellard S. *Emery's Elements of Medical Genetics*. Philadelphia, PA: Elsevier Churchill Livingstone; 2011. .
64. Watson JD, Crick FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* 1953;171:737-8.
65. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921.
66. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science* 2001;291:1304-51.
67. International Human Genome Sequencing C. Finishing the euchromatic sequence of the human genome. *Nature* 2004;431:931-45.
68. Moulin F, Ponte B, Pruijm M, Ackermann D, Bouatou Y, Guessous I, Ehret G, Bonny O, Pechere-Bertschi A, Staessen JA, Paccaud F, Martin PY, Burnier M, Vogt B, Devuyst O, Bochud M. A population-based approach to assess the heritability and distribution of renal handling of electrolytes. *Kidney international* 2017.
69. Keller BJ, Martini S, Sedor JR, Kretzler M. A systems view of genetics in chronic kidney disease. *Kidney international* 2012;81:14-21.
70. Smyth LJ, Duffy S, Maxwell AP, McKnight AJ. Genetic and epigenetic factors influencing chronic kidney disease. *American journal of physiology Renal physiology* 2014;307:F757-76.
71. Kottgen A. Genome-wide association studies in nephrology research. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2010;56:743-58.
72. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308:385-9.
73. National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI). GWAS catalogue [Internet]. 2017 [Accessed August 10th 2017]. Available from: <https://www.ebi.ac.uk/gwas/>
74. Wuttke M, Kottgen A. Insights into kidney diseases from genome-wide association studies. *Nat Rev Nephrol* 2016;12:549-62.
75. Hwang SJ, Yang Q, Meigs JB, Pearce EN, Fox CS. A genome-wide association for kidney function and endocrine-related traits in the NHLBI's Framingham Heart Study. *BMC medical genetics* 2007;8 Suppl 1:S10.

76. Kottgen A, Kao WH, Hwang SJ, Boerwinkle E, Yang Q, Levy D, Benjamin EJ, Larson MG, Astor BC, Coresh J, Fox CS. Genome-wide association study for renal traits in the Framingham Heart and Atherosclerosis Risk in Communities Studies. *BMC medical genetics* 2008;9:49.
77. Kottgen A, Glazer NL, Dehghan A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. *Nature genetics* 2009;41:712-7.
78. Kottgen A, Pattaro C, Boger CA, et al. New loci associated with kidney function and chronic kidney disease. *Nature genetics* 2010;42:376-84.
79. Li M, Li Y, Weeks O, et al. SOS2 and ACP1 Loci Identified through Large-Scale Exome Chip Analysis Regulate Kidney Development and Function. *Journal of the American Society of Nephrology : JASN* 2017;28:981-94.
80. Pattaro C, Kottgen A, Teumer A, et al. Genome-wide association and functional follow-up reveals new loci for kidney function. *PLoS genetics* 2012;8:e1002584.
81. Gorski M, Tin A, Garnaas M, et al. Genome-wide association study of kidney function decline in individuals of European descent. *Kidney international* 2015;87:1017-29.
82. National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI). GWAS Catalogue Diagram [Internet]. 2017 [Accessed August 10th 2017]. Available from: <https://www.ebi.ac.uk/gwas/diagram>
83. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk to disease from genome-wide association studies. *Genome research* 2007;17:1520-8.
84. Rebholz CM, Crews DC, Grams ME, Steffen LM, Levey AS, Miller ER, 3rd, Appel LJ, Coresh J. DASH (Dietary Approaches to Stop Hypertension) Diet and Risk of Subsequent Kidney Disease. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2016;68:853-61.
85. Foster MC, Hwang SJ, Massaro JM, Jacques PF, Fox CS, Chu AY. Lifestyle factors and indices of kidney function in the Framingham Heart Study. *American journal of nephrology* 2015;41:267-74.
86. Ma J, Jacques PF, Hwang SJ, Troy LM, McKeown NM, Chu AY, Fox CS. Dietary Guideline Adherence Index and Kidney Measures in the Framingham Heart Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2016;68:703-15.
87. Effects of dietary protein restriction on the progression of moderate renal disease in the Modification of Diet in Renal Disease Study. *Journal of the American Society of Nephrology : JASN* 1996;7:2616-26.
88. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics* 2001;69:89-95.
89. IARC International Agency for Research on Cancer. Rothman N, Hainaut P, Schulte P, Smith M, Boffetta P, Perera F. *Molecular Epidemiology: Principles and Practices*. Lyon, France: IARC International Agency for Research on Cancer; 2011.
90. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx* 2004;1:182-8.
91. Srivastava S, Gopal-Srivastava R. Biomarkers in cancer screening: a public health perspective. *The Journal of nutrition* 2002;132:2471S-5S.

92. Wu I, Parikh CR. Screening for kidney diseases: older measures versus novel biomarkers. *Clinical journal of the American Society of Nephrology : CJASN* 2008;3:1895-901.
93. Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 1975;258:577-80.
94. National Center for Biotechnology Information. PENK proenkephalin [Homo sapiens (human)] [Internet]. 2017 [Accessed August 12th 2017]. Available from: <https://www.ncbi.nlm.nih.gov/gene/5179>
95. Denning GM, Ackermann LW, Barna TJ, Armstrong JG, Stoll LL, Weintraub NL, Dickson EW. Proenkephalin expression and enkephalin release are widely observed in non-neuronal tissues. *Peptides* 2008;29:83-92.
96. Hook V, Funkelstein L, Lu D, Bark S, Wegrzyn J, Hwang SR. Proteases for processing proneuropeptides into peptide neurotransmitters and hormones. *Annu Rev Pharmacol Toxicol* 2008;48:393-423.
97. Lu WD, Funkelstein L, Toneff T, Reinheckel T, Peters C, Hook V. Cathepsin H functions as an aminopeptidase in secretory vesicles for production of enkephalin and galanin peptide neurotransmitters. *Journal of neurochemistry* 2012;122:512-22.
98. Funkelstein L, Hook V. The novel role of cathepsin L for neuropeptide production illustrated by research strategies in chemical biology with protease gene knockout and expression. *Methods in molecular biology* 2011;768:107-25.
99. Johannig K, Juliano MA, Juliano L, Lazure C, Lamango NS, Steiner DF, Lindberg I. Specificity of prohormone convertase 2 on proenkephalin and proenkephalin-related substrates. *The Journal of biological chemistry* 1998;273:22672-80.
100. Peinado JR, Li H, Johannig K, Lindberg I. Cleavage of recombinant proenkephalin and blockade mutants by prohormone convertases 1 and 2: an in vitro specificity study. *Journal of neurochemistry* 2003;87:868-78.
101. Slominski AT, Zmijewski MA, Zbytek B, Brozyna AA, Granese J, Pisarchik A, Szczesniwski A, Tobin DJ. Regulated proenkephalin expression in human skin and cultured skin cells. *The Journal of investigative dermatology* 2011;131:613-22.
102. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol* 2010;11:23-36.
103. Wei C, Moller CC, Altintas MM, Li J, Schwarz K, Zacchigna S, Xie L, Henger A, Schmid H, Rastaldi MP, Cowan P, Kretzler M, Parrilla R, Bendayan M, Gupta V, Nikolic B, Kalluri R, Carmeliet P, Mundel P, Reiser J. Modification of kidney barrier function by the urokinase receptor. *Nature medicine* 2008;14:55-63.
104. Yoo TH, Pedigo CE, Guzman J, Correa-Medina M, Wei C, Villarreal R, Mitrofanova A, Leclercq F, Faul C, Li J, Kretzler M, Nelson RG, Lehto M, Forsblom C, Groop PH, Reiser J, Burke GW, Fornoni A, Merscher S. Sphingomyelinase-like phosphodiesterase 3b expression levels determine podocyte injury phenotypes in glomerular disease. *Journal of the American Society of Nephrology : JASN* 2015;26:133-47.
105. Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maiguel D, Karumanchi SA, Yap HK, Saleem M, Zhang Q, Nikolic B, Chaudhuri A, Daftarian P, Salido E, Torres A, Salifu M, Sarwal MM, Schaefer F, Morath C, Schwenger V, Zeier M, Gupta V, Roth D, Rastaldi MP, Burke G, Ruiz P, Reiser J. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nature medicine* 2011;17:952-60.

106. Wei C, Trachtman H, Li J, Dong C, Friedman AL, Gassman JJ, McMahan JL, Radeva M, Heil KM, Trautmann A, Anarat A, Emre S, Ghiggeri GM, Ozaltin F, Haffner D, Gipson DS, Kaskel F, Fischer DC, Schaefer F, Reiser J, PodoNet, Consortia FCS. Circulating suPAR in two cohorts of primary FSGS. *Journal of the American Society of Nephrology* : JASN 2012;23:2051-9.
107. Borne Y, Persson M, Melander O, Smith JG, Engstrom G. Increased plasma level of soluble urokinase plasminogen activator receptor is associated with incidence of heart failure but not atrial fibrillation. *Eur J Heart Fail* 2014;16:377-83.
108. Edsfeldt A, Nitulescu M, Grufman H, Gronberg C, Persson A, Nilsson M, Persson M, Bjorkbacka H, Goncalves I. Soluble urokinase plasminogen activator receptor is associated with inflammation in the vulnerable human atherosclerotic plaque. *Stroke; a journal of cerebral circulation* 2012;43:3305-12.
109. Persson M, Ostling G, Smith G, Hamrefors V, Melander O, Hedblad B, Engstrom G. Soluble urokinase plasminogen activator receptor: a risk factor for carotid plaque, stroke, and coronary artery disease. *Stroke; a journal of cerebral circulation* 2014;45:18-23.
110. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, Schultz MJ. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. *Intensive care medicine* 2012;38:1418-28.
111. de Bock CE, Wang Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. *Med Res Rev* 2004;24:13-39.
112. Angiari S, Constantin G. Regulation of T cell trafficking by the T cell immunoglobulin and mucin domain 1 glycoprotein. *Trends Mol Med* 2014;20:675-84.
113. GTEx portal *hepatitis A virus cellular receptor 1 gene expression* Available from: <http://www.gtexportal.org/home/gene/HAVCR1> [Accessed 29th May 2017].
114. Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, Sanicola M. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *The Journal of biological chemistry* 1998;273:4135-42.
115. Humphreys BD, Xu F, Sabbisetti V, Grgic I, Movahedi Naini S, Wang N, Chen G, Xiao S, Patel D, Henderson JM, Ichimura T, Mou S, Soeung S, McMahan AP, Kuchroo VK, Bonventre JV. Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. *The Journal of clinical investigation* 2013;123:4023-35.
116. Nielsen SE, Andersen S, Zdunek D, Hess G, Parving HH, Rossing P. Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy. *Kidney international* 2011;79:1113-8.
117. Nielsen SE, Reinhard H, Zdunek D, Hess G, Gutierrez OM, Wolf M, Parving HH, Jacobsen PK, Rossing P. Tubular markers are associated with decline in kidney function in proteinuric type 2 diabetic patients. *Diabetes research and clinical practice* 2012;97:71-6.
118. Vaidya VS, Niewczasz MA, Ficociello LH, Johnson AC, Collings FB, Warram JH, Krolewski AS, Bonventre JV. Regression of microalbuminuria in type 1 diabetes is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and N-acetyl-beta-D-glucosaminidase. *Kidney international* 2011;79:464-70.
119. Hsu CY, Xie D, Waikar SS, Bonventre JV, Zhang X, Sabbisetti V, Mifflin TE, Coresh J, Diamantidis CJ, He J, Lora CM, Miller ER, Nelson RG, Ojo AO, Rahman M, Schelling JR, Wilson FP, Kimmel PL, Feldman HI, Vasan RS, Liu KD, Investigators CS,

- Consortium CKDB. Urine biomarkers of tubular injury do not improve on the clinical model predicting chronic kidney disease progression. *Kidney international* 2016.
120. Panduru NM, Sandholm N, Forsblom C, Saraheimo M, Dahlstrom EH, Thorn LM, Gordin D, Tolonen N, Waden J, Harjutsalo V, Bierhaus A, Humpert PM, Groop PH, FinnDiane Study G. Kidney injury molecule-1 and the loss of kidney function in diabetic nephropathy: a likely causal link in patients with type 1 diabetes. *Diabetes care* 2015;38:1130-7.
 121. Nowak N, Skupien J, Niewczas MA, Yamanouchi M, Major M, Croall S, Smiles A, Warram JH, Bonventre JV, Krolewski AS. Increased plasma kidney injury molecule-1 suggests early progressive renal decline in non-proteinuric patients with type 1 diabetes. *Kidney international* 2016;89:459-67.
 122. Coca SG, Nadkarni GN, Huang Y, Moledina DG, Rao V, Zhang J, Ferket B, Crowley ST, Fried LF, Parikh CR. Plasma Biomarkers and Kidney Function Decline in Early and Established Diabetic Kidney Disease. *Journal of the American Society of Nephrology : JASN* 2017.
 123. Sabbiseti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL, Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS, Bonventre JV. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *Journal of the American Society of Nephrology : JASN* 2014;25:2177-86.
 124. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, Mattisson I, Berglund G. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation* 2001;10:489-99.
 125. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. *Journal of internal medicine* 1993;233:45-51.
 126. Rosvall M, Persson M, Ostling G, Nilsson PM, Melander O, Hedblad B, Engstrom G. 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;239:615-21.
 127. Pero RW, Olsson A, Berglund G, Janzon L, Larsson SA, Elmstahl S. The Malmo biological bank. *Journal of internal medicine* 1993;233:63-7.
 128. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012;491:56-65.
 129. O'Connell J, Gurdasani D, Delaneau O, Pirastu N, Ulivi S, Cocca M, Traglia M, Huang J, Huffman JE, Rudan I, McQuillan R, Fraser RM, Campbell H, Polasek O, Asiki G, Ekoru K, Hayward C, Wright AF, Vitart V, Navarro P, Zagury JF, Wilson JF, Toniolo D, Gasparini P, Soranzo N, Sandhu MS, Marchini J. A general approach for haplotype phasing across the full spectrum of relatedness. *PLoS genetics* 2014;10:e1004234.
 130. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS genetics* 2009;5:e1000529.
 131. Callmer E, Riboli E, Saracci R, Akesson B, Lindgarde F. Dietary assessment methods evaluated in the Malmo food study. *Journal of internal medicine* 1993;233:53-7.
 132. Elmstahl S, Riboli E, Lindgarde F, Gullberg B, Saracci R. The Malmo Food Study: the relative validity of a modified diet history method and an extensive food frequency

- questionnaire for measuring food intake. *European journal of clinical nutrition* 1996;50:143-51.
133. Riboli E, Elmstahl S, Saracci R, Gullberg B, Lindgarde F. The Malmo Food Study: validity of two dietary assessment methods for measuring nutrient intake. *International journal of epidemiology* 1997;26 Suppl 1:S161-73.
 134. Ludvigsson JF, Andersson E, Ekblom A, Feychting M, Kim JL, Reuterwall C, Heurgren M, Olausson PO. External review and validation of the Swedish national inpatient register. *BMC public health* 2011;11:450.
 135. Ernst A, Kohrle J, Bergmann A. Proenkephalin A 119-159, a stable proenkephalin A precursor fragment identified in human circulation. *Peptides* 2006;27:1835-40.
 136. Kofoed K, Schneider UV, Scheel T, Andersen O, Eugen-Olsen J. Development and validation of a multiplex add-on assay for sepsis biomarkers using xMAP technology. *Clinical chemistry* 2006;52:1284-93.
 137. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson AC, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS one* 2014;9:e95192.
 138. OLINK Kidney Injury Molecule (KIM1) Available from: <http://www.olink.com/products/cvd-ii-panel/biomarkers/?biomarkerId=426> [Accessed 7th June 2017].
 139. Orho-Melander M. Genetics of coronary heart disease: towards causal mechanisms, novel drug targets and more personalized prevention. *Journal of internal medicine* 2015;278:433-46.
 140. Sonestedt E, Wirfalt E, Gullberg B, Berglund G. Past food habit change is related to obesity, lifestyle and socio-economic factors in the Malmo Diet and Cancer Cohort. *Public Health Nutr* 2005;8:876-85.
 141. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Statistics in medicine* 2008;27:157-72; discussion 207-12.
 142. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics* 2007;81:559-75.
 143. Schulz CA, Christensson A, Ericson U, Almgren P, Hindy G, Nilsson PM, Struck J, Bergmann A, Melander O, Orho-Melander M. High Level of Fasting Plasma Proenkephalin-A Predicts Deterioration of Kidney Function and Incidence of CKD. *Journal of the American Society of Nephrology : JASN* 2017;28:291-303.
 144. Schulz CA, Persson M, Christensson A, Hindy G, Almgren P, Nilsson PM, Melander O, Engstrom G, Orho-Melander M. Soluble Urokinase-type Plasminogen Activator Receptor (suPAR) and Impaired Kidney Function in the Population-based Malmo Diet and Cancer Study. *Kidney Int Rep* 2017;2:239-47.
 145. Kieneker LM, Hartmann O, Struck J, Bergmann A, Gansevoort RT, Joosten MM, van den Berg E, de Boer RA, Bakker SJL. Plasma Proenkephalin and Poor Long-Term Outcome in Renal Transplant Recipients. *Transplant Direct* 2017;3:e190.
 146. Zoccali C, Ciccarelli M, Mallamaci F, Maggiore Q, Lotti M, Zucchelli GC. Plasma met-enkephalin and leu-enkephalin in chronic renal failure. *Nephrology, dialysis,*

transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 1987;1:219-22.

147. Smith R, Grossman A, Gaillard R, Clement-Jones V, Ratter S, Mallinson J, Lowry PJ, Besser GM, Rees LH. Studies on circulating met-enkephalin and beta-endorphin: normal subjects and patients with renal and adrenal disease. *Clinical endocrinology* 1981;15:291-300.
148. McLaughlin PJ, Zagon IS. The opioid growth factor-opioid growth factor receptor axis: homeostatic regulator of cell proliferation and its implications for health and disease. *Biochemical pharmacology* 2012;84:746-55.
149. Cheng F, McLaughlin PJ, Verderame MF, Zagon IS. The OGF-OGFr axis utilizes the p16INK4a and p21WAF1/CIP1 pathways to restrict normal cell proliferation. *Molecular biology of the cell* 2009;20:319-27.
150. Zagon IS, Donahue RN, McLaughlin PJ. Opioid growth factor-opioid growth factor receptor axis is a physiological determinant of cell proliferation in diverse human cancers. *American journal of physiology Regulatory, integrative and comparative physiology* 2009;297:R1154-61.
151. Jefferson JA, Shankland SJ. The pathogenesis of focal segmental glomerulosclerosis. *Advances in chronic kidney disease* 2014;21:408-16.
152. Thuno M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers* 2009;27:157-72.
153. Sutton TA, Fisher CJ, Molitoris BA. Microvascular endothelial injury and dysfunction during ischemic acute renal failure. *Kidney international* 2002;62:1539-49.
154. Myers BD, Chui F, Hilberman M, Michaels AS. Transtubular leakage of glomerular filtrate in human acute renal failure. *The American journal of physiology* 1979;237:F319-25.
155. Sutton TA. Alteration of microvascular permeability in acute kidney injury. *Microvascular research* 2009;77:4-7.
156. O'Seaghdha CM, Fox CS. Genome-wide association studies of chronic kidney disease: what have we learned? *Nat Rev Nephrol* 2011;8:89-99.
157. Ledo N, Ko YA, Park AS, Kang HM, Han SY, Choi P, Susztak K. Functional genomic annotation of genetic risk loci highlights inflammation and epithelial biology networks in CKD. *Journal of the American Society of Nephrology : JASN* 2015;26:692-714.
158. Maurano MT, Humbert R, Rynes E, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012;337:1190-5.
159. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation* 2007;115:928-35.
160. Cook NR. Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. *Clinical chemistry* 2008;54:17-23.
161. Boger CA, Heid IM. Chronic kidney disease: novel insights from genome-wide association studies. *Kidney & blood pressure research* 2011;34:225-34.
162. Pattaro C, Aulchenko YS, Isaacs A, Vitart V, Hayward C, Franklin CS, Polasek O, Kolcic I, Biloglav Z, Campbell S, Hastie N, Lauc G, Meitinger T, Oostra BA, Gyllenstein U, Wilson JF, Pichler I, Hicks AA, Campbell H, Wright AF, Rudan I, van Duijn CM, Riegler P, Marroni F, Pramstaller PP, Consortium E. Genome-wide linkage analysis of

- serum creatinine in three isolated European populations. *Kidney international* 2009;76:297-306.
163. Hostetter TH, Meyer TW, Rennke HG, Brenner BM. Chronic effects of dietary protein in the rat with intact and reduced renal mass. *Kidney international* 1986;30:509-17.
 164. Levey AS, Greene T, Beck GJ, Caggiula AW, Kusek JW, Hunsicker LG, Klahr S. Dietary protein restriction and the progression of chronic renal disease: what have all of the results of the MDRD study shown? Modification of Diet in Renal Disease Study group. *Journal of the American Society of Nephrology : JASN* 1999;10:2426-39.
 165. Lew QJ, Jafar TH, Koh HW, Jin A, Chow KY, Yuan JM, Koh WP. Red Meat Intake and Risk of ESRD. *Journal of the American Society of Nephrology : JASN* 2017;28:304-12.
 166. Gopinath B, Harris DC, Flood VM, Burlutsky G, Brand-Miller J, Mitchell P. Carbohydrate nutrition is associated with the 5-year incidence of chronic kidney disease. *The Journal of nutrition* 2011;141:433-9.
 167. Xu H, Huang X, Riserus U, Krishnamurthy VM, Cederholm T, Arnlov J, Lindholm B, Sjogren P, Carrero JJ. Dietary fiber, kidney function, inflammation, and mortality risk. *Clinical journal of the American Society of Nephrology : CJASN* 2014;9:2104-10.
 168. Diaz-Lopez A, Bullo M, Basora J, Martinez-Gonzalez MA, Guasch-Ferre M, Estruch R, Warnberg J, Serra-Majem L, Aros F, Lapetra J, Ros E, Pinto X, Covas MI, Salas-Salvado J. Cross-sectional associations between macronutrient intake and chronic kidney disease in a population at high cardiovascular risk. *Clin Nutr* 2013;32:606-12.
 169. Herber-Gast GC, van Essen H, Verschuren WM, Stehouwer CD, Gansevoort RT, Bakker SJ, Spijkerman AM. Coffee and tea consumption in relation to estimated glomerular filtration rate: results from the population-based longitudinal Doetinchem Cohort Study. *The American journal of clinical nutrition* 2016;103:1370-7.
 170. Nakajima K, Hirose K, Ebata M, Morita K, Munakata H. Association between habitual coffee consumption and normal or increased estimated glomerular filtration rate in apparently healthy adults. *The British journal of nutrition* 2010;103:149-52.
 171. Kim BH, Park YS, Noh HM, Sung JS, Lee JK. Association between Coffee Consumption and Renal Impairment in Korean Women with and without Diabetes: Analysis of the Fourth Korea National Health and Nutrition Examination Survey in 2008. *Korean J Fam Med* 2013;34:265-71.
 172. Kotani K, Sakane N, Yamada T, Taniguchi N. Association between coffee consumption and the estimated glomerular filtration rate in the general Japanese population: preliminary data regarding C-reactive protein concentrations. *Clinical chemistry and laboratory medicine : CCLM / FESCC* 2010;48:1773-6.
 173. Glasscock R, Delanaye P, El Nahas M. An Age-Calibrated Classification of Chronic Kidney Disease. *JAMA : the journal of the American Medical Association* 2015;314:559-60.

Copyright references

Page 22 and Figure 1; page 23 and Figure 2

Eckardt, J Coresh, O Devuyst, RJ Johnson, A Köttgen, AS Levey, A Levin. Evolving importance of kidney disease: from subspecialty to global health burden. *Lancet* 2013;382:158-69.

Republished with permission of [Elsevier Science and Technology Journals The Lancet], from [Evolving importance of kidney disease: from subspecialty to global health burden, KU Eckardt, J Coresh, O Devuyst, RJ Johnson, A Köttgen, AS Levey, A Levin, 382, 9887,2013]; permission conveyed through Copyright Clearance Center, Inc.

Page 25 Figure 4

Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney international* 2014;85:49-61.

Reprinted from *Kidney international*, 85 /1, Levin A, Stevens PE, Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward, 49-61., Copyright (2014), with permission from Elsevier.

Page 29 Figure 5

Glasscock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nat Rev Nephrol* 2017;13:104-14.

Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Nephrology] (Glasscock RJ, Warnock DG, Delanaye P. The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nat Rev Nephrol* 2017;13:104-14.), copyright (2016)

Page 30 Figure 6

James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. *Lancet* 2010;375:1296-309

Republished with permission of [Elsevier Science and Technology Journals The Lancet], from [Early recognition and prevention of chronic kidney disease., James MT, Hemmelgarn BR, Tonelli M, 375, 9722, 2010]; permission conveyed through Copyright Clearance Center, Inc.

*Page 54 Figure 9; page 56 Figure 10; page 57 Figure 11,
Paper I appended*

Schulz, CA, Christensson, A, Ericson, U, Almgren, P, Hindy, G, Nilsson, PM, Struck, J, Bergmann, A, Melander, O, Orho-Melander, M: High Level of Fasting Plasma Proenkephalin-A Predicts Deterioration of Kidney Function and Incidence of CKD. *Journal of the American Society of Nephrology* : JASN, 28: 291-303, 2017.

Republished with permission of [American Society of Nephrology], from [High Level of Fasting Plasma Proenkephalin-A Predicts Deterioration of Kidney Function and Incidence of CKD, Schulz CA, Christensson A, Ericson U, Almgren P, Hindy G, Nilsson PM, Struck J, Bergmann A, Melander O, Orho-Melander M, 28, 9, 2016]; permission conveyed through Copyright Clearance Center, Inc.

*Page 59 Figure 12; page 60 Table 3,
Paper II appended*

Schulz CA, PM, Christensson A, Hindy G, Almgren P, Nilsson PM, Engström G, Orho-Melander M.: Soluble Urokinase-type Plasminogen Activator Receptor (suPAR) and Impaired Kidney Function in the Population-based Malmö Diet and Cancer Study. *KI Reports*, 2017.

© 2016 International Society of Nephrology. Published by Elsevier Inc.

Popular Science summary

Today, more than one out of ten persons worldwide is suffering from chronic kidney disease (CKD). It is a serious health issue, which may in the end lead to renal failure and death. However, awareness for CKD is relatively low. It is considered a ‘silent’ epidemic, which often develops over many years as a decreased kidney function may be unrecognized for a long time. Typically, symptoms arise after a lengthy latent period, when complications of decreased kidney function occur. In the long run an impaired kidney function increases the risk for cardiovascular diseases, hospitalization and death. Thus, it is a serious public health issue.

The kidneys are interconnected with many organs including the heart, lung, and brain, and are involved in several important physiological processes such as the water and electrolyte balance, hormone production, the acid-base balance and the Vitamin D metabolism. Each kidney contains around 1 million nephrons. These are the important functional components of the kidney, responsible for filtering and urine production. Every day they are filtering around 180 liters of plasma. Through this fine-tuned process, needed substances are returned to the blood, whereas waste products (primarily urea, uric acid and creatinine) are excreted in the urine. If kidney function is impaired, these waste products accumulate in the body. Creatinine is a commonly used marker for kidney function. In addition, measuring the amount of the protein albumin in urine is used to assess kidney function, as it should not be present in urine under normal physiological conditions. However, both markers are rather insensitive when it comes to assess the long-term kidney function. Thus, new biomarkers are needed for prediction of future kidney function.

Therefore, this PhD-thesis has studied three novel biomarkers (pro-ENK, suPAR and KIM-1) in relation to long-term kidney function. In the three conducted studies we found that higher levels of these biomarkers in plasma associated with greater decline in kidney function and increased risk of incident CKD in a general population of apparently healthy middle-aged participants. Thus, we concluded that these novel biomarkers may be clinically useful in estimating future kidney function in addition to traditional risk factors.

Complex diseases, such as diabetes and cardiovascular disease, are multifactorial which means that several different factors contribute to the disease. Such complex diseases have both a heritable component and environmental risk factors. In the last decades mutations have been identified in more than 110 genes that cause different monogenetic kidney diseases. These diseases are very rare and one mutation in one gene causes a

disease. In addition, more than 50 genetic variants have been identified that increase the risk for the much more common multifactorial kidney diseases. These genetic variants do not cause the disease, but they together with environmental risk factors play a role in the disease etiology. However, little is known about if information on genetic predisposition for impaired kidney function may be useful in predicting future CKD in addition to traditional risk factors.

Therefore, we have studied if adding a genetic risk score consisting of 53 genetic variants, previously identified to associate with kidney function, affect the risk of incident CKD. We found that individuals with high number of genetic risk variants were more likely to have CKD. Thus, we concluded that adding information on genetic predisposition to a risk model with traditional risk factors may be useful in identification of individuals with an increased risk for CKD in the future.

Not only genetic but also environmental factors may influence the risk of incident CKD. The current guidelines for evaluation, classification, and management of CKD include recommendations in regards to dietary intake for patients with CKD. It is suggested that protein intake shall be limited to $\leq 0.8\text{g/kg/d}$ with appropriate education in patients with severe CKD. Moreover, for adults at risk for progression of CKD it is suggested that high protein intake ($>1.3\text{ g/kg/d}$) is avoided. However, little is known about how diet may influence future kidney function of individuals with a “normal” kidney function. Therefore, we investigated the role of diet and studied how reported intake of macronutrients, three beverages and 15 food groups related to long-term kidney function. We observed that higher intake of sucrose associated with decreased kidney function, whereas higher intakes of fiber and coffee associated a beneficial long term kidney function.

The results of this thesis can be summarized as follows: the novel circulating biomarkers (pro-ENK, suPAR, and KIM-1) as well as the genetic biomarkers may be clinically useful in estimating future kidney function. In addition, our results highlight the importance of dietary intakes in longitudinal kidney function.

Populärvetenskaplig sammanfattning

I dag lider mer än en av tio personer i världen av kronisk njursjukdom. Det är ett allvarligt hälsoproblem som även kan leda till njursvikt och död. Medvetenheten om kronisk njursjukdom är däremot relativt låg. Den betraktas som en "tyst" epidemi, som ofta utvecklas under många år, eftersom nedsatt njurfunktion kan förbli oupptäckt under lång tid. Vanligtvis uppstår symtom efter en längre latent period när komplikationerna av nedsatt njurfunktion uppträder. På sikt ökar en nedsatt njurfunktion risken för hjärt-kärlsjukdomar, hospitalisering, och död. Det är därför ett allvarligt folkhälsoproblem.

Njurarna är sammanlänkade med många organ, som hjärta, lunga och hjärna. De är involverade i flera viktiga fysiologiska processer som vatten- och elektrolytbalansen, hormonproduktionen, syra-basbalansen och vitamin D-metabolismen. Varje njure innehåller ungefär 1 miljon nefroner, som genom att ansvara för filtrering och urinproduktion utgör viktiga funktionella komponenter i njuren. Varje dag filtrerar de omkring 180 liter plasma. Genom denna fint trimmade process återupptas nödvändiga ämnen till blodet, medan avfallsprodukter (främst urea, urinsyra och kreatinin) utsöndras i urinen. Om njurfunktionen är nedsatt ackumuleras dessa avfallsprodukter istället i kroppen. Kreatinin är en vanligt förekommande markör för njurfunktion. Man mäter även mängden proteinalbumin i urinen för att bedöma njurfunktionen, eftersom proteinet albumin inte bör finnas i urin under normala fysiologiska förhållanden. Båda markörerna är dock relativt okänsliga när det gäller att bedöma njurfunktionen på längre sikt och det finns därför ett behov av nya biomarkörer för bättre prognos av framtida njurfunktion.

I avhandlingen studeras därför tre nya biomarkörer (pro-ENK, suPAR och KIM-1) i relation till långvarig njurfunktion. I de tre genomförda studierna fann vi att högre nivåer av dessa biomarkörer i plasma visade på samband med försämrad njurfunktion och ökad risk för förekomst av kronisk njursjukdom hos en genomsnittlig population av friska medelålders deltagare. Vi drog således slutsatsen att dessa nya biomarkörer kan vara kliniskt användbara för att uppskatta framtida njurfunktion, utöver användandet av traditionella riskfaktorer.

Komplexa sjukdomar, som diabetes och hjärt-kärlsjukdomar, är multifaktoriella vilket innebär att flera olika faktorer bidrar till sjukdomen. Sådana komplexa sjukdomar har både en ärftlig komponent och miljöbetingade riskfaktorer. Under de senaste decennierna har mutationer identifierats i mer än 110 gener som kan orsaka olika monogena njursjukdomar. Dessa sjukdomar är mycket sällsynta och en mutation i en enda gen kan orsaka sjukdomen. Därutöver har mer än 50 genetiska varianter

identifierats som ökar risken för de mycket vanligare multifaktoriella njursjukdomarna. Dessa genetiska varianter orsakar inte sjukdomen, men tillsammans med miljöbetingade riskfaktorerna spelar de en roll i sjukdomens etiologi. Lite är emellertid känt om ifall information om genetisk predisposition för nedsatt njurfunktion kan användas som tillägg till traditionella riskfaktorer för att förutsäga framtida kronisk njursjukdom. En s.k. genetisk risk score konstruerades genom att kombinera 53 risk varianter som tidigare kopplats till njurfunktion och studera om dessa påverkar risken för förekomst av kronisk njursjukdom. Resultatet visade att individer med ett fler genetiska riskvarianter hade större sannolikhet för att drabbas av kronisk njursjukdom. Vi drog därför slutsatsen att genom att inkludera information om genetisk predisposition till en riskmodell med traditionella riskfaktorer, kan vi bättre identifiera individer med ökad risk för framtida kronisk njursjukdom.

Inte bara genetiska faktorer utan även miljöfaktorer kan påverka risken för förekomst av kronisk njursjukdom. De rådande riktlinjerna för utvärdering, klassificering och hantering av kronisk njursjukdom inbegriper rekommendationer om kostintag för patienter med kronisk njursjukdom. Rekommendationerna är att proteinintaget ska begränsas till $\leq 0,8$ g/kg/d tillsammans med lämplig utbildning för patienter med svår kronisk njursjukdom. Vidare rekommenderas att vuxna som riskerar att utveckla kronisk njursjukdom undviker högproteinintag ($>1,3$ g/kg/d). Lite är dock känt om hur kost kan påverka framtida njurfunktion hos individer med en "normal" njurfunktion. Vi undersökte därför kostens betydelse och studerade hur rapporterat intag av makronäringsämnen, tre olika drycker och 15 olika livsmedelsgrupper relaterade till njurfunktion på längre sikt. Vi observerade att ett högre intag av socker visade på samband med nedsatt njurfunktion, medan högre intag av fibrer och kaffe visade en gynnsam effekt på njurfunktionen på längre sikt.

Resultaten av avhandlingen kan sammanfattas enligt följande: de nya cirkulerande biomarkörerna (pro-ENK, suPAR och KIM-1) kan, liksom de genetiska biomarkörerna, vara kliniskt användbara för att förutsäga framtida njurfunktion. Dessutom framhäver våra resultat kostens betydelse för njurfunktionen över längre tid.



Christina-Alexandra obtained a Diploma degree in nutrition and household Science (2010) from Bonn University and graduated with a Master in Public Health from Ludwig-Maximilian-University in Munich (2012). During her time as a PhD-student at Lund University (2013-2017) she has been involved in clinical research focused on kidney function.

This thesis briefly describes the concept of chronic kidney disease, a public health issue estimated to occur in more than 10% of the population globally. In a longitudinal cohort setting it explores the clinical potential of three novel circulating biomarkers, and the applicability of a genetic risk score for prediction of future chronic kidney disease. In addition, it investigates the role of dietary intake on long-term kidney function.

In her free time Christina-Alexandra is a passionate runner, enjoys to cook, and cherishes to read.

