The kidney and the brain

Role of vascular dysfunction

Sanaz Sedaghat

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Role of vascular dysfunction

De nieren en het brein

De rol van vasculaire dysfunctie

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CHAPTER 1

General introduction

Vascular dysfunction; a systemic phenomenon

An increasing body of evidence suggests that vascular dysfunction is a systemic disease.[1] In other words, when one organ is affected by vascular dysfunction, it is likely that other organs are also involved.[1,2] Simultaneous occurrence of vascular dysfunction in multiple organs increases with age and accelerates with exposure to vascular risk factors.[2,3] In fact, similar pathological processes damaging vascular beds in different organs contribute to advanced vascular aging.[3] High flow and low resistant organs such as the kidney and brain are more sensitive to consequences of vascular dysfunction such as arterial stiffness, atherosclerosis, and small vessel disease.[4] Patients with impaired kidney function are at increased risk for accelerated vascular aging due to the presence of uremia and high plasma levels of asymmetric dimethylarginine and homocysteine. Accelerated vascular aging, on the other hand, can lead to a further decline in kidney function and affect vasculatures of other organs.[5,6] Previous studies showed that cerebrovascular and neurodegenerative disorders, particularly those with vascular features, are more common in different stages of kidney disease compared to the general population.[7-11] In line with that, vascular-type dementia rather than Alzheimer type dementia is reported to be more prevalent in patients with kidney disease.[8] Despite this evidence, the contributions and interactions of different organs in development and progression of systemic vascular dysfunction remain to be addressed. This thesis focuses on two major organs, the kidney and the brain, which both contribute and are affected by systemic vascular dysfunction. Findings of this thesis shed further light on the mechanisms increasing the risk of neurovascular impairment in relation to decreased kidney function.

Vascular dysfunction and decline in kidney function

Chronic kidney disease (CKD) is an emerging worldwide public health problem with the global prevalence of 8–16%.[5] Kidney is a highly vascularized organ which receives more than 20% of the cardiac output, around1584 L/24h in a 70-kg adult male.[12] This enormous blood supply is not only necessary for glomerular filtration but also essential for maintenance of vascular hemostasis in the body.[13] Adequate blood supply of the kidneys is dependent on the intact structure and function of the extra- and intra-renal arteries.[14,15]

Dysfunction in extra-renal arteries can manifest as arterial stiffness.[13,16,17] Stiffness in the large arteries impairs buffer capacity of the vessels and transmits high pulsatile flow and shear stress to the kidneys and this contributes to dysregulation of kidney blood flow auto-regulation leading to a decline in glomerular filtration rate.[13] Previous studies showed a strong association between arterial stiffness and kidney impairment in patients with kidney disease.[18-20] However, results from population-based studies have been conflicting.[19,21-23]

On the other hand, integrity of the intra-renal arteries, which are mainly medium and small size vessels, are crucial for filtration function of the kidney as they serve as barriers between systemic circulation and glomerulus.[24,25] Impaired structural integrity of intra-renal arteries can lead to hypo-perfusion of the kidney and impairs glomerular integrity.[24] Therefore the kidney is a very susceptible organ to ischemic and thrombotic events which compromises its microcirculation.[24] While previous animal experiments and studies in patient groups suggest that a prothrombotic state increases risk of kidney function,[25-28] whether this link extends to individuals from general populations remains to be elucidated.

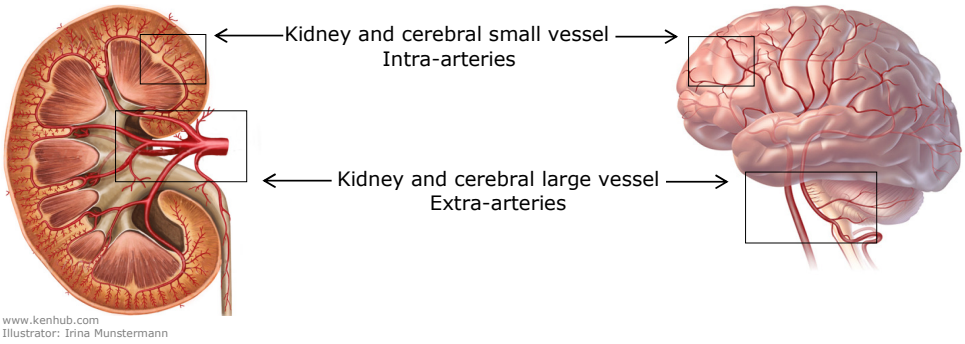

Figure 1. The kidney and brain arteries

Vascular dysfunction and brain neurovascular integrity

Cerebral hypo-perfusion has been associated with the development of cerebrovascular and neurodegenerative disorders.[29] Cerebral blood flow is under a tight regulation by cerebral auto-regulation to ensure enough supply of oxygen and glucose to the brain.[29-31] Cerebral blood flow is not only dependent on mean arterial pressure but also on intact integrity of both intra and extracranial vessels.[32]

Extracerebral arteries play an important role in handling hemodynamic stress to the brain.[32] Similar to kidneys, the high-flow and low-resistance nature of the brain vasculature makes it vulnerable to pulsatile hemodynamic stress throughout each cardiac cycle.[14,33] With stiffening of central elastic arteries and decreasing in the capacity of the brain vessels to buffer pressure and flow pulsations, risk of microvascular damage increases substantially.[4] Microvascular damage leads to ischemia, impairment in neurovascular integrity, and subsequently to a wide range of clinical and subclinical cerebrovascular disorders.[4] Previous studies showed a strong association between aortic stiffness and cerebrovascular events.[33] Nonetheless, it is not clear whether stiffness in carotid arteries, which carry the major part of the blood to the brain, has similar

or even stronger association with the brain outcomes.[34] Furthermore, carotid stiffness can directly contribute to the development of rupture-prone atherosclerotic plaques in the internal carotid artery and increase the risk of stroke.[35] Therefore, stiffness in the carotid artery might differentially associate with risk of stroke and it might have a better predictive value for stroke incidence compared with stiffness in the aorta.

Vascular dysfunction and subsequently microvascular damage in the brain increases the risk of cerebral small vessel disease such as microbleeds, lacunes, and white matter lesions.[36] Previous studies showed that cerebral small vessel disease is related not only to cerebrovascular events but also to other adverse health outcomes and higher risk of mortality.[37] Several studies reported a link between presence of white matter lesions and shorter survival.[37,38] Recently a new technique, namely diffusion-MRI, has been developed which enables us to detect and quantify early subtle changes in integrity of white matter.[39] Given the important role of white matter integrity in the brain function and maintenance of health through homeostasis and human behavior, it is important to study whether early changes in white matter microstructure before development of irreversible white matter lesions are associated with mortality.[40] This will open new avenues targeting early changes in the brain structure to reduce the risk of neurodegenerative disorders and promoting healthy aging in the middle age and older populations.

Vascular interactions between the kidney and brain

Despite shorter survival of patients with CKD, it has been shown that these patients are at a greater risk for developing cerebrovascular events and dementia.[9] Incident rates of stroke are 1.9 to 7.6 times higher in CKD patients compared to subjects without kidney disease.[9] Likewise, individuals even at early stages of CKD have higher risk of developing dementia compared to general population.[41] Nevertheless, mechanisms underlying higher prevalence of brain disorders in patients with kidney disease are

not fully understood. Current evidence suggests that this link could be mediated through vascular mechanisms.[1,8] Several putative mechanisms can explain the vascular association between the kidney and brain. The juxtamedullary afferent arterioles in the kidney and the perforating arteries in the brain have a similar structure. They are believed to be evolutionally developed to deliver blood from large arteries and maintain the perfusion of vital tissues such as nephrons and the brainstem.[4] As a consequence, these vessels are exposed to high pressure from large arteries.[25] Vascular dysfunction in these arteries induced by shared risk factors, such as high blood pressure, arterial stiffness, and diabetes, may lead to microcirculatory damage, impair the auto-regulation capacity and expose both the kidney and brain tissues to hypo-perfusion.[14] Besides the shared risk factors, intact kidney function is crucial for regulation of total blood volume and vascular tone.[5] Hence, impairments in kidney function can disturb regulation of blood flow in the brain. Furthermore, impaired kidney function with alterations in water and electrolytes balance, and promoting chronic inflammation and sympathetic overactivity can contribute to vascular injury and endothelial dysfunction in the brain.[42] Considering these potential biological pathways, the exact mechanism underlying the association between kidney function and cerebrovascular and neurodegenerative disorders need to be further studied.

Outline of this thesis

Chapter 2 of this thesis is devoted to the association between vascular dysfunction and decrease in kidney function. **Chapter 2.1** examines the role of blood pressure in the association between uric acid and incidence of CKD. The aim of **chapter 2.2** is to test whether genetic variants related to uric acid are causally related to blood pressure. In **chapter 2.3**, the cross-sectional association between kidney function and indices of arterial stiffness is evaluated. **Chapter 2.4** presents the independent role of arterial stiffness in risk of decline in kidney function. Finally, in **chapter 2.5** we examined the

role of prothrombotic factors in risk of decline in kidney function.

Chapter 3 focuses on the association of vascular dysfunction and brain abnormalities and their influence on survival. In **chapter 3.1**, the influence of carotid stiffness on risk of stroke is examined in both population-based and patient-based cohorts. **Chapter 3.2** presents the association between subtle changes in white matter integrity and risk of mortality.

In **chapter 4** of this thesis, we investigated the link between kidney function and various subclinical changes of the brain including subtle changes of the brain white matter integrity **(chapter 4.1)**, different types of cerebral small vessel diseases **(chapter 4.2)**, and levels of cerebral blood flow **(chapter 4.3)**.

Finally in **chapter 5**, key findings of this thesis will be presented and our findings will be discussed in the context of the current literature.

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CHAPTER 2

Vascular dysfunction and the kidney

2.1 Serum uric acid and chronic kidney disease: The role of hypertension

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There are inconsistent findings on the role of hyperuricemia as an independent risk factor for chronic kidney disease (CKD). Hypertension has been implicated as a factor influencing the association between serum uric acid and CKD. In this population-based study we investigated the association between serum uric acid and decline in renal function and tested whether hypertension moderates this association. We included 2601 subjects aged 55 years and over from the Rotterdam Study. Serum uric acid and estimated glomerular filtration rate (eGFR) were assessed at baseline. After average 6.5 years of follow-up, second eGFR was assessed. CKD was defined as eGFR < 60 ml/min/1.73 m^2. All associations were corrected for socio-demographic and cardiovascular factors. Each unit (mg/dL) increase in serum uric acid was associated with 0.19 ml/min per 1.73 m^2 faster annual decline in eGFR. While the association between serum uric acid and incidence of CKD was not significant in our study population (Hazard Ratio: 1.12, 95% confidence interval [CI]: 0.98-1.28), incorporating our results in a meta-analysis with eleven published studies revealed a significant association (Relative Risk: 1.18, 95%CI: 1.15-1.22). In the stratified analyses, we observed that the associations of serum uric acid with eGFR decline and incident CKD were stronger in hypertensive subjects (P for interaction =0.046 and 0.024, respectively). Our findings suggest that hyperuricemia is independently associated with a decline in renal function. Stronger association in hypertensive individuals may indicate that hypertension mediates the association between serum uric acid and CKD.

Background

The incidence of chronic kidney disease (CKD) is steadily increasing.[1] Affected individuals have high rates of cardiovascular morbidity and mortality and often require costly treatments such as dialysis and kidney transplantation.[2] Identifying novel risk factors for CKD may improve preventative programs that would eventually decrease the burden caused by this disease.[3]

One of the recently proposed risk factors for CKD is hyperuricemia.[4] Although elevated serum uric acid is associated with CKD, it is not clear whether hyperuricemia plays a detrimental role in developing CKD or if it merely is a consequence of lower glomerular filtration rate prior to CKD.[5] To this end, different prospective studies investigated the association between serum uric acid and incident CKD.[4,6-18] However, these studies showed inconsistent findings.[19-21] Furthermore, a growing body of evidence supports a role for uric acid in developing hypertension, a well-established risk factor for CKD.[22,23] We hypothesize that hypertension might mediate the effect of uric acid on renal function. If so, the association between serum uric acid and CKD should be stronger in hypertensive individuals.

In this study we investigated the association of serum uric acid with decline in estimated glomerular filtration rate (eGFR) and incident CKD in the Rotterdam Study, a prospective cohort study of individuals 55 years and older. Moreover, we performed a meta-analysis to provide a reliable estimate of the effect of serum uric acid on risk of CKD. Finally, we studied whether this association differs in hypertensive and normotensive individuals.

Methods

Population

The Rotterdam Study is a population-based cohort study, including 7,983 participants living in Ommoord, a district of Rotterdam, The Netherlands. All participants aged

55 and over, were invited to this study (n = 10,275). The Rotterdam Study started in the early 1990s and periodical examinations were performed every 3 to 5 years. In addition, participants were continuously followed for vital status, obtaining information regularly from the municipal health authorities in the Rotterdam area. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center and written informed consent was obtained from all participants. [24,25]

Uric acid

Serum uric acid was measured once at baseline. Values of serum uric acid were obtained from baseline non-fasting blood samples which were centrifuged for 10 minutes at 3000 rotations per minute and subsequently stored at -20°C for one week. Uric acid activity was ascertained with Kone Diagnostica reagent kit and Kone autoanalyzer.[26] In order to check the calibration, for every 10 samples, 3 control samples were included. In each run (100 samples), if the average values of the control samples were not within 2.5% of the true value, the run was repeated. Calibration was also done on day-by-day variation, which had to be within 5%.[27]

eGFR decline and incident CKD

At baseline visit serum creatinine was determined using non-kinetic alkaline picrate (Jaffé) method. At follow up visit serum creatinine was determined using an enzymatic assay method.[28] In order to calibrate, we aligned the mean values of serum creatinine with serum creatinine values of the participants of the Third National Health and Nutrition Examination Survey (NHANES III) in different gender and age groups (<60, 60-69, ≥70). Measurements were done for 5280 individuals at baseline (1989 - 1993) and 3867 individuals at the follow up visit (1997 - 1999). eGFR was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation which is recommended by the National Kidney Foundation.[28] CKD was defined as eGFR <60

ml/min per 1.73 m^2. To calculate the annual eGFR decline, we first subtracted the eGFR estimates of the follow up examination from the eGFR estimates at baseline and then divided by the time between the two visits. These two examinations were on average 6.5 years apart. Incident cases were defined among the individuals free of CKD at baseline (eGFR >60 ml/min per 1.73 m^2), who had a decline in eGFR to less than 60 ml/min per 1.73 m^2 between the two periodical examinations. To estimate the censoring date of the cases, we assumed a linear decrease in eGFR. Given this assumption, the date that each case had passed the eGFR threshold of 60 ml/min per 1.73 m^2 was taken as the censoring date and it was used to calculate the follow up time for incident cases. For controls, the time spent between the two examinations was used as the follow up time.

Covariates

Body mass index was calculated by dividing weight in kilograms by height in meters squared. Serum total cholesterol and high density lipoprotein cholesterol levels was determined using an automated enzymatic method. Information on smoking and alcohol consumption was acquired from the questionnaires. Participants were asked for the average daily consumption of alcohol. Coronary heart disease was considered as experiencing myocardial infarction or coronary revascularization procedures. Diabetes mellitus was defined as the use of blood glucose lowering drugs or a random non-fasting glucose above 11.1 mmol/l. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of blood pressure lowering medication with hypertension as the indication. Medication use information is based on home interview. Data for all the covariates were obtained once at baseline.

Population for analysis

As depicted in Figure 1, from 7983 participants at baseline, 5139 individuals had

available data at baseline. We further excluded 164 participants using antigout medications (allopurinol, probenecid, benzbromarone, and colchicine). Among 4975 subjects 1132 died during the 6.5 years of follow-up time, 62 participants were not able to participate in the follow up visit, 947 subjects did not participate in the follow up visit and 233 did not have second serum creatinine measurements. This resulted in a sample of 2601 participants for the longitudinal analyses on eGFR decline. Furthermore, we excluded subjects with baseline CKD (n=196) for the analyses on the incidence of CKD.

Statistical analysis

A linear regression model was used to evaluate the association between serum uric acid (mg/dL) and eGFR decline. The Cox proportional hazard model was applied to calculate the hazard ratio (HR) for the association between serum uric acid and incidence of CKD. All analyses were adjusted for age, sex and baseline eGFR. In a multivariate analysis, we additionally adjusted for potential confounders including systolic blood pressure, diabetes mellitus, body mass index, high density lipoprotein, alcohol consumption, smoking, total cholesterol, coronary heart disease, and use of diuretics , beta blockers, calcium channel blockers, and ACE inhibitors. Analysis was further done in subgroups of hypertensive and normotensive individuals. Interaction was assessed, by adding an interaction term in the regression model. The interaction term was the product of the interacting factor and serum uric acid. R version 2.13.0 was used to calibrate creatinine values at baseline and follow up. All the other analyses were carried out using SPSS 17.0.2 for windows.

Meta-analysis

We searched for studies published in MEDLINE (PubMed), EMBASE and Web of Science using the common key words related to incidence of chronic kidney disease and serum

uric acid including “renal disease” or “renal insufficiency” or “kidney disease” and “blood urate” or “serum uric acid” or “hyperuricemia”. We restricted the language of the search to English. Population-based studies which evaluated the association between serum uric acid and incidence of CKD were included in our meta-analysis. Some of the eligible studies had used different outcome definitions and measurements of serum uric acid. Therefore, we contacted nine authors to obtain results consistent with our definitions and adjustments. Authors were asked to adjust the analysis for the following variables: age, sex, smoking, alcohol consumption, body mass index, diabetes, hypertension, total cholesterol, baseline kidney function, and proteinuria. From 12 studies, 6 studies provided HR and 6 studies used odds ratio (OR) to report the effect size. Since the incidence of CKD is relatively low,[29] we accepted OR as a proxy for HR and combined them in the meta-analysis. Data used for the meta analysis is available in supplementary document (Table S1).The heterogeneity assumption was investigated using a commonly used statistical method, namely the I-square statistic.[30] Publication bias was evaluated using the Egger’s test.[31] The statistical analyses were performed using the “meta” package of the statistical software R, version 2.13.0. Moreover, we tested the publication bias using STATA version 10.

Results

As depicted in Figure 1 among 4975 individuals at risk of developing CKD at baseline, 2374 subjects either died or were lost to follow up during 6.5 years of follow up. Comparing them with the population included in the analysis, they were significantly older and had higher C-reactive protein level, systolic blood pressure, and serum uric acid. They also had lower eGFR, alcohol consumption, total cholesterol and body mass index. Finally, they were more likely to use antihypertensive medication and to have diabetes mellitus, CKD, and coronary heart disease (Table S2).

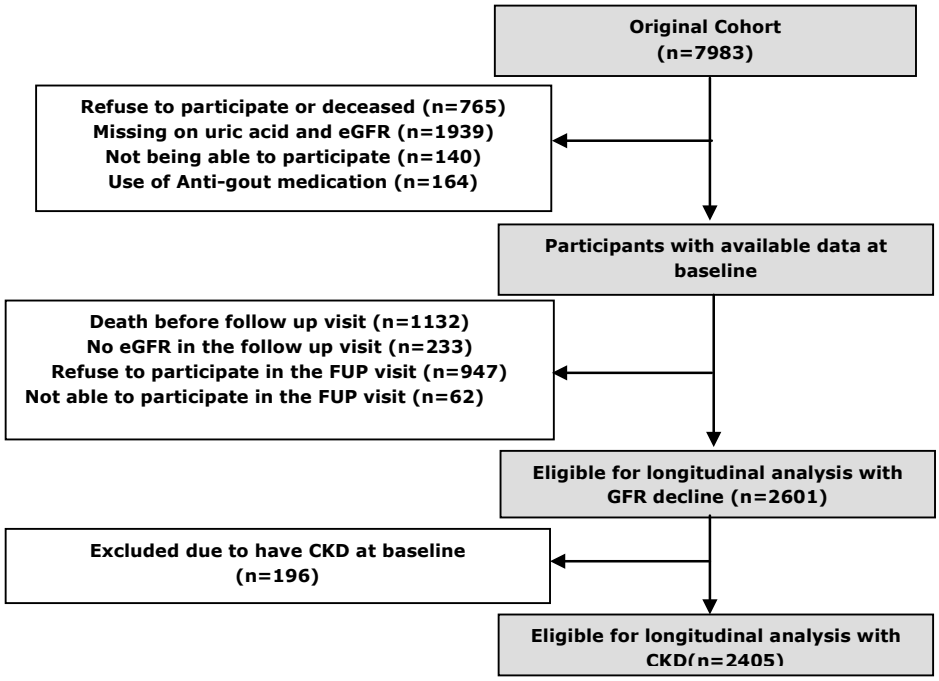

Figure 1. Population for analysis

Baseline characteristics

The study population had an average age of 70.4 years, serum uric acid level of 5.38 mg/dL, and eGFR of 77.15 ml/min per 1.73 m^2. As shown in Table 1, subjects in the higher quartiles of serum uric acid were older and more likely to be male, diabetic, alcohol drinker, and former smoker. They had a significantly higher body mass index, systolic and diastolic blood pressure, C-reactive protein level, history of coronary heart disease and prevalence of antihypertensive medications use. In addition, they had lower levels of eGFR and HDL cholesterol.

Serum uric acid and eGFR decline

Average annual eGFR decline was 0.92 ml/min per 1.73 m^2 (SD=2.21) for all participants. For each unit increase in serum uric acid, the annual eGFR decline was

higher by 0.19 ml/min per 1.73 m^2 (95% confidence interval [CI]: 0.13 - 0.26) in the age and sex adjusted model and 0.18 ml/min per 1.73 m^2 (95%CI: 0.10 - 0.26) in the multivariate adjusted model (Table 2). We repeated the analyses in subgroups of hypertensive and normotensive participants. In both models, the association was stronger in hypertensive subjects compared to normotensive subjects (P-value for interaction = 0.020). Hypertensive participants in the highest quartile of serum uric acid had 1.12 higher risk of annual eGFR decline per one unit increase in serum uric acid compared to those in the lowest quartile (Figure 2-A).

Serum uric acid and incidence of CKD

As shown in Table 3, serum uric acid was associated with incident CKD in age and sex adjusted model (HR: 1.22; 95%CI: 1.10 - 1.35). After further adjusting for potential confounders, the direction of the association remained the same; however, the strength of the association attenuated and it was no longer statistically significant (1.12; 95%CI: 0.98 - 1.28). We further examined the association between serum uric acid and CKD separately in hypertensive and normotensive participants. In both models, the association was present in hypertensive subjects (HR: 1.29, 95%CI: 1.14 - 1.46) but absent in normotensive subjects (HR: 1.03, 95%CI: 0.85 - 1.24) (P-value for interaction= 0.030). Hypertensive participants in the highest quartile of serum uric acid had more than three times higher risk of developing CKD compared with those in the lowest quartile (Figure 2-B). To further explore whether other metabolic factors, including high density lipoprotein, diabetes, and waist circumference, could influence the association of uric acid with incidence of CKD, we performed a series of stratified analyses. These stratified analyses showed that there was no statistical difference, in the association of serum uric acid and incidence of CKD, between different groups of participants (Figure S1).

Table1. Baseline characteristics of the participants in different quartiles of uric acid levels

	Uric Acid Quartiles (mg/dL*)				
	≤4.5 (n=1257)	**4.5-5 (n=1210)**	**5-6 (n=1263)**	**>6 (n=1245)**	**P for trend****
Age, mean (SD†), y	69.1(8.9)	70.4(9.2)	70.4(9.0)	71.5(9.4)	<0.001
Men (%)	208 (16.5)	374(30.9)	586(46.4)	705(56.6)	<0.001
Smoking					
Current (%)	312(24.8)	277(22.9)	313(24.8)	245(19.7)	0.001
Former (%)	366(29.1)	429(35.5)	529(41.9)	614(49.3)	
Alcohol Intake, median (IR), g/d	4.5(0.7-13.6)	5.0(1.0-14.8)	9.3(1.7-21.2)	11.2(2.5-27.9)	<0.001
Body mass index, mean (SD), kg/m^2	25.2(3.4)	25.7(3.5)	26.6(3.6)	27.3(3.8)	<0.001
Total cholesterol, mean (SD), mmol/L	6.6(1.2)	6.5(1.2)	6.6(1.3)	6.6(1.2)	0.472
HDL cholesterol, mean (SD), mmol/L	1.4(0.3)	1.3(0.3)	1.3(0.3)	1.2(0.3)	<0.001
eGFR, mean (SD),ml/min per 1.73 m^2	82.9(16.8)	78.3(15.4)	76.1(16.3)	71.1(18.2)	<0.001
SBP, mean (SD), mm Hg	137.3(22.2)	138.3(21.7)	139.3(21.5)	141.2(22.4)	<0.001
DBP, mean (SD), mm Hg	72.6(11.5)	72.8(11.6)	73.4(11.6)	74.0(12.1)	0.091
Chronic kidney disease (%)	65(5.2)	116(9.6)	173(13.7)	331(26.6)	<0.001
Diabetes Mellitus (%)	136(10.9)	109(9.1)	125(9.9)	178(14.4)	<0.001
History of coronary heart disease (%)	88(7.1)	121(10.2)	193(15.4)	264(21.6)	<0.001
Diuretics (%)	101(8.0)	125(10.3)	189(15.0)	413(33.2)	<0.001
Calcium channel blockers (%)	52(4.1)	46(3.8)	75(5.9)	131(10.5)	<0.001
Beta-blockers (%)	103 (8.2)	134(11.1)	192(15.2)	293(23.5)	<0.001
ACE inhibitors (%)	41(3.3)	33(2.7)	61(4.8)	126(10.1)	<0.001

*To convert to SI unit multiply by 59.48
**P-value adjusted for age and sex for continuous measure of uric acid
†Standard Deviation

Table 2. The association between serum uric acid (mg/dL) and decline in estimated glomerular filtration rate (ml/min per 1.73 m^2)

	Minimally adjusted model **				Multivariate adjusted model †			
	N	Annual decline	95% CI*	P-value	N	Annual decline	95% CI	P-value
Total population	2601	0.19	0.13, 0.26	1.2×10^{-10}	2312	0.18	0.10 , 0.26	1.0×10^{-5}
Normotensive	1312	0.13	0.03 , 0.23	0.008	1167	0.14	0.03 , 0.25	0.014
Hypertensive	1275	0.24	0.14 , 0.33	7.8×10^{-9}	1145	0.20	0.08, 0.31	0.001
P for interaction				0.020				0.046

*CI: confidence interval
**Adjusted for age, sex, and baseline eGFR
†Adjusted for age, sex, systolic blood pressure, body mass index, alcohol consumption, smoking, high density lipoprotein, diabetes mellitus, coronary heart disease, total cholesterol, and the use of diuretics, beta blockers, calcium channel blockers, ACE inhibitors, and baseline eGFR

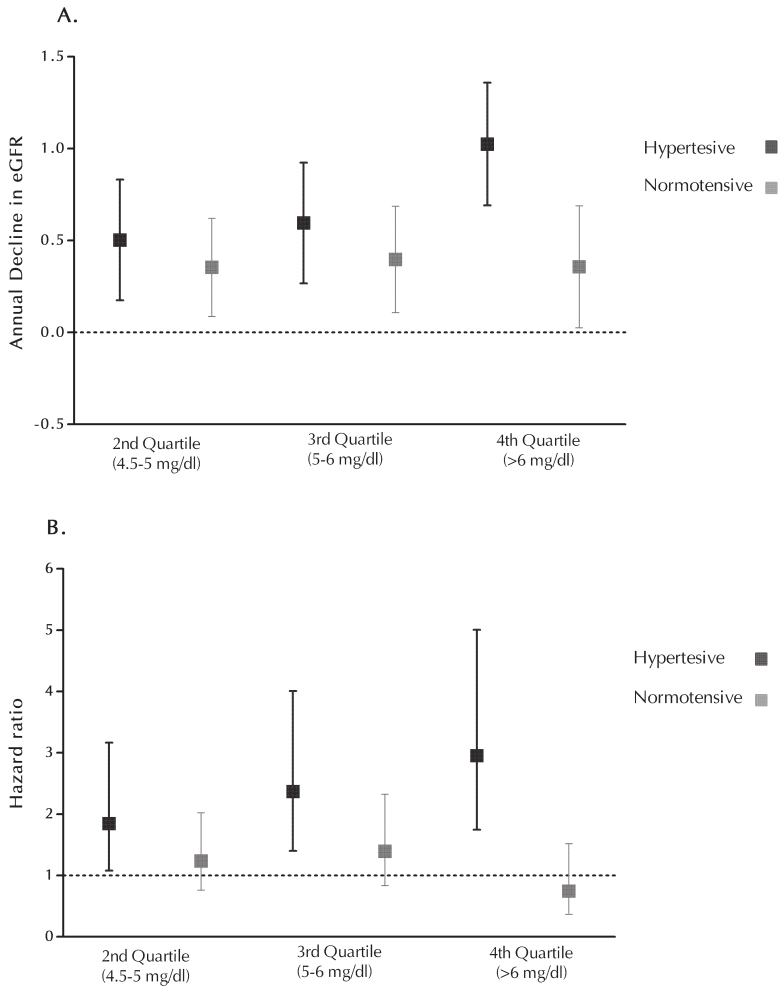

Figure 2. Association of serum uric acid with kidney function in normotensive and hypertensive subjects (A) Annual decline in eGFR in relation to serum uric acid quartiles in hypertensive and normotensive participants. Analyses are adjusted for age, sex and baseline eGFR. Quartiles are compared with participants in the first quartile of serum uric acid (<4.5 mg/dL) (B) Risk of incident CKD in relation to quartiles of serum uric acid level in hypertensive and normotensive participants. Analyses are adjusted for sex, age and baseline eGFR. All odds ratios are compared with participants in the first quartile of serum uric acid (<4.5 mg/dL).

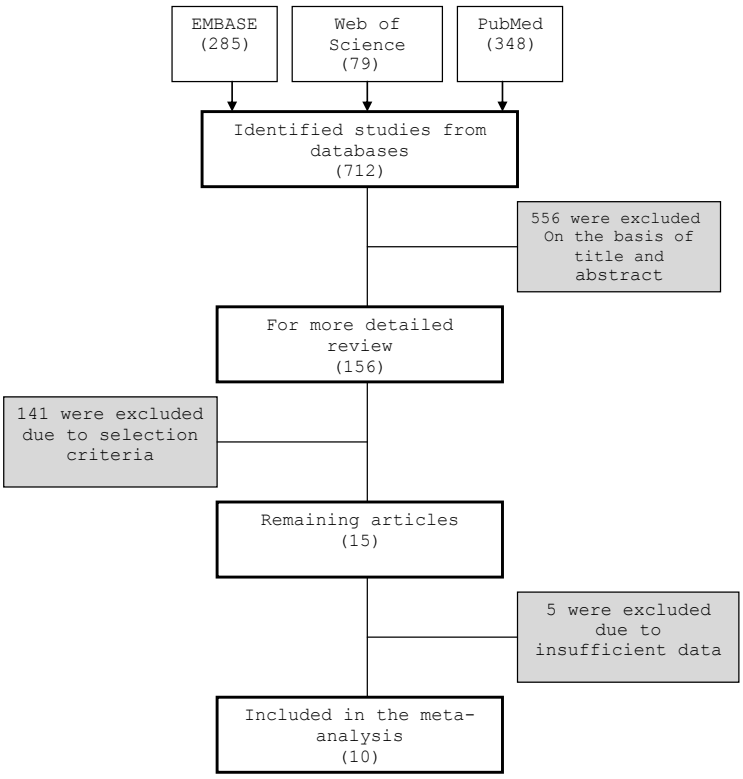

Figure 3. Flow diagram of studies through the different phases of the meta-analysis

Meta-analysis of serum uric acid and incidence of CKD

Figure 3 shows the flow diagram for inclusion of the relevant studies in our meta-analysis.[6,7,12,17,18,32-37] We performed a fixed effect model meta-analysis on 12 studies including 11 published studies and the current analysis from the Rotterdam Study (Figure 4). The overall relative risk was 1.18 (95%CI: 1.15 - 1.22) for incidence of CKD per 1 mg/dL higher level of serum uric acid. There was no evidence of publication bias (Egger's test P-value = 0.583) and test for heterogeneity resulted in moderate estimates (I^2 = 57.7% [19.7% - 77.7%]).

Table 3. The association between serum uric acid (mg/dL) and incidence of chronic kidney disease

	Minimally adjusted model †				Multivariate adjusted model ††			
	N(case)	HR*	95% CI**	P-value	N(case)	HR*	95% CI	P-value
Total population	2405 (289)	1.22	1.10 , 1.35	1.8×10^{-2}	2154 (249)	1.12	0.98 , 1.28	0.079
Normotensive	1243 (112)	1.03	0.85 , 1.24	0.747	1111(102)	0.96	0.78 , 1.19	0.758
Hypertensive	1149 (175)	1.29	1.14 , 1.46	4.4×10^{-3}	1043(147)	1.23	1.04 , 1.45	0.016
P for interaction				0.030				0.024

*HR: hazard ratio
**CI: confidence interval
†Adjusted for age, sex, and baseline eGFR
††Adjusted for age, sex, systolic blood pressure, body mass index, alcohol consumption, smoking, high density lipoprotein,diabetes mellitus, coronary heart disease, total cholesterol, diuretics, beta blockers, calcium channel blockers, ACE inhibitors, and baseline eGFR.

Discussion

In this prospective cohort study, we found that high levels of serum uric acid are associated with faster decline in eGFR and increased incidence of CKD. Moreover, combining our finding with 11 published population-based studies in a meta-analysis suggested a role for serum uric acid as an independent predictor of incident CKD. Finally, we observed that the association is more pronounced in hypertensive subjects compared to normotensive individuals.

A number of recent studies evaluated the relation between serum uric acid and incidence of CKD.[4,7-17,38] In agreement with our findings, some of them introduced high serum uric acid as a risk factor for the development of CKD.[4,6-13,17] Weiner et al. pooled data from Atherosclerosis Risk in Communities (ARIC) study and Cardiovascular Health Study (CHS) and found an odds ratio of 1.11 for CKD incidence per one unit (mg/dl) increase in serum uric acid.[13] However, these findings were contradicted with some other reports. A large cohort of 28,745 young participants (age 20-49 years) in Taiwan showed a weak correlation between serum uric acid and eGFR (Pearson correlation= -0.22).[20] Moreover, Sturm et al. analyzed 227 patients with primary non-diabetic CKD and found that the association between high serum uric acid and progression of CKD disappeared after adjustment for potential confounders.[21] Given these inconsistencies, we performed a meta-analysis to combine the results of published studies on serum uric acid and incident CKD. Our meta-analysis, based on 12 studies including the current study, confirmed the association between high serum uric acid and CKD incidence. This finding is in accordance with the results of trials that have shown a slower progression of CKD after treatment with allopurinol.[39,40]

We found evidence for moderate heterogeneity in the association between serum uric acid and CKD in our meta-analysis. One possible explanation is that potential intermediate factors are not evenly distributed among these studies. Hypertension is

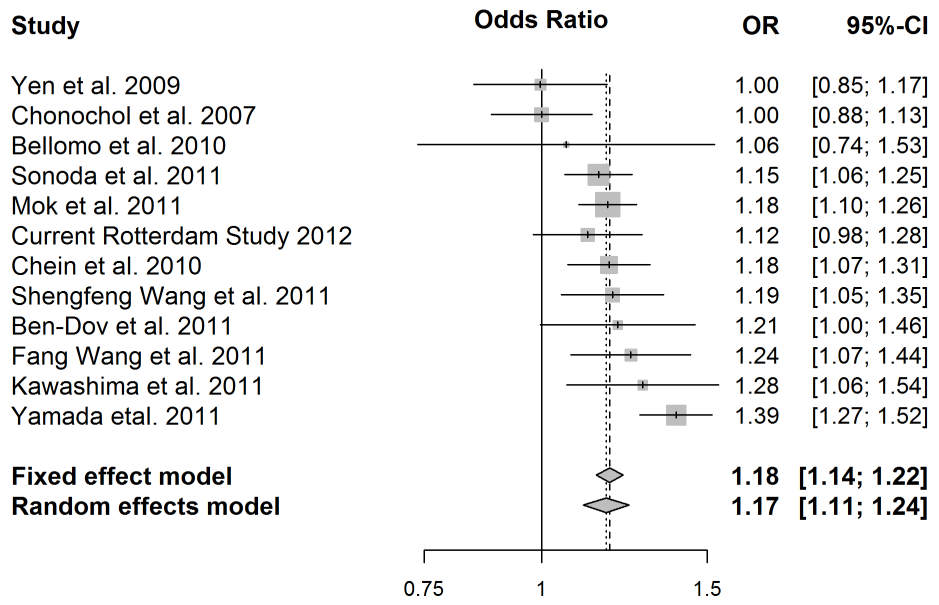

Figure 4. Forest plot of multivariate adjusted relative risk for CKD incidence associated with continuous values of serum uric acid (mg/dL)

one of the factors that may play an important role in the association between serum uric acid and CKD. The correlation between serum uric acid and hypertension is well-documented [41,42] and a large number of studies have reported linear and dose dependent associations between serum uric acid and Blood pressure. In current study we observed that the association between high serum uric acid and CKD was stronger in hypertensive individuals compared to normotensives. In agreement with this finding, in the study of normotensive adults no association was found between high serum uric acid and incidence of CKD.[7] These findings may offer an explanation for the heterogeneity between studies and may provide evidence for possible role of hypertension in mediating the association between high serum uric acid and CKD.

Different explanations can be presented for our observation regarding the association of serum uric acid with risk of CKD and the role of hypertension. Uric acid can increase

the risk of CKD directly through inhibition of endothelial nitric oxide bioavailability, activation of the renin-angiotensin system, and increase in renal microvascular damage.[43,44] Moreover, it is possible that high serum uric acid leads to kidney damage through conventional risk factors for CKD such as hypertension. It has been shown that high serum uric acid increases the risk of developing hypertension by enhancing salt sensitivity. [23] Another possible explanation might be that antihypertensive medications such as diuretics increase serum uric acid level in hypertensive individuals,[45] and consequently elevated serum uric acid may directly result in kidney damage.[46] In this study we adjusted our analyses for different types of antihypertensive medications including diuretics; therefore it is unlikely that the associations were influenced by the medication use. We adjusted the analyses for the use of any diuretic; however, we acknowledge that different types of diuretics have differential impact on serum uric acid concentrations.

Our study has several strengths. First, the Rotterdam Study is a large population-based cohort study, which on one hand provides sufficient statistical power to answer our research question and on the other hand could be generalized to general population. Second, we controlled for several potential confounders such as different types of antihypertensive medications, and performed the analyses separately in subgroups of hypertensives and normotensives. Third, we performed a meta-analysis that provides a robust estimate of the association. Different limitations of this study should also be acknowledged. First, no data on albuminuria were available, which is an important element in defining CKD. However, CKD definition of eGFR <60 ml/min per 1.73 m^2 is a well-accepted definition in population-based research settings.[47] Second, 2374 participants were lost to follow up, mainly because they died before the follow up visit. These subjects were older, had lower eGFR, higher serum uric acid level, and more often had hypertension and diabetes mellitus. Since subjects who dropped out from our study during follow up period had higher uric acid levels and were more likely to

develop CKD, we may have underestimated the association between serum uric acid and risk of CKD.

Conclusion

We have demonstrated that serum uric acid is independently associated with the risk of CKD. This association was significantly stronger in hypertensive individuals. Future studies are needed to test whether better monitoring or even lowering of serum uric acid levels in hypertensive patients can slow down the progression of CKD.

Online supplement
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0076827#s5

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2.2 Association of uric acid genetic risk score with blood pressure: The Rotterdam Study

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High levels of serum uric acid are associated with hypertension in observational studies. The aim of this study is to investigate the association of uric acid gene variants with blood pressure. We studied 5,791 participants aged 55 years and older from the Rotterdam Study. Thirty gene variants identified for serum uric acid level were used to compile genetic risk score. We used linear regression models to investigate the association of the uric acid genetic risk score with systolic and diastolic blood pressure in the whole study population, and separately in participants with and without co-morbidities and medication use. In the age and sex adjusted model, each standard deviation increase in uric acid genetic risk score was associated with 0.75 mmHg lower systolic blood pressure (95% CI: -1.31, -0.19) and 0.42 mmHg lower diastolic blood pressure (95%CI: -0.72, -0.13). The association did not attenuate after further adjustment for anti-hypertensive medication use, and conventional cardiovascular risk factors. In subgroup analysis, the association of uric acid genetic risk score with systolic blood pressure was significantly stronger in participants (n= 885) on diuretic treatment (P for interaction: 0.007). In conclusion, we found that higher uric acid genetic risk score is associated with lower systolic and diastolic blood pressure. Diuretics treatment may modify the association of uric acid genetic risk and systolic blood pressure. Our study suggests that genome wide association study's findings can be associated with an intermediate factor or have a pleiotropic role and therefore should be applied for Mendelian Randomization with caution.

Background

Hypertension represents the highest proportion of attributable mortality amongst all global risk factors.[1,2] The pathogenesis of hypertension is complex and cardio-metabolic factors might play a role.[3] One of the recently proposed risk factors for hypertension is hyperuricemia.[4] Observational studies have consistently confirmed the association between serum uric acid and incidence of hypertension.[5,6] However, high serum uric acid is associated with co-morbidities such as cardiovascular disease, kidney disease, or diabetes and antihypertensive medications that can influence the association of serum uric acid and blood pressure.

In recent years genetic information has been repeatedly used in order to assess causality in the pathogenesis of complex disorders. This approach is based on the fact that alleles are allocated randomly during gamete formation; therefore, genetic variants are inherited independent of potential confounding factors.[7] One of the limitations of this approach is lack of power that is a result of using a single risk allele. Recently, genome wide association studies (GWAS) have identified numerous gene variants for many complex disorders. Therefore, using the combined effect of all single nucleotide polymorphism (SNP)s related to a phenotype, collectively named a genetic risk score (GRS), might improve the power. For serum uric acid nearly 30 gene variants have been identified which explain ~7% of variation in serum uric acid.[8] In this study we aimed to use these genetic loci to compile a uric acid GRS and investigate the association between uric acid GRS and blood pressure components in the Rotterdam Study, a large prospective population-based cohort study of elderly Caucasian subjects. We further evaluated whether this association differs by key population characteristics including comorbidities and medication intake.

Methods

Population for analyses

This study was performed within the Rotterdam Study, a population-based cohort study designed to assess the occurrence and risk factors for chronic diseases in older subjects. In 1990, all inhabitants of Ommoord, a district of Rotterdam in the Netherlands, aged 55 years or older were invited, of whom 7983 agreed to participate. Genotyping data was available for 5974 participants. For this study we included 5791 participants with data available for genotyping and blood pressure components. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[9]

Measurement of serum uric acid and blood pressure

Values of serum uric acid were obtained from non-fasting blood samples which were centrifuged for 10 minutes at 3000 rotations per minute and subsequently stored in -20°C for one week. Uric acid activity was ascertained with Kone Diagnostica reagent kit and Kone autoanalyzer. In order to check the calibration, for every 10 samples, 3 control samples were included. In each run (100 samples), if the average values of the control samples were not within 2.5% of the true value, the run was repeated. Calibration was also done on day-by-day variation, which had to be within 5%.[10]

After a resting period of five minutes, blood pressure was measured twice in a single visit using a random-zero sphygmomanometer (cuff size of 32× 17) on the right arm of participants in the sitting position. The average of two measurements, separated by a count of the pulse rate, was used in the analyses.[11]

Table 1. Characteristics of the 5,791 study participants

Characteristics	
Age, mean (SD), y	69.4 (9.1)
Men (%)	2427 (40.6)
Systolic blood pressure, mean (SD),mmHg	139.2 (22.2)
Diastolic blood pressure, mean (SD),mmHg	73.7 (11.5)
Alcohol Intake, median (IR), g/d	3.5 (0.1-14.9)
Body mass index, mean (SD), kg/m^2	26.3 (3.7)
Total cholesterol, mean (SD), mmol/L	6.6 (1.2)
HDL cholesterol, mean (SD), mmol/L	1.3 (0.3)
GFR, mean (SD), ml/min per 1.73 m2	77.1 (17.2)
Chronic kidney disease n(%)	600 (10.0)
Diabetes Mellitus n(%)	631 (10.6)
History of cardiovascular disease n(%)	777 (13.0)
Diuretics n(%)	923 (15.5)
Anti gout medication n(%)	196 (3.3)
ACE inhibitors n(%)	354 (6.2)
Calcium channel blocker n(%)	372 (6.2)
Beta blocker n(%)	861(14.4)
Serum uric acid, mean (SD), µmol/l	321.8 (81.5)

Abbreviations
SD: Standard deviation
IR: Interquartile range
GFR: Glomerular filtration rate

Covariates

Body mass index was calculated by dividing weight in kilograms by height in meters squared. Information on alcohol consumption was acquired from the questionnaires. Participants were asked for the average daily consumption of alcohol. Medication use information was based on home interview. Diabetes mellitus was defined as the use of blood glucose lowering medications or a random non-fasting glucose above 11.1 mmol/l.[12] Coronary heart disease was considered as having experienced myocardial infarction or undergone coronary revascularization procedures.[13] Serum creatinine was determined using non-kinetic alkaline picrate (Jaffé) method. Estimated glomerular

filtration rate (eGFR) was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation.[14]

Genotyping

Genotyping was conducted using the Illumina 550K array. Subjects were excluded if they had excess autosomal heterozygosity, mismatch between called and phenotypic sex, or recognized as being outlier with IBS clustering analysis. Moreover, SNPs with allele frequency ≤1%, Hardy-Weinberg equilibrium (HWE) $p<10^{-5}$, or SNP call rate ≤90% were excluded. Imputation was done with reference to HapMap release 22 CEU using the maximum likelihood method implemented in MaCH (version 1.0.15).

Statistical analyses

In this study, we selected SNPs previously reported to have an association with serum uric acid from a GWAS of more than 140,000 European-ancestry individuals.[8] A GRS was compiled using 30 SNPs associated with serum uric acid (Table S1). We calculated a weighted GRS by multiplying the number of risk alleles at each locus by the corresponding reported beta coefficient from the previous GWAS and then summing the products. The total score was then divided by the average effect size, multiplied by 100 to rescale the scores to a range between 0 and 100. Association of continuous measures of uric acid GRS with systolic blood pressure and diastolic blood pressure were evaluated using linear regression models. We estimated the betas and 95% confidence interval (CI) per standard deviation (SD) increase in uric acid GRS. All analyses were adjusted for age and sex. In the second model, we additionally adjusted for eGFR, body mass index, alcohol consumption, diabetes mellitus, history of cardiovascular diseases, anti-gout medication, and different type of antihypertensive medications (diuretics, beta blockers, ACE inhibitors, and calcium channel blockers). To investigate whether the association of uric acid GRS with systolic and diastolic blood

pressure differs in participants with various comorbidities (diabetes, cardiovascular disease, chronic kidney disease and hypertension) and antihypertensive medication use (diuretics, beta blockers, ACE inhibitors, and calcium channel blockers), we assessed the interaction of uric acid GRS and aforementioned characteristics by adding an interaction term in the regression model. The interaction term was the product of the interacting factor and serum uric acid GRS. In addition, we performed a series of stratified analyses by separately studying the association of uric acid GRS with systolic and diastolic blood pressure in participants with and without comorbidities and antihypertensive medication use.

Results

Fifty nine percent of participants were female. Average age of participants was 69 years, with mean systolic blood pressure of 140.6 mmHg and mean serum uric acid of 321.8 µmol/l (Table 1). Figure 1 shows adjusted (age and sex) mean levels of serum uric acid, systolic and diastolic blood pressure in different categories of uric acid GRS. We observed a positive linear association between serum uric acid and uric acid GRS. The variance in serum uric acid concentrations explained by uric acid genetic risk score was 4.2% in our study.

As shown in Table 2, each SD increase in uric acid GRS was associated with 0.75 mmHg lower systolic blood pressure in age, and sex adjusted model (95% CI -1.31, -0.19). Further adjustments for eGFR, cardiovascular risk factors and medications did not substantially change the association between uric acid GRS and systolic blood pressure. Each SD increase in uric acid GRS was associated with 0.42 mmHg lower diastolic blood pressure (95%CI -0.72, -0.13). Similarly, further adjustment did not change the associations (Table 2). Adjusting for serum level of uric acid did not change the association of uric acid GRS with systolic and diastolic blood pressure (Table S2).

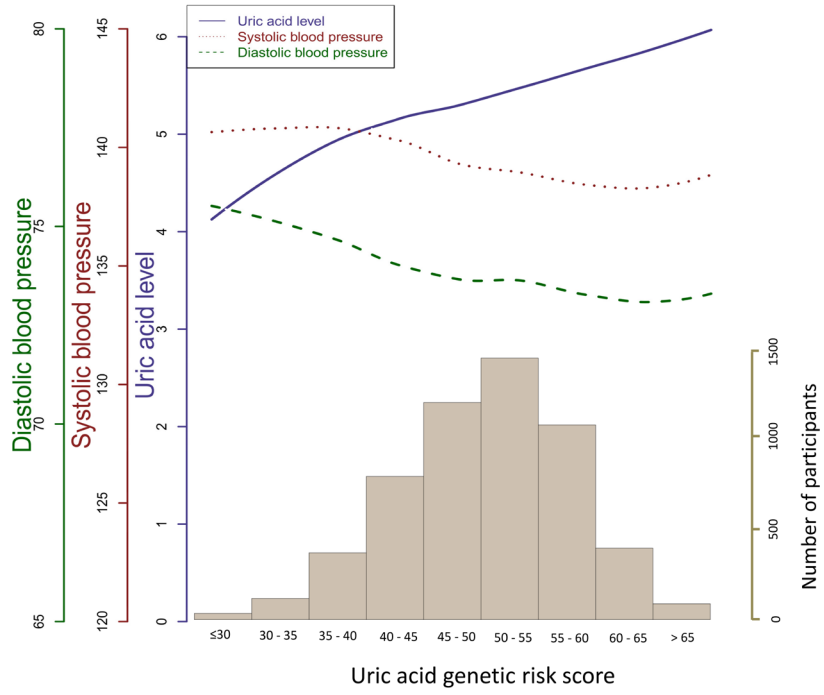

Figure 1. Mean levels of serum uric acid, systolic and diastolic blood pressure in different categories of uric acid genetic risk score. Mean values are adjusted for age, and sex.

In the stratified analyses, there was no statistical difference in the strength of the association between uric acid GRS and systolic blood pressure in subgroups of participants with and without diabetes, cardiovascular disease, chronic kidney disease and hypertension (Figure 2, Table S3). However, the association was stronger in participants who reported to use diuretics (P for interaction: 0.007). We did not find such a difference for other types of antihypertensive medications including calcium channel blockers, ACE inhibitors, and beta blockers or any type of antihypertensive medications (Figure 2, Table S3). To find out if the interaction is driven by one or a number of SNPs, we investigated the interaction of each uric acid SNPs with diuretics treatment in relation to systolic blood pressure (Table S4). After Bonferroni correction

Table 2. Association of uric acid genetic risk score with systolic and diastolic blood pressure

Models	Systolic blood pressure			Diastolic blood pressure		
	Beta *	95 % CI	P-value	Beta	95 % CI	P-value
Model 1 (n=5791)	-0.75	-1.31,-0.19	0.008	-0.42	-0.72, -0.13	0.005
Model 2 (n=3471)	-0.92	-1.62,-0.23	0.009	-0.55	-0.92,-0.18	0.004

CI: Confidence interval
*Betas are per standard deviation increase in uric acid GRS
Model 1: Adjusted for age and sex
Model 2: Adjusted for age, sex, eGFR, body mass index, alcohol consumption, diabetes mellitus, history of cardiovascular diseases, anti-gout medication, diuretics, beta blockers, ACE inhibitors, calcium channel blockers

a SNP in SLC2A9 gene (rs12498742) showed a significant interaction with diuretics treatment in relation to systolic blood pressure (P for interaction: 0.0009) (Table S4). The variance in serum uric acid concentrations explained by SLC2A9 gene was 2.3% in our study. We further estimated adjusted means (age and sex) of systolic blood pressure across different genotypes of rs12498742 in participants with and without diuretics use. In participants reported to use diuretics, there was a significant association between systolic blood pressure and different genotypes of rs12498742 SNP (P for trend 0.001) (Figure 3). Among participants on diuretic treatment, participants with AA genotype had lower mean systolic blood pressure. There was no association between systolic blood pressure and different genotypes of rs12498742 SNP in participants who were not on diuretics treatment (P for trend 0.598). After excluding this SNP from the uric acid GRS the association was not present anymore, however the direction of the effect estimates remained the same (Table S5). To explore whether the association of uric acid GRS with systolic and diastolic blood pressure is influenced by the genes that have been shown to be related to blood pressure, [15] we performed an extra analysis by excluding variants in ATXN2 and OVOL1 genes from uric acid GRS. The associations

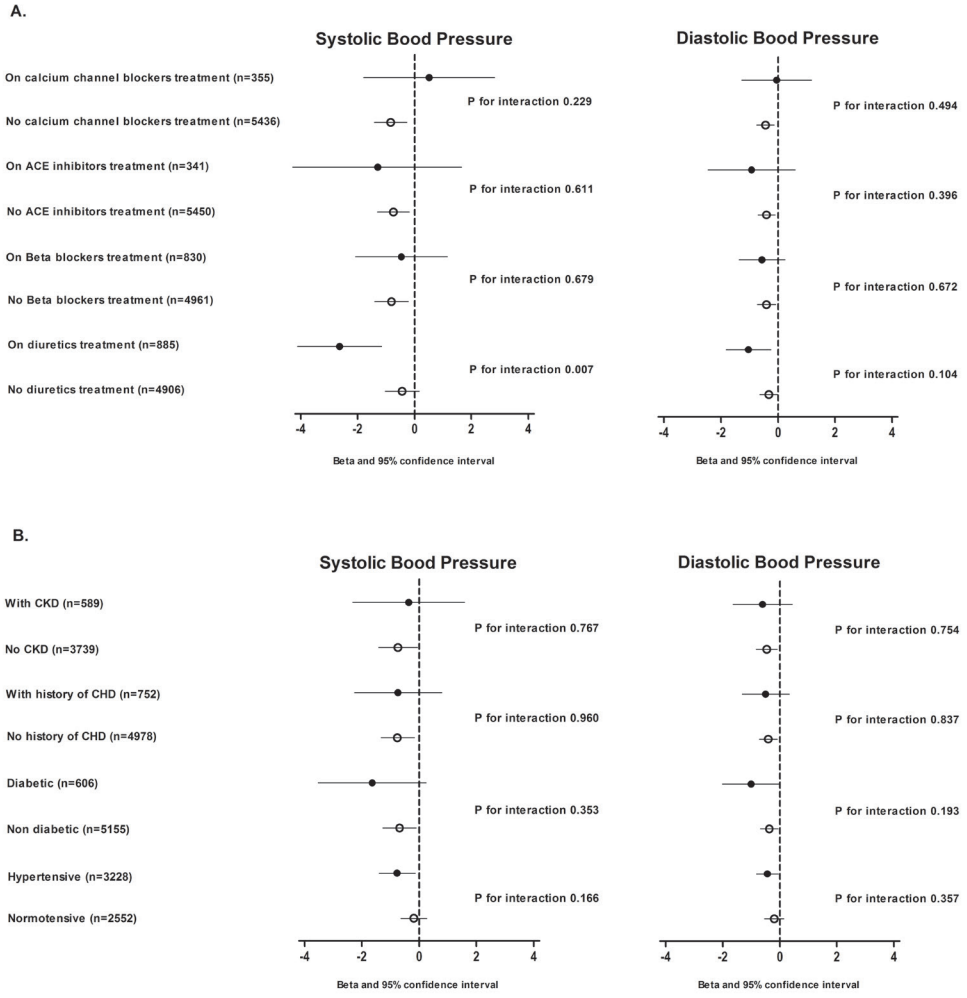

Figure 2. A) Association of uric acid genetic risk score with systolic and diastolic blood pressure in subgroups of the participants with and without antihypertensive medications. B) Association of uric acid genetic risk score with systolic and diastolic blood pressure in subgroups of the participants with and without co-morbidities. All betas are calculated per standard deviation increase in uric acid genetic risk score. All analyses are adjusted for age, and sex.

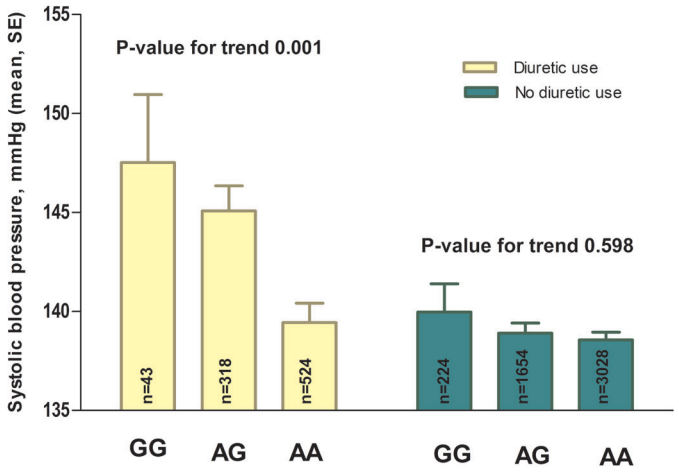

Figure 3. Mean and standard error of systolic blood pressure in different genotypes of rs12498742 SNP (SLC2A9 gene) in participants with and without diuretics use. Mean values are adjusted for age and sex.

remained similar in this analysis (Table S6).

In a restricted analysis excluding participants on anti-gout and antihypertensive medications, we observed that the association of uric acid GRS with systolic and diastolic blood pressure did not essentially change (Table S7). Furthermore, the effect estimate in diabetic participants was larger than the effect estimate in non-diabetic subjects (P for interaction was not significant).

Discussion

In contrast to the findings of the previous studies, in this cross sectional population-based study we found that a uric acid GRS, composed of 30 known uric acid genetic variants, is associated with lower systolic and diastolic blood pressure. The association was more pronounced in those who were using diuretics.

SLC2A9 gene was the first gene identified for serum uric acid by GWAS and confers the strongest association with uric acid levels. Parsa et al. investigated the association

between a missense SNP in the SLC2A9 gene and blood pressure.[16] They found that a decrease in serum uric acid due to the above-mentioned SNP is associated with lower levels of systolic blood pressure, depending on salt intake.[16] In addition, a recent paper by Mallamaci et al. showed that rs7345555 variant in SLC2A9 gene is associated with systolic blood pressure in 449 cardiovascular complication-free individuals.[17] In another Mendelian Randomization study, Palmer et al. studied the association of a variant in SLC2A9 gene with systolic and diastolic blood pressure. They found no strong evidence for the causal association between uric acid and blood pressure.[18]

Combining small effect of all genes associated with serum uric acid, we found that uric acid GRS is associated with lower levels of systolic and diastolic blood pressure. The association is not likely to be mediated through elevated uric acid levels since adjusting for serum uric acid did not change the effect estimates. Our findings are in agreement with Yang et al., who found a negative association (borderline significant) between a uric acid GRS (based on 8 SNPs) and systolic blood pressure.[19] The association was not present after excluding participants that were treated with antihypertensive medication.[19]

The discrepancy between our findings and other studies [5,6,16,18,20] could be explained in different ways. First, subjects with high genetic susceptibility had a higher probability to develop hypertension and therefore receive antihypertensive medications. Thus, the association between high uric acid GRS and lower levels of blood pressure could be at least partially explained by the use of these medications. However, adjusting for antihypertensive medications did not change the association. Moreover, this does not explain why we only observe the association in those who use diuretics and not in those who are using other types of anti-hypertensive medications. Second, hyperuricemia is known to be a side effect of diuretics. It is possible that some of the genes that have identified in uric acid GWAS are the genes that interact with diuretics treatment to decrease blood pressure. Therefore, individuals who carry the risk allele respond better

to diuretics and are more likely to be treated with diuretics which will lead to the development of hyperurecimia as a side effect of diuretics. In agreement with this conjecture, we observed a significant association between systolic blood pressure and different genotypes of rs12498742 SNP in participants reported to use diuretics while such association was not present in participants who were not on diuretic treatment. This finding is supported by Hasannejad et al., who showed that solute carrier family 22 may play an important role in the basolateral uptake of thiazides and uptake of loop diuretics. [21] We did not observe an interaction between uric acid GRS and diuretics in relation to diastolic blood pressure. This may be due to the fact that diuretics lower the blood pressure by inducing sodium and fluid loss, which is more affecting systolic blood pressure.[22,23] In addition, excluding the rs12498742 SNP from the uric acid GRS did not change the direction of the effect estimates, therefore it is possible that this gene has pleiotropic effects. Third explanation could be that uric acid might offer protective effects against vascular endothelium using its antioxidant properties. It has been suggested that moderate elevation of serum uric acid due to genetic susceptibility might be associated with lower blood pressure.[24,25, 26]

We performed the analyses in a large population-based prospective cohort study, which enables us to see the small effects of the gene variants, test for interactions, and control for several potential confounders. Nevertheless, as a limitation, we built up our uric acid GRS only based on common variants (which explain only ~7% of total genetic variance) and we were unable to assess the potential contribution of rare variants.

Conclusions

We found that a higher uric acid genetic risk score is associated with lower systolic and diastolic blood pressure. Diuretics treatment may moderate the association of uric acid GRS and systolic blood pressure. Vast amount of genes are stemming from the GWA studies; however, findings of this study suggest that some of the genetic variants

can be associated with an intermediate factor or have a pleiotropic role. Therefore, due to pleiotropic effects of the genes, results from the Mendelian Randomization approach based on GRS should be interpreted with caution. Future studies are needed to elucidate the mechanisms behind these associations.

Online supplement
http://hyper.ahajournals.org/content/early/2014/09/02/HYPERTENSIONAHA.114.03757/suppl/DC1

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2.3 Association of renal function with vascular stiffness in older adults: The Rotterdam Study

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Arterial stiffening is a marker of vascular aging and an independent risk factor for cardiovascular disease. A potential mechanism linking cardiovascular disease to chronic kidney disease might be the change in arterial elasticity. We aim to determine the association between renal function and arterial stiffness in older subjects. We included 3279 subjects from 1997 to 1999 with a mean age of 71.9 years from the Rotterdam Study, a population-based cohort study. Estimation of glomerular filtration rate (eGFR) was used to assess renal function. Aortic pulse wave velocity and carotid distensibility coefficient were used as measures of arterial stiffness. Each standard deviation increase in eGFR, adjusting for age and sex, was associated with 0.14 m/s lower pulse wave velocity (95% confidence interval (CI): -0.23, -0.05). Further adjustments for socio-demographic and cardiovascular risk factors, did not change the association (Beta: -0.16 m/s; 95% CI: -0.26, -0.06). There was a linear association between mean values of pulse wave velocity and quartiles of glomerular filtration rate (p for trend= 0.006). There was no association between decreased renal function and carotid distensibility. There was no statistical difference in the strength of the association between renal function and pulse wave velocity in subgroups of participants with and without cardiovascular risk factors. In this large population-based study of elderly subjects, our findings suggest that renal impairment is associated with aortic stiffness. This association is independent of cardiovascular risk factors.

Background

Patients with chronic kidney disease (CKD) are at an increased risk for cardiovascular disease compared to general population.[1] The mechanism underlying this association is not well understood. Early kidney dysfunction through rise in deposition of mineral and glycation products can lead to increased vascular remodelling and arterial stiffness.[2] Arterial stiffness, a known marker of vascular aging,[3] has been identified as an independent risk factors for cardiovascular disease. Therefore, change in arterial elasticity might be one of the mechanisms in the pathway linking CKD to cardiovascular disease.

Several studies have suggested an association between arterial stiffness and renal function.[4-10] Most of these studies have been conducted in high risk populations with co-morbid conditions such as diabetes and chronic renal failure with inconsistent findings.[4,5,7-10] Since both arterial stiffness and renal function are closely associated with cardiovascular risk factors and cardiovascular diseases,[11] at least part of the controversy may originate from inclusion of high risk populations. Therefore, in this study we aimed to investigate the possible association between renal function and measures of arterial stiffness in a general population of older subjects. Furthermore, we investigated whether this association differs between participants with and without cardiovascular risk factors.

Methods

Study population

This study was performed in the framework of the Rotterdam Study, a population-based prospective study comprising 7983 participants aged 55 years and over living in Ommoord, a district of Rotterdam, the Netherlands. All participants were invited every three to four years to the research center for follow-up examinations. Arterial stiffness

was measured in the random subset of the third visit (1997-1999) of the Rotterdam Study. From 4797 individuals who participated in this visit data was available for 3279 for analysis of carotid-femoral pulse wave velocity (PWV) and for 2470 individuals for analysis of carotid distensibility coefficient. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.[12]

Renal function

Serum creatinine was assessed by enzymatic assay method.[13]. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[14] The equation for the eGFR is as follow; if female and serum creatinine (Scr) ≤ 0.7 mg/dl: $144\times(Scr/0.7)^{-0.329}\times0.993^{age}$, if female and Scr >0.7 mg/dl: $144\times(Scr/0.7)^{-1.209}\times0.993^{age}$, if male and Scr ≤ 0.9 mg/dl: $141\times(Scr/0.9)^{-0.411}\times0.993^{age}$, and if male and Scr >0.9 mg/dl: $141\times(Scr/0.9)^{-1.209}\times0.993^{age}$.

Measures of arterial stiffness

Carotid-femoral PWV was measured with the subjects in supine position. PWV was assessed with an automatic device (Complior Artech Medicla)[15] measuring the time delay between the rapid upstroke at the base of simultaneously recorded pulse waves in the carotid and the femoral arteries. PWV was calculated as the ratio between the carotid and the femoral arteries distance and the base-to-base time delay.[16]

The carotid distensibility was assessed with the subjects in supine position, with the head tilted to the contra-lateral side. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system, as described by Hoeks.[16] After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb

of the carotid artery was identified with the use of B-mode ultrasound. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were calculated as the mean of 4 cardiac cycles of 3 successive recordings.[17] The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient= 2ΔD/(D × pulse pressure) (10^{-3}/kPa).[17] Mean arterial pressure (MAP) and pulse rate was recorded simultaneously for PWV and carotid distensibility coefficient.

Cardiovascular risk factors

A trained interviewer using a computerized questionnaire collected information on current health status, medical history, drugs use, and smoking behaviour from the subjects at the third examination round (1997-1999). Serum glucose, total serum cholesterol and high-density lipoprotein cholesterol were measured using standard laboratory techniques. Diabetes mellitus patients were defined by a history of either the disease, use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer, after the participant had been seated for at least 5 minutes. The mean of both blood pressure values was used in the analyses. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or use of blood pressure lowering medication with hypertension as the indication.

Statistical analysis

Multivariate linear regression was used with PWV and carotid distensibility as the dependent variables and eGFR as the independent variable. We ran the analyses in two models. Analyses in the first model were adjusted for age, sex, continuous measures of mean arterial pressure, and heart rate. In a second model, we further adjusted

the analyses for continuous measures of body mass index, total serum cholesterol, high-density lipoprotein cholesterol, as well as diabetes mellitus, smoking, and use of antihypertensive medication (diuretics, beta blockers, ACE inhibitors, and calcium channel blockers). We estimated the difference per standard deviation increase in eGFR values. Additionally, we performed an analysis of covariance (ANCOVA) where mean values of PWV were compared across eGFR quartile levels. Cut-off points for quartiles of GFR were 65.22 mL/min, 74.91 mL/min and 84.72 mL/min. We performed a series of stratified analyses, to explore whether the associations between eGFR and markers of arterial stiffness is consistent in different participants with and without cardiovascular risk factors (diabetes, cardiovascular disease, hypertension, and body mass index >25). Analyses were adjusted for age, sex, mean arterial pressure and heart rate. In addition, interaction was assessed, by adding an interaction term in the regression model. The interaction term was the product of the interacting factor and eGFR. All the analyses were performed using SPSS statistical package (IMB® SPSS® Statistics, version 20).

Results

The study population had a mean age (±SD) of 71.9±6.8 years, and eGFR of 73.9± 14.01 ml/min per 1.73 m^2. As shown in Table 1, subjects with lower eGFR were older. In addition, subjects with lower eGFR had higher systolic blood pressure, mean arterial pressure, pulse pressure, body mass index, history of coronary heart disease and prevalence of antihypertensive medications use (all $p<0.05$). Subjects with lower eGFR had lower HDL-C and prevalence of diabetes mellitus (all $p<0.05$).

Table 2 shows the association of eGFR with markers of arterial stiffness. In model 1, one standard deviation increase in eGFR, was associated with 0.14 m/s lower PWV (95%CI: -0.23, -0.05). Adjusting for other cardiovascular risk factors in model 2 did not change the association (Beta: -0.16, 95%CI: -0.26, -0.06). Full models including the effect sizes of the other covariates are presented in the Table S1. In addition, we

repeated the analyses on the association of renal function and pulse wave velocity per 0.8 m/s increase in measures of PWV. This further analysis showed similar findings (Table S2). There was no significant association between renal function and carotid distensibility (Beta: 0.10 10^{-3}/kPa, 95%CI: -0.04, 0.23).

We calculated adjusted mean values of arterial stiffness markers in quartiles of eGFR (Figure S1). Analyses were adjusted for cardiovascular risk factors. There was a linear association between mean values of PWV and quartiles of eGFR (p for trend= 0.006). Mean values of PWV was higher in first quartile of eGFR compared to the fourth quartile (13.97 m/s vs.13.28 m/s) (Figure S1a). We did not observe a linear association between mean values of carotid ditensibility and quartiles of eGFR (Figure S1b). In the stratified analyses, there was no statistical difference in the strength of neither the association between eGFR and PWV nor eGFR and carotid distensibility in subgroups of participants with and without cardiovascular risk factors (Figure 1).

Discussion

In this study, we found an association between renal function and PWV. Subjects with decreased renal function had higher PWV measures, hence stiffer aorta. This association was independent of cardiovascular risk factors. We did not observe an association between decreased renal function and carotid distensibility. Several studies have reported the association of kidney function with arterial stiffness; however most of these studies were conducted either on patients' population or were based on health checkup data.[4,6-8,10,18] A population-based study performed by Peralta, et al. showed that early kidney dysfunction in older adults with mean age of 62 years was associated with decreased arterial elasticity in small arteries but they did not find an association between renal function and aortic distensibility. [9] In contrast, we observed that impaired renal function is associated with increase in aortic PWV in population-based sample of older adults with mean age of 72 years. A recent study by Lui et al. found an inverse

Table 1. Baseline characteristics of the study population (n=3279)

	eGFR Quartiles (ml/min per 1.73 m²)				
	≤65.22 (n=819)	**65.27-74.91 (n=821)**	**74.96-84.72 (n=820)**	**>84.72 (n=819)**	**P- value for trend**
Age, years	75.1(6.7)	72.4(6.8)	71.4(6.5)	68.3(4.8)	<0.001
Men	338(41.3)	331(40.4)	377(46.0)	347(42.4)	0.249
SBP, mmHg	146.5(21.9)	143.5(20.1)	142.6(20.9)	141.8(21.5)	<0.001
DBP, mmHg	75.5(11.3)	75.9(11.1)	74.6(10.9)	75.9(10.9)	0.040
MAP, mmHg	108.0(13.2)	106.7(12.5)	105.4(12.7)	105.8(12.5)	<0.001
Pulse pressure, mmHg	70.0(18.1)	67.2(17.3)	66.3(17.6)	63.9(16.1)	<0.001
Heart rate, bpm	73.9(14.9)	75.2(14.2)	75.5(14.6)	75.5(14.1)	0.071
Body mass index, kg/m²	27.2(3.7)	26.7(3.9)	26.5(3.7)	26.5(3.8)	<0.001
Current smokers	102(12.6)	94(11.6)	138(17.0)	173(21.2)	<0.001
Total cholesterol, mmol/L	5.8(1.0)	5.8(0.9)	5.7(0.9)	5.8(0.9)	0.107
HDL-cholesterol, mmol/L	1.3(0.4)	1.4(0.4)	1.4(0.4)	1.4(0.4)	<0.001
Diabetes mellitus	121(15.5)	80(10.2)	108(13.6)	133(16.9)	0.001
Cardiovascular disease	159(19.4)	100(12.2)	105(12.8)	89(10.9)	<0.001
Antihypertensive medication	444(54.2)	304(37.1)	278(33.9)	236(28.8)	<0.001

High density lipoprotein, eGFR: estimated glomerular filtration rate, MAP: mean arterial pressure
Categorical variables: number (percentage), Continuous variables: mean (standard deviation)

Table 2. Multiple linear regression (difference and 95% confidence intervals) describing the association between renal function and arterial stiffness

		PWV (m/s)		Carotid distensibility (10^{-3}/kPa)	
		Difference*	95% CI	Difference	95% CI
eGFR	Model 1	-0.14	-0.23, -0.05	0.10	-0.04, 0.23
	Model 2	-0.16	-0.26, -0.06	0.05	-0.09, 0.19

*Differences are beta coefficients from multiple linear regression models. Betas are per standard deviation of eGFR values.
CI: confidence interval, PWV: Aortic pulse wave velocity, eGFR: estimated glomerular filtration rate
Model 1: Adjusted for age, sex, mean arterial pressure, and heart rate
Model 2: Adjusted for age, sex, mean arterial pressure, heart rate, body mass index, serum total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking, antihypertensive medication.

correlation between eGFR and brachial ankle PWV in the elderly aged 60 years and older.[19] Brachial ankle PWV appears to be an index for peripheral stiffness, rather than central stiffness.[20] In this study we used carotid femoral PWV, a gold standard measure of central arterial stiffness, that has been shown to be consistently associated with cardiovascular diseases.[21] Briet, et al. observed that vascular stiffness measured by carotid distensibility was higher in CKD patients compared to normotensive individuals. In our population-based study, we did not find an association between kidney function and carotid distensibility which might be explained by different types of population under study with different stages of renal function.[4]

In this study we found an association between a decreased renal function and aortic stiffness in the older adults. It is speculated that such an association between eGFR and PWV could be driven by cardiovascular risk factors such as age, hypertension and diabetes mellitus.[2,22] Nevertheless, adjustments for those factors did not affect the association. In addition, we did not observe a statistical difference in the strength of the association between eGFR and PWV in participants with and without cardiovascular risk factors. Collectively, we showed that renal function could play a role on the arterial functional properties independent of cardiovascular risk factors.

Some pathophysiologic mechanisms might explain our results. Arterial stiffness is

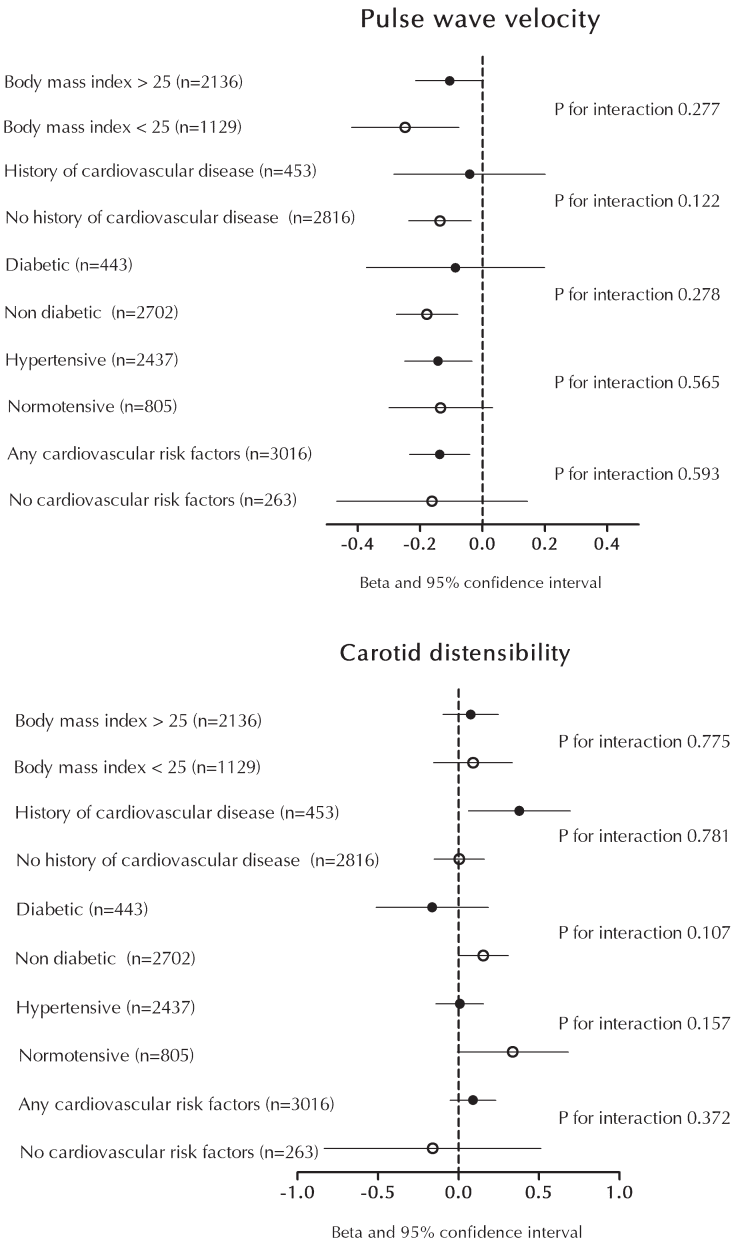

Figure 1. Association of eGFR with pulse wave velocity and carotid distensibility in subgroups of the participants with and without cardiovascular risk factors. All betas are calculated per standard deviation increase in eGFR. All analyses are adjusted for age, sex, mean arterial pressure and heart rate.

accelerated in patients with decreased renal function and in these patients arterial stiffening seems to be related to arterial wall thickening.[5,6,23] Arterial wall properties are influenced not only by age, genetics, hypertension, diabetes, but also by factors associated with the presence of uremia[4,24] and arterial calcification which are common in CKD.[25] Furthermore, high plasma levels of asymmetric dimethylarginine and hemocysteine may increase arterial stiffness through endothelial dysfunction.[10] In contrast, increased arterial stiffness through elevated intrarenal pulse pressure may lead to glomerular damage.[26] In this study, we could not clarify the cause and effect relationship between renal function and arterial stiffness.

We found no association between decreased renal function and carotid distensibility. This could be explained by the differences in anatomic and functional properties between aorta and carotid arteries. Carotid distensibility is a local measure of stiffness that gives information on one specific elastic artery, while the aortic pulse wave velocity reflects the vessel wall stiffness of several territories providing also information on both elastic and muscular arteries.[27] We cannot exclude that the difference in findings for the distensibility coefficient and aortic pulse wave velocity is due to differences in the validity or reproducibility of the measurement. In a reproducibility study performed among 47 subjects, the intraclass correlation coefficient was 0.80 for both the PWV and the carotid distensibility coefficient.[28] Therefore we do not think that this is a likely explanation for our results.

Our study has some strengths, the Rotterdam Study is a large population-based cohort study, which on one hand provides sufficient statistical power to answer our research question and on the other hand could be generalized to general population. Furthermore, we controlled for several potential confounders, and performed the analyses separately in subgroups of participants with and without cardiovascular risk factors. Our study has some limitations. First, the cross-sectional design could limit our ability to infer the directionality of the association between arterial stiffness and renal

function. Second, measures of renal function and vascular stiffness were measured only once; therefore intra-individual variation could not be taken into account, however such variation will likely result in an underestimation of the true relationship.[29] Third, measures of renal function and vascular stiffness were not available for all participants. However, this was due to logistical reasons and therefore random, thus, we believe that this did not bias the results. Fourth, for computing the carotid distensibility we used the brachial pulse pressure rather than the carotid pulse pressure. Information on comparisons between carotid and brachial pulse pressures indicates that the difference between these pressures is 8 mm Hg in a presumed healthy population and 2.6 mm Hg in patients with severe coronary artery disease.[30] These findings indicate that using brachial artery pulse pressure instead of carotid artery pulse pressure may lead to an underestimation of the distensibility.

In conclusion, in this large population based study we found that in elderly subjects a decreased renal function is associated with increased aortic stiffness independent of other cardiovascular risk factors. Therefore, increased arterial stiffness may underlie at least in part the elevated cardiovascular risk in individuals with impaired renal function. Future longitudinal studies are needed to study the parallel deterioration of renal function and arterial stiffness.

Online supplement
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2.4 Arterial stiffness and the risk of chronic kidney disease

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The independent link between arterial stiffness and chronic kidney disease (CKD) has remained unknown. We investigated the association of indicators of arterial stiffness with decline in kidney function. We studied 3666 participants (mean age 65 years; 58% women) from the Rotterdam Study. Pulse pressure, carotid stiffness, and pulse wave velocity (PWV) were measured as indicators of arterial stiffness. We created genetic risk scores for pulse pressure and PWV using genetic variants from GWAS. Annual decline in kidney function and incident CKD were assessed using eGFR. In the basic model, each standard deviation (SD) higher pulse pressure was associated with 0.15 ml/min/1.73 m^2 steeper annual eGFR decline (95%CI: 0.10, 0.20) and 11% increased risk of incident CKD (CI: 1.05, 1.18). Each SD greater carotid stiffness was associated with 0.08 steeper annual eGFR decline (95%CI: 0.04, 0.13) and 13% increased risk of incident CKD (95%CI: 1.05, 1.22). Each SD higher PWV was associated with 7% increased risk of incident CKD (95%CI: 1.00, 1.14). Incorporating our findings in a meta-analysis of population-based studies, each SD higher pulse pressure and pulse velocity were associated with 16% (95%CI: 1.12, 1.21) and 8% (95%CI: 1.03, 1.14) higher risk of incident CKD. Each SD higher pulse pressure genetic risk score was associated with 0.06 steeper annual eGFR decline (95%CI: 0.01, 0.10), and 8% increased risk of incident CKD (95%CI: 1.03, 1.14). There was no association between PWV genetic risk score and decline in kidney function. We found that higher indices of arterial stiffness are associated with steeper decline in kidney function. This suggests that vascular stiffness could be considered as a target for delaying decline in kidney function in particular in patients with early stage kidney disease.

Background

Considerable proportions of patients with CKD carry multiple cardiovascular risk factors and die from cardiovascular causes.[1] Accumulating evidence suggests a strong association between cardiovascular pathology and CKD. Nevertheless, exact mechanisms linking cardiovascular diseases with kidney impairment remain to be elucidated.[2]

Vascular risk factors such as age, smoking, and hypertension have been proposed in the association between cardiovascular disease and CKD.[3] One of the novel risk factors proposed for cardiovascular disease is arterial stiffness.[4] Arterial stiffness, independent of mean arterial pressure, results in end-organ damage by imposing hemodynamic stress on vascular beds.[5] Aortic stiffening especially in older people facilitates transmission of excessive pressure and flow pulsatility into the microvascular beds of the kidneys, a high flow organ, which will potentially lead to microvascular ischemia and tissue damage.[6]

Several studies have investigated whether an independent association exists between arterial stiffness and decline in kidney function, but these results have been inconsistent.[7-14] Heterogeneity in the study populations and the limited power of the individual studies could underlie the inconsistent findings. In addition, all observational studies are subject to confounding and reverse causation. Relevant genetic variants could potentially be used to overcome these flaws.[15]

We aimed to investigate the association between arterial stiffness as well as genetic variations related to arterial stiffness with the risk of decline in kidney function in the Rotterdam Study, a population based study of individuals 55 years and older. Moreover, to put our finding in the context of the literature, we performed a meta-analysis of population-based studies on the association of arterial stiffness markers and risk of kidney disease.

Methods

Population for analysis

This study was performed within the framework of the population-based Rotterdam Study. The cohort originated in 1990, including 7983 participants from inhabitants of Ommoord, a district of Rotterdam in the Netherlands, aged 55 years or older (RS-I). In 2000 the first extension of the Rotterdam Study (RS-II) started, adding 3011 new participants. Arterial stiffness was evaluated at the third visit of RS-I and the first visit of RS-II. All individuals with available data on arterial stiffness markers at baseline and repeated creatinine measurements (at baseline and at the next visit) were included in the analyses. The mean follow up time elapsed between two creatinine measurements was 10.9 years. This resulted in 2950 participants with available data on brachial pulse pressure, 2665 with pulse wave velocity data and 2344 with carotid stiffness data. DNA was extracted from sample taken at the first visit of RS-I and at the first visit of RS-II (n=8131). Among them, 3666 had repeated measurements of creatinine for longitudinal assessment of kidney function. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[16]

Measurement of arterial stiffness

Aortic stiffness was measured as the carotid femoral pulse wave velocity with subjects in supine position with an automatic device (Complior Artech Medical, Pantin, France) that measures the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery.[17] The distance between the recording sites in the carotid and the femoral artery was measured with a tape over the surface of the body. Pulse wave velocity was calculated as the ratio

between distance and the foot-to-foot time delay, and was expressed in meters per second.

Common carotid stiffness was assessed with the subjects in supine position, with the head tilted slightly to the contralateral side for the measurement in the common carotid artery.[18] The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system.[18,19] After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified with the use of B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radiofrequency signals originating from 2 selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of 4 cardiac cycles of 3 successive recordings. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient=2ΔD/(D×pulse pressure) (10^{-3}/kPa) [18,20]. Lower carotid distensibility represents greater carotid stiffness. Heart rate was measured simultaneously with arterial stiffness measurements. Three observers performed all measurements. In a reproducibility study performed among 47 subjects who were invited twice exactly 1 week apart, the intraclass correlation coefficient was 0.80 for both the pulse wave velocity and the carotid distensibility coefficient.[19,21] After five minutes of rest, systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer and the mean was taken as the subject's reading. Pulse pressure was estimated as the difference between systolic and diastolic blood pressure.

Genetic risk score

Genotyping was conducted using the Illumina 550K array among self-reported

Caucasian individuals. Imputation was done with reference to HapMap release 22 CEU using the maximum likelihood method implemented in MaCH (version 1.0.15). We selected SNPs reported in GWAS to be associated with pulse pressure and pulse wave velocity.[22,23] There is no GWAS available on carotid stiffness. Genetic risk score was formed using 10 SNPs associated with pulse pressure and 9 SNPs associated with pulse wave velocity (Table S1). For variants in the same locus, the variant with the smallest p-value was selected. We calculated a weighted genetic risk score by multiplying the number of risk alleles at each locus by the corresponding reported beta coefficient from the previous GWASs and summing the products. The total score was then divided by the average effect size, multiplied by 100 to rescale the scores to a range between 0 and 100.

Measurement of estimated glomerular filtration rate (eGFR)

Serum creatinine was determined using an enzymatic assay method. Inter-assay and intra-assay coefficient variations were <0.92% and <1.37%, respectively. We calibrate creatinine measurements by aligning the mean values of creatinine with creatinine values of the participants of the NHANES III in different gender and age groups (<60, 60-69, ≥70).[24] eGFR was calculated according to the CKD-EPI formula.[25] To calculate the annual eGFR decline, we first subtracted the eGFR estimates of the follow-up examination from the eGFR estimates at baseline and then divided by the time between the two visits. CKD was defined as eGFR<60ml/min/1.73m^2. Incident cases were defined among the individuals free of CKD at baseline (eGFR>60ml/min/1.73m^2), who had a decline in eGFR to less than 60 between the two periodical examinations.[26]

Statistical analysis

Association of measures of arterial stiffness with annual decline in eGFR and incidence of CKD was evaluated using linear regression models and log-binomial regressions,

respectively. Betas were estimated per standard deviation (SD) increase for pulse wave velocity and pulse pressure. For carotid distensibility betas were estimated per negative SD increase of its measures which represents greater carotid stiffness. In the first model analyses were adjusted for age, sex, mean arterial pressure, heart rate, baseline eGFR, and follow up time (for analyses on incidence of CKD). In the second model, we further adjusted for body mass index, alcohol consumption, smoking, high-density lipoprotein cholesterol, total cholesterol, history of diabetes mellitus and coronary heart disease, and different type of antihypertensive medications (diuretics, beta blockers, ACE inhibitors, and calcium channel blockers). Missing values on covariates were imputed using the expectation maximization method (single imputation). Percentage of missing values on covariates was not substantial and ranged from 0.2% to 13.3%. In addition, since an interaction between blood pressure and PWV as biomarkers and indicators of hemodynamic status has been suggested previously,[27] we assessed the interaction of PWV and systolic and diastolic blood pressure by adding an interaction term in the regression model. The interaction term was the product of the PWV and systolic or diastolic blood pressure. In an extra analysis, we adjusted the associations of arterial stiffness genetic risk scores with decline in kidney function for measures of pulse pressure and PWV. All analyses were adjusted for the effect of the two Rotterdam Study cohorts and were carried out using STATA 13.1, or R version 2.15.0.

Meta-analysis

We searched for studies published in MEDLINE, EMBASE, Web of Science, and Google Scholar using the common key words related to arterial stiffness and incident CKD (Supplemental document, Appendix). Population-based studies evaluating the association between indicators of arterial stiffness and incidence of CKD were included (Table S2).[10-14] Figure S1 shows the flow diagram for inclusion of the relevant studies in our meta-analyses. Incident CKD was defined as eGFR<60 in all included

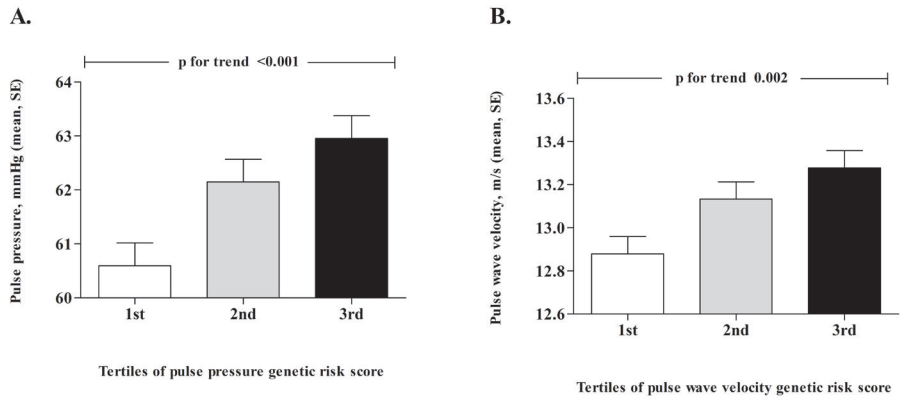


Figure 1. (A) Mean and standard error of pulse pressure in tertiles of pulse pressure genetic risk score. (B) Mean and standard error of pulse wave velocity in tertiles of pulse wave velocity genetic risk score. Analyses are adjusted for age, and sex.

studies except one study that eGFR loss of >3ml/min/1.73m^2 was used.[13] We excluded one study from the meta-analysis of pulse pressure since the outcome was reported continuously for each ml/min/1.73m^2 decline in eGFR.[14] We performed random and fixed effect meta-analysis including the current Rotterdam Study. The heterogeneity assumption was investigated using a commonly used statistical method, namely the I-square statistic. There was no evidence of publication bias using Egger's test.

Results

Baseline characteristics of participants are presented in Table 1. Mean age of the participants was 65±6.7 years, and 58.3 percent were female. Figure 1 shows the mean and standard errors of pulse pressure and pulse wave velocity in tertiles of pulse pressure and the pulse wave velocity genetic risk scores. Table 2 shows the association between indicators of arterial stiffness and kidney function. In the first model, we observed that higher pulse pressure and greater carotid stiffness was associated with steeper annual decline in eGFR and increased risk of incident CKD.

Table 1. Baseline characteristics of participants

Characteristics	n=3666
Age, mean (SD), year	65.0 (6.7)
Women, n (%)	2139 (58.3)
Body mass index, mean (SD), kg/m^2	26.6 (3.6)
Total cholesterol, mean (SD), mmol/L	6.4 (1.2)
HDL cholesterol, mean (SD), mmol/L	1.3 (0.3)
Alcohol Intake, median (IQR), g/d	4.8 (0.33- 16.6)
Smoking, n (%)	
Current	755 (20.6)
Former	1649 (45.0)
Systolic blood pressure, mean (SD), mmHg	137.3 (19.9)
Diastolic blood pressure, mean (SD), mmHg	75.4 (10.9)
Pulse rate, mean (SD), beats/minute	72.2 (11.4)
Mean arterial pressure, mean (SD), mmHg	96.0(12.6)
Pulse pressure, mean (SD), mmHg*	62.5 (15.5)
Pulse wave velocity, mean (SD), m/s*	12.2 (2.5)
Carotid distensibility coefficient, mean (SD), 10^{-3}/kPa*	12.9 (4.6)
Glomerular filtration rate, mean (SD), ml/min /1.73 m^2	79.3 (13.7)
Diabetes Mellitus, n (%)	279 (7.6)
History of coronary heart disease, n (%)	327 (8.9)
Antihypertensive medication, n (%)	
Diuretics,	355 (9.7)
ACE inhibitors	204 (5.6)
Calcium channel blocker	177 (4.8)
Beta blocker	503 (13.7)

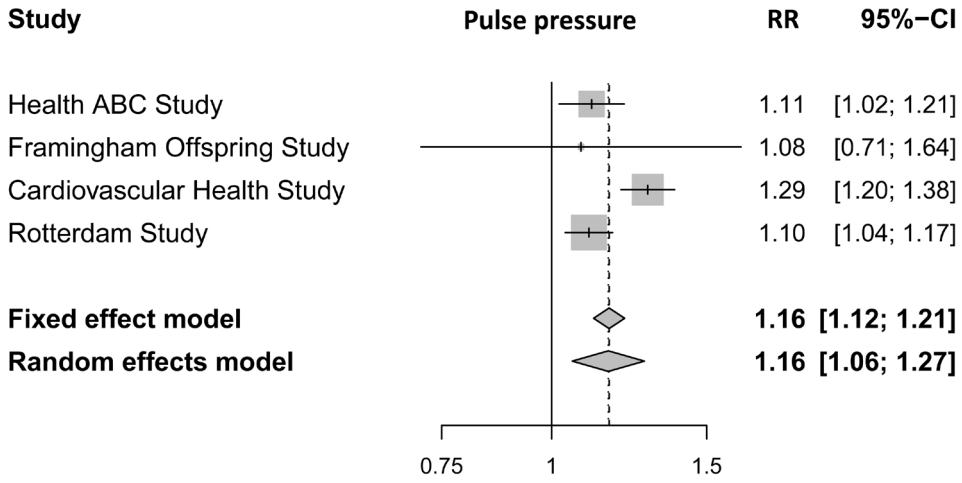
*Data is based on the correspondence sample size (pulse pressure n=2950, pulse wave velocity n=2665, and carotid distensibility n=2344)
Abbreviations: SD standard deviation, IQR interquartile range, HDL high-density lipoprotein

Adjusting for further potential confounders, in the second model, did not substantially change the association. There was no association between pulse wave velocity and annual decline in eGFR. Higher pulse wave velocity was associated with increased risk of inciednt CKD. Furthermore, we did not observe any statistically significant

interaction between PWV and systolic or diastolic blood pressure (Table S3). However, the association was not present after adjustment for potential confounders in the second model (Table 2).

To provide more reliable estimates, we performed a meta-analysis of the available studies (including the present study) reporting the association of pulse pressure and pulse wave velocity with incident CKD (Figure 2). Combining the effect estimates of our study with three previous population-based studies, we observed the overall relative risk of 1.16 (95%CI: 1.12, 1.21) for each SD increase in pulse pressure in respect to incident CKD. Test for heterogeneity resulted in moderate estimates (I^2=75% [30.6%; 91%]). Excluding the study with outcome defined as eGFR loss of > 3 mml/min per1.73 m^2 resulted in no heterogeneity (Figure S2). Regarding pulse wave velocity, we observed the overall relative risk of 1.08 (95%CI: 1.03, 1.14) for incident CKD per each SD increase in pulse wave velocity. Test for heterogeneity, resulted in moderate estimates (I^2 =59.5% [0%; 86.5%]). Excluding the study with carotid brachial pulse wave velocity measures reduced the heterogeneity (I^2 =24% [0%; 92.1%]) (Figure S2). Pulse pressure genetic risk score was associated with steeper annual decline in eGFR and increased risk of incident CKD (RR: 1.08; 95%CI: 1.03, 1.14) (Table 3). There was no association between pulse wave velocity genetic risk score and kidney function. Adjusting the associations for pulse pressure and PWV measurements changed the associations minimally (Table 4). Given the correlation between pulse pressure and blood pressure, we investigated if any of the pulse pressure genes are associated with systolic or diastolic blood pressure in our sample (Table S2). After Bonferroni correction (adjusted p-value at 0.002) none of the variants were significantly associated with blood pressure measures; however, a SNP in the PIK3CG gene and a SNP in PLCE-1 gene were suggestively associated with systolic blood pressure (p=0.003). Excluding these SNPs from the genetic risk score of pulse pressure did not essentially change the associations (Table S4).

A)



B)

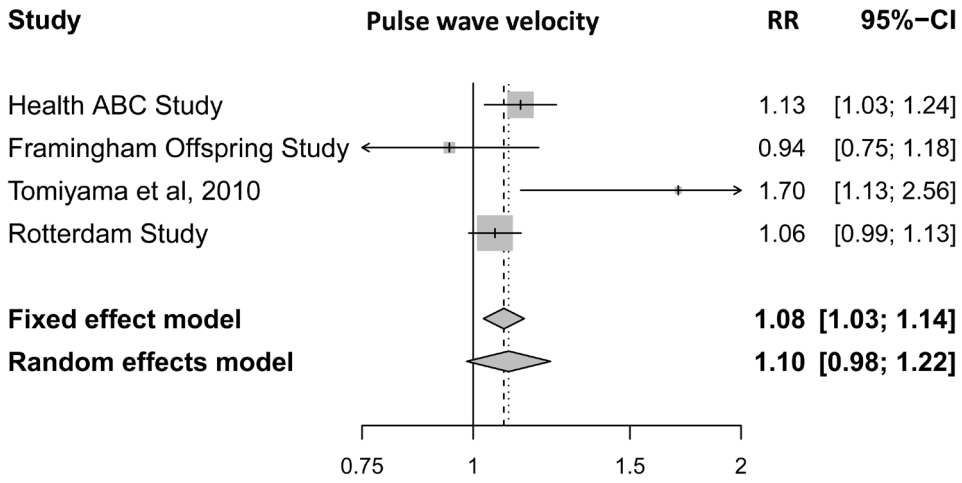


Figure 2. Forest plot of multivariate adjusted relative risk for the association of each standard deviation (A) pulse pressure and (B) pulse wave velocity with incident CKD.

Discussion

We showed that markers of arterial stiffness are independently associated with future decline in kidney function. In addition, we provided further evidence for the association between pulse pressure and decline in kidney function, using genetic variability in

pulse pressure.

Previous studies showed inconsistent findings regarding the association between arterial stiffness and decline in kidney function.[7,9-14,28] In a study including patients with chronic kidney disease, Ford et al. showed that higher pulse wave velocity but not pulse pressure was associated with the rate of change in kidney function.[9] Similarly, in a Japanese cohort with normal kidney function, an association was observed between higher brachial pulse wave velocity and steeper decline in eGFR.[11] In contrast, results of the Framingham offspring cohort showed that pulse wave velocity is associated with the incidence of albuminuria but not with mild to moderate CKD.[12] Briet et al. reported the association between pulse pressure and higher risk of ESRD, but not with pulse wave velocity.[7] In this study, we observed an association between pulse pressure and decline in kidney function but the association between pulse wave velocity and CKD disappeared after adjustment for cardiovascular risk factors. To improve the power, increase generalizability and decrease heterogeneity, we combined our results with the effect estimates of the previous population-based studies and provided further support for an independent link between pulse wave velocity and decline in kidney function.

Pulse pressure and PWV are two commonly used measures of arterial stiffness. Arterial stiffness is not uniform along the arterial tree; therefore, assessment of arterial stiffness at different sites in relation to clinical outcomes is important.[29] We have previously shown that arterial stiffness measured as carotid femoral PWV is associated with cardiovascular morbidity and mortality; however, we did not observe such an association with stiffness in the carotid artery. Previous studies showed that local carotid arterial stiffness is associated with brain outcomes (direct organ supplies by carotid arteries). In this study, we showed an independent association between carotid stiffness and decline in kidney function. Both kidney and brain are low resistance, high flow end-organs which renders them vulnerable to pulsatile changes in the blood flow. This might suggest that systemic pulsatile pressure can cause vascular injury in both

Table 2. Association of measures of arterial stiffness with decline in eGFR and incidence of CKD

	eGFR decline			Incident CKD		
	Difference	**95%CI**	**P-value**	**RR**	**95%CI**	**P-value**
Pulse pressure N=2950						
Model 1	0.15	0.10, 0.20	<0.001	1.11	1.05, 1.18	<0.001
Model 2	0.13	0.09, 0.18	<0.001	1.10	1.03, 1.17	0.002
Carotid stiffness N= 2342						
Model 1	0.08	0.04, 0.13	<0.001	1.13	1.05, 1.22	0.001
Model 2	0.07	0.02, 0.11	0.002	1.13	1.05, 1.22	0.001
Pulse wave velocity N= 2665						
Model 1	0.04	-0.00, 0.09	0.07	1.07	1.01, 1.14	0.04
Model 2	0.02	-0.02, 0.07	0.33	1.05	0.99, 1.31	0.10

Abbreviation: CI 95% Confidence interval; RR Relative risk
Differences (beta) and relative risks are calculated per each standard deviation of arterial stiffness measures.
Model 1: Adjusted for age, sex, mean arterial pressure, heart rate, baseline eGFR, and follow up time (for analyses on incidence of CKD)
Model 2: Additionally adjusted for body mass index, alcohol consumption, smoking, high-density lipoprotein cholesterol, total cholesterol, diuretics, ACE inhibitors, beta blockers, calcium channel blockers, and history of diabetes and coronary heart disease.

Table 3. Association of genetic risk scores for measures of arterial stiffness with annual decline in eGFR and incidence of CKD.

	eGFR decline			Incident CKD		
	Difference	**95%CI**	**P-value**	**RR**	**95%CI**	**P-value**
Pulse pressure GRS N= 3666						
Model 1	0.06	0.01, 0.10	0.01	1.08	1.03, 1.14	<0.01
Model 2	0.05	0.01, 0.11	0.02	1.07	1.02, 1.13	<0.01
Pulse wave velocity GRS N= 3666						
Model 1	-7.4×10^{-4}	-0.04, 0.04	0.99	1.03	0.98, 1.08	0.18
Model 2	3.6×10^{-3}	-0.04, 0.05	0.87	1.03	0.98, 1.08	0.17

Abbreviations: CI 95% Confidence interval; GRS Genetic risk score; RR Relative risk
Differences (beta) are per standard deviation of pulse pressure GRS and pulse wave velocity GRS.
Model 1: Adjusted for age, sex, mean arterial pressure, heart rate, eGFR baseline and follow up time (for analyses on incidence of CKD).
Model 2: Additionally adjusted for body mass index, alcohol consumption, smoking, high-density lipoprotein cholesterol, total cholesterol, diuretics, ACE inhibitors, beta blockers, calcium channel blockers, and history of diabetes and coronary heart disease.

organs. Future studies are needed to investigate the mechanism behind the association between carotid stiffness and decline in kidney function.

We observed that pulse pressure genetic variants are associated with kidney function decline. However, we did not find such an association with pulse wave velocity. Our findings can be explained by relatively more power for pulse pressure given the stronger association of pulse pressure with kidney function compared with pulse wave velocity. It is also known that pulse pressure is not only the indicator of arterial stiffness, but it is also influenced by peak systolic blood pressure.[30] Some of the genes found for pulse pressure are known to be associated with blood pressure variation.[31-33] In our study, adjustment for blood pressure and excluding SNPs with suggestive association with systolic blood pressure did not change the associations. Studies with more power are needed to investigate the association of genetic variants of pulse wave velocity with kidney function.

There are different putative mechanisms suggesting a role for arterial stiffness in the deterioration of kidney function. A plausible mechanism is that arterial stiffness increases circumferential and shear stresses and increases the pressure and flow in the arterial lumen. This hemodynamic stress on the vasculature of the kidney may result in endothelial dysfunction and microvascular ischemia leading to kidney injury.[34] Other possible mechanisms include chronic inflammation, oxidative stress, and activation of the renin-angiotensin system.[12]

We performed the analyses in a large population-based study, which enables us to control for several potential confounders and see the small effects of the genes. In addition, we performed a meta-analysis to provide a more precise estimate of the association. We confirmed the association between pulse pressure and kidney function, using genetic variants as the less biased proxy for the arterial stiffness parameters. Limitations of this study should be acknowledged. First, data on albuminuria were unavailable, which is an important element in defining CKD. However, eGFR<60ml/min/1.73 m^2

is a well-accepted definition for CKD in population-based research settings.[35] Second, adjustments for pulse pressure changed the association between genetic variants of pulse pressure and kidney disease only minimally; this might indicate that our findings with genetic risk score of pulse pressure could be partially explained by pleiotropic effects in the genetic risk score such as blood pressure genetic variants. Third, in computing the carotid distensibility coefficient, we used the brachial pulse pressure rather than the carotid pulse pressure. Substantial differences has been reported between carotid and brachial pulse pressures which can lead to an underestimation of the distensibility measurements and subsequently an underestimation of the association with the disease.[4]

We have shown that higher arterial stiffness is independently associated with future decline in kidney function. Currently major strategies to prevent CKD are focused on conventional cardiovascular risk factors while in this study we showed that vascular stiffness independent of cardiovascular risk factors is associated with decline in kidney function. This highlights that vascular stiffness can be considered as a target for prevention of CKD in old age.

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Supplementary document

Table S1. Alleles used for making the genetic risk score [1,2]

SNP	Closest gene	Coded allele frequency	Coded allele	Beta
Pulse Pressure				
rs13002573	FIGN	0.203	G	-0.31
rs871606	CHIC2	0.850	T	0.429
rs17477177	PIK3CG	0.717	T	-0.418
rs2071518	NOV	0.167	T	0.312
rs11222084	ADAMTS-8	0.375	T	0.337
rs1173756	NPR3	0.525	T	-0.267
rs9663362	PLCE-1	0.533	G	-0.271
rs3824755	CYP17A1-NT5C3	0.933	G	0.477
rs17249754	ATP2B1	0.892	G	0.392
rs17608766	GOSR2	0.908	T	-0.534
Pulse Wave Velocity				
rs1381289	C14orf64	0.436	T	-0.073
rs10764094	C10orf112	0.471	C	0.057
rs4778983	EFTUD1	0.301	C	0.057
rs6485690	CKAP5	0.308	A	-0.056
rs7959220	ELK3	0.027	G	0.266
rs6472483	SLCO5A1	0.452	T	-0.05
rs6101837	MAFB	0.416	C	-0.05
rs6947805	CADPS2	0.050	T	0.117
rs3742207	COL4A1	0.361	G	-0.025

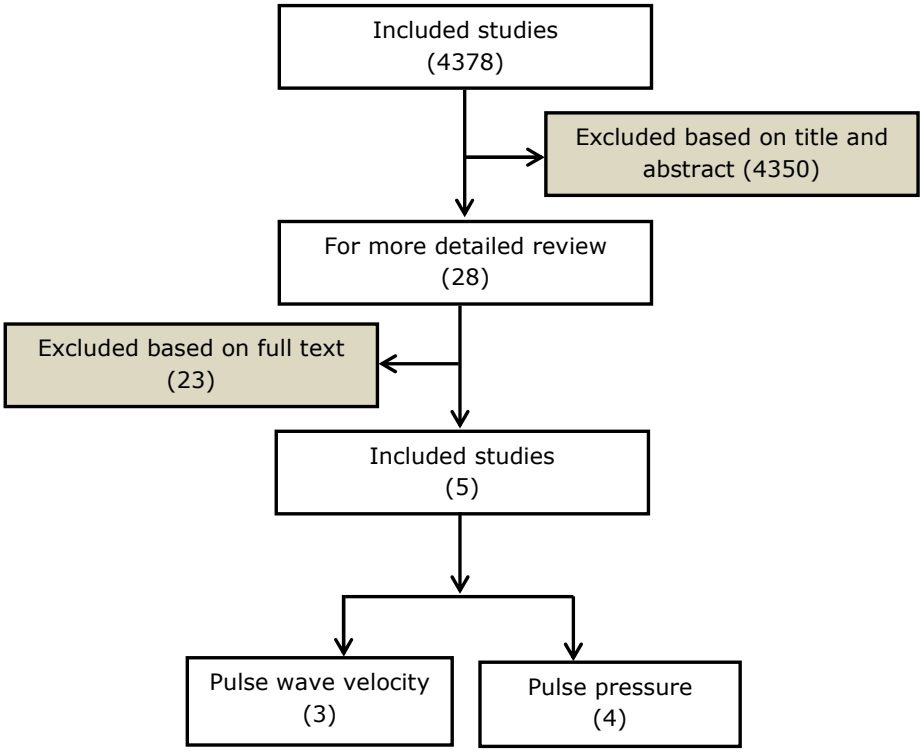


Figure S1. Follow diagram of studies through the different phases of the meta-analyses

Table S2. Included studies investing the association of markers of arterial stiffness and CKD incident in the general population

Study reference	Sample size	Study population	Country	Mean age	Stiffness index	Modelling of stiffness index	Outcome(s)	Fully adjusted effect estimate(s)	Follow-up time	Adjusted confounders
Pulse Wave Velocity										
Madero, 2013	2129	Population-based Health ABC	USA	74y	cfPWV	Doubling of PWV	-GFRcys loss of > 3 mml/min per1.73 m^2 -eGFR< 60 mml/min per1.73 m^2	OR: 1.16 (0.89,1.52) IRR: 1.39 (1.09,1.77)	8.5 years	Age, sex, race, site, anti HTN medication, DM, smoking, LDL, HDL, HF, baseline GFR
Upadhyay, 2009	1252 1675	Population-based Framingham Study	USA	47y	cfPWV	per SD SD PWV= 3.1	-UACR>17men, >25 women -eGFR< 60 mml/min per1.73 m^2	OR: 1.14 (0.94, 1.42) OR: 0.94 (0.75, 1.19)	7-10 years	Age, sex, MAP, HR, BMI, DM, fasting glucose, total / HDL cholesterol ratio, triglycerides, CHD, anti-hypertensive and(or) lipid-lowering medication, smoking, hormone replacement therapy, baseline UACR or GFR
Tomiyama, 2010	2053	Occupational cohort	Japan	40y	baPWV	1 m/s	-GFRcr loss of > 3 mml/min per1.73 m^2 -eGFR< 60 mml/min per1.73 m^2	OR: 1.15 (1.03, 1.29) OR: 1.36 (1.09, 1.70)	5-6 years	Baseline GFR, age, sex, BMI, alcohol, smoking, BP, HR, CHOL, HDL, TG, glucose, medication, CHD, stroke, HTN, DM, dyslipidaemia

Abbreviations: SD standard deviation, PP pulse pressure, cfPWV carotid-femoral pulse wave velocity, baPWV brachial-ankle pulse wave velocity, eGFR estimated glomerular filtration rate, OR odds ratio, IRR incident rate ratio, HTN hypertension, DM diabetes mellitus, LDL low density lipoprotein, HDL high density lipoprotein, HF heart failure, MAP mean arterial pressure, HR heart rate, CHD coronary heart disease, UACR, urine albumin creatinine ratio, BP blood pressure, TG triglyceride, SBP systolic blood pressure

Table S2. Included studies investing the association of markers of arterial stiffness and CKD incident in the general population

Study reference	Sample size	Study population	Country	Mean age	Stiffness index	Modelling of stiffness index	Outcome(s)	Fully adjusted effect estimate(s)	Follow-up time	Adjusted confounders
Pulse Pressure										
Madero, 2013	2129	Population-based Health ABC	USA	74y	Brachial PP	10 mmHg	-GFRcys loss of > 3 mml/min per1.73 m^2 -eGFR< 60 mml/min per1.73 m^2	OR: 1.10 (1.04,1.16) IRR: 1.06 (1.01,1.11)	8.5 years	Age, sex, race, site, anti HTN medication, DM, smoking, LDL, HDL, HF, baseline GFR
Upadhyay 2009	1252 1675	Population-based Framingham Study	USA	47y	Central PP	per SD SD PP= 14.5 mmHg	- ACR>17men, >25 women -eGFR< 60 mml/min per1.73 m^2	OR: 1.33 (0.92, 1.93) OR: 1.08 (0.71, 1.64)	7-10 years	Age, sex, MAP, HR, BMI, DM, fasting glucose, total / HDL cholesterol ratio, triglycerides, CHD, anti-hypertensive and(or) lipid-lowering medication, smoking, hormone replacement therapy, baseline UACR or GFR
Rifkin, 2013	4365	Population-based Cardiovascular Health Study	USA	72y	Brachial PP	10 mmHg	-GFRcys loss of > 3 mml/min per1.73 m^2 -delta GFR	OR: 1.15 (1.11, 1.20) Beta: -0.15 (-0.21, -0.09)	5-6 years	Baseline GFR, age, sex, BMI, alcohol, smoking, BP, HR, CHOL, HDL, TG, glucose, medication, CHD, stroke, HTN, DM, dyslipidaemia
Peralta, 2012	4853	Population-based MESA	USA	60y	Brachial PP	per SD SD PP= 16mmHg	Decline in GFR	Beta: 0.35 (-0.43 , -0.28)	4.76 years	Age, sex, race, education, BMI, DM, smoking, anti HTN medication, LDL, HDL, CRP, UACR, SBP

Abbreviations: SD standard deviation, PP pulse pressure, cfPWV carotid-femoral pulse wave velocity, baPWV brachial-ankle pulse wave velocity, eGFR estimated glomerular filtration rate, OR odds ratio, IRR incident rate ratio, HTN hypertension, DM diabetes mellitus, LDL low density lipoprotein, HDL high density lipoprotein, HF heart failure, MAP mean arterial pressure, HR heart rate, CHD coronary heart disease, UACR, urine albumin creatinine ratio, BP blood pressure, TG triglyceride, SBP systolic blood pressure

Table S3. Association of the pulse pressure genes with systolic and diastolic blood pressure

SNP name	Closest gene	Coded Allele	Systolic blood pressure		Diastolic blood pressure	
			Difference	P value	Difference	P value
rs13002573	FIGN	G	0.653	0.117	-0.061	0.781
rs871606	CHIC2	T	0.051	0.930	-0.345	0.252
rs17477177	PIK3CG	T	-1.303	0.003	0.087	0.709
rs2071518	NOV	T	-0.087	0.831	0.213	0.315
rs11222084	ADAMTS-8	T	-0.329	0.379	0.461	0.018
rs1173756	NPR3	T	0.074	0.832	0.209	0.253
rs9663362	PLCE-1	G	1.031	0.003	0.368	0.046
rs3824755	CYP17A1-NT5C3	G	0.721	0.246	0.156	0.633
rs17249754	ATP2B1	G	0.742	0.125	0.252	0.319
rs17608766	GOSR2	T	0.610	0.226	0.186	0.480

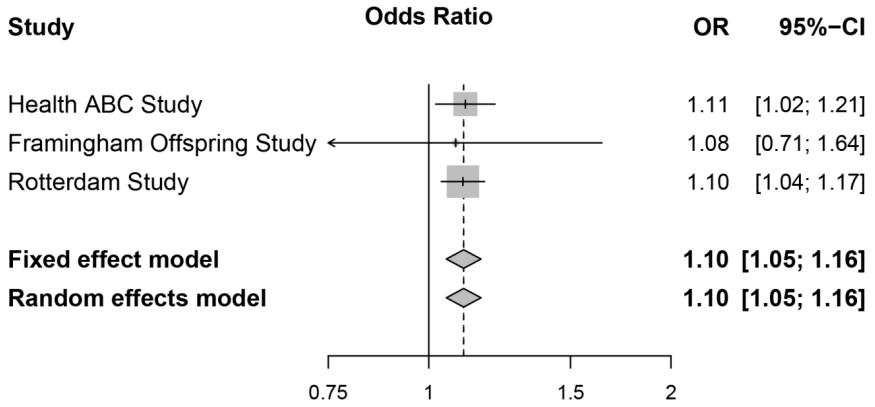
To account for multiple testing, we used Bonferroni corrected P-value (0.05/ number of SNPs ×2 (10×2) = 0.002).

Table S4. Association of pulse pressure genetic risk score excluding PIK3CG and PLCE-1 gene with annual decline in eGFR and incidence of CKD.

	eGFR decline			Incident CKD		
	Difference	**95%CI**	**P-value**	**RR**	**95%CI**	**P-value**
Pulse pressure GRS						
Model 1	0.06	0.01, 0.11	0.01	1.08	1.03,1.14	<0.01
Model 2	0.06	7.9×10^{-3}, 0.11	0.01	1.08	1.02, 1.14	<0.01

Abbreviations: CI Confidence interval, GRS Genetic risk score, RR Relative risk
Differences (beta) are per standard deviation.
Model 1: Adjusted for age, sex, mean arterial pressure, heart rate, eGFR baseline and follow up time (for analyses on incidence of CKD).
Model 2: Additionally adjusted for diuretics, ACE inhibitors, beta blockers, calcium channel blockers, body mass index, alcohol consumption, smoking, high density lipoprotein cholesterol, total cholesterol, history of diabetes and coronary heart disease, and follow up time (for analyses on incidence of CKD).

A)



B)

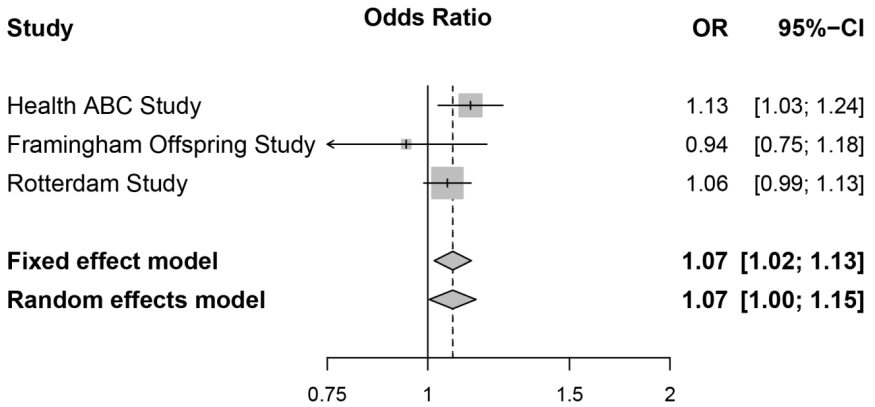


Figure S2. (A) Forest plot of multivariate adjusted relative risk for the association of pulse pressure with new onset CKD excluding the study with outcome definition of GFR loss of > 3 ml/min/1.73 m^2
(B) Forest plot of multivariate adjusted relative risk for the association of pulse wave velocity with new onset CKD excluding the study with ankle brachial pulse wave velocity.

Appendix. Search terms for the association between markers of arterial stiffness and incidence kidney disease in the population-based study.

Embase
('arterial stiffness'/de OR 'pulse pressure'/de OR 'arterial pressure'/de OR 'blood vessel compliance'/exp OR 'blood vessel calcification'/exp OR 'augmentation index'/de OR 'Young modulus'/de OR 'pulse wave'/de OR 'pulsatile flow'/de OR (((aort* OR arter* OR vascul* OR vessel*) NEAR/6 (stiff* OR complian* OR calcif*)) OR (wave NEXT/1 (velocit* OR reflection*)) OR ('internal carotid' NEAR/3 index) OR (pulse NEAR/3 (pressure* OR tension*)) OR distensibil* OR augmentation OR 'stiffness index' OR ((capacit* OR oscillat*) NEAR/3 complian*) OR ((elastic* OR young) NEXT/1 modul*) OR PWV OR CPP OR 'pulsatile flow'):ab,ti) AND (kidney/exp OR 'kidney function'/exp OR 'kidney function test'/exp OR 'kidney disease'/de OR 'chronic kidney disease'/exp OR 'chronic kidney failure'/exp OR microalbuminuria/exp OR ('albumin'/exp AND 'creatinine'/exp) OR 'cystatin C'/exp OR (kidney* OR renal OR nephro* OR glomeru* OR ckd OR microalbuminuri* OR (micro NEXT/1 albuminuri*) OR (albumin* NEAR/3 creatinin*) OR 'cystatin C'):ab,ti) AND ('cohort analysis'/exp OR 'longitudinal study'/exp OR 'prospective study'/exp OR 'follow up'/exp OR (population* OR cohort* OR longitudinal* OR prospectiv* OR 'follow up')) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Conference Paper]/lim OR [Editorial]/lim)
Medline (OvidSP)
("Vascular Stiffness"/ OR exp Elasticity/ OR "Arterial Pressure"/ OR "Vascular Calcification"/ OR "Pulse Wave Analysis"/ OR "pulsatile flow"/ OR (((aort* OR arter* OR vascul* OR vessel*) ADJ6 (stiff* OR complian* OR calcif*)) OR (wave ADJ (velocit* OR reflection*)) OR ("internal carotid" ADJ3 index) OR (pulse ADJ3 (pressure* OR tension*)) OR distensibil* OR augmentation OR "stiffness index" OR ((capacit* OR oscillat*) ADJ3 complian*) OR ((elastic* OR young) ADJ modul*) OR PWV OR CPP OR "pulsatile flow").ab,ti.) AND (exp kidney/ OR exp Kidney Function Tests/ OR kidney diseases/ OR Renal Insufficiency, Chronic/ OR (albumins/ AND creatinine/) OR cystatin C/ OR (kidney* OR renal OR nephro* OR glomeru* OR ckd OR microalbuminuri* OR (micro ADJ albuminuri*) OR (albumin* ADJ3 creatinin*) OR cystatin C).ab,ti.) AND (exp Cohort Studies/ OR (population* OR cohort* OR longitudinal* OR prospectiv* OR "follow up")) NOT (exp animals/ NOT humans/) AND english.la. NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.
Web-of-science
TS=(((((aort* OR arter* OR vascul* OR vessel*) NEAR/6 (stiff* OR complian* OR calcif*)) OR (wave NEAR/1 (velocit* OR reflection*)) OR ("internal carotid" NEAR/3 index) OR (pulse NEAR/3 (pressure* OR tension*)) OR distensibil* OR augmentation OR "stiffness index" OR ((capacit* OR oscillat*) NEAR/3 complian*) OR ((elastic* OR young) NEAR/1 modul*) OR PWV OR CPP OR "pulsatile flow")) AND ((kidney* OR renal OR nephro* OR glomeru* OR ckd OR microalbuminuri* OR (micro NEAR/1 albuminuri*) OR (albumin* NEAR/3 creatinin*) OR "cystatin C")) AND ((population* OR cohort* OR longitudinal* OR prospectiv* OR "follow up"))) AND DT=(Article) AND LA=(english)
PubMed publisher
("Vascular Stiffness"[mh] OR Elasticity[mh] OR "Arterial Pressure"[mh] OR "Vascular Calcification"[mh] OR "Pulse Wave Analysis"[mh] OR "pulsatile flow"[mh] OR (((aort*[tiab] OR arter*[tiab] OR vascul*[tiab] OR vessel*[tiab]) AND (stiff*[tiab] OR complian*[tiab] OR calcif*[tiab])) OR (wave ADJ (velocit*[tiab] OR reflection*[tiab])) OR ("internal carotid" AND index) OR (pulse AND (pressure*[tiab] OR tension*[tiab])) OR distensibil*[tiab] OR augmentation OR "stiffness index" OR ((capacit*[tiab] OR oscillat*[tiab]) AND complian*[tiab]) OR ((elastic*[tiab] OR young) ADJ modul*[tiab]) OR PWV OR CPP OR "pulsatile flow")) AND (kidney[mh] OR Kidney Function Tests[mh] OR kidney diseases[mh] OR Renal Insufficiency, Chronic[mh] OR (albumins[mh] AND creatinine[mh]) OR cystatin C[mh] OR (kidney*[tiab] OR renal OR nephro*[tiab] OR glomeru*[tiab] OR ckd OR microalbuminuri*[tiab] OR (micro ADJ albuminuri*[tiab]) OR (albumin*[tiab] AND creatinin*[tiab]) OR cystatin C)) AND (Cohort Studies[mh] OR (population*[tiab] OR cohort*[tiab] OR longitudinal*[tiab] OR prospectiv*[tiab] OR "follow up")) NOT (animals[mh] NOT humans[mh]) AND english[la] AND publisher[sb]
Google scholar
"arterial| pulse| aorta| aortic| artery stiffness| pressure| compliance| calcification"|"wave velocity|reflection"|distensibility|augmentation|"pulsatileflow"kidney|renal| glomerular cohort |longitudinal|prospective|"follow up"

Supplementary references

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2.5 Von Willebrand factor, ADAMTS13 activity and decline in kidney function

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Altered levels of Von Willebrand factor and ADAMTS13 can promote thrombosis and disturb blood flow in kidney microcirculations. In this study, we investigated the association of serum Von Willebrand factor antigen (VWF:Ag), ADAMTS13 activity and their ratio in relation to decline in kidney function. We included 5291 individuals with a mean age of 63 years (43% men) from the prospective population-based Rotterdam Study. Annual decline in kidney function and new onset chronic kidney disease (CKD) during a median follow up of 11 years were assessed using the estimated glomerular filtration rate (eGFR). Higher VWF-to-ADAMTS13 ratio was associated with steeper annual decline in eGFR (0.12 ml/min; 95%CI: 0.04, 0.21) and higher risk of new onset CKD (OR: 1.34; 95%CI: 1.02, 1.75). Likewise, each standard deviation increase in VWF:Ag was associated with 0.04 ml/min steeper annual decline in eGFR (95%CI: 0.01, 0.08) and 14% higher risk of new onset CKD (OR: 1.14 ; 95%CI: 1.01, 1.28). Each standard deviation lower ADAMTS13 activity was associated with 0.05 ml/min steeper annual decline in eGFR (95% CI: 0.01, 0.09). There was no association between ADAMTS13 activity and risk of new onset CKD. In this population-based study, we observed that VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity are associated with decline in kidney function over time. This finding suggests a role of elevated prothrombotic factors in the development and progression of kidney disease.

Background

von Willebrand factor (VWF) is a multimeric glycoprotein which mediates platelet adhesion and aggregation.[1] VWF function is partly regulated by the VWF protease, ADAMTS13.[1] ADAMTS13 cleaves ultra-large VWF multimers into smaller multimers that are less procoagulant.[1,2] Therefore, the imbalance between VWF and ADAMTS13 is an important indicator of a prothrombotic state.[3]

Evidence on the association between prothrombotic factors and kidney function is scarce.[3-6] Given the dependency of kidney function on the adequate blood flow to the glomerulus, the kidney is one of the most susceptible organs to thrombotic events in its microcirculation.[7] The imbalance between VWF and ADAMTS13 may promote thrombosis in kidney vessels, leading to disturbances in kidney circulation and thereby contributing to the decline in kidney function.[5] This is one of the hallmark clinical characteristics of thrombotic thrombocytopenic purpura (TTP), which is caused by a complete deficiency of ADAMTS13. In TTP patients, ADAMTS13 deficiency results in microthrombi formation in the circulation as well as the small vessels of the kidney, contributing to renal insufficiency.[8] While previous animal studies [7] and studies in patient groups [3, 5] suggest a link between VWF and ADAMTS13 with kidney function, whether this link extends to individuals from general populations remains to be elucidated. We investigated the association of VWF-to-ADAMTS13 ratio, VWF, and ADAMTS13 activity with decline in kidney function in the population-based Rotterdam Study.

Methods

Study population

The present study is embedded within the framework of the population-based Rotterdam Study. The design of the Rotterdam Study has been described previously.[9]

In brief, the cohort started in 1990, consisting of 7983 participants aged 55 years or older living in Ommoord, a district of Rotterdam in the Netherlands (RS-I). In 2000, the first extension of the Rotterdam Study (RS-II) started, adding 3011 new participants. VWF antigen (VWF:Ag) and ADAMTS13 activity were evaluated at the third visit of RS-I (1997-1999) and the first visit of RS-II (2000-2001). We included 6249 participants with both VWF:Ag and ADAMTS13 measurements. Among them, 6106 had serum creatinine measurements and 6024 cystatin C measurements. Repeated measurements of creatinine for the evaluation of longitudinal kidney function were available in 2885 participants. Repeated measurements of cystatin C were not available. The median time elapsed between the two creatinine measurements was 11 years (range: 7.8-13.6). The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[9]

Measurement of VWF:Ag and ADAMTS13 activity

Fasting venous blood samples were taken at the research center and collected in citrated tubes. Samples were stored at -80°C. VWF:Ag was determined with an in-house ELISA with polyclonal rabbit antihuman VWF antibodies (DakoCytomation, Glostrop, Denmark) for catching and tagging.[10] The intra-assay coefficient of variation was 5.8% and the interassay coefficient of variation was 7.8%.[10] ADAMTS13 activity was measured using the Fluorescence Resonance Energy Transfer Substrate VWF 73 kinetic assay (FRETS-VWF73).[11] Samples of VWF and ADAMTS13 were measured against a reference curve of serial dilutions of normal human plasma, calibrated against the international standard (Siemens, Germany).[11]

Measurement of estimated glomerular filtration rate (eGFR)

Serum creatinine was determined using an enzymatic assay method. Creatinine values were standardized to isotope-dilution mass spectrometry–traceable (IDMS) measurements. In order to calibrate, we aligned the mean values of serum creatinine with serum creatinine values of the participants of the Third National Health and Nutrition Examination Survey (NHANES III) in different gender and age groups (<60, 60-69, ≥70).[12] $eGFR_{creatinine}$ was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[13] To calculate the annual $eGFR_{creatinine}$ decline, we first subtracted the $eGFR_{creatinine}$ values of the follow-up examination from the $eGFR_{creatinine}$ values at baseline and then divided by the time, in years, between the two visits. Chronic kidney disease (CKD) was defined as $eGFR_{creatinine}$ < 60 ml/min/1.73 m^2. New onset cases were defined among the individuals free of CKD at baseline (eGFR >60 ml/min/1.73 m^2), who had a decline in $eGFR_{creatinine}$ to less than 60 ml/min/1.73 m^2 between the two periodical examinations. Cystatin C was measured at baseline with a particle-enhanced immunonephelometric assay. $eGFR_{cystatin}$ was calculated according to the CKD-EPI formula.[13]

Covariates

Body mass index was calculated by dividing weight in kilograms by height in meters squared. Information on smoking and alcohol consumption was acquired from questionnaires. Participants were asked for the average daily consumption of alcohol and data is presented as grams per day. Smoking was categorized in never, former and current smoking. Blood pressure was measured twice by an oscillometric device after five minutes of rest and the mean was taken as the subject's reading. Information on medication use was based on home interview. Serum total cholesterol and high-density lipoprotein cholesterol levels were determined using an automated enzymatic method. Coronary heart disease was considered as experiencing myocardial infarction

or coronary revascularization procedures. Diabetes mellitus was defined by the use of blood glucose lowering drugs and/or a random non-fasting glucose above 11.1 mmol/l or a fasting serum glucose level equal to or greater than 7.0 mmol/l at baseline. Blood group was defined based on rs687289 variant, which discriminates blood group O from non-O status.[14]

Statistical analysis

The association of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity with $eGFR_{creatinine}$, $eGFR_{cystatin}$, and annual decline in eGFR was evaluated using linear regression models. Logistic regressions were used to estimate the odds ratio (OR) for the association of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity with prevalent CKD, and the new onset CKD. Betas were estimated per standard deviation (SD) increase for VWF:Ag and ADAMTS13 activity. Since measures of VWF-to-ADAMTS13 ratio and VWF:Ag were not normally distributed, they were natural log transformed. We performed analyses using two models. In the first model analyses were adjusted for age, sex, cohort effect, and baseline eGFR (only for longitudinal analyses). In the second model, we further adjusted the analyses for body mass index, alcohol consumption, smoking, high-density lipoprotein cholesterol, total cholesterol, history of diabetes mellitus and coronary heart disease, blood group (O or non-O), and antihypertensive and antithrombotic medications. All analyses with new onset CKD as an outcome were adjusted for the follow up time elapsed between the two measurements of creatinine. We divided participants into tertiles of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 and compared participants from the second and third tertile with participants from the first tertile (reference category). To investigate whether the association of prothrombotic factors and decline in kidney function differs based on gender, age, and blood group, we assessed the interaction of the prothrombotic factors and the aforementioned characteristics by adding an interaction term in the model. The

interaction term was the product of the interacting factor and prothrombotic factors. In addition, we performed a series of stratified analyses by separately studying the association of prothrombotic factors and decline in kidney function in participants with blood group O and non-O, in men and women and in participants younger and older than 65 years. Evaluating linearity assumption, there was neither departure from linearity for the linear regression models and nor for logistic regressions, using fractional polynomials. We performed multiple imputation for missing data in the covariates (< 8% for all covariates), using a Markov Chain Monte Carlo method. The calibration of GFR measurements and the evaluation of linearity assumptions were done using R version 2.15.0. All other analyses were carried out using SPSS 20.0.2 for windows.

Results

The characteristics of 5291 study participants are presented in Table 1. The average age of the participants was 69 years and 43% were male. Participants had average VWF:Ag level of 120% and average ADAMTS13 activity of 91.7 %. The correlation between VWF:Ag and ADAMTS13 activity was minimal (r= -0.08, p <0.01).

Cross-sectional analysis

The cross-sectional association of VWF-to-ADAMTS13 ratio, VWF:Ag level, and ADAMTS13 activity with kidney function is presented in Table 2. In model I, higher VWF-to-ADAMTS13 ratio was associated with lower $eGFR_{creatinine}$ (-3.58 mL/min; 95% confidence interval [CI]: -4.33, -2.83) and a higher prevalence of CKD (2.08; 95% CI: 1.74, 2.50). Each standard deviation (SD) higher VWF:Ag was associated with 1.36 mL/min/1.73 m^2 lower $eGFR_{creatinine}$ (95% CI: -1.69, -1.04) and a higher prevalence of CKD (OR: 1.33; 95%CI: 1.23, 1.44). Likewise, each SD lower ADAMTS13 activity was associated with 0.90 mL/min/1.73 m^2 lower $eGFR_{creatinine}$ (95% CI: 0.55, 1.26) and a

Table 1. Baseline characteristics of study participants

Characteristics	n= 5291
Age, years	69.2 (8.1)
Men	2265 (42.8)
Systolic blood pressure, mmHg	143.2 (21.1)
Diastolic blood pressure, mmHg	76.5 (11.1)
Body mass index, kg/m^2	26.9 (3.9)
Alcohol, g/day	4.3 (0.5-17.1)
Current smoker	967 (18.3)
Total cholesterol, mmol/l	5.8 (0.9)
HDL cholesterol, mmol/l	1.3 (0.4)
Blood group O	2340 (44.2)
History of diabetes mellitus	658 (12.4)
History of coronary heart disease	448 (8.5)
Antithrombotic agents	998 (18.9)
Antihypertensive medication	1768 (33.4)
Estimated glomerular filtration rate (creatinine), mL/min/1.73 m^2	74.5 (14.8)
Estimated glomerular filtration rate (cystatin C), mL/min/1.73 m^2	72.1 (18.0)
Von Willebrand factor antigen, %	120.0 (92.0-159.0)
ADAMTS13 activity, %	91.7 (17.5)
VWF-to-ADAMTS13 ratio	1.3 (0.9-1.8)

Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and von willebrand factor antigen, VWF-to-ADAMTS13 ratio and alcohol intake are presented as medians (interquartile ranges)
The following variables had missing data: cystatin C (n=75), body mass index (n= 29), cholesterol (n=1), HDL cholesterol (n= 46), Systolic and diastolic blood pressure (n= 18), alcohol (n=463), smoking (n=26), Antithrombotic and antihypertensive medication (n=200), History of diabetes (n= 70), history of coronary heart disease (n=130), blood group (n=127).

higher prevalence of CKD (OR: 1.20; 95%CI: 1.10, 1.31). Adjustments for cardiovascular risk factors, medications, and blood group in model II, did not essentially change the associations. Performing the analyses with $eGFR_{cystatin}$ as the outcome yielded similar findings (Table S1).

Table 2. Cross sectional association of von Willebrand factor antigen, ADAMTS13 activity, and VWF- to-ADAMTS13 ratio with kidney function

	$eGFR_{creatinine}$ N= 5291			CKD N (case) =5291(858)		
	Beta	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
VWF-to-ADAMTS13 ratio						
Model I	-3.58	-4.33, -2.83	<0.001	2.08	1.74, 2.50	<0.001
Model II	-3.64	-4.37, -2.90	<0.001	2.31	1.90, 2.80	<0.001
VWF:Ag						
Model I	-1.36	-1.69, -1.04	<0.001	1.33	1.23, 1.44	<0.001
Model II	-1.32	-1.64, -1.00	<0.001	1.38	1.27, 1.51	<0.001
ADAMTS13						
Model I	0.90	0.55, 1.26	<0.001	0.83	0.76, 0.91	<0.001
Model II	1.10	0.74, 1.45	<0.001	0.79	0.72, 0.87	<0.001

Betas/odds ratios and 95% CI are calculated per standard deviation measures of VWF:Ag and ADAMTS13 activity.
Betas/odds ratios and 95% CI are calculated for log-transformed values of VWF:Ag and VWF-to-ADAMS13 ratio.
Model I: Adjusted for age, sex and the cohort effect.
Model II: Additionally adjusted for systolic blood pressure, antihypertensive medication, antithrombotic agents, alcohol intake, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, body mass index, and blood group.
Abbreviation: CI: confidence interval, OR: odds ratio, CKD: chronic kidney disease, $eGFR_{creatinine}$: creatinine based estimated glomerular filtration rate, VWF:Ag: von willebrand factor antigen.

Annual eGFR-decline and new-onset CKD

The association of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity with annual decline in eGFR and new onset CKD is presented in Table 3. Higher VWF-to-ADAMTS13 ratio, in model I was associated with steeper annual decline in eGFR (0.12 mL/min; 95%CI: 0.04, 0.21) and a higher risk of developing CKD (1.34; 95%CI: 1.02, 1.75). Adjustment for potential confounders did not change the associations. Each SD higher VWF:Ag, in model I, was associated with 0.04 mL/min (95%: 0.01, 0.08) unit steeper annual decline in eGFR and 14% (95%CI: 1.01, 1.28) higher risk of developing CKD. Similarly, adjustments for potential confounders did not change the associations.

Table 3. Longitudinal association of von Willebrand factor antigen, ADAMTS13 activity, and VWF- to-ADAMTS13 ratio with annual decline in eGFR and new onset CKD

	Annual eGFR decline N= 2479			New onset CKD N (case) =2272(500)		
	Beta	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
VWF-to-ADAMTS13 ratio						
Model I	0.12	0.04, 0.21	<0.01	1.34	1.02, 1.75	0.03
Model II	0.12	0.04, 0.21	<0.01	1.36	1.01, 1.83	0.04
VWF:Ag						
Model I	0.04	0.01, 0.08	0.01	1.14	1.01,1.28	0.02
Model II	0.04	0.00, 0.07	0.04	1.11	0.98, 1.27	0.08
ADAMTS13						
Model I	-0.03	-0.07, 0.01	0.07	0.98	0.87, 1.11	0.78
Model II	-0.05	-0.09, -0.01	0.01	0.92	0.81, 1.04	0.19

Betas/odds ratios and 95% CI are calculated per standard deviation measures of VWF:Ag and ADAMTS13 activity.
Betas/odds ratios and 95% CI are calculated for log-transformed values of VWF:Ag and VWF-to-ADAMS13 ratio.
Model I: Adjusted for age, sex, cohort effect and baseline $eGFR_{creatinine}$.
Model II: Additionally adjusted for systolic blood pressure, antihypertensive medication, antithrombotic agents, alcohol intake, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index, blood group (O and non-O), and follow up time (for analyses with new onset CKD).
Abbreviation: CI: confidence interval, $eGFR_{creatinine}$: creatinine based estimated glomerular filtration rate, VWF:Ag: von willebrand factor antigen, CKD: chronic kidney disease, OR: odds ratio.

Each SD lower ADAMTS13 activity was associated with 0.05 ml/min unit steeper annual decline in eGFR (95% CI: 0.01, 0.09), after adjusting for potential confounders in model II. There was no association between ADAMTS13 and risk of new onset CKD.

Analysis by tertiles

Analyses of the tertiles of the prothrombotic variables and decline in eGFR and new onset CKD are presented in Figure 1. Participants in the third tertile of the VWF-to-ADAMTS13 ratio and VWF:Ag compared to participants in the first tertile had steeper decline in eGFR and higher risk of developing new onset CKD (P for trend <0.05).

Participants in the highest tertile of ADAMTS13 had lower decline in eGFR compared to the participants in first tertile (P for trend 0.03). We did not observe a linear trend between tertiles of ADAMTS13 and risk of developing new onset CKD (P value for trend 0.27).

In the stratified analyses, there was no statistically significant difference in the strength of the association of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity with annual decline in eGFR or risk of new onset CKD in subgroups of participants based on their blood group, gender, and age (Figure S1).

Discussion

In this population-based study, we found that higher VWF-to-ADAMTS13 ratio, higher VWF:Ag, and lower ADAMTS13 activity are associated with steeper decline in kidney function independent of potential confounders.

A limited number of studies investigated a potential role for VWF and ADAMTS13 in relation to kidney function.[15-17] In agreement with our cross-sectional findings, previous studies reported higher levels of VWF and lower ADAMTS13 activity in patients with chronic kidney disease and end stage renal disease.[15, 17] Apart from the cross-sectional observations, few studies reported an association between higher levels of VWF:Ag and progression of CKD.[18-20] These studies were either limited to patients or performed in a small group of healthy individuals.[18] Regarding the role of ADAMTS13, the link between its activity and CKD development has been investigated only in small groups of patients.[3-6] Ono et al., found that lower ADAMTS13 activity was associated with higher serum creatinine levels and future risk of kidney injury.[5] This study was performed in patients with sepsis and severe deficiency in ADAMTS13 activity. In the current large population-based study we observed a clear association between VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity and decline in kidney function. The association was evident even after excluding individuals with extreme levels of

these measures, suggesting that the associations hold even within normal ranges of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity. Of note, although the effect estimates indicate a slight increase in kidney disease risk, previous studies showed that even trivial declines in eGFR are associated with considerable risk of future end stage renal disease.[21]

The plasma concentration of VWF and ADAMTS13 has been shown to be influenced by cardiovascular risk factors and differ based on certain characteristics.[3, 10, 22-24] For example, individuals with type O blood group have 25 percent lower VWF than those with non O blood group.[22] It is reported that VWF level and ADAMTS13 activity differs between men and women,[24] and in different age ranges.[25] It is also well-known that cardiovascular risk factors can influence the kidney function.[26] Therefore, the association of VWF:Ag, ADAMTS13 activity and their ratio with decline in kidney function may be confounded by these factors. In this study, adjustments for cardiovascular risk factors, medications and blood group did not change our findings. In addition, we did not observe any differences in the association of prothrombotic factors and decline in kidney function in different subgroups of participants, indicating that the associations of VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity with decline in kidney function are independent of cardiovascular risk factors and blood group.

VWF is known as an endothelial function marker.[1] Patients with CKD are more prone to endothelial damage and hence higher levels of VWF.[21] Therefore, it could be speculated that the steeper kidney function decline is a reflection of existing endothelial dysfunction at baseline. However, the prospective nature of our findings, adjustment of longitudinal analyses for baseline eGFR, as well as excluding participants with baseline eGFR less than 60 mL/min/1.73 m^2 rule out this conjecture.

Further evidence to support the etiologic role of ADAMTS13 on progression of kidney function can be provided by genetic variants in the ADAMTS13 gene. A Pro618Ala polymorphism in ADAMTS13 is shown to be predictive of renal events

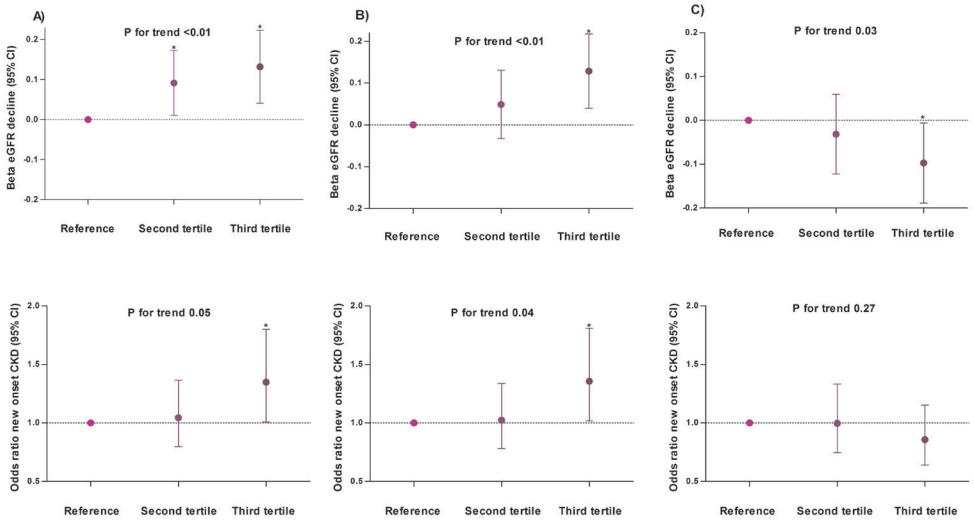

Figure 1. Association of A) VWF- to-ADAMTS13 ratio, B) von Willebrand factor antigen, and C) ADAMTS13 activity tertiles with annual decline in eGFR and new onset CKD
VWF-to-ADAMTS13 ratio tertiles (reference: < 0.1, second: 0.01-0.02, third: ≥0.02)
VWF:Agtertiles (reference: < 101%; second: 102-104%, third: ≥143%)
ADAMTS13 activity tertiles (reference: < 84%, second: 84-98%, third: ≥ 98%)
*represents a p-value<0.05 when a tertile was compared to the reference category (first tertile).

in normoalbuminuric type 2 diabetic patients.[6] In addition, in a porcine model of Escherichia coli sepsis, decreased ADAMTS13 activity and increased large VWF multimers, was reported along with glomerular microthrombi enriched with platelets and VWF, and acute kidney injury.[7] Furthermore, severe deficiency in ADAMTS13 caused by auto-antibodies or defects in the ADAMTS13 gene is the cause of TTP and, in fact, acute kidney injury occurs in over 50% of TTP patients.[4, 8, 27] Taken together, this suggests a potential causal role for VWF, ADAMTS13 and particularly the imbalance between them in relation to decline in kidney function.

We observed a stronger association between VWF-to-ADAMTS13 ratio and decline in kidney function compared to levels of VWF:Ag or ADAMTS13 activity, separately. It is known that ultra-large VWF multimers are more procoagulant; however, measuring ultra-large VWF is technically difficult and laborious.[3] In line with our observation,

several studies have indicated that the imbalance between VWF concentration and ADAMTS13 activity, rather than levels of VWF:Ag or ADAMTS13 activity, may allow a better evaluation of the prothrombotic state.[3, 28, 29]

The population-based design of this study, the large sample size, prospective setting, and the availability of extensive data on various socio-demographic and cardiovascular risk factors that enabled us to control for several potential confounders, can be marked as the main strengths of this study. Limitations of this study should also be acknowledged. No data on albuminuria were available, which is an important element in defining CKD. In addition, although the definition of CKD based on KDIGO criteria requires two values of eGFR less than 60 ml/min/1.73m^2 at least 90 days apart, we only had a single measurement of eGFR. However, eGFR < 60 ml/min /1.73 m^2 is a well-accepted definition for CKD in population-based research setting.[30]

In conclusion, we observed that VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity are independently associated with decline in kidney function in the general population setting. Future studies are needed to explore whether monitoring VWF, ADAMTS13 and more specifically the imbalance between them, would be a useful addition to the traditional risk factors to prevent decline in kidney function.

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Supplementary document

Table S1. Cross sectional association of von Willebrand factor antigen, ADAMTS13 activity, and VWF- to-ADAMTS13 ratio with estimated glomerular filtration rate based on cystatin C

	$eGFR_{cystatin}$ N= 5217		
	Beta	**95% CI**	**p-value**
VWF-to-ADAMTS13 ratio			
Model I	-6.98	-7.81, -6.14	<0.001
Model II	-6.57	-7.38, -5.75	<0.001
VWF:Ag			
Model I	-2.66	-3.02, -2.29	<0.001
Model II	-2.44	-2.80, -2.09	<0.001
ADAMTS13			
Model I	1.65	1.25, 2.05	<0.001
Model II	1.75	1.36, 2.15	<0.001

Betas/odds ratios and 95% CI are calculated per standard deviation measures of VWF:Ag and ADAMTS13. Betas/odds ratios and 95% CI are calculated for log-transformed values of VWF:Ag andVWF-to-ADAMS13 ratio.
Model I: Adjusted for age, sex, and cohort effect.
Model II: Additionally adjusted for systolic blood pressure, antihypertensive medication, antithrombotic agents, alcohol intake, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index.
Abbreviation: CI: confidence interval, $eGFR_{cystatin}$: cystatin C based estimated glomerular filtration rate, VWF:Ag: von willebrand factor antigen.

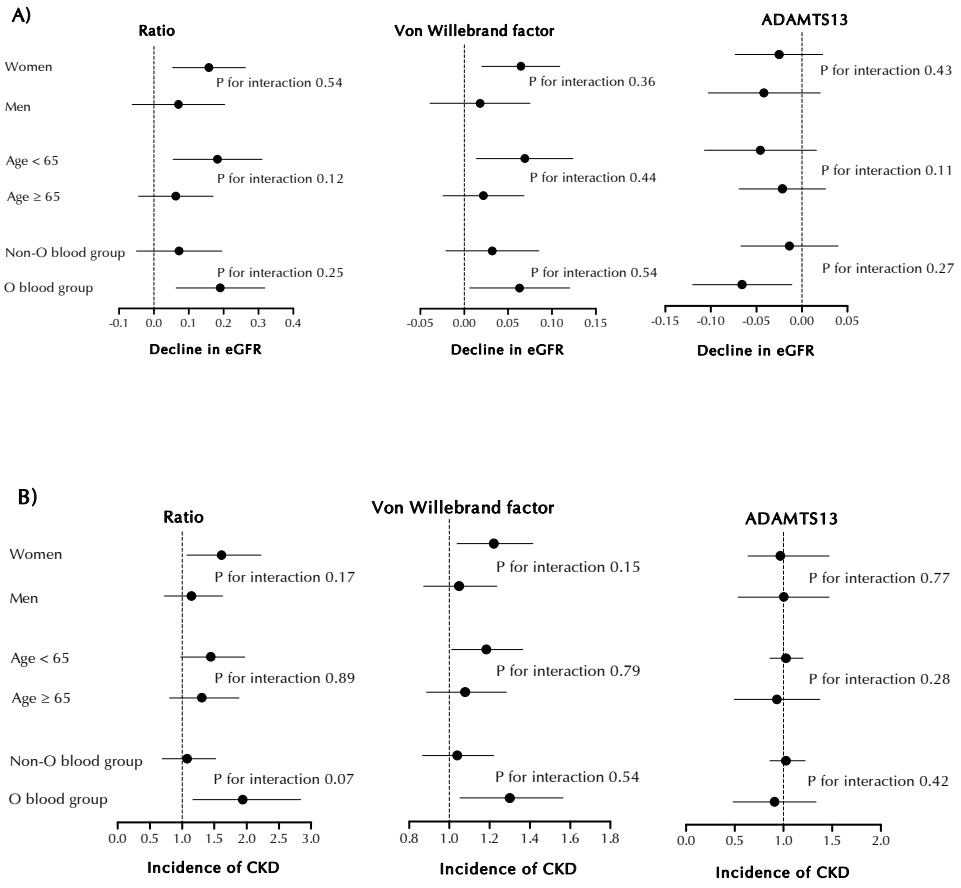

Figure S1. Association of von Willebrand factor antigen, ADAMTS13 activity, and VWF- to-ADAMTS13 ratio with A) decline in eGFR and B) new onset CKD stratified based on blood group, gender, and age. All betas are calculated per SD increase in natural logarithm of VWF:Ag and ADAMTS13 activity and per natural logarithm of VWF- to-ADAMTS13 ratio. All analyses are adjusted for age, sex, cohort effect, baseline eGFR, and follow up time (for analyses with new onset CKD).

CHAPTER 3

Vascular dysfunction and the brain

3.1 Carotid stiffness is associated with incident stroke: A systematic review and individual participant data meta-analysis

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We investigated whether carotid stiffness is associated with incident stroke, and whether this association is independent of aortic stiffness as estimated by carotid-femoral pulse wave velocity (cfPWV). In addition, we evaluated the incremental value of carotid stiffness for stroke risk prediction beyond Framingham risk factors and cfPWV. We performed systematic review and meta-analyses of aggregate and individual participant data (IPD). We searched MEDLINE and EMBASE for prospective studies on carotid stiffness and incident cardiovascular events and/or mortality, published between inception and March 2015. For the IPD meta-analysis, we requested individual level data of all studies with available data on carotid stiffness and cfPWV. Ten studies (n=22,472) were included in the aggregate data meta-analysis and four (n=4,540) in the IPD meta-analysis. The aggregate data meta-analysis showed that, after adjustment for cardiovascular factors, greater carotid stiffness (per SD) was associated with stroke (HR 1.18 [95%CI 1.05;1.33]). In addition, carotid stiffness was associated with total cardiovascular events 1.16 [1.07;1.26]) and cardiovascular (1.30 [1.15;1.46]) and all-cause mortality (1.22 [1.12;1.34]), but not with coronary heart disease events (1.03 [0.98;1.10]). The IPD meta-analysis showed that additional adjustment for cfPWV did not materially change these associations. In addition, carotid stiffness improved stroke risk prediction beyond Framingham and cfPWV (integrative discrimination improvement: 0.4%-point [0.1;0.6%-point] and continuous net reclassification improvement: 18.6% [5.8;31.3%]). Carotid stiffness is associated with incident stroke independently of aortic stiffness and cardiovascular factors. In addition, carotid stiffness improves stroke risk prediction beyond Framingham and aortic stiffness.

Background

Stroke is one of the leading causes of disability and mortality worldwide.[1] The global burden of stroke has greatly increased in the last decades, and will continue to increase in the coming years.[1, 2] Therefore, effective prevention strategies need to be developed, which requires a better understanding of the risk factors for stroke.[1]

Ageing and cardiovascular disease (CVD) risk factors lead to stiffening of the common carotid artery,[3] which can be quantified non-invasively by measuring local distensibility.[3, 4] Stiffening of carotid arteries impairs their cushioning function and increases pressure and flow pulsatility, which transmit distally into the cerebral circulation and, thus, may increase the risk of stroke.[5, 6] In addition, carotid stiffening may lead to stroke through development of (rupture-prone) atherosclerotic carotid plaques.[7]However, results of studies[6, 8-10] on the association between carotid stiffness and incident stroke have not been consistent. One study[8] reported a statistically significant association between carotid stiffness and incident stroke, whereas three additional, smaller, studies[6, 9, 10] did not.

We therefore performed a systematic review and aggregate data meta-analysis of cohort studies on the association between carotid stiffness and incident stroke. Carotid-femoral pulse wave velocity (cfPWV), a measure of aortic stiffness,[3] is the most often used arterial stiffness measurement and is associated with incident CVD,[11,12] we, therefore, additionally performed an individual participant data (IPD) meta-analysis with data from cohorts with measures of both carotid stiffness and cfPWV, and evaluated whether the association between carotid stiffness and stroke (if any) is independent of cfPWV. In addition, to evaluate whether carotid stiffness has any potential of being used as a risk predictor of stroke, we quantified the incremental value of carotid stiffness for stroke risk prediction beyond Framingham stroke risk score factors and cfPWV. Finally, we evaluated the association between carotid stiffness and other cardiovascular

outcomes than stroke, including coronary heart disease (CHD) events, nonfatal and fatal cardiovascular events, and all-cause mortality.

Methods

This review is reported in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement.[13]

Evaluation procedure

Two independent reviewers selected all relevant studies based on title and abstract, retrieved selected full texts, performed an eligibility assessment, extracted data and assessed risk of bias (described below). Any disagreements between the reviewers were resolved by consensus. A third independent reviewer was available to solve any persisting disagreements.

Data sources and search strategy

We identified relevant studies through a search of Medline and Embase from inception to March 16, 2015, without any language restriction. In addition, we identified studies by reviewing the reference lists of all relevant articles identified and by discussion with experts in the field to identify unpublished data.

Eligibility criteria and study selection

For the systematic review and aggregate data meta-analysis, we considered eligible all prospective cohort studies in humans (of any age) that investigated the association between, on the one hand, carotid stiffness and, on the other, (nonfatal and/or fatal) incident stroke, CHD events and/or total cardiovascular events, and/or all-cause mortality. We selected all studies that measured common carotid artery properties (diameter and distention) by ultrasound, together with brachial or local carotid pulse

pressure (PP), and calculated carotid artery distensibility coefficient (DC), Young's elastic modulus (YEM), compliance coefficient (CC) or beta-stiffness index (SI). DC represents arterial stiffness (the lower DC, the greater the stiffness).[3, 4] The other indices are closely related to the DC: higher YEM represents greater stiffness of the arterial wall material; lower CC represents lower arterial buffering capacity; and higher SI represents greater stiffness and takes into account the nonlinear relation between pressure and carotid artery diameter.[3, 4]

Data extraction

We used a predesigned data extraction form to collect information on the following items: study size, location, population characteristics, measures of arterial stiffness, follow-up duration, type and number of events, reported risk estimates, and variable(s) that were adjusted for in the analyses. In the case of multiple publications,[6, 14-16] we included the most up-to-date or comprehensive information.For the aggregate data meta-analysis, additional information for two studies[17, 18] was requested from corresponding authors; none provided the requested data.

Risk of bias assessment

Risk of bias was evaluated with the Newcastle-Ottawa Scale (NOS).[19] The NOS includes items on participant selection, validity of measurements, whether or not results were adjusted for age and blood pressure, plus duration and completeness of follow-up.

Individual participant data meta-analysis

For the IPD meta-analysis, we requested individual level data of all studies eligible for the aggregate data meta-analysis with available data on cfPWV. All four eligible studies provided the requested data. Individual data from these studies were collected and harmonized for the statistical analysis using PASW statistics (version 21).

Outcome definitions

In both the aggregate data and IPD meta-analysis, outcome definitions were used as reported in the originally published articles. Stroke included nonfatal and fatal cerebral infarction and intracerebral hemorrhage; CHD events included nonfatal and fatal acute myocardial infarction, angina pectoris, coronary artery bypass grafting, percutaneous coronary intervention and sudden death; total cardiovascular events included nonfatal and fatal CHD events, stroke, congestive heart failure and peripheral arterial disease; cardiovascular mortality included all fatal cardiovascular events (as defined above); and all-cause mortality included death from any cause.

Data synthesis and analysis

All analyses were performed with Cochrane Review Manager (version 5.2) and R statistical software (version 2.15).

Aggregate data meta-analysis

Results were pooled for the association between one standard deviation (SD) greater carotid stiffness and incident stroke. In addition, we evaluated the association of carotid stiffness with CHD events, total cardiovascular events, and cardiovascular and all-cause mortality. Results were included for lower DC or, if not available, higher YEM, lower CC or higher SI. For studies that reported results on carotid stiffness calculated with brachial as well as local PP, we included the results on carotid stiffness calculated with brachial PP in the main analysis, because these were available in the largest number of participants. In a sensitivity analysis, results were pooled for carotid stiffness calculated with local PP. All included studies calculated hazard ratios (HRs), except one study[20] which calculated an odds ratio. We treated this odds ratio as a HR. Pooled HRs were calculated using the random-effects inverse variance method. For each study,

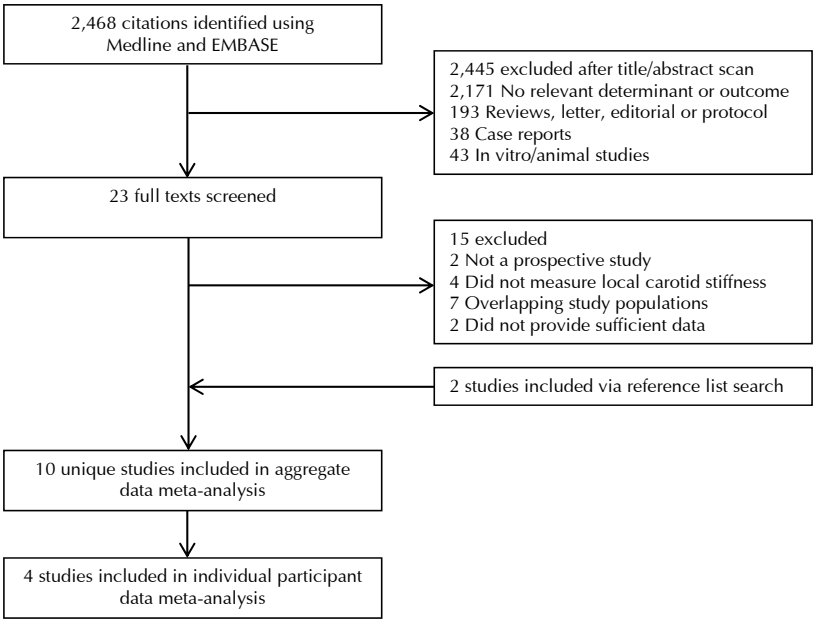

Figure 1. Flow chart of selection process of eligible studies

we included the fully adjusted value for the HR. Heterogeneity between studies was investigated with Higgins I^2 statistic. Several sensitivity analyses were done: analyses were repeated after exclusion of studies with a relatively high risk of bias (NOS score <7); analyses were repeated with studies which obtained carotid stiffness data by echotracking, which is considered the "gold standard" measurement technique to assess common carotid artery properties[3, 21, 22]; and results were pooled for each stiffness index separately (for lower DC, higher YEM, lower CC, and higher SI, respectively).

Individual participant data meta-analysis

Missing values on covariates were imputed using the expectation maximization method (single imputation) for each cohort separately. Percentage of missing values on covariates was minimal (total 2·0%). We first used a two-stage analysis approach[23] with

estimates of association calculated separately within each study before pooling across studies by the random-effects inverse variance method. We used Cox proportional hazard models with one SD lower carotid DC as the determinant and incident stroke as the outcome. Additionally, we evaluated the association of carotid stiffness with CHD events, total cardiovascular events, and cardiovascular and all-cause mortality. The associations were first adjusted for the following potential confounders (selected based on previous literature and previous knowledge): age, sex, mean arterial pressure, heart rate, body mass index, total / high density lipoprotein cholesterol ratio, triglycerides, current smoking, diabetes, prior CVD, the use of anti-hypertensive and lipid-modifying medication (model 1); and additionally for cfPWV (model 2). We checked whether the associations of carotid stiffness with outcomes were linear by visual inspection of graphs of carotid stiffness quartiles against the corresponding HR and formal testing for nonlinearity using cubic restricted splines.[24] The proportional hazards assumption was assessed by tests and visual inspection of graphs based on Schoenfeld residuals.[24] We used interaction terms to explore whether any association with incident stroke differed according to age, sex, hypertension and/or diabetes. In addition, we evaluated the association between the individual elements of the stiffness indices (PP, distension and diameter) and stroke.

We then evaluated whether carotid stiffness has any potential of being used as a risk predictor of stroke. We used the integrated discrimination improvement (IDI) and the continuous (category-free) net reclassification index (NRI) to quantify the incremental value of carotid DC for prediction of stroke risk beyond Framingham risk score factors and cfPWV. These indices quantify any reclassification, irrespective of (clinically relevant) cutoffs. We used a one-stage approach.[23] The IDI is a measure that reflects the average improvement, in percent point, in predicted probabilities summed across events and nonevents.[25] The continuous NRI is a measure of reclassification that quantifies the sum of the percentages, for events and nonevents separately, of

individuals in whom the directional change in predicted risk was consistent with observed events; values can range between -200% and +200%.[25] These analyses were done in individuals without a prior CVD (at baseline) and limited to a time horizon of 10 years. We first fitted a Cox proportional hazards model to the data using the Kaplan-Meier estimate[26] on the basis of cfPWV and the Framingham stroke risk score factors,[27] i.e. age, sex, systolic blood pressure, total and high density lipoprotein cholesterol, current smoking, diabetes, the use of anti-hypertensive medication and left ventricular hypertrophy (atrial fibrillation was not used in this model, because atrial fibrillation was an exclusion criterium for arterial stiffness measurements in each study). We refer to this model as the "base model". This base model was then extended by carotid DC, and the base and extended model were compared using the IDI and continuous NRI. Additionally, we calculated the (change in) C-statistic, a measure of risk discrimination.[24] Confidence intervals for the IDI, NRI and C-statistic were calculated by bootstrapping (1,000 repetitions). Finally, we evaluated the incremental value of carotid DC beyond Framingham cardiovascular risk score factors[28] (i.e. age, sex, systolic blood pressure, total and high density lipoprotein cholesterol, current smoking, diabetes and the use of anti-hypertensive medication) and cfPWV for risk prediction of CHD events, total cardiovascular events, and cardiovascular and all-cause mortality.

Results

Aggregate data meta-analysis: study characteristics

Figure 1 shows the selection process of included studies, out of 2,468 references initially identified, 10 were included. Of the ten studies[6, 8-10, 15, 20, 29-32] included, four[6, 8-10] evaluated stroke (n=17,662 with 898 events), five[6, 8-10, 30] CHD events (n=21,080 with 2,113 events), ten[6, 8-10, 15, 20, 29-32] any cardiovascular events (n=22,214 individuals with

3,010 events), seven[6, 9, 10, 15, 29, 31, 32] cardiovascular mortality (n=8,534 with 806 events) and five[6, 10, 15, 29, 32] all-cause mortality (n=5,991 with 2,062 events). For the Rotterdam Study[10] and the study of Blacher et al.[15]the original investigators were able to provide an update of previously published results with unpublished data on a higher number of participants and longer follow-up duration. The updated results of the Rotterdam Study were based on 4,713 individuals and a median follow-up duration of 12.0 years (previously published results[10] were based on n=2,835 and 4.1 years follow-up), and the updated results of Blacher et al. were based on 156 individuals and a median follow-up duration of 5.1 years (previously published results[15] were based on n=110 and 4.4 years follow-up). The studies included were conducted in the general population (five studies[6, 8, 10, 30, 32]), or in individuals with chronic kidney disease (four studies[15, 20, 29, 31]) or prior CVD (one study[9]). The follow-up duration ranged from 2.8 to 13.8 years.

Individual participant data meta-analysis: study characteristics

Four studies (the study of Blacher et al.,[15] and the Rotterdam,[10] Hoorn[6] and Nephrotest[29] Studies) had data available on cfPWV and were included in the IPD meta-analysis. Of these, two studies (Rotterdam and Hoorn Studies) had data available on incident stroke (n=4,075 with 351 events) and all four had data available on total cardiovascular events (n=4,395 with 763 events).

Risk of bias of individual studies

Risk of bias among the included studies was evaluated. Overall, risk of bias was low (mean NOS score was 7 out of 8).

Aggregate data meta-analysis

Greater carotid stiffness (per SD) was associated with a higher stroke incidence (HR 1.18 [95%CI 1.05; 1.33]) (Figure 2, Panel A). In addition, greater carotid stiffness was

associated with a higher incidence of total cardiovascular events (1.16 [1.07; 1.26]), and with greater cardiovascular (1.30 [1·15; 1.46]) and all-cause mortality (1.22 [1.121.34]), but not with CHD events (1.03 [0.98; 1.10]) (Figure 2, Panels B to E). The statistical heterogeneity between studies was low to moderate (range of I^2 was 0% to 55%; see also Figure 2, Panels A to E). Results did not materially change when data were pooled of carotid stiffness calculated with local PP; after exclusion of studies with a relatively high risk of bias; or when data were pooled of studies which obtained carotid stiffness data by echotracking(Figure S1). In addition, results were qualitatively similar for each carotid stiffness index, except for carotid CC, which was not statistically significantly associated with stroke or any of the other outcomes (Figure S1).

Individual participant data meta-analysis

After adjustment for potential confounders, lower carotid DC (per SD) was associated with higher stroke incidence (1.24 [1.05; 1.47]) (Table 2, panel A, model 1). Further adjustment for cfPWV did not materially change this association (1.24 [1.05; 1.46]) (model 2). In addition, lower carotid DC was associated with a higher incidence of total cardiovascular events, and greater cardiovascular and all-cause mortality, but not with CHD events (Table S1). We found no interaction of carotid DC with incident stroke according to sex, age, hypertension or diabetes (P-value for interaction >·24). Higher PP, lower distension and greater carotid diameter were associated with a higher stroke incidence (Figure S2). The baseline stroke risk was high as estimated by the base model (including Framingham stroke risk score factors and cfPWV) for individuals included in the IPD meta-analysis (i.e. 50.2% of individuals included had an estimated stroke risk higher than 5·0%). This was due to the inclusion of older individuals,[6, 10] and/or individuals with diabetes[6] or chronic kidney disease.[15, 29] When carotid DC was added to the base model, the IDI and continuous NRI for incident stroke improved statistically significantly with 0·4%-point (95% confidence interval 0.1 to 0.6%-point) and 18.6%

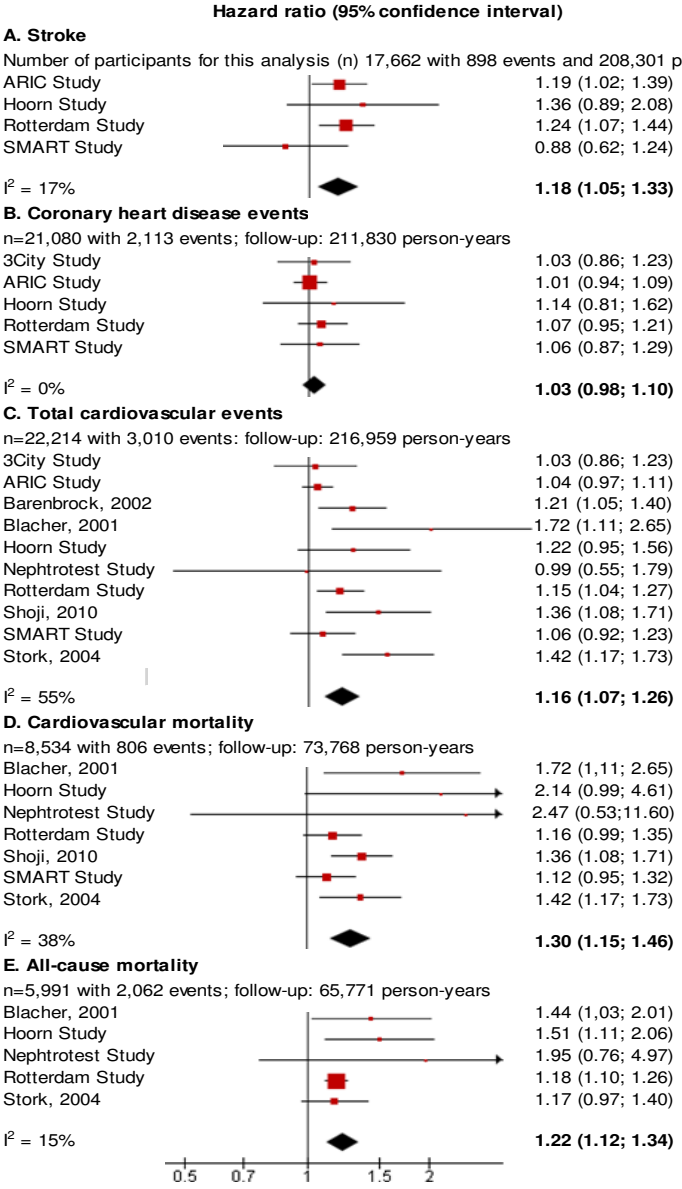

Figure 2. Results of the aggregate data meta-analysis. Forest plot for the association between, on the one hand, one standard deviation (SD) greater carotid stiffness and, on the other, incident stroke (A), coronary heart disease events (B), total cardiovascular events (C), cardiovascular mortality (D) and all-cause mortality (E).
For each study, the hazard ratio was pooled for, if available, one SD lower carotid distensibility coefficient; if not available, the hazard ratio was pooled for one SD higher Young's elastic modulus (for SMART Study9; 3City Study30; and Stork, 200432), or one SD higher beta-stiffness index (for Shoji, 201031). Abbreviations: ARIC = atherosclerosis risk in communities; SMART = second manifestations of arterial disease.

Table 1. Results of individual participant data meta-analysis. Association between carotid stiffness and incident stroke: additional adjustments for carotid-femoral pulse wave velocity (cfPWV) (A) and analysis of risk improvement (B)

Models	**Carotid DC (per one lower SD) as the determinant and incident stroke as the outcome***
A. Cox regression analysis	Hazard ratio (95% confidence interval)
Model 1†	1.24 (1.05; 1.47)
Model 1† + cfPWV	1.24 (1.05; 1.46)
B. Risk improvement analysis‡	Effect estimate (95% confidence interval)
IDI (%-point)	0.4 (0.1; 0.6)
Continuous NRI (%)	18.6 (5.8; 31.3)
C-statistic base model	0.747 (0.710; 0.784)
C-statistic extended model	0.750 (0.713; 0.787)
Change in C-statistic	0.003 (-0.003; 0.009)

*Number of participants for this analysis (n) 4,075 with 351 events and 47,881 person-years of follow-up.
†Model 1: results adjusted for age, sex, mean arterial pressure, heart rate, body mass index, smoking habits, diabetes, triglycerides, total / high density lipoprotein cholesterol ratio, prior cardiovascular disease, the use of lipid-modifying and anti-hypertensive medication.
‡Base model for risk improvement analysis included Framingham stroke risk score factors and cfPWV. Model was extended by carotid distensibility coefficient (DC) (per one lower standard deviation, SD).
Abbreviations: IDI = integrated discrimination improvement; NRI = net reclassification index.

(5.8 to 31.3%), respectively (Table 2, panel B). The C-statistic also improved, but this was not statistically significant (Table 2, panel B). In addition, the IDI and continuous NRI improved statistically significantly for cardiovascular mortality (0.3%-point [0.1 to 0.5%-point] and 17.5% [3.2 to 31.7%], respectively) and all-cause mortality (0.6%-point [0.4 to 0.9%-point] and 19.0% [12.3 to 25.7%], respectively), but not for CHD events (-0.0%-point [-0.1 to 0.1%-point] and 3.9% [-7.3 to 15.1%], respectively) and total cardiovascular events (0·1%-point [-0.2 to 0.3%-point] and 5.0% [-4.6 to 15.0%], respectively) (Table S1). The C-statistic did not statistically significantly improve for any of these outcomes (Table 3).

Discussion

The present systematic review and meta-analysis of aggregate and individual participant data showed that greater carotid stiffness was associated with a higher stroke incidence. This association was independent of age, sex, blood pressure and other CVD risk factors, and did not materially change after adjustment for aortic stiffness (measured as cfPWV). In addition, estimation of carotid stiffness modestly improved stroke risk prediction beyond Framingham stroke risk score factors and cfPWV, as indicated by a statistically significant improvement of the IDI and continuous NRI. Finally, carotid stiffness was associated with a higher incidence of total cardiovascular events, and greater cardiovascular and all-cause mortality, but not with CHD events.

This is the first systematic review and meta-analysis on the association between carotid stiffness and incident cardiovascular disease and mortality. The findings are in agreement with, and further extend, previous observational studies[6, 8, 20, 29] that reported an association between carotid stiffness and incident CVD,[6, 8, 20, 29] including stroke.[8] The aggregate data meta-analysis enabled us to examine these associations in greater detail with enhanced power. In addition, the IPD meta-analysis allowed us to do a comprehensive range of additional analyses, including adjustment for cfPWV and quantification of stroke risk improvement beyond Framingham risk score factors and cfPWV.

Some methodological issues warrant consideration. Firstly, the results were consistent across different study populations notwithstanding differences in methods to quantify carotid stiffness, and were not related to the risk of bias of included studies, which strengthens the validity of the findings. Secondly, the results were consistent for all carotid stiffness indices, except for carotid CC, which was not statistically significantly associated with stroke. To further explore this finding, we evaluated the association between individual elements of the stiffness indices (PP, distension and diameter) and

stroke. The results showed that greater carotid diameter, lower distension and higher PP were each associated with a higher stroke incidence. The association between greater carotid diameter and incident stroke is in accordance with previous studies,[33, 34] and may reflect arterial remodeling in response to atherosclerosis or increased arterial stiffness.[14] However, arterial diameter enlargement leads to greater compliance, and this may explain that we did not find an association between (lower) carotid CC and stroke. Thirdly, the present study had insufficient power to formally test the potential influence of publication bias.[35] Nevertheless, a broad systematic search was done to identify all relevant studies, and we were able to include published as well as unpublished data. This limits the possibility of the presence of (substantial) publication bias.

The present study showed that greater carotid stiffness is associated with a higher stroke incidence, independently of aortic stiffness, and supports the concept that carotid stiffening is important in the pathogenesis of stroke.[6] The underlying mechanism may be that stiffening of the carotid artery (or of other elastic arteries for which the carotid artery may serve as a proxy) leads to a higher pulsatile pressure and flow load on the brain.[3, 4,36] This increased load can penetrate distally into the cerebral microcirculation and may directly cause cerebral ischemia and hemorrhage.[5, 36,37] In addition, the increased pulsatile load may induce a hypertrophic remodeling response and rarefaction of small cerebral arteries, which, in turn, may lead to chronic ischemia. Furthermore, stiffening of the carotid artery may lead to stroke through local development of rupture-prone atherosclerotic plaques. Indeed, previous studies[7, 38] have shown that arterial stiffness is associated with presence[7, 38] and a rupture-prone phenotype[7] (e.g. intraplaque hemorrhage) of atherosclerotic plaques in the internal carotid artery.

In the present study, carotid stiffness, in contrast to aortic stiffness (as determined by cfPWV),[11,12] was not associated with incident CHD events. A possible explanation for these observations may be that stiffening of the aorta, but not of the carotid artery, leads to a higher left ventricular load and reduced diastolic coronary perfusion.[3, 4]

In addition, carotid stiffness was associated with total (nonfatal and fatal) cardiovascular events and with all-cause mortality not explained quantitatively by stroke. This suggests that stiffening of carotid arteries additionally increases the risk of diseases other than stroke. For example, it is conceivable that stiffening of the carotid artery is associated with risk of congestive heart failure, as stiffening of the carotid artery could act as a proxy for stiffening of the proximal elastic segment of the aorta, which increases cardiac afterload and is associated with risk of congestive heart failure.[39, 40] In addition, carotid stiffness may be a marker of biological aging and, thus, be associated with mortality of age-related diseases other than cardiovascular disease.[6] These possibilities require further investigation.

The observation that carotid stiffness was associated with incident stroke independently of aortic stiffness could have clinical relevance, as this identifies carotid stiffness as a potential separate target for stroke risk lowering therapy. CVD risk factors have different impacts on stiffening of elastic versus muscular arteries.[41, 42] This may be attributed to the marked differences in the architecture of these arteries, and suggests that stiffness of elastic arteries may be specifically targeted. Currently, no effective clinical therapy is available that specifically targets stiffness of elastic arteries.

In the present study, carotid stiffness improved risk prediction of stroke beyond Framingham stroke risk score factors and cfPWV, as indicated by improvement of IDI and continuous NRI. This finding provides proof of principle that carotid stiffness can have additional value as a risk predictor of stroke. The improvement of stroke risk prediction by carotid stiffness was, however, modest, and, in high-risk populations such as those included in the current analyses, such an improvement may not be clinically relevant.[43] Nevertheless, the current data provide a framework for investigating whether assessment of carotid stiffness can improve stroke risk prediction in younger individuals and in those at intermediate risk for stroke, in whom improvement of risk prediction may be of greater importance.[44]

A limitation of the present study is that (unavoidable) survival bias may have led to an underestimation of the associations observed. In addition, we could not evaluate the association between carotid stiffness and stroke subtypes, i.e. ischemic versus hemorrhagic. However, it is likely that stiffening of the carotid artery increases the risk of both ischemic and hemorrhagic stroke.[5, 36, 37]

In conclusion, the present study shows that greater carotid stiffness is associated with a higher stroke incidence independently of cfPWV. In addition, carotid stiffness modestly improved risk prediction of stroke beyond Framingham stroke risk score factors and cfPWV. This identifies carotid stiffness as a potential separate target for prevention strategies of stroke. Further studies are needed to quantify the predictive value of carotid stiffness in individuals at intermediate risk for stroke in whom reclassification improvement may be of greatest clinical importance.

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Supplementary document

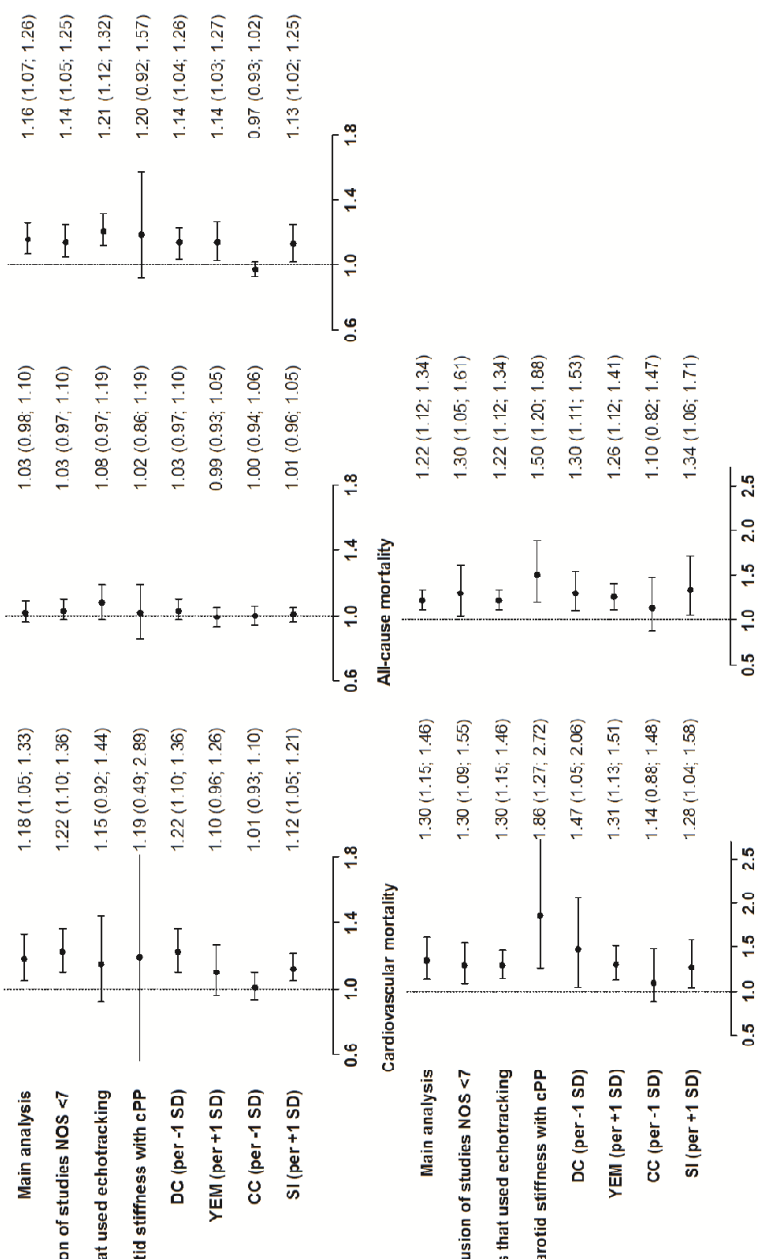

Figure S1. Results of the aggregate data meta-analysis.Main and sensitivity analyses of the association between carotid stiffness and incident cardiovascular events and mortality. Data represent hazard ratios (and corresponding 95% confidence intervals) for one SD higher carotid stiffness. For each study included in the analyses, the hazard ratio was pooled for, if available, one SD lower carotid distensibility coefficient (DC); if not available, the hazard ratio was pooled for one SD higher Young's elastic modulus (YEM) or one SD higher beta-stiffness index (SI). In addition, the hazard ratios were pooled for each stiffness index separately (for lower DC, higher YEM, lower compliance coefficient (CC) and higher SI, respectively). Abbreviations: NOS = Newcastle-Ottowa scale; cPP = local carotid pulse pressure.

Table S1. Results of the individual participant meta-analysis. Association between one SD lower carotid distensibility coefficient (DC) and incident cardiovascular events and mortality*: additional adjustments for carotid-femoral pulse wave velocity (cfPWV) (panel A) and analysis of risk improvement (panel B)

Models	Coronary heart disease events	Total cardiovascular events	Cardiovascular mortality	All-cause mortality
A. Cox regression analysis	Hazard ratio (95% confidence interval)			
Model 1†	1.02 (0.90; 1.16)	1.14 (0.94; 1.37)	1.36 (0.95; 1.95)	1.34 (1.11; 1.62)
Model 1† + cfPWV	1.02 (0.90; 1.16)	1.10 (0.92; 1.33)	1.39 (0.92; 2.10)	1.31 (1.10; 1.57)
B. Risk improvement analysis‡	Effect estimate (95% confidence interval)			
IDI (%-point)	-0.0 (-0.1; 0.1)	0.1 (-0.2; 0.3)	0.3 (0.1; 0.5)	0.6 (0.4; 0.9)
Continuous NRI (%)	3.9 (-7.3; 15.1)	5.0 (-4.6; 15.0)	17.5 (3.2; 31.7)	19.0 (12.3; 25.7)
C-statistic base model	0.698 (0.673; 0.723)	0.721 (0.690; 0.752)	0.812 (0.773; 0.851)	0.778 (0.760; 0.796)
C-statistic extended model	0.699 (0.674; 0.724)	0.721 (0.690; 0.752)	0.813 (0.774; 0.852)	0.779 (0.761; 0.797)
Change in C-statistic	0.001 (-0.003; 0.005)	0.001 (-0.003;0.004)	0.001 (-0.003; 0.004)	0.001 (-0.001; 0.003)

*Number of participants (n) for incident coronary heart disease events: 4,114 with 482 events and 40,207 person-years of follow-up; for total cardiovascular events: n=4,395 with 763 events and 41,060 person-years of follow-up; for cardiovascular mortality: n=4,540 with 351 events and 50,711 person-years of follow-up; and for all-cause mortality: n=4,545 with 1,545 events and 52,622 person-years of follow-up.
†Model 1: results adjusted for age, sex, mean arterial pressure, heart rate, body mass index, smoking habits, diabetes, triglycerides, total / high density lipoprotein cholesterol ratio, prior cardiovascular disease, the use of lipid-modifying and anti-hypertensive medication.
‡Base model for risk improvement analysis included Framingham cardiovascular risk score factors and cfPWV .Model was extended by carotid DC (per one lower standard deviation, SD).
Abbreviations: IDI = integrated discrimination improvement; NRI = net reclassification index.

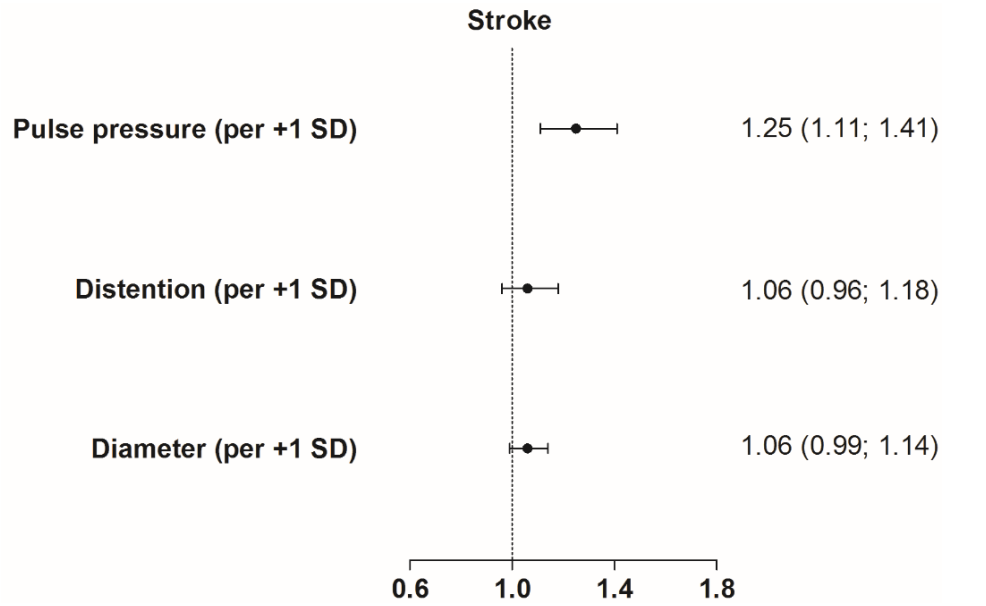

Figure S2. Results of the individual participant data meta-analysis.
Association between the individual elements of the stiffness indices (pulse pressure, distension and diameter) and incident stroke.
Data represent hazard ratios and corresponding 95% confidence intervals.

3.2 Microstructural brain white matter integrity and the risk of mortality

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While several studies reported a link between presence of white matter lesions and shorter survival, it is not yet clear whether this link extends to more subtle cerebral white matter changes. We investigated the independent association of the cerebral white matter microstructural integrity with risk of mortality. We included 4294 stroke and dementia free individuals (mean age 63.6 years, 44% male) from the population-based Rotterdam Study. Diffusion-magnetic resonance imaging was used to assess the microstructural integrity of the normal-appearing white matter. All-cause, cardiovascular and non-cardiovascular mortality was recorded. Cox regression models, adjusted for age, sex, cardiovascular risk factors and macrostructural MRI changes, were used to estimate hazard ratios. White matter in the population had an average fractional anisotropy (FA) of 0.34±0.01 and an average mean diffusivity (MD) of 0.74±0.03 10^{-3} mm^2/s. During the median follow up time of 5.4 years, 216 (5%) participants died from all-causes. For cause-specific mortality, during the median follow up time of 3.6 years, 25 (0.7%) participants died of cardiovascular causes and 71 (1.7%) individuals died of non-cardiovascular causes. Each standard deviation (SD) decrease in FA and each SD increase in MD was associated with 1.37 fold (95%CI: 1.20, 1.57) and 1.49 fold (95%CI: 1.28, 1.75) higher risk of all-cause mortality, respectively. The associations were more prominent with cardiovascular mortality rather than non-cardiovascular mortality. In tract-specific analyses, we observed that association tracts are more prominently related to mortality. Changes in the microstructure of cerebral white matter are related to higher risk of mortality in particular cardiovascular related mortality.

Background

Brain white matter plays a major role in brain functioning e,g. in cognitive function, motor function and physiological processes.[1, 2] One of the manifestations of white matter damage is the emergence of white matter lesions, a common finding on magnetic resonance imaging (MRI) of older people.[3] Several studies reported a link between presence of white matter lesions and adverse health outcomes, as well as shorter survival.[4-6] However, it seems that white matter lesions are only the "tip of the iceberg" of white matter pathology, and changes in the microstructure of cerebral white matter develop long before appearance of white matter lesions on a brain MRI.[7] Given the prominent role of white matter lesions in not only development and progression of brain disorders but also in poor survival; it is of great importance to study early changes in white matter before irreversible white matter lesions form.[8] Therapeutic approaches and life style changes in early phase might help to prevent further progression of impairments in white matter microstructural integrity.[8, 9] Diffusion-MRI is a sensitive MRI technique which can detect and quantify subtle changes in the cerebral white matter microstructure.

In 4294 stroke and dementia free individuals, we aimed to investigate the independent association of cerebral white matter microstructural integrity in relation to risk of mortality. Moreover, we assessed whether regional differences in white matter microstructural integrity had differential effects in respect to mortality risk.

Methods

Population

The present study is embedded within the framework of the Rotterdam Study, an on-going prospective population-based cohort study, among inhabitants of Ommoord, a

district of Rotterdam in The Netherlands. The design of the Rotterdam study has been described previously.[10] Since 2005, brain MRI is implemented into the study protocol of the Rotterdam Study. Out of 5430 eligible participants, 4841 persons (non-demented and without MRI-contraindications) underwent a structural and diffusion-MRI of the brain. We excluded 53 scans due to incomplete acquisitions, 112 scans due to artifacts hampering automated processing, 135 scans due to failed tissue segmentation, and 160 scans due to presence of cortical infarcts (MRI-defined). We additionally excluded 87 participants with clinical stroke. This resulted in 4294 participants for the analyses. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[10]

MRI acquisition and processing

Brain MRI scanning was performed on a 1.5 tesla MRI scanner (GE Signa Excite). Scan protocol and sequence details are described extensively elsewhere.[11] For the diffusion scan, a single shot, diffusion weighted spin echo echo-planar (maximum b-value was 1000 s/mm^2 in 25 non-collinear directions, three volumes were acquired without diffusion weighting (b-value = 0 s/mm^2)) sequence was performed.[11] An automated segmentation approach, based on a conventional k-nearest-neighbor classifier, which was extended with a post-processing white matter lesion (WML) segmentation, was used to segment scans into grey matter, white matter, WML, cerebrospinal fluid (CSF) and background tissue.[12, 13] Supratentorial intracranial volume (ICV) was estimated by summing grey and white matter and CSF volumes.[12] Cortical infarcts were rated on structural sequences, and in case of involvement of grey matter, they were classified as cortical infarcts. Lacunes were defined as focal lesions (size ≥3 and < 15 mm) with the signal intensity of CSF on all sequences, and when located supratentorially with a

hyperintense rim on fluid-attenuated inversion recovery (FLAIR) sequence. The presence of cerebral microbleeds was rated on a three-dimension T2*-weighted gradient-recalled echo MRI by 1 of 5 trained research physicians, blinded to the clinical data.

Diffusion-MRI processing and tractography

All diffusion data were pre-processed using a standardized pipeline (including correction for motion and Eddy currents), obtaining global mean fractional anisotropy (FA), mean diffusivity (MD), and axial and radial diffusivities.[13, 14] Next, white matter tracts were segmented using a probabilistic diffusion tractography approach described previously.[14] We segemented 14 different white matter tracts (11 tracts were present in the left and right hemispheres), and obtained median FA and MD inside each white matter tract, with subsequent combination of left and right measures,[15] where after standardization of these tract-specific diffusion-MRI parameters.[15] Tracts were categorized into brainstem tracts, projection tracts, association tracts, limbic system tracts, and callosal tracts.[15] To obtain tracts-specific white matter volumes, tract segmentation were used and by combining tissue and tract segmentations to obtain tract-specific WML volumes. Due to a skewed distribution of WMLs, we natural-log transformed tract-specific WML volumes.

Mortality

Deaths were continuously reported through automatic linkage of general practitioner files. In addition, municipal records were checked bimonthly for information on vital status. The follow-up for total mortality was complete until July 4, 2014. Information about cause of death was obtained from general practitioner and hospital records. For cause-specific mortality, research physicians reviewed all available information and coded the events according to the International Classification of Diseases, 10th edition (ICD-10). Death due to cardiovascular mortality was classified as ICD-10 codes

I00-I99 and death due to other reasons was recorded as non-cardiovascular mortality. A consensus panel, led by a physician with expertise in cardiovascular disease, decided the final cause of death according to ICD-10 codes using standardized definitions. The follow-up for cause specific mortality was complete until January 1, 2012.[16]

Cardiovascular risk factors

After a resting period of five minutes, blood pressure was measured twice in a single visit using a random-zero sphygmomanometer on the right arm of participants in the sitting position. The average of two measurements, separated by a count of the pulse rate, was used in the analyses. Serum total and high density lipoprotein (HDL) cholesterol levels were determined using an automated enzymatic method. Information on antihypertensives and lipid lowering medication was based on home interviews. Information related to smoking was acquired through personal interviews using questionnaires. Smoking was categorized in never, former and current smoking. Cardiovascular disease was considered as a history of myocardial infarction, or coronary revascularization procedures.[16] Diabetes mellitus was defined by use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l.[7] Body mass index was calculated by dividing weight in kilograms by height in meters squared.

Statistical analysis

Associations of white matter microstructural integrity (global and tract-specific) with all-cause, cardiovascular and non-cardiovascular mortality were evaluated using cox proportional hazard models. We performed the analyses in three models. In the first model we adjusted for age, and sex. In the second model, analyses were adjusted for age, sex, and cardiovascular risk factors including systolic blood pressure, diastolic blood pressure, antihypertensive medication, total and high density

lipoprotein cholesterol, lipid-lowering medication, smoking, history of coronary heart disease, diabetes mellitus and body mass index. In the third model, we adjusted for macrostructural white matter changes (white matter volume, and the logarithm of white matter lesion volume), intracranial volume, presence of microbleeds, and presence of lacunar infarcts. In all analyses, we treated different cohorts of the Rotterdam Study as a potential confounder.[14] Since the cerebellum could not be fully incorporated in the diffusion scan which might lead to a varying field of view to cover the brainstem tracts (mainly medial lemniscus), we additionally controlled for this factor in the tract-specific analyses of the medial lemniscus. Linearity and proportionality assumptions were met for all the analyses.

To take into account the differences in age and sex, tertiles of FA and MD were made based on the unstandardized residuals of FA and MD regressed against age and sex. We then inspected the mortality rates per 1000 person years in three categories of FA and MD. We additionally used the Kaplan-Meier method to estimate cumulative mortality curves of all-cause mortality associated with tertiles of FA and MD. We performed series of sensitivity analyses. First, participants with interim dementia or stroke were excluded to investigate the influence of new cases of dementia and stroke during the follow up on the association of FA and MD with all-cause mortality. Second, we repeated the association of FA and MD with cardiovascular mortality after excluding all death from stroke. We used Šidák correction to correct for multiple comparisons, after estimating the number of independent tests,[17](Print resulting in $p < 0.0037$ as the significance threshold. All analyses were carried out using SPSS 20.0.2 for windows or R version 2.15.0.

Results

Baseline characteristics of the participants are presented in Table 1. Average age of the participants was 63.6±11, and 44 % were male. Participants had a mean FA of

0.34±0.01 and a mean MD of 0.74±0.03 10^{-3} mm^2/s. During the median follow up time of 5.4 [3.6-5.9] years, 216 (5%) participants died. For cause -specific mortality, during the median follow up time of 3.6 [2.3-4.7] years, 25 (0.7%) individuals died of cardiovascular causes and 71 (1.7%) participants died from non-cardiovascular causes. All-cause mortality rates in participants with low, middle and high categories of FA was 21.8, 8.9, and 4.6 per 1000 person years, respectively. All-cause mortality for low, middle and high categories of MD was 3.3, 5.4, and 30.2 per 1000 person years, respectively (Figure 1).

Table 2 presents the association of white matter microstructural integrity with all-cause mortality. In model I adjusted for age and sex, each SD decrease in FA was associated with 1.37 fold higher risk of all-cause mortality (95% confidence interval [CI]: 1.20, 1.57). Likewise, each SD increase in MD was associated with 1.49 fold higher risk of mortality (95%CI: 1.28, 1.75). Adjustments for macrostructural MRI changes and cardiovascular risk factors attenuated the associations minimally. The associations of white matter microstructural integrity with cardiovascular and non-cardiovascular mortality are shown in Table 3.

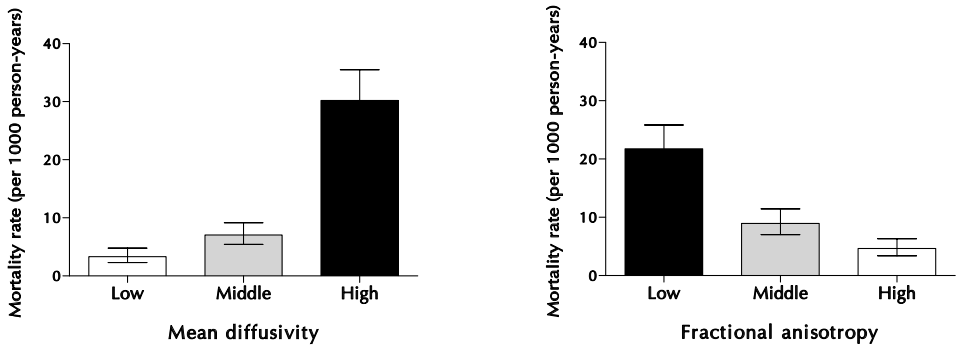


Figure 1. Mortality rate per 1000 person-years in tertiles of fractional anisotropy (FA) and mean diffusivity (MD)
Tertiles of FA and MD are calculated based on the unstandardized residuals of FA and MD regressed against age, and sex. Cut off points for tertiles are:
FA: low (0.32- 0.33), middle (0.33- 0.34), high (0.34- 0.35)
MD: low (0.71- 0.73), middle (0.73- 0.75), high (0.75- 0.80)

Table 1. Baseline characteristics

Baseline characteristic	n= 4294
Age, years	63.6 (11.0)
Men	1906 (44.4)
Systolic blood pressure, mmHg	139.0 (21.5)
Diastolic blood pressure, mmHg	83.1 (10.8)
Antihypertensive medication	1463 (34.1)
Total cholesterol, mmol/l	5.5 (1.0)
HDL cholesterol, mmol/l	1.4 (0.4)
Lipid-lowering medication	1014 (23.6)
Smoking	
Current	884 (20.6)
Former	2060 (48.0)
History of coronary heart disease	256 (6.0)
Diabetes mellitus	393 (9.2)
Body mass index, kg/m^2	27.4 (4.1)
White matter volume, mL	403.3 (60.8)
White matter lesion volume, mL	4.4 (2.4,8.8)
Intracranial volume, mL	1340.0 (132.9)
Cerebral microbleeds	779 (18.1)
Lacunar infarcts	93 (2.2)
Fractional anisotropy	0.34 (0.01)
Mean diffusivity, 10^{-3} mm^2/s	0.74 (0.03)

Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and white matter lesion volume is presented as median (interquartile range).
The following variables had missing data: blood pressure (n=90), blood pressure lowering medication (n=78), smoking (n=69), lipid-lowering medication (n= 48), HDL: high density lipoprotein cholesterol (n=520), total cholesterol (n= 518), body mass index (n=82), diabetes (n=125), history of coronary heart disease (n=44).

In the age and sex adjusted model, each SD decrease in FA was associated with 1.86 fold increased risk of cardiovascular mortality (95% CI: 1.29, 2.69). Similarly, each SD higher MD was associated with 1.90 fold increased risk of cardiovascular mortality (95%CI: 1.25, 2.88). Adjustment for cardiovascular risk factors attenuated the associations of FA and MD with cardiovascular mortality. After adjustment for macrostructural MRI changes, the associations of FA and MD with cardiovascular mortality were not present anymore (p 0.088 and 0.186, respectively).

Table 2. The association between white matter microstructural ntegrity and risk of mortality

	All-cause mortality	
	Hazard ratio* (95% CI) N=4294 (216)	**P value**
Fractional anisotropy		
Model I	1.37 (1.20, 1.57)	<0.001
Model II	1.31 (1.15, 1.49)	<0.001
Model III	1.27 (1.10, 1.48)	0.001
Mean diffusivity		
Model I	1.49 (1.28, 1.75)	<0.001
Model II	1.40 (1.20, 1.64)	<0.001
Model III	1.39 (1.17, 1.66)	<0.001

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.
Model I: Adjusted for age, and sex.
Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index.
Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.
Abbreviation: CI: confidence interval.

There was no association between FA and non-cardiovascular mortality (all p>0.05). Higher MD, however, was associated with increased risk of non-cardiovascular mortality (HR: 1.42, 95%CI: 1.08, 1.87), but the association attenuated after adjusting for cardiovascular risk factors and disappeared after controlling for macrostructural white matter changes (p 0.153).

Kaplan-Meier survival curves showed that individuals with low FA and high MD had the highest cumulative mortality rate (Figure 2).

Associations of microstructural organization of specific white matter tracts and mortality are presented in Table 4. In the tract-specific analyses, we observed that for all tracts, MD was more prominently related to mortality than FA. For MD, association between tract-specific white matter loss and mortality seemed to be present throughout the brain; however, it was more prominent in the association tracts. Excluding participants

Table 3. The association of white matter microstructural integrity and risk of cardiovascular and non-cardiovascular mortality

	Cardiovascular mortality		Non-cardiovascular mortality	
	Hazard ratio* (95% CI)	**P value**	**Hazard ratio* (95% CI)**	**P value**
	N=4294 (25)		**N=4294 (71)**	
Fractional anisotropy				
Model I	1.86 (1.29, 2.69)	0.001	1.24 (0.98, 1.57)	0.072
Model II	1.75 (1.19, 2.56)	0.004	1.19 (0.94, 1.51)	0.148
Model III	1.42 (0.95, 2.13)	0.088	1.11 (0.86, 1.44)	0.401
Mean diffusivity				
Model I	1.90 (1.25, 2.88)	0.002	1.42 (1.08, 1.87)	0.012
Model II	1.79 (1.15, 2.79)	0.010	1.35 (1.02, 1.79)	0.034
Model III	1.38 (0.85, 2.26)	0.186	1.25 (0.92, 1.72)	0.153

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.
Model I: Adjusted for age, and sex.
Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index.
Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.
Abbreviation: CI: confidence interval.

with interim dementia or stroke did not change our observations (Table S1). Similarly, excluding stroke death did not change the association of FA and MD with cardiovascular mortality (Table S2).

Discussion

We showed that stroke and dementia free individuals with loss of microstructural integrity of cerebral white matter are at increased risk for mortality, independent of cardiovascular risk factors and macrostructural MRI changes. The association was more prominent with cardiovascular mortality rather than non-cardiovascular mortality. Furthermore, we found that loss of white matter microstructure in the association tracts most strongly related to the risk of mortality.

Table 4. Associations of tract-specific diffusion-MRI parameters and risk of mortality

	Fractional anisotropy	Mean diffusivity
	Hazard ratio* (95% CI)	**Hazard ratio* (95% CI)**
Tracts in brainstem		
Middle cerebellar peduncle	0.96 (0.79, 1.16)	1.08 (0.95, 1.22)
Medial lemniscus [a]	0.97 (0.82, 1.15)	**1.21 (1.06, 1.37)**
Projection tracts		
Corticospinal tract	0.94 (0.82, 1.08)	**1.29 (1.11, 1.49)**
Anterior thalamic radiation	0.95 (0.81, 1.12)	1.17 (1.00, 1.37)
Superior thalamic radiation	0.94 (0.82, 1.07)	1.21 (1.05, 1.38)
Posterior thalamic radiation	0.84 (0.70, 1.00)	1.10 (0.95, 1.27)
Association tracts		
Superior longitudinal fasciculus	0.83 (0.71, 0.97)	**1.28 (1.13, 1.46)**
Inferior longitudinal fasciculus	0.92 (0.77, 1.09)	**1.28 (1.09, 1.49)**
Inferior fronto-occipital fasciculus	0.80 (0.67, 0.96)	1.24 (1.05, 1.45)
Uncinate fasciculus	0.79 (0.67, 0.94)	**1.35 (1.15, 1.57)**
Limbic system tracts		
Cingulate gyrus part of cingulum	0.92 (0.78, 1.07)	**1.27 (1.10, 1.47)**
Parahippocampal part of cingulum	0.88 (0.77, 1.03)	1.00 (0.91, 1.10)
Callosal tracts		
Forceps major	0.78 (0.66, 0.94)	1.08 (0.96, 1.23)
Forceps minor	0.81 (0.67, 0.98)	1.24 (1.06, 1.45)

*Hazard ratios and 95% CI, calculated per SD increase in fractional anisotropy and mean diffusivity, adjusted for age, sex, intracranial volume, white matter volume, white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.
Results in bold are significant after correction for multiple testing ($p < 3.7 \times 10^{-3}$).

White matter is a key component of the brain consisting of glial cells and myelinated axons that transmit signals between various regions of the brain and spinal cord.[2] White matter accounts for nearly half of the brain volume and its microstructural organization is crucial for normal brain function.[7] White matter damage represented as white matter lesions and white matter atrophy are reported to be related to a higher risk of mortality.[4, 18, 19] However, the association between minor microstructural changes in

white matter, that can provide a better insight into the cerebral white matter pathology, has not been investigated in the population-based setting. Previous studies in specific patient groups showed that minor microstructural changes in white matter are linked to a worse cardiorespiratory and metabolic profile and an increased risk of mortality.[20] In this study we showed that this finding can be extended to the general population free of dementia and stroke.

We observed that worse white matter microstructural integrity was associated with a higher risk of all-cause, cardiovascular, and non-cardiovascular mortality, but most prominently with cardiovascular mortality. A possible explanation for this observation could be that white matter is more prone to vascular insults.[21, 22] Shared cardiovascular risk factors such as hypertension and diabetes might play a role in this association. However, our findings persisted after adjustments for conventional cardiovascular risk factors, indicating that loss of white matter microstructure may represent cardiovascular risk factors that could not be measured, or represent the very initial phase of atherosclerosis.

Available literature suggests that damage to cerebral white matter is not only associated with deterioration in cerebral functions such as cognition, sensory or motor function loss but also with disturbances in regulation of endocrine and autonomic nervous systems throughout the body.[23] Disturbances in the white matter microstructural integrity may therefore influence a variety of functions throughout the body that may result in a higher risk of mortality also due to causes other than neurological diseases.[6, 23] This is in line with our observation that adjusting for macrostructural-MRI changes and excluding neurological diseases (interim stroke and dementia) did not change our findings. Moreover, the association between white matter microstructure and cardiovascular mortality persisted even after excluding cerebrovascular related mortality, indicating that the association was not mainly driven by neurovascular diseases. Further research is needed to investigate the exact mechanisms underlying the association of white

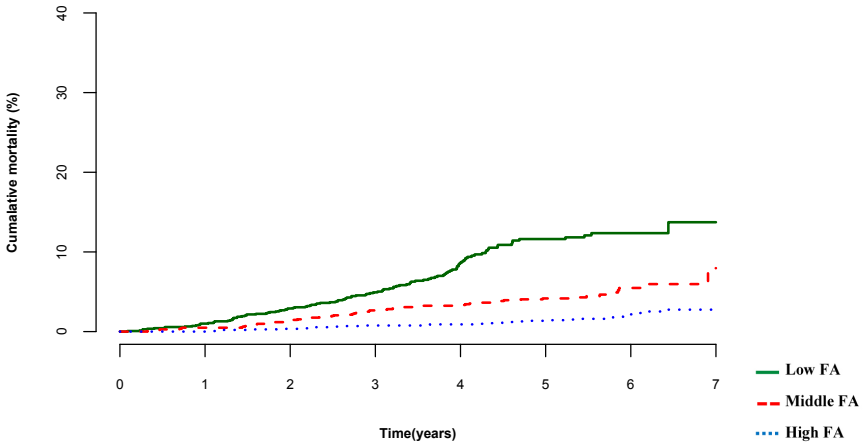
matter microstructure and higher risk of mortality.

In the tract specific analyses, we observed that MD is more prominently associated with mortality rather than FA. For FA, loss of microstructural integrity in association tracts is most strongly related to mortality. For MD, the spatial distribution was relatively widespread; however, also more apparent to the association tracts. Previous research has shown that the microstructure of association tracts is mainly influenced by age and cardiovascular risk factors,[22] and this may explain why we found the stronger relation between association tracts and mortality.

Strengths of this study include the large sample size and its population-based design consisting of dementia and stroke free population. Furthermore, availability of extensive data on cardiovascular risk factors and macrostructural MRI-markers enabled us to control for potential confounders. Limitations of this study should be acknowledged. In the tracts-specific analysis we used median FA and MD and this disposes spatial information compared to a voxel-based technique. Furthermore, varying field of views were used to cover the cerebellum, resulting in less reliable conclusions on brain stem tracts.

In conclusion, we observed that early stage changes in white matter microstructure are independently associated with poor survival. Our findings suggest that impairments in cerebral white matter, even at early stages, are not limited to adverse brain outcomes and they can put individuals at higher risk of mortality from both cardiovascular and non-cardiovascular causes. Diffusion-MRI parameters may be markers reflecting the overall health status and may be a clinical tool to assess the prognosis of individuals.

A) Fractional anisotropy



B) Mean diffusivity

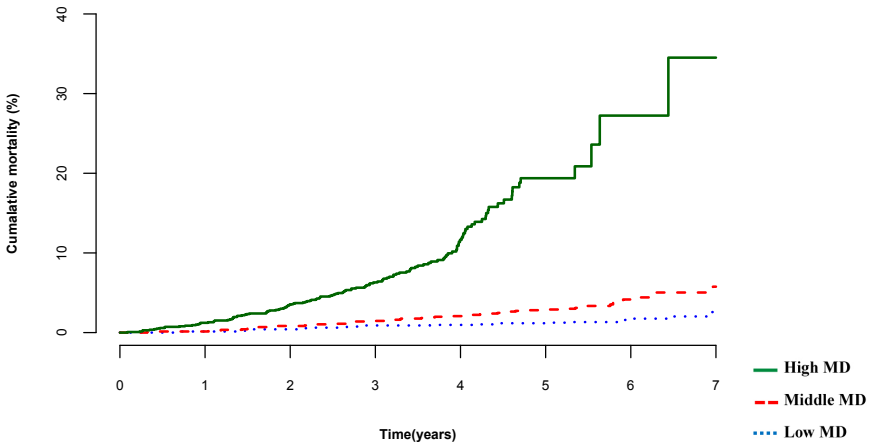


Figure 2. Cumulative mortality rate in tertiles of A) fractional anisotropy (FA) and B) mean diffusivity (MD)
Tertiles of FA and MD are calculated based on the unstandardized residuals of FA and MD regressed against age, and sex. Cut off points for tertiles are:
FA: low (0.32- 0.33), middle (0.33- 0.34), high (0.34- 0.35)
MD: low (0.71- 0.73), middle (0.73- 0.75), high (0.75- 0.80)

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Supplementary document

Table S1. The association of white matter microstructural integrity and risk of mortality excluding participants with interim stroke and dementia

	Total mortality	
	Hazard ratio* (95% CI) N=4219 (198)	**P value**
Fractional anisotropy		
Model I	1.35 (1.17, 1.56)	<0.001
Model II	1.27 (1.09, 1.49)	0.003
Model III	1.31 (1.14, 1.51)	<0.001
Mean diffusivity		
Model I	1.50 (1.27, 1.78)	<0.001
Model II	1.42 (1.18, 1.71)	<0.001
Model III	1.45 (1.22, 1.73)	<0.001

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.
Model I: Adjusted for age, and sex.
Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index.
Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.

Table S2. The association of white matter microstructural integrity and risk of cardiovascular mortality excluding death from stroke

	Cardiovascular mortality	
	Hazard ratio* (95% CI) N=4286 (20)	**P value**
Fractional anisotropy		
Model I	1.63 (1.07, 2.48)	0.023
Model II	1.32 (0.83, 2.09)	0.234
Model III	1.49 (0.97, 2.30)	0.069
Mean diffusivity		
Model I	1.97 (1.22, 3.19)	0.005
Model II	1.53 (0.88, 2.66)	0.133
Model III	1.83 (1.09, 3.05)	0.021

*Hazard ratios and 95% CI are calculated per standard deviation decrease in fractional anisotropy and per standard deviation increase in mean diffusivity.
Model I: Adjusted for age, and sex.
Model II: Model I + systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of coronary heart disease, and body mass index.
Model III: Model I + intracranial volume, white matter volume, logarithm of white matter lesion volume, presence of microbleeds, and presence of lacunar infarcts.

CHAPTER 4

The kidney and the brain

4.1 Kidney function and microstructural integrity of brain white matter

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We investigated the association of kidney function with white matter microstructural integrity. We included 2726 participants with a mean age of 56.6 years (45% men) from the population-based Rotterdam Study. Albumin-to-creatinine ratio, and glomerular filtration rate (eGFR), using serum cystatin C (eGFRcys) and creatinine (eGFRcr), were measured to evaluate kidney function. Diffusion-MRI was used to assess microstructural integrity of the normal-appearing white matter. Multiple linear regression models, adjusted for macrostructural MRI-markers and cardiovascular risk factors were used to model the association of kidney function with white matter microstructure. Participants had average eGFRcr of 86.1 mL/min/1.73 m^2, average eGFRcys of 86.2 mL/min/1.73 m^2, and median albumin-to-creatinine ratio of 3.4 mg/g. Lower eGFRcys was associated with worse global white matter microstructural integrity, reflected as lower fractional anisotropy (FA) (standardized difference per SD: -0.053, 95%CI: -0.092, -0.014) and higher mean diffusivity (MD) (0.036, 95%CI: 0.001, 0.070). Similarly, higher albumin-to-creatinine ratio was associated with lower FA (-0.044, 95%CI: -0.078, -0.011). There was no linear association between eGFRcr and white matter integrity. Subgroup analyses showed attenuation of the associations after excluding subjects with hypertension. The associations with global DTI-measures didn't seem to be driven by particular tracts, but rather spread across multiple tracts in various brain regions. Reduced kidney function is associated with worse white matter microstructural integrity. Our findings highlight the importance for clinicians to consider concomitant macro- and microstructural changes of brain in subjects with impaired kidney function.

Background

The brain and the kidney are both vulnerable to vascular and hemodynamic alterations due to similar high flow and low resistance circulation.[1] Therefore, vascular damage in the kidney could mirror cerebrovascular changes in the brain.[1] Accordingly, a higher prevalence of cerebrovascular diseases such as stroke and vascular dementia among patients with chronic kidney disease (CKD) has been reported.[2, 3] Beyond clinically evident cerebrovascular diseases, previous studies showed an association between kidney function and subclinical cerebrovascular diseases including brain atrophy and white matter lesions.[2, 4, 5] However, subclinical cerebrovascular diseases have a wide spectrum and conventional MRI sequences are not capable of capturing this entire spectrum. Diffusion tensor imaging (DTI) is an advanced MRI technique that provides quantitative information of microscopic changes of the cerebral white matter. Recognition of early changes in white matter structural integrity is of importance as it might help to prevent further progression of brain pathologies before reaching an irreversible stage.[6] Despite the current evidence indicating that advanced impairments in kidney function are associated with brain pathologies,[2, 3] it is unknown whether changes in kidney function and glomerular integrity are linked to more subtle, microstructural changes in the brain. In this study, we hypothesized that loss of kidney function is associated with microstructural changes of the white matter.

Methods

Population

The present study is embedded within the second extension of the population-based Rotterdam Study (2005-2009), including participants of 45 years and older. For the current study, we included 2825 individuals with DTI data, of whom 2680 had urine albumin and urine creatinine measurements, 2717 had serum creatinine measurements,

and 2726 had available data on serum cystatin C measurements (Figure S1).

Standard Protocol Approvals, Registrations, and Patient Consents

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.[7]

Kidney function

Estimated glomerular filtration rate (eGFR) was calculated for creatinine (eGFRcr) and cystatin C (eGFRcys) measurements separately as well as for both measurements combined (eGFRcrcys), according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[8] Albumin-to-creatinine ratio was estimated by dividing albumin by creatinine (mg/g).[9] Since measures of albumin-to-creatinine ratio were not normally distributed, albumin-to-creatinine ratio values were natural log transformed (further details about kidney function measurements in supplemental material). We defined three categories of kidney function using information from both eGFR and albumin-to-creatinine ratio. This categorization is based on the cutoffs of the Kidney Disease: Improving Global Outcomes (KDIGO) 2013,[10] and has been applied in the research setting before.[11] Categories were defined on the basis of two criteria: eGFRcrcys > 60 mL/min/1.73 m^2 and albumin-to-creatinine ratio < 30 mg/g. First category included participants that met both criteria. Second category included participants that met only one criterion. Participants that met none of the criteria were classified as the third category. [11]

Brain DTI-MRI

Brain MRI scanning was performed on a 1.5 tesla MRI scanner (GE Signa Excite). Scan

protocol and sequence details are described extensively elsewhere.[12] For DTI, we performed a single shot, diffusion-weighted spin echo echo-planar imaging sequence. Maximum b-value was 1000 s/mm^2 in 25 non-collinear directions; three volumes were acquired without diffusion weighting (b-value = 0 s/mm^2).[12] All diffusion data were pre-processed using a standardized pipeline, including correction for motion and eddy currents, estimation of the diffusion tensor, and registration to tissue segmentation to obtain global mean DTI-measures in the normal-appearing white matter. These measures includes fractional anisotropy (FA), mean diffusivity (MD), and axial and radial diffusivities.[13] In general, lower values of FA and higher values of MD are indicative of worse microstructural integrity of the white matter.

Next, white matter tracts were segmented using a diffusion tractography approach described previously.[14] We identified 14 different white matter tracts (11 tracts were defined for left and right hemispheres) in subject native space. Tracts were categorized into brainstem tracts, projection fibers association fibers, limbic system fibers and callosal fibers.[15] Tract-specific measurements of microstructure were obtained by taking median measures inside each white matter tract, with subsequent combination of left and right measures.[14] DTI values, both global and tract-specific, were measured using fully automated methods (no readers involved). Since these measures are not observer dependent, no observer bias was introduced. However, there might be some random measurement noise in the scan protocol. The average tract-specific reproducibility of our multi-step method was 87%. More details about the reproducibility of tract-specific DTI-parameters are provided elsewhere.[14]

Cardiovascular risk factors

Information related to smoking and alcohol consumption was acquired using questionnaires. Alcohol consumers were categorized into non, moderate and heavy drinkers. Smoking was categorized in never, former and current smoking. Information

on medication use was based on home interviews. Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥ 90 mmHg or the use of antihypertensive medication. Cardiovascular disease was considered as a history of myocardial infarction, stroke or coronary revascularization procedures.[16] Diabetes mellitus was defined by use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l.

Statistical analysis

Associations between kidney function markers and DTI-parameters were evaluated using multiple linear regression models. Subject-specific global and tract-specific DTI-parameters were standardized to z scores. Betas and 95% confidence intervals (CI) for difference in DTI parameters were estimated per standard deviation increase of measures of the kidney function. We performed the analyses in four steps. The first model was performed unadjusted. In the second model analyses were adjusted for age, sex, and macrostructural MRI-markers including white matter volume, intracranial volume, and WML (also known as white matter hyperintensities) volume. In the third model we additionally adjusted the analyses for cardiovascular risk factors (systolic blood pressure, diastolic blood pressure, alcohol intake, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, history of cardiovascular disease, and body mass index) and antihypertensive and lipid-lowering medication. In the fourth model, analyses with eGFR as determinants were adjusted for albumin-to-creatinine ratio, and analyses with albumin-to-creatinine ratio as determinant were further adjusted for eGFRcrcys.

Based on previous literature[17] suggesting a U-shaped association between serum creatinine and brain outcomes, we further checked the non-linear association of eGFRcr with DTI-parameters of white matter integrity by including the quadratic term in the model. We performed an analysis of covariance where mean values of

FA and MD were compared across three categories of kidney function. Moreover, we performed a series of sensitivity analyses, excluding subjects with chronic kidney disease (defined as eGFRcrcys < 60 mL/min/1.73 m^2), diabetes mellitus, hypertension, and with a history of cardiovascular disease. To investigate whether the association between kidney function and white matter integrity differs in participants with and without hypertension, we assessed the interactions between kidney function markers and hypertension in relation to DTI-parameters. In exploratory analyses, we evaluated if the associations between kidney function and white matter integrity is independent of C-reactive protein levels. Furthermore, to compare the magnitude of the association with age, as an established risk factor for impairments in white matter integrity, we calculated the effect estimates for the association of age with FA and MD. Then, we divided the betas (per standard deviation) of kidney function markers by the betas of age in relation to DTI-parameters and reported the corresponding ratios.

In all analyses, we treated the phase encoding direction of the diffusion scan as a potential confounder. In tract specific analyses of the medial lemniscus, we additionally corrected for its varying coverage inside the field of view of the diffusion acquisition across participants. All analyses were carried out using SPSS 20.0.2 for windows or R version 2.15.0.

Results

Table 1 presents the characteristics of the 2726 study participants. Average age of the participants was 56 ±6.4 years and 45 % were male. Table 2 presents baseline characteristics of participants in different categories based on participants' kidney function. The association between kidney function markers and global DTI-parameters of white matter microstructural integrity are presented in Table 3. In the unadjusted model, higher albumin-to-creatinine ratio was associated with lower FA and higher MD (standardized difference FA: -0.102, 95% confidence interval (CI): -0.139, -0.066;

Table 1. Population characteristics

Characteristics	n= 2726
Age, years	56.6 (6.4)
Men	1225 (44.9)
Systolic blood pressure, mmHg	131.9 (18.5)
Diastolic blood pressure, mmHg	82.3 (10.8)
Antihypertensive medication	595 (21.8)
Alcohol	
Moderate drinker	1640 (60.4)
Heavy drinker	806 (29.6)
Smoking	
Current	715 (26.2)
Former	1190 (43.7)
Total cholesterol, mmol/l	5.6 (1.0)
HDL cholesterol, mmol/l	1.4 (0.4)
Lipid-lowering medication	570 (20.9)
Diabetes mellitus	214 (7.9)
History of cardiovascular disease	128 (4.7)
Hypertension	1191 (43.7)
Body mass index, kg/m^2	27.5 (4.3)
Albumin-to-creatinine ratio, mg/g	3.4 (2.2, 6.2)
eGFRcr, mL/min/1.73 m^2	86.1 (13.5)
eGFRcys, mL/min/1.73 m^2	86.2 (16.0)
eGFRcrcys, mL/min/1.73 m^2	86.3 (13.4)
Intracranial volume, mL	1128.6 (122.0)
White matter volume, mL	416.8 (59.9)
White matter lesion volume, mL	2.0 (1.3, 3.5)
Fractional anisotropy	0.33 (0.01)
Mean diffusivity, 10^{-3} mm^2/s	0.74 (0.02)
Axial diffusivity, 10^{-3} mm^2/s	1.01 (0.03)
Radial diffusivity, 10^{-3} mm^2/s	0.60 (0.02)

The following variables had missing data: blood pressure (n=9), smoking (n=5), alcohol (n=12), lipid-lowering medication (n= 24), antihypertensive medication (n=24), HDL: high density lipoprotein cholesterol (n=7), Total cholesterol (n= 5), albumin-to-creatinine ratio (n=141), history of cardiovascular disease (n=28), body mass index (n=2), hypertension (n=24)
Abbreviations: eGFRcys: cystatin C based estimated glomerular filtration rate, eGFRcr: creatinine based estimated glomerular filtration rate, eGFRcrcys: creatinine and cystatin C based estimated glomerular filtration rate

Table 2. Population characteristics in categories of kidney function

Characteristics*	First category	Second category	Third category
	eGFR>60 and ACR <30 N=2320	eGFR<60 or ACR >30 N=179	eGFR<60 and ACR >30 N=14
Age, years	56.4 (5.9)	60.5 (9.6)	65.4 (9.2)
Men	1040 (44.8)	76 (42.5)	7 (50.0)
SBP, mmHg	131.4 (18.1)	139.6 (21.1)	145.5 (23.5)
DBP, mmHg	82.1 (10.7)	85.1 (12.0)	85.1 (11.2)
Anti-HTN Med	476 (20.5)	68 (38.0)	11 (78.6)
Alcohol			
Moderate drinker	1393 (60.0)	111 (62.0)	6 (42.9)
Heavy drinker	694 (29.0)	45 (25.1)	4 (28.6)
Smoking			
Current	590 (25.4)	49 (27.4)	4 (28.6)
Former	1014 (43.7)	80 (44.7)	7 (50.0)
Total chol, mmol/l	5.6 (1.0)	5.4 (1.1)	5.8 (1.3)
HDL chol, mmol/l	1.4 (0.4)	1.3 (0.4)	1.4 (0.5)
Lipid lowering Med	469 (20.2)	53 (29.6)	6 (42.9)
DM	155 (6.7)	34 (19.0)	2 (14.3)
CVD	99 (4.3)	19 (10.6)	2 (14.3)
HTN	982 (42.3)	116 (64.8)	14 (100)
BMI, kg/m^2	27.4 (4.2)	29.0 (5.1)	28.4 (4.6)
ACR, mg/g	3.3 (2.1, 5.5)	39.0 (8.4, 74.4)	130.1 (73.4, 229.1)
eGFRcr, mL/min/1.73 m^2	87.0 (11.9)	77.3 (18.9)	44.8 (16.7)
eGFRcys,mL/min/1.73 m^2	87.4 (14.3)	74.2 (21.4)	38.1 (13.3)
eGFRcrcys, mL/min/1.73 m^2	87.4 (11.7)	75.6 (20.0)	40.2 (13.9)
ICV, mL	1127.6 (121.1)	1125.3 (136.9)	1103.7 (133.5)
WMV, mL	417.3 (58.7)	408.7 (68.5)	385.9 (93.1)
WMLV, mL	2.0 (1.3, 3.4)	2.9 (1.6, 5.5)	8.9 (3.1, 13.0)
FA	0.33 (0.01)	0.33(0.01)	0.32 (0.02)
MD, 10^{-3} mm^2/s	0.74 (0.02)	0.75 (0.03)	0.76 (0.03)
Axial diffusivity, 10^{-3} mm^2/s	1.01 (0.03)	1.02 (0.03)	1.03 (0.03)
Radial diffusivity, 10^{-3} mm^2/s	0.60 (0.02)	0.61 (0.03)	0.63 (0.04)

*Sample size in this table is based on participants with available data for both eGFRcrcys and ACR (n=2513).
Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and white matter lesions and albumin-to-creatinine ratio are presented as medians (interquartile ranges).

standardized difference MD: 0.096, 95%CI: 0.061, 0.132). Higher albumin-to-creatinine ratio was also associated with higher radial diffusivity (0.063, 95%CI: 0.027, 0.100) and higher axial diffusivity (0.103, 95%CI: 0.067, 0.139). Adjustments for age, sex, and macrostructural MRI-markers (white matter volume, intracranial volume, and WML volume) attenuated the association of albumin-to-creatinine ratio with FA, MD, and radial diffusivity (Table 3).There was no association between albumin-to-creatinine ratio and axial diffusivity in the second model. After further adjustments for cardiovascular risk factors, in the third model, associations of albumin-to-creatinine ratio with FA and radial diffusivity did not change, but the association with MD became non-significant (p: 0.09). Adjustment for eGFRcrcys in the fourth model didn't change the results (Table 3).

In the unadjusted model, each standard deviation higher eGFRcys was associated with higher FA and lower MD (FA: 0.204, 95% CI: 0.168, 0.240; MD: -0.248, 95%CI: -0.283, -0.213). Likewise, each standard deviation higher eGFRcys was associated with lower radial diffusivity (-0.268, 95%CI: -0.303, -0.233) and lower axial diffusivity (-0.157, 95%CI: -0.194, -0.120). The association of eGFRcys with FA, MD, and radial diffusivity attenuated after adjustment for age, sex, and macrostructural MRI-markers. There was no association between eGFRcys and axial diffusivity in the second model. Further adjustments for cardiovascular risk factors, in the third model, did not change the associations of eGFRcys with DTI-parameters of white matter integrity. After adjustment for albumin-to-creatinine ratio in the fourth model, the association of eGFRcys with FA and MD attenuated and became non-significant (p: 0.061 and 0.068, respectively) (Table 3).

Each standard deviation higher eGFRcr was associated with higher FA, lower MD, and lower radial and axial diffusivity (all $p < 0.05$). There was no linear association between eGFRcr and DTI-parameters of white matter integrity after adjustment for age, sex, and macrostructural MRI-markers (all $p>0.05$) (Table 3). Including the quadratic

term of eGFRcr in the model suggested a U-shaped association between eGFRcr and markers of white matter integrity (all P-values <0.05 for test of quadratic term) (Table S1). After excluding the participants with 4 standard deviation lower/higher eGFRcr (n=9) the U-shaped association was not present anymore. Performing the analyses with eGFRcrcys as the determinant, the effect estimates were intermediate between two separate equations of eGFRcr and eGFRcys and resulted in borderline significant findings (Table S2).

Figure 1 shows age and sex adjusted means of FA and MD in three categories of kidney function. We observed a linear trend between different categories of kidney function and white matter microstructural DTI-parameters, reflecting worse white matter microstructure in persons with worse kidney function (p for trend 0.001 for FA and 0.002 for MD). Excluding individuals with chronic kidney disease, diabetes mellitus, or history of cardiovascular disease yielded similar findings. Excluding individuals with hypertension attenuated the association of kidney function with DTI parameters (Figure S2). In the stratified analyses, the association between eGFRcys and MD was stronger in hypertensive participants (P for interaction, 0.001). A similar, borderline significant trend was observed for the interaction between albumin-to-creatinine ratio and hypertension in relation to FA (P for interaction 0.059) (Table S3). Adjusting the association between markers of kidney function and DTI-parameters of white matter integrity for C-reactive protein didn't substantially change the associations (data not shown).

In an extra analysis, we observed that each year increase in age was associated with lower FA and higher MD (Table S4). Comparing the effect estimates of age with eGFRcys in relation to FA, we showed that each standard deviation lower eGFRcys had the magnitude equal to 4.1 years of increase in age. Similarly, each standard deviation lower eGFRcys in relation to MD had the magnitude equal to 1.5 years increase in age. Associations between kidney function and tract-specific diffusion measures are

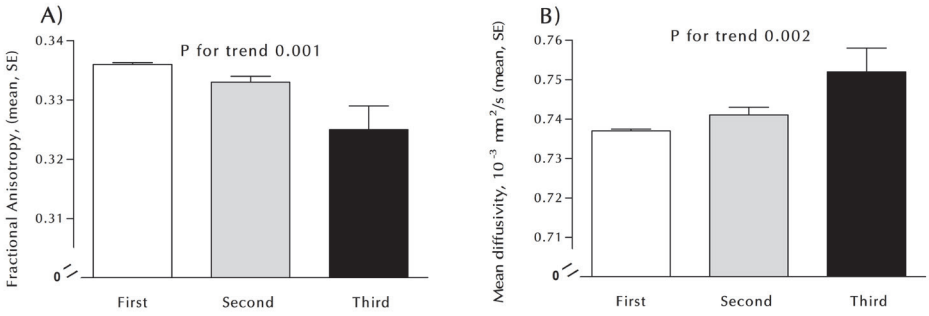

Figure 1. Means of A) fractional anisotropy B) and mean diffusivity in different categories of kidney function

First category: indicates eGFRcrcys > 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio < 30 mg/g (N=2320)
Second category: indicates eGFRcrcys > 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio > 30 mg/g OR eGFRcrcys < 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio < 30 mg/g (N=179)
Third category: indicates eGFRcrcys < 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio > 30 mg/g (N= 14)
Mean values are adjusted for age and sex.

presented in Figure 2 and Table S5. Higher albumin-to-creatinine ratio was associated with higher MD in all tracts (projection fibers, association fibers, limbic system fibers, and callosal fibers). Likewise, higher eGFRcys was related to MD in all tracts, except for brain stem tracts. We did not observe any linear association between eGFRcr and tract-specific DTI-measures.

Discussion

In this cross-sectional study, we demonstrated that reduced kidney function was independently associated with worse microstructural integrity of cerebral white matter. The associations did not seem to be confined to specific white matter tracts.

Most of previous studies that investigated the association of kidney function with brain outcomes have focused either on cerebrovascular accidents, conventional macrostructural MRI-markers such as brain atrophy, or manifestations of cerebral small

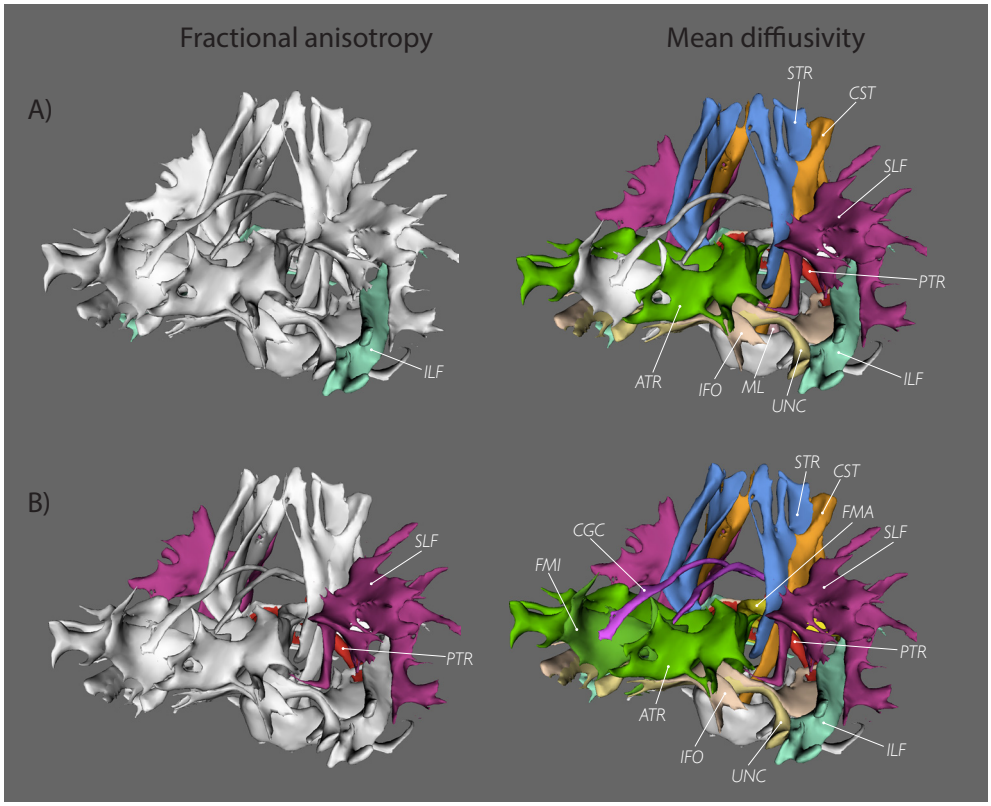

Figure 2. Association of A) ACR and B) eGFRcys with FA and MD in specific tracts.
Adjusted for age, sex, white matter volume, intracranial volume, and log transformed white matter lesion volume.
Tracts with significant associations (after Bonferroni correction: $p<3.6\times10^{-3}$) are labeled and colored.

vessel disease.[2, 4, 5] In this study we show that reduced kidney function is related to loss of white matter microstructural integrity, which may be considered a more sensitive or subtle measure of white matter disease compared to macrostructural MRI-markers. The associations persisted after adjusting for history of stroke and conventional MRI-markers, indicating that decrease in kidney function might reflect early brain microstructural changes independent of co-existing comorbidities and pathologies.

A linear association between eGFRcys and white matter microstructure was present; however, we observed a U-shaped association between eGFRcr and DTI-parameters

of white matter integrity. This discrepancy could be explained by the fact that subjects with very low creatinine are also at increased risk because of malnutrition and loss of muscle mass.[18] In line with this explanation, Seliger et al. reported a linear association between higher levels of serum cystatin C and prevalence of subclinical brain infarcts.[17] In contrast, they found that the prevalence of subclinical brain infarcts was higher in both subjects with low and high serum creatinine suggesting a U-shaped association.[17] Levels of serum cystatin C are reported to be independent of muscle mass; nevertheless, some cardiovascular risk factors such as obesity and diabetes can be non-GFR determinants of serum cystatin C levels.[19] In addition, previous reports proposed that underlying inflammation can influence levels of cystatin C.[19] Since inflammation itself is a vascular risk factor, cystatin C may reflect inflammatory status independent of eGFR. However, in this study adjusting for cardiovascular risk factors as well as an inflammatory marker (C-reactive protein) did not change the associations. Several explanations can be proposed for the association of kidney function with degeneration of white matter. First, kidney and the brain share several cardiovascular risk factors. Therefore, existence of these risk factors can simultaneously damage the vasculature of both kidney and brain.[1] While in this study adjusting for conventional cardiovascular risk factors such as blood pressure and excluding participants with diabetes, and cardiovascular disease did not change our findings, excluding participants with hypertension attenuated the associations. This might indicate that history of hypertension plays a role as an effect modifier and not as a confounder in the association between kidney function and white matter integrity. Future studies are needed to explore whether subjects with hypertension are more vulnerable to detrimental influences of impaired kidney function on white matter microstructural integrity. Second, it has been suggested that vascular damage is a diffuse phenomenon.[20] Given that the brain and kidney have similar circulation systems, and endothelial cells play a crucial role in both blood brain barrier and glomerular integrity,[1] it is possible that this association can originate from

Table 3. The association of kidney function parameters with DTI-parameters of white matter microstructural integrity

	Fractional anisotropy	Mean diffusivity	Axial diffusivity	Radial diffusivity
	Difference*(95% CI)	Difference*(95% CI)	Difference*(95% CI)	Difference*(95% CI)
ACR				
N= 2680				
Model I	-0.102 (-0.139, -0.066)	0.096 (0.061, 0.132)	0.063 (0.027, 0.100)	0.103 (0.067, 0.139)
Model II	-0.049 (-0.081, -0.016)	0.033(0.004, 0.062)	0.018 (-0.015, 0.050)	0.037 (0.008, 0.066)
Model III	-0.044 (-0.078, -0.011)	0.026(-0.004, 0.056)	0.010 (-0.024, 0.044)	0.031 (0.001, 0.061)
Model IV	-0.043 (-0.078, -0.008)	0.027 (-0.004, 0.058)	0.013 (-0.022, 0.049)	0.031 (0.001, 0.062)
eGFRcys				
N=2726				
Model I	0.204 (0.168, 0.240)	-0.248 (-0.283, -0.213)	-0.157 (-0.194, -0.120)	-0.268 (-0.303, -0.233)
Model II	0.053(0.016, 0.090)	-0.040 (-0.073, -0.007)	-0.001 (-0.038, 0.036)	-0.057 (-0.091, -0.024)
Model III	0.053(0.014, 0.092)	-0.036 (-0.070, -0.001)	0.006 (-0.033, 0.045)	-0.056 (-0.090, -0.021)
Model IV	0.039 (-0.002, 0.079)	-0.033 (-0.069, 0.003)	0.003 (-0.038, 0.044)	-0.050 (-0.087, -0.014)
eGFRcr				
N= 2717				
Model I	0.100 (0.063, 0.136)	-0.136 (-0.171, -0.100)	-0.092 (-0.129, -0.055)	-0.143 (-0.179, -0.107)
Model II	-0.007 (-0.041, 0.027)	0.011 (-0.020, 0.041)	0.015 (-0.020, 0.049)	0.007 (-0.024, 0.038)
Model III	0.002 (-0.033, 0.036)	0.003 (-0.028, 0.034)	0.008 (-0.027, 0.043)	-0.001 (-0.032, 0.031)
Model IV	-0.013 (-0.049, 0.023)	0.009 (-0.023, 0.041)	0.008 (-0.029, 0.044)	0.009 (-0.024, 0.041)

Model I: Crude model.
Model II: Adjusted for age, sex, intracranial volume, white matter volume, and log transformed white matter lesion volume.
Model III: Additionally adjusted for systolic blood pressure, diastolic blood pressure, antihypertensive medication, alcohol intake, smoking, total cholesterol, high density lipoprotein cholesterol, lipid-lowering medication, diabetes mellitus, history of cardiovascular disease, and body mass index.
Model IV: Additionally adjusted for eGFRcrcys in analyses with ACR as determinant, and additionally adjusted for ACR in analyses with eGFRcr or eGFRcys.

a systemic process affecting vascular beds in both organs.[20] Third, it is possible that impaired kidney function can lead to brain vascular injury via increase in pro-inflammatory factors and decrease in serum nitric oxide.[5] Nitric oxide regulates the function of the cerebral microcirculation and the blood brain barrier, which can contribute to cerebral hypoperfusion and subsequently white matter damage.[1]

In the tract-specific analyses, MD seemed to be a more sensitive marker for loss of white matter microstructural integrity in relation to kidney function. This might be explained by the prevailing theory that less uniform changes in FA are seen in deterioration of crossing fibers regions,[21] in contrast to MD that may show more consistent changes in areas of white matter loss. This is further supported by the considerable estimates for the prevalence of crossing fiber regions in the brain.[21]

The integrity of white matter is of great importance in normal functioning of the brain. Complex cognitive tasks are coordinated by interactions between different regions of the brain and intact white matter integrity plays an essential role in these interactions.[14, 22] In line with this notion, we previously showed that early changes in the white matter microstructure, as reflected in DTI-measures of the white matter, are associated with impaired cognitive function.[22] These associations were independent of macrostructural brain abnormalities such as white matter lesions. Our findings might suggest that impairments in white matter integrity can be an underlying mechanism behind the association between impaired kidney function and cognitive dysfunction.

Strengths of our study include the large sample size and availability of extensive data on various cardiovascular risk factors, and macrostructural MRI-markers that enabled us to control for several potential confounders. Nevertheless, a number of limitations should be acknowledged. First, due to the cross-sectional design of the study, we cannot draw conclusions about the directionality and causality. Second, the cerebellum was not being fully incorporated in the field of view of the diffusion scan, making conclusion on brain stem tracts less reliable. In addition, our findings are based on a population-

based study including relatively young and healthy individuals. Prevalence of kidney disease in this population is low and hence the associations are based on minor degrees of kidney dysfunction. Therefore, it is possible that the associations would be more prominent in older populations and in patients with kidney disease. In this study we did not have enough power to perform the analyses in participants with eGFR<60 mL/min/1.73 m^2.Further patient-based studies are needed to investigate this link in high risk individuals with lower eGFR levels.

In this study we showed that kidney function is related to impaired white matter microstructural integrity, suggesting that both kidney biomarkers and microstructural changes of white matter are biologically related. Our findings suggest that clinicians need to consider concomitant macro- and microstructural changes in the brain among subjects with impaired kidney function.

Online supplement
http://www.neurology.org/content/suppl/2015/06/17/WNL.0000000000001741.DC1/Supplemental_Material.pdf

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4.2 Kidney function and cerebral small vessel disease in the general population

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Anatomic and hemodynamic similarities between renal and cerebral vessels suggest a tight link between kidney disease and brain disease. Although several distinct markers are used to identify subclinical kidney and brain disease, a comprehensive assessment of how these markers link damage at both end-organs is lacking. In 2,526 participants of the population-based Rotterdam Study, we measured urinary albumin-to-creatinine ratio, and estimated glomerular filtration rate (eGFR) based on serum creatinine and cystatin C. All participants underwent brain MRI. We assessed presence of cerebral small vessel disease by calculating white matter lesion (WML) volumes and rating the presence of lacunes and cerebral microbleeds. We used multivariable linear and logistic regression to investigate the association between kidney function and cerebral small vessel disease. Worse kidney function was consistently associated with a larger WML volume (mean difference per SD increase in albumin-to-creatinine ratio: 0.09, 95% CI 0.05; 0.12; per SD decrease in creatinine-based eGFR: -0.04, 95% CI -0.08;-0.01, and per SD decrease in cystatin C-based eGFR: -0.09, 95% CI -0.13;-0.05). Persons with higher albumin-to-creatinine ratio or lower cystatin C-based eGFR levels had a higher prevalence of lacunes (odds ratio per SD increase in albumin-to-creatinine ratio: 1.24, 95% CI 1.07;1.43). Only participants in the highest quartile of albumin-to-creatinine ratio had a higher frequency of microbleeds compared to the lowest quartile. Worse kidney function is associated with cerebral small vessel disease. Of all measures of kidney function, in particular albumin-to-creatinine ratio is related to cerebral small vessel disease.

Background

Small blood vessels in the kidney and the brain are closely linked because of anatomical and hemodynamic similarities.[1,2] These resemblances highlight the likelihood of a shared pathogenesis of renal and cerebrovascular disease. Since small vessel disease is considered a systemic disorder, pathology in one end-organ may provide information on coexistent or future damage in another end-organ. Indeed, worse kidney function has been related to cerebral small vessel disease, [3-6] which may appear as white matter lesions (WML), lacunes, and cerebral microbleeds on magnetic resonance imaging (MRI).

While the association between kidney function and cerebral small vessel disease has been studied before, there have been inconsistent reports. The inconsistencies can partly be explained by differences in population characteristics, morbidities, and ethnicities. Also, studies have typically correlated only a single marker of kidney dysfunction to markers of cerebral small vessel disease. Kidney function measures (i.e., albuminuria, estimated glomerular filtration rate (eGFR) based on creatinine or cystatin C) reflect damage to glomerular vessels of numerous kind, and may associate differently with the ischemic and hemorrhagic subtypes of cerebral small vessel disease. Thus far, no study has examined how the various serum and urinary markers of kidney function associate with the entire spectrum of subclinical cerebral small vessel lesions within one single population. Such a study would add to current literature because it allows for direct comparison of kidney markers without being limited by heterogeneity of study populations.

In a middle-aged and elderly population, we therefore investigated whether measures of kidney function – i.e., urinary albumin-to-creatinine ratio and eGFR based on serum creatinine or cystatin C - were associated with cerebral small vessel disease on MRI.

Methods

Study population

The Rotterdam study is an ongoing prospective population-based cohort designed to investigate chronic diseases in the middle-aged and elderly population.[7] The cohort originated in 1990 and comprised 7,983 participants aged 55 years and older. In 2000 and 2006 the cohort was expanded and now counts 14,926 participants aged ≥45 years.[7] Brain MRI was implemented from 2005 onwards,[8] and a subset of the cohort had urine and serum samples collected around the time of MRI. Urine and blood samples were collected in 3,181 participants, and all three kidney function measures were obtained in 2,596 participants. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants. Participants with eGFR <15 or > 200 mL/min/1.73m^2 were excluded from all analyses (N=3). Additionally, participants who showed cortical infarcts on MRI (N=67) were excluded because we solely aimed to study cerebral small vessel pathology and because gliosis around cortical infarcts may cause automated WML segmentation to become unreliable. This resulted in 2,526 participants who were included in our analyses. In the analyses involving WML, we additionally excluded 34 participants because the automated WML volume tissue segmentation was rendered unreliable.

Kidney function measures

For the assessment of albuminuria, participants were asked to collect (overnight) timed urine samples. Urinary albumin and creatinine were determined by a turbidimetric method and measured by a Hitachi MODULAR P analyzer (Roche/Hitachi Diagnostics, Mannheim, Germany).[9] Albumin-to-creatinine ratio was calculated by dividing albumin

(grams) by creatinine (mol). Because albumin-to-creatinine ratio was not normally distributed we used natural log-transformed values and added 1 gram/mol to the non-transformed values to account for zero values of albuminuria (Ln[albumin-to-creatinine ratio +1.0 gram/mol]). Although serum creatinine is generally used to estimate GFR, cystatin C has been proposed to be a more stable marker of kidney function in an elderly population.[10] We therefore calculated both measures in our study. Serum creatinine was measured using an enzymatic assay method.[11] Creatinine was calibrated by aligning mean values of serum creatinine from our cohort with those of the Third National Health and Nutrition Examination Survey (NHANES III) for men and women separately in age categories of <50, 50-59, 60-69, ≥70. Cystatin C was measured with a particle-enhanced immunonephelometric assay using a Roche/Hitachi cobas c 501 analyzer which calculates the analyte concentration of each sample automatically in mg/L.[12] Estimated GFR was calculated based on either serum creatinine or serum cystatin C using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula.[13] In accordance, chronic kidney disease (CKD) was defined as eGFR <60 mL/min/1.73 m^2.

Brain MRI and markers of cerebral small vessel disease

Participants were scanned on a 1.5-Tesla MRI scanner.[8] We performed a T1-weighted, proton-density weighted, fluid-attenuated inversion recovery (FLAIR), and T2*-weighted gradient-recalled echo sequence (3D T2* GRE), as described in detail before.[8] Automated tissue segmentation, including conventional k-nearest-neighbor brain tissue classifier extended with WML segmentation[14] was used to segment brains into grey matter volume, white matter volume, WML volume, and cerebrospinal fluid. All scans were rated by 1 of 5 trained research-physicians to determine presence and location of infarcts (lacunes and cortical infarcts) and microbleeds. Lacunes were rated on FLAIR, proton-density-weighted and T1-weighted sequences, and were defined as focal lesions ≥3mm and <15mm in size, with the same signal intensity as cerebrospinal fluid on

all sequences and a hyperintense rim on the FLAIR (when located supratentorially).[15] Infarcts showing involvement of cortical grey matter were classified as cortical infarcts. Microbleeds were visually rated as small, focal, round to ovoid areas of signal loss on 3D T2* GRE images.[15]

Assessment of cardiovascular risk factors

Cardiovascular risk factors were assessed by interview, laboratory and physical examinations during the same visits in which kidney function measures were examined. Body mass index (BMI) was calculated by dividing the weight (in kilograms) by the height squared (in meters). Systolic and diastolic blood pressures were measured twice with a random-zero sphygmomanometer in sitting position at the right arm. Total and high-density lipoprotein (HDL) cholesterol (mmol/L) was determined using an automated enzymatic procedure (Hitachi analyser, Roche Diagnostics). Diabetes mellitus was defined as fasting blood glucose of ≥7.0mmol/l, and/or the use of any glucose lowering medication. During home interviews, participants were asked about their smoking status (ever versus never smoking), and whether they used antihypertensive and/or lipid-lowering medication.

Statistical analysis

Urinary albumin-to-creatinine ratio (g/mol) was modelled continuously per standard deviation (SD) increase. For serum creatinine (mg/dL) and serum cystatin C (mg/L) we calculated the eGFR (mL/min/1.73m^2), which was also modelled continuously per SD increase. Additionally, participants were dichotomized as having CKD yes or no (eGFR<60 mL/min/1.73m^2 used as cut off for CKD).[16] WML volume was natural log transformed because of its skewed distribution and investigated continuously. Lacunes and microbleeds were investigated dichotomously (present versus absent). In accordance with their presumed etiological background, cerebral microbleeds were

categorized by location into strictly lobar versus deep or infratentorial (with or without the presence of lobar microbleeds).[15] We used multivariable linear regression to obtain mean differences in WML volumes for every SD increase in kidney function measures and for CKD presence. Logistic regression was used to calculate odds ratios to study the relation of kidney function with lacunes and microbleeds. All models were adjusted for age and sex (model I), and additionally for cardiovascular risk factors that were considered confounders (model II). As potential confounders we took into account: BMI, systolic and diastolic blood pressure, total and HDL cholesterol, diabetes mellitus, smoking, antihypertensive and lipid-lowering medication. All analyses involving WML volume were also adjusted for intracranial volume. In sensitivity analyses we also adjusted for C-reactive protein and a history of clinical cardiovascular disease (stroke or coronary heart disease) because these factors may confound the association between kidney function and cerebral small vessel disease. Additionally, we adjusted the kidney function measurements for each other to estimate the independent association of every kidney function marker on cerebral small vessel disease. Finally, we computed quartiles of kidney function measures and compared the highest to the lowest quartiles, and studied the linear trends of these measurements in relation to markers of cerebral small vessel disease.

All analyses were done using R version 2.15.0 and IBM SPSS Statistics for Windows version 21.0, using an alpha-value of 0.05.

Table 1. Characteristics of the study population

Characteristics	N=2,526
Age, years	56.9 (6.3)
Female	1406 (55.6)
Albumin-to-creatinine ratio in urine, g/mol	0.4 (0.2-0.7)
Serum creatinine, mg/dL	0.9 (0.2)
Creatinine-based eGFR, mL/min/1.73m^2	86.2 (12.9)
Serum cystatin C, mg/dL	0.9 (0.2)
Cystatin C-based eGFR, mL/min/1.73m^2	86.4 (15.6)
White matter lesion volume, mL	2.0 (1.3-3.5)
Lacunes	92 (3.6)
Microbleeds	318 (12.6)
Strictly lobar microbleeds	233 (9.2)
Deep or infratentorial microbleeds	85 (3.4)
Body mass index, kg/m^2	27.5 (4.3)
Systolic blood pressure, mmHg	132.2 (18.7)
Diastolic blood pressure, mmHg	82.4 (10.8)
Total cholesterol, mmol/L	5.9 (1.05)
High-density lipoprotein, mmol/L	1.4 (0.4)
Diabetes mellitus	182 (7.2)
Smoking	1752 (69.3)
Antihypertensive medication	636 (25.2)
Lipid-lowering medication	530 (21.0)

Data presented as mean (standard deviation) for continuous variables and number (percentages) for categorical variables. Albumin-to-creatinine ratio and white matter lesion volume presented as median (interquartile range).
The following variables had missing data: body mass index (N=2), blood pressures (N=6), high-density lipoprotein cholesterol (N=1), diabetes mellitus (N=20), smoking (N=5), antihypertensive and lipid-lowering medication (N=21).
White matter lesion volumes were measured in N= 2,492 participants (excluding 34 scans in which tissue segmentation measures could not be obtained reliably).

Results

Characteristics of the study population are presented in Table 1. Of the 2,526 participants, 55.6% were women and mean age was 56.9 years (SD 6.3). Median albumin-to-creatinine-ratio was 0.4 (interquartile range 0.2 to 0.7) g/mol. Mean estimated GFR was 86 mL/min/1.73m^2, measured either with serum creatinine or cystatin C. Based on

serum creatinine levels, there were 64 CKD cases (eGFR <60 mL/min/1.73m^2) versus 128 CKD cases based on serum cystatin C levels. Median WML volume was 2.0 mL. In total, 3.6% of the participants had one or more lacunes, and 12.6% had one or more microbleeds.

In Table 2, we show the association between kidney function and markers of cerebral small vessel disease. Participants with worse kidney function - i.e., higher urinary albumin-to-creatinine ratio, lower creatinine or lower cystatin C-based eGFR- had more WML volume (age and sex adjusted mean difference for WML volume per SD increase in albumin-to-creatinine ratio 0.09, 95% confidence interval [CI] 0.05;0.12). Participants with a higher urinary albumin-to-creatinine ratio and higher serum cystatin C-based eGFR also had a higher prevalence of lacunes (odds ratio for lacunes per SD increase in albumin-to-creatinine ratio 1.24, 95% CI 1.07;1.43). No association was found between continuous measures of kidney function and cerebral microbleeds. Similar associations were found when investigating the relation between CKD and imaging markers of cerebral small vessel disease (Table S1). Participants with CKD had larger volumes of WML and more lacunes compared to those without CKD. No association was found for microbleeds. Additional adjustments for potential cardiovascular confounders did not alter any of the above-mentioned results. Also, adjusting for C-reactive protein, a history of clinical cardiovascular disease or adjusting kidney function measurements for each other did not change the interpretation of our results (data not shown).

Figure 1 shows the association of kidney function measures in quartiles with WML volume, lacunes, and microbleeds (in any location). Participants in the highest quartile of albumin-to-creatinine ratio had more cerebral small vessel disease compared to those in the lowest quartiles (P-values for linear trend tests were <0.05 in relation to all markers of cerebral small vessel disease). Also, those in the lowest quartile of cystatin C-based eGFR had more WML volume and lacunes compared to those in the highest

quartile (P-values for linear trend test were <0.05 in relation to WML and lacunes).

Discussion

In this population-based study we investigated the association between various markers of kidney function and cerebral small vessel disease. Of all renal markers, albumin-to-creatinine ratio showed strongest associations with cerebral small vessel disease presence. Higher albumin-to-creatinine ratio, and lower eGFR based on creatinine and cystatin C were all associated with larger WML volumes. A higher albumin-to-creatinine ratio and decreased eGFR based on cystatin C were related to presence of lacunes, whereas only a higher albumin-to creatinine ratio was associated with presence of microbleeds.

Strengths of our study are the population-based character, with a large number of participants, and the extensive phenotyping within the Rotterdam Study that enabled us to investigate both urine and serum markers of kidney function in relation to several imaging markers of cerebral small vessel disease. We also acknowledge several limitations of our study. First, our study was performed cross-sectionally and we cannot draw conclusions regarding causality. Second, markers of kidney function were only measured once and this may have biased our results towards the null. Third, although we adjusted for important and evident potential cardiovascular risk factors in the association between kidney disease and cerebral small vessel disease we cannot rule out residual confounding because of unmeasured confounders. Several hypotheses have been proposed to explain the link between renal and cerebral small vessel disease. First, small vessels in kidney and brain are both exposed to high blood flow volumes during the entire cardiac cycle.[1] It is very likely that kidney disease and cerebral small vessel disease are both signs of systemic small vessel disease affecting different end-organs with anatomical and hemodynamic similarities. Shared risk factors, in particular high arterial blood pressure, may cause concurrent vascular damage to afferent arterioles in

Table 2. Kidney function and cerebral small vessel disease

	Mean difference (95% CI)		Odds ratios (95% CI)	
	White matter lesions	Lacunes	Strictly lobar microbleeds	Deep or infratentorial microbleeds
Model I				
Albumin-to-creatinine ratio	0.09 (0.05;0.12)	1.24 (1.07;1.43)	0.99 (0.87;1.14)	1.14 (0.96;1.35)
Creatinine-based eGFR	-0.04 (-0.08;-0.01)	1.08 (0.86;1.35)	1.06 (0.92;1.23)	0.98 (0.77;1.23)
Cystatin C-based eGFR	-0.09 (-0.13;-0.05)	0.70 (0.56;0.89)	0.94 (0.81;1.10)	0.82 (0.64;1.05)
Model II				
Albumin-to-creatinine ratio	0.07 (0.04;0.11)	1.19 (1.02;1.39)	0.96 (0.84;1.11)	1.11 (0.93;1.33)
Creatinine-based eGFR	-0.04 (-0.08;-0.004)	1.07 (0.85;1.34)	1.07 (0.92;1.25)	0.96 (0.77;1.21)
Cystatin C-based eGFR	-0.08 (-0.12;-0.04)	0.74 (0.58;0.95)	0.96 (0.82;1.13)	0.83 (0.65;1.07)

White matter lesions: values represent mean differences (95% CI) in white matter lesion volume per standard deviation increase in measures of kidney function.
Lacunes: values represent odds ratios (95% CI) for lacunes per standard deviation increase in measures of kidney function.
Microbleeds: values represent odds ratios (95% CI) for microbleeds per standard deviation increase in measures of kidney function.
Model I: adjusted for age and sex.
Model II: as Model I, additionally adjusted for body mass index, systolic and diastolic blood pressure, total and high-density lipoprotein cholesterol, diabetes mellitus, smoking, antihypertensive and lipid-lowering medication. Analyses involving white matter lesions were additionally adjusted for intracranial volume. Analyses in Model II were performed as a complete case analysis.
Participants with scans in which tissue segmentation measures could not be obtained reliably were excluded from the analyses of white matter lesion volume (N=34).

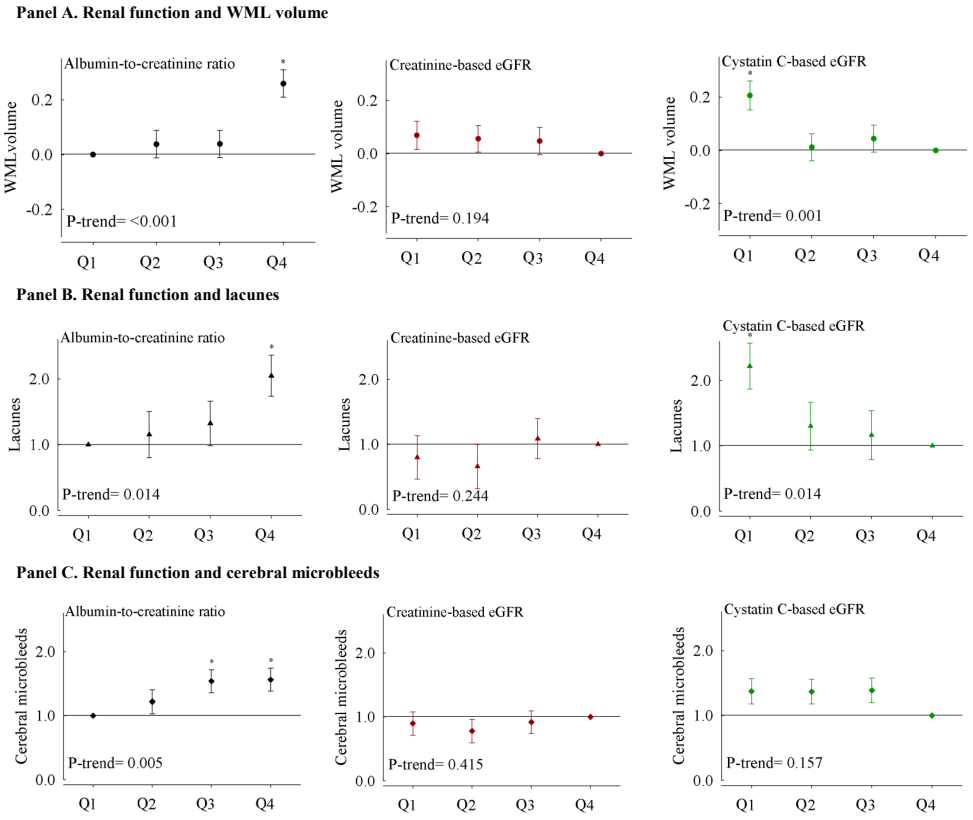

Figure 1. Quartiles of kidney function measures and markers of cerebral small vessel disease.
On the x-axis of all panels: quartiles of standardized kidney function measures. For urinary albumin-to-creatinine ratio the lowest quartile was used as reference category. For serum creatinine-based eGFR and serum cystatin C-based eGFR the highest quartile was used as reference category.
Panel A: circles represent the age, sex, and intracranial volume adjusted mean differences in WML volume. Panel B: triangles represent age and sex adjusted odds ratios for lacunes. Panel C: diamonds represent age and sex adjusted odds ratios for cerebral microbleeds.
Bars represent the standard error. * Represents a p-value<0.05 when a quartile was compared to the reference category. P-values for linear trends are presented per kidney function measure.
The range of albumin-to-creatinine ratio for each quartile was: Q1=0.00-0.28;Q2= 0.21-0.44; Q3=0.32-0.74; Q4=0.61-148.43, range of creatinine-based eGFR for each quartile was: Q1=17.11-82.93; †Q2=67.09-92.27; Q3=73.46-102.51; Q4=83.93-180.09, range of cystatin C-based eGFR for each quartile was: Q1=20.62-78.33; Q2=72.16-88.98; Q3=81.46-101.29; Q4=91.12-138.20.
†For lacunes and cerebral microbleeds, range of creatinine-based eGFR for Q2= 65.00-92.27.

the kidney and the brain as these vessels branch off large arteries that maintain high vessel tone to ensure perfusion of kidney and brain tissue.[2] Second, endothelial dysfunction, regardless of the cause, leads to leakage of proteins into interstitial space in both kidney and brain.[17] Various markers have been suggested to cause endothelial dysfunction, including nitric oxide. Kidney dysfunction is known to induce nitric oxide deficiency due to disturbances in L-arginine metabolism or an increase in endogenous nitric oxide synthase inhibitors,[18] and may cause problems in maintaining microcirculation and blood brain barrier function.[18] Third, inflammatory processes, including the direct or indirect effects of lipoprotein phospholipase A2, myeloperoxidase and/or C-reactive protein, have been shown to affect both renal and brain vessels, and are also known for their role in endothelial dysfunction.[19-22]

Among the kidney function measures we investigated, we found that albuminuria was strongest associated with small vessel disease in the brain. This association has been described in the past, both in the general population and in patients with hypertension or acute stroke.[4,17,23-26] Although the role of albuminuria in cerebrovascular disease is not well understood, endothelium dysfunction throughout the entire body is the most appealing explanation.[27,28] Since albuminuria associates with different phenotypes of cerebral small vessel disease - both ischemic and hemorrhagic - it may suggest that endothelial dysfunction acts as a stressor and activates pathways that induce both thrombi and small vessel fragility. However, one could also argue that WML, lacunes, and microbleeds reflect markers of a single pathologic continuum. Either way, microalbuminuria may serve as an important surrogate marker for the presence of cerebral small vessel disease.

We found that a worse kidney function measured with either creatinine or cystatin C was associated with WML. This is in agreement with previous studies in the general population.[3,29-31] Although some recent studies suggest otherwise,[32-34] cystatin C is generally thought to be a more stable measurement for kidney function in the elderly

than creatinine,[10] because cystatin C is hypothetically less affected by aging, loss of muscle mass and gender. We indeed found that, compared to creatinine, GFR based on cystatin C correlated better with the presence of lacunes. This confirms previous findings from the Cardiovascular Health Study.[6] In contrast to previous studies in stroke patients,[5,35-37] we did not find an association between creatinine or cystatin C and microbleeds. Potential explanations for these observations are first, a lack of statistical power in our study. Second, differences in ethnicity of study populations yield different cardiovascular risk. Third, during the acute phase of stroke, levels of creatinine and cystatin C may be affected and consequently this could have influenced the results of the studies performed in acute stroke patients. Fourth, the pathology underlying microbleeds is thought to differ according to their location in the brain.[15] The majority of microbleeds in our study were located in lobar regions and are thought to reflect cerebral amyloid angiopathy; an angiopathy typically known not to affect organs outside the brain.

In conclusion, we found that of all kidney function measures, a higher albumin-to-creatinine ratio was strongest associated with the presence of imaging markers of cerebral small vessel disease. Our study emphasizes that albumin-to-creatinine ratio is a particularly helpful marker to identify presence of or risk for subclinical cerebral small vessel disease in people with generalized small vessel disease.

Online supplement
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4.3 Kidney function and cerebral blood flow: The Rotterdam Study

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Chronic kidney disease is linked with various brain disorders. While the brain integrity is dependent on cerebral perfusion, the association between kidney function and cerebral blood flow is yet to be determined. The study was performed in the framework of the population-based Rotterdam Study including 2645 participants with mean age of 56.6 years (45% men). Estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio was used to assess kidney function. Phase-contrast magnetic resonance imaging of basilar and carotid arteries were performed to measure cerebral blood flow. Participants had average (standard deviation) eGFR of 86.3(13.4) mL/min/1.73 m^2, and median [Interquartile range] albumin-to-creatinine ratio of 3.4 [2.2-6.1] mg/g. In age and sex adjusted models, higher albumin-to-creatinine ratio was associated with lower levels of cerebral blood flow (Difference in cerebral blood flow (mL/min/100mL) per doubling of albumin-to-creatinine ratio -0.31; 95%CI: -0.58, -0.03). The association was not present after adjustment for cardiovascular risk factors (P = 0.10). Each standard deviation lower eGFR was associated with 0.42 mL/min/100mL lower cerebral blood flow (95%CI: 0.01, 0.83), adjusted for cardiovascular risk factors. In this population-based study, we observed that lower eGFR is independently associated with lower cerebral blood flow.

Background

Cerebrovascular diseases and dementia occur more often in patients with chronic kidney disease (CKD)[1]. Incident rates of stroke are 1.9 to 7.6 times higher in CKD patients compared to subjects without kidney disease, depending on age and population studied.[2] Likewise, individuals at all stages of CKD have higher risk of developing dementia than general population.[3] An increasing body of evidence suggests that the link between impairment in kidney function and cognitive impairment is mediated through vascular mechanisms.[4, 5] In line with this notion Seliger et al. showed that higher serum creatinine was related to vascular-type dementia rather than Alzheimer type dementia.[5] Intact kidney function is crucial for regulation of total blood volume and vascular tone.[6] Therefore, impairments in kidney function can lead to disturbances in regulation of blood flow in organs that are critically dependent on constant and adequate blood flow such as the brain.[2] Cerebral hypo-perfusion has been implicated in the development of vascular and neurodegenerative disorders of the brain.[7, 8] While cerebral circulation is of great importance in control of adequate brain perfusion, previous literature suggests that systemic factors also play a role in regulation of cerebral blood flow.[9] Different hemodynamic disturbances have been reported in patients with chronic kidney disease.[10, 11] Nevertheless, it is not clear whether impaired kidney function is associated with lower cerebral blood flow in a general population. Therefore, we aimed to investigate the association between different measures of kidney function and cerebral blood flow in a population-based cohort of individuals 45 years and older. In addition, we investigated the association between kidney function and cerebrovascular diseases (stroke or dementia) in subjects with different levels of cerebral blood flow.

Methods

Population

The study is performed in the third cohort of the Rotterdam Study (2005-2009), including 3932 participants 45 years and older living in Ommoord, a district of Rotterdam, The Netherlands. From 3932 individuals participated in the study, cerebral blood flow using brain MRI was measured in 2956 participants of whom five participants with three standard deviation higher or lower values for cerebral blood flow were excluded (mainly due to cortical infarcts or vessel occlusions). This resulted in 2951 participants of whom 2797 had data on albumin-to-creatinine ratio, 2840 on serum creatinine levels, 2724 on serum cystatin C levels, and 2645 had both creatinine and cystatin C measurements (Figure 4). The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.[27]

Kidney function

Serum creatinine and cystatin C were measured with an enzymatic assay method and a particle-enhanced immunonephelometric assay, respectively. Creatinine values were standardized to isotope-dilution mass spectrometry–traceable (IDMS) measurements. eGFR, based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, was calculated for creatinine (eGFRcr) and cystatin C (eGFRcys) measurements separately and for both measurements combined (eGFRcrcys).[25] Participants collected timed overnight urine one day before the examination. Urine albumin and creatinine were determined by a turbidimetric method and measured by a Hitachi MODULAR P analyzer (Roche/Hitachi Diagnostics, Mannheim, Germany).[28] Albumin-to-creatinine ratio (mg/g) was estimated by dividing albumin by creatinine. Since albumin-to-

creatinine ratio was not normally distributed, we used log-transformed values to obtain values per doubling of albumin-to-creatinine ratio. We added 1 to the non-transformed values to account for those who did not have albuminuria.We defined three categories of kidney function using information from both eGFR and albumin-to-creatinine ratio. Kidney function was defined according to two criteria: eGFRcrcys > 60 mL/min/1.73 m^2 and albumin-to-creatinine ratio < 30 mg/g.[23] First category included participants that met both criteria. Participants that met only one criterion were categorized to the second category, and participants included in the third category met none of the criteria.

Cerebral blood flow

Magnetic resonance imaging (MRI) of the brain was performed on a 1.5-T MRI scanner (Signa Excite II, General Electric Healthcare, Milwaukee, WI, USA).[29] An eight-channel head coil was used for reception of the signal. For flow measurement, two dimensional phase-contrast imaging was performed.[29] First, a sagittal 2D phase-contrast MRI angiographic scout image was performed (repetition time (TR) = 24 ms, echo time (TE) = 9 ms, field of view (FOV) = 32 cm2, matrix = 256 160, flip angle = 101, number of excitations (NEX) = 1, bandwidth (BW) = 8.06 kHz, velocity encoding = 60 cm/sec, slice thickness = 60 mm). Acquisition time was 12 secs. On this scout image, a transverse imaging plane perpendicular both to the precavernous portion of the internal carotid arteries and to the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (TR = 20 ms, TE = 4 ms, FOV= 19 cm2, matrix = 256 160, flip angle = 81, NEX= 8, BW= 22.73 kHz, velocity encoding = 120 cm/sec, slice thickness = 5 mm). Acquisition time was 51 secs, and no cardiac gating was performed.[30] Acquisition time was 51 secs, and no cardiac gating was performed.[31] For the assessment of brain volumes, the structural MRI scans (T1-weighted, proton density weighted, and fluid attenuated inversion recovery) were used. Details including

preprocessing steps and the classification algorithm have been described elsewhere.[30] Flow (in mL/sec) was calculated by multiplying the average velocity with the cross-sectional area of the vessel.[31] To calculate global cerebral blood flow (in mL/min), flow rates for the carotid arteries and the basilar artery were summed and multiplied by 60 secs/min. To measure parenchymal cerebral blood flow in ml/min/100 ml brain tissue, values were divided by each individual's brain volume (mL) and the obtained results were multiplied by 100 (Figure S5). Retest-reliability was performed by two independent experienced technicians in 533 scans for all manual drawing around both carotids and the basilar artery and subsequent flow measurements. This showed inter-rater correlations > 0.94 for all vessels, indicating an excellent agreement.[29]

Covariates

Information related to smoking (past/current/never) and alcohol consumption was based on interviews using questionnaires. Alcohol consumers were categorized into moderate (less than 15 g/day) and heavy (more than 15 g/day) drinkers. Information on lipid lowering medication and antihypertensive medication use was based on home interview. Serum total and high density lipoprotein cholesterol levels were measured using an automated enzymatic method. Blood pressure was measured twice in a single visit and the average of two measurements, separated by a count of the pulse rate, was used in the analyses. History of coronary heart disease was considered as experiencing myocardial infarction or coronary revascularization procedures. Diabetes mellitus were defined by use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l. Information on stroke was acquired through digital record linkage with general practitioners and medical specialists in the research area.[32] To define dementia cases, individuals with the Mini Mental State Examination (MMSE) <26 or the Geriatric Mental Schedule (GMS) organic level >0 underwent an examination and informant interview with the Cambridge Examination

for Mental Disorders in the Elderly (CAM DEX). Further neurological testing was applied for participants who were suspected of having dementia. Covariates were selected based on the previous knowledge and literature. We checked the absence of co-linearity between covariates in the model using Variance Inflation Factor (VIF). We did not observe any co-linearity between the covariates included in the models.

Statistical analysis

Associations of measures of kidney function (albumin-to-creatinine ratio, eGFRcr, eGFRcys, and eGFRcrcys) with cerebral blood flow were evaluated using multiple linear regression models. Betas and 95% CI were estimated per standard deviation SD decrease for measures of kidney function. First, all analyses were adjusted for age, and sex. Then we further adjusted the analyses for systolic blood pressure, diastolic blood pressure, body mass index, alcohol consumption, smoking, total cholesterol, high density lipoprotein cholesterol, Mini-Mental State Examination (MMSE), coronary heart disease, diabetes mellitus, lipid lowering medication, and blood pressure lowering medications (diuretics, beta blockers, ACE inhibitors, and calcium channel blockers). In the third model, analyses with eGFR as determinants were further adjusted for albumin-to-creatinine ratio, and analysis with albumin-to-creatinine ratio as determinant was further adjusted for eGFRcrcys. There was no departure from linearity in the association between kidney function markers and cerebral blood flow. We performed the analysis of covariance where adjusted mean values of cerebral blood flow were compared across three categories of kidney function. In addition, we performed a series of sensitivity analyses, excluding subjects with chronic kidney disease (eGFRcrcys < 60 mL/min/1.73 m^2), stroke, or dementia. To explore whether the association between kidney function and cerebral blood flow was consistent between right and left carotid internal and basilar artery, we assessed the association between kidney function measures and cerebral blood flow in different arteries separately.

Furthermore, to compare the magnitude of the association with age, as an established risk factor for brain disorders, we calculated the effect estimates for the association of age with cerebral blood flow. We then divided the betas of kidney function markers by the beta of age in relation to cerebral blood flow and reported the corresponding ratios. To investigate whether the link between kidney function and cerebrovascular diseases is mediated through lower cerebral blood flow, we evaluated the interaction between kidney function and cerebral blood flow in relation to presence of clinical stroke or clinical dementia. In addition, in the stratified analysis, we investigated the association of eGFRcrcys and albumin-to-creatinine ratio with stroke or dementia in two groups of participants with low and high cerebral blood flow. To evaluate whether subjects with both low eGFRcrcys and cerebral blood flow have worse performance in cognitive test, we calculated mean values of different cognitive domains (memory, executive function, processing speed, and motor speed) across different categories of eGFRcrcys and cerebral blood flow in non-demented individuals. Cognitive function was assessed with the following neuropsychological test battery: the Mini-Mental State Examination, a 15-word verbal recall learning test, the Stroop test, the Letter-Digit Substitution Task (LDST), the Purdue Pegboard Test, and a word fluency test. The compound score for memory was the average of the z scores for the immediate and delayed recall of the 15-word verbal learning test. Executive function was constructed by averaging the z scores for the Stroop tests, the LDST, and the word fluency test. Information processing speed was the average of the z scores for the Stroop reading and Stroop color naming test and the LDST. Details of the cognitive assessments are provided elsewhere.[33] All analyses were carried out using SPSS 20.0.2 for windows or R version 2.15.0.

Results

Table 1 presents characteristics of the study population in categories of estimated glomerular filtration rate (eGFRcrcys). Participants' characteristics based on their albumin-to-creatinine values are presented in Table S1.

Table 2 shows the association of measures of kidney function with cerebral blood flow. In age and sex adjusted model, higher log-transformed albumin-to-creatinine ratio was associated with 0.31 mL/min/100mL lower cerebral blood flow (95% confidence interval (CI): -0.58, -0.03). After adjusting for potential confounders in the second model, the association was not present (p=0.10). Each standard deviation (SD) higher eGFRcrcys was associated with 0.42 mL/min/100mL higher cerebral blood flow (95% CI: 0.01, 0.83) after adjusting for cardiovascular risk factors. Each SD higher eGFR based on creatinine (eGFRcr) was associated with 0.48 mL/min/100mL higher cerebral blood flow (95% CI: 0.11, 0.85). Further adjustments did not change the association. We did not observe any association between eGFR based on cystatin C (eGFRcys) and cerebral blood flow (p>0.05). Adjustment for eGFRcrcys in the analysis with albumin-to-creatinine ratio as determinant, and for albumin-to-creatinine ratio in the analyses with eGFR as determinant, did not alter our findings (Table 2). The association between kidney function markers and cerebral blood flow in different arteries were consistent (Figure S1).

We observed a linear trend between different categories of kidney function and cerebral blood flow, indicating lower cerebral blood flow in persons with worse kidney function (Figure 1). In a series of sensitivity analyses, exclusion of individuals with chronic kidney disease, stroke, or dementia did not change our findings (Figure S2).

Comparing the effect estimates of age with albumin-to-creatinine ratio in relation to cerebral blood flow, we showed that doubling of albumin-to-creatinine ratio corresponds to 1.7 years of increase in age. Likewise, each SD lower eGFRcrcys in

Table 1. Population characteristics

Characteristics	Total Population	eGFRcrcys (mL/min/1.73 m²)		
	(n= 2645)	<60 (n=80)	60 - 90 (n=1459)	>90 (n=1106)
Age, years	56.6 (6.4)	66.8(10.1)	57.8(6.3)	54.3(4.8)
Men	1186 (44.8)	31(38.8)	621(42.6)	534(48.3)
Systolic blood pressure, mmHg	132.0 (18.5)	143.6(23.3)	132.5(18.4)	130.5(17.2)
Diastolic blood pressure, mmHg	82.3 (10.8)	84.2(11.3)	82.2(11.1)	82.3(10.3)
Body mass index, kg/m²	27.5 (4.3)	29.3(5.0)	27.7(4.3)	26.9(4.1)
Alcohol				
Moderate drinker	1593 (60.2)	46(57.5)	900(61.7)	647(58.5)
Heavy drinker	783 (29.6)	17(21.3)	407(27.9)	359(32.5)
Smoking				
Current	689 (26.0)	19(23.8)	384(26.3)	286(25.9)
Former	1157 (43.7)	42(52.5)	627 (43.0)	488(44.1)
Total cholesterol, mmol/l	5.6 (1.0)	5.4(1.1)	5.6(1.0)	5.5(1.0)
HDL, mmol/l	1.4 (0.4)	1.3(0.3)	1.4(0.4)	1.4(0.4)
MMSE, point	28 [27, 29]	28 [27, 29]	28 [27, 29]	28 [27, 29]
Diabetes mellitus	206 (7.8)	18(22.5)	96(6.6)	92(8.3)
History of coronary heart disease	92 (3.5)	8(10.0)	54(3.7)	30(2.7)
History of stroke	35 (1.3)	4(5.0)	21(1.4)	10(0.9)
Dementia	9 (0.3)	3 (3.8)	5 (0.3)	1(0.1)
Antihypertensive medication	582 (22.0)	48(60.0)	340(23.3)	194(17.5)
Lipid lowering medication	556 (21.0)	28(35.0)	329(22.5)	199(18.0)
GFRcr, mL/min/1.73 m²	86.1 (13.3)	53.5(13.0)	80.6(10.1)	95.8(8.1)
GFRcys, mL/min/1.73 m²	86.2 (15.8)	47.4(10.2)	78.4(10.8)	99.3(8.8)
GFRcrcys, mL/min/1.73 m²	86.3 (13.4)	49.4(10.0)	79.0(7.2)	98.5(6.2)
Albumin-to-creatinine ratio, mg/g	3.4 [2.2, 6.1]	5.9[2.8, 19.0]	3.4[2.2,6.1]	3.5[2.1,5.9]
CBF, mL/min/100mL brain volume	58.5 (9.7)	54.7(10.2)	58.4(9.7)	59.0(9.7)

Categorical variables: number (percentage), continuous variables: mean (standard deviation), albumin-to-creatinine ratio was presented as median [Interquartile range]
The following variables had missing data: blood pressure (n=9), smoking (n=5), alcohol (n=12), lipid-lowering medication (n= 24), antihypertensive medication (n=24), HDL: high density lipoprotein cholesterol (n=7), Total cholesterol (n= 5), history of cardiovascular disease (n=28), body mass index (n=2), hypertension (n=24), dementia (n=3).
Abbreviations: eGFRcr = Estimated glomerular filtration rate based on creatinine; eGFRcys = Estimated glomerular filtration rate based on cystatin C; eGFR/eGFRcrcys = Estimated glomerular filtration rate based on creatinine and cystatin

relation to cerebral blood flow corresponds to 2 years increase in age (Table S2).
In subjects with low cerebral blood flow, each SD lower eGFRcrcys was associated with higher prevalence of stroke or dementia (OR: 1.62; 95%CI: 1.01, 2.36) while there was not such an association in subjects with high cerebral blood flow (P for interaction=0.02). There was no difference between subjects with high or low cerebral blood flow in the association between albumin-to-creatinine ratio and higher prevalence of stroke or dementia (P for interaction=0.14) (Table 3). In addition, we observed that subjects with both low eGFRcrcys and low cerebral blood flow performed worse in cognitive tests compared with subjects with both high eGFRcrcys and cerebral blood flow (Figure S3).

Discussion

In this population-based cross-sectional study, we observed that lower eGFRcrcys and lower eGFRcr are independently associated with lower cerebral blood flow. The association between higher albumin-to-creatinine ratio and lower cerebral blood flow was not independent of cardiovascular factors. Previous studies have reported a close link between kidney function and brain outcomes.[3, 12, 13] Lee et al, in a meta-analysis, showed that individuals with eGFR<60 ml/min/1.73 m^2 have a higher risk for stroke.[14] Similarly, epidemiological studies established a strong association between kidney function and dementia.[3] Beyond clinically evident cerebrovascular disorders, prevalence of MRI-defined microvascular damages such as cerebral microbleeds, lacunar infarcts, and white matter lesions is higher among individuals with impaired kidney function.[12] Comparing five hemodialysis patients with six healthy individuals, Pierro et al. showed that blood transit time in the cerebral microcirculation is significantly longer in hemodialysis patients.[15] Given the important role of cerebral hypo-perfusion in occurrence of brain abnormalities, in this study we studied the association of kidney

Table 2. Association of measures of kidney function with cerebral blood flow (mL/min/100mL brain volume)

	Cerebral blood flow (mL/min/100mL brain volume)	
	Difference* (95% CI)	**P-value**
Albumin-to-creatinine ratio n= 2797		
Model I	-0.31 (-0.58, -0.03)	0.02
Model II	-0.24 (-0.52, 0.05)	0.10
Model II	-0.18 (-0.48, 0.12)	0.24
eGFRcrcys n= 2645		
Model I	0.37 (-0.03, 0.77)	0.07
Model II	0.42 (0.01, 0.83)	0.04
Model III	0.49 (0.06, 0.93)	0.02
eGFRcr n= 2840		
Model I	0.48 (0.11, 0.85)	0.01
Model II	0.48 (0.11, 0.87)	0.01
Model III	0.58 (0.18, 0.97)	<0.01
eGFRcys n=2724		
Model I	0.09 (-0.32, 0.51)	0.65
Model II	0.12 (-0.31, 0.55)	0.58
Model III	0.16 (-0.29, 0.61)	0.49

Differences (betas) are calculated per each standard deviation increase in eGFR. Beta values for albumin-to-creatinine ratio presents cerebral blood flow values (mL/min/100mL brain volume) per doubling of albumin-to-creatinine ratio.
Model I: Adjusted for age and sex.
Model II: Additionally adjusted for systolic blood pressure, diastolic blood pressure, body mass index, alcohol consumption, smoking, total cholesterol, high density lipoprotein cholesterol, cardiovascular disease, diabetes mellitus, MMSE, antihypertensive medication, and lipid lowering medication.
Model III: Additionally adjusted for eGFRcrcys in analyses with albumin-to-creatinine ratio as determinant, and additionally adjusted for albumin-to-creatinine ratio in analyses with eGFR as determinant.
Abbreviations: eGFRcr = Estimated glomerular filtration rate based on creatinine; eGFRcys = Estimated glomerular filtration rate based on cystatin C; eGFRcrcys = Estimated glomerular filtration rate based on creatinine and cystatin C; CI=Confidence interval
Each standard deviation eGFRcrcys=13.4, eGFRcys =13.8, eGFRcr= 13.3

Table 3. Association of kidney function markers with prevalence of stroke and dementia in categories of cerebral blood flow

	Stroke or dementia prevalence OR(95%CI)*			P for interaction
	Total population	Low CBF	High CBF	
eGFRcrcys				
Model I	1.38 (1.02, 1.87)	1.62 (1.11, 2.36)	1.02 (0.58, 1.79)	0.02
Model II	1.35 (0.98, 1.86)	1.63 (1.07, 2.48)	1.05 (0.60, 1.84)	0.03
Albumin-to-creatinine ratio				
Model I	1.18 (1.01, 1.37)	1.21 (1.01, 1.45)	1.09 (0.82, 1.45)	0.14
Model II	1.07 (0.90, 1.27)	1.07 (0.87, 1.32)	1.05 (0.76, 1.44)	0.53

*ORs are presented per each negative standard deviation of eGFRcrcys and per doubling of albumin-to-creatinine ratio.
Cut off for cerebral blood flow is 57 mL/min/100mL brain volume.
Model I: Adjusted for age and sex.
Model II: Additionally adjusted for systolic blood pressure, diastolic blood pressure, body mass index, alcohol consumption, smoking, total cholesterol, high density lipoprotein cholesterol, cardiovascular disease, diabetes mellitus, MMSE, antihypertensive medication, and lipid lowering medication.
Abbreviations: CBF= cerebral blood flow, eGFRcrcys = Estimated glomerular filtration rate based on creatinine and cystatin C, CI=Confidence interval, OR=Odds ratio
Each standard deviation eGFRcrcys=13.4

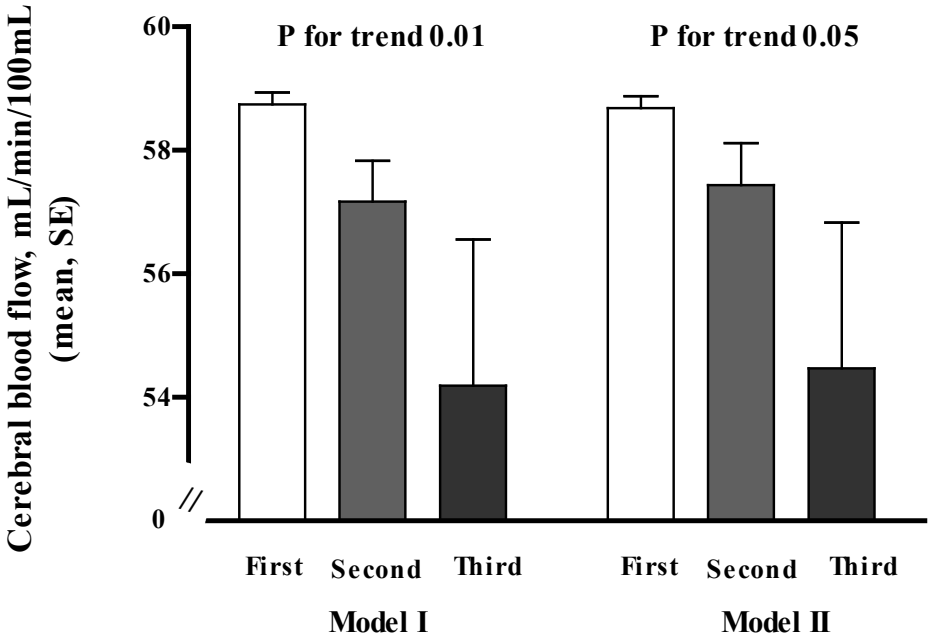

Figure 1. Adjusted means and standard errors of cerebral blood flow (mL/min/100mL) in different categories of kidney function
First category: indicates eGFRcrcys > 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio < 30 mg/g (n=2315);Second category: indicates eGFRcrcys > 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio > 30 mg/g OR eGFRcrcys < 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio < 30 mg/g (n=210);Third category: indicates eGFRcrcys < 60 mL/min/1.73 m^2 AND albumin-to-creatinine ratio > 30 mg/g (n=16)
Model I: Adjusted for age and sex.
Model II: Additionally adjusted for systolic blood pressure, diastolic blood pressure, body mass index, alcohol consumption, smoking, total cholesterol, high density lipoprotein cholesterol, cardiovascular disease, diabetes mellitus, MMSE, antihypertensive medication, and lipid lowering medication.
Abbreviations: eGFRcrcys= creatinine and cystatin C based estimated glomerular filtration rate

function and cerebral blood flow. In agreement with the previous evidences, we showed that worse kidney function is associated with lower levels of cerebral blood flow. We also found a more prominent association between eGFR and prevalence of stroke or dementia in participants with lower cerebral blood flow. In addition, we observed that subjects with both impaired kidney function and low cerebral blood flow have lowest cognitive function. These findings might suggest that cerebral blood flow levels might play a role in the association of kidney function with brain outcomes.

However, given the cross-sectional setting of our study, future longitudinal studies are needed to examine the potential role of cerebral blood flow in the relation between kidney function and brain outcomes.

Intact cerebral auto-regulation is dependent on preserve endothelial function and vasoreactivity.[16] In subjects at risk for accelerated vascular endothelial damage, such as patients with impaired kidney function, this regulatory mechanism might fall short and put the brain at the risk of hypoperfusion.[15] In line with this notion, it is reported that impaired cerebral auto-regulation is related to not only cerebrovascular disorders and dementia, but also to higher risk of mortality.[17-19] Association between kidney function and cerebral blood flow can be explained in different ways. Brain and kidney share common traditional cardiovascular risk factors such as hypertension and diabetes which can lead to a vascular injuries in both organs.[13] Impaired kidney vascular integrity as reflected in albuminuria might show a vascular damage not only in the kidney but also in the other organs with similar vascular bed like brain. [20] Additionally, impaired kidney function with alterations in water and electrolytes balance, vascular resistance and promoting chronic inflammation and sympathetic nerve over reactivity can contribute to vascular injury and endothelial dysfunction in the brain.[12] Another explanation could be that accumulation of vasoactive species such as asymmetric dimethyl arginine (ADMA) in kidney impairment results in vasoconstriction of cerebral vessels which ultimately decrease perfusion to the brain.[21] Future studies are required to address the mechanisms behind the association between kidney function and brain outcomes.

We observed an association between eGFRcr and cerebral blood flow and not with eGFRcys. This finding is in contrast to previous studies evaluating the link between kidney function and brain outcomes.[5, 22] For instance in the study by Darsie et al., eGFR based on cystatin C demonstrates larger effect size in relation to decline in cognitive function compared with eGFR based on creatinine.[22] These discrepancies

might suggest the role of factors independent of glomerular filtration rate influencing the association between eGFRcr and cerebral blood flow. [23] In addition, eGFR based on cystatin C levels is reported to be more accurate in populations with lower creatinine production, such as the elderly, and people with co-morbidities.[24] Given the relatively young population in our study with high levels of eGFR and low prevalence of comorbidities, eGFR based on creatinine might be a better marker for evaluating the kidney function. We also observed an association between albumin-to-creatinine ratio as well as the combined creatinine-cystatin C eGFR equation and cerebral blood flow. eGFR based on both creatinine and cystatin C measurements is reported to be more precise and perform better than either of the markers alone.[25] However, given the high correlation between combined creatinine-cystatin C eGFR, and either of the markers it is also possible that measures of eGFRcrcys are influenced by eGFRcr levels. Previous studies showed that range of cerebral blood flow in healthy middle-aged and older subjects is between 56 to 60 mL/min/100mL.[26] In this study average value of cerebral blood flow was 58.5±9.7 which indicates that majority of our study population had a normal cerebral blood flow. Despite our relatively young and healthy population-based sample, significant associations were observed between kidney function and cerebral blood flow. Therefore, we could expect that the magnitude of the association would be larger in patient populations.

Limitations of this study should be acknowledged. First, urinary albumin and creatinine was based on a single spot-urine sample, which is a common practice in epidemiological research setting.[1, 23] Second, although we adjusted the analyses for conventional cardiovascular risk factors, the potential roles of unmeasured cardiovascular risk factors cannot be excluded. Third, the cross-sectional design of this study limits our ability to infer directionality of the associations. Nevertheless, the population-based design of this study, large sample size, and availability of extensive data on various socio-demographic and cardiovascular factors which enables us to control for several

potential confounders, can be marked as the main strengths of this study.

Overall, we observed that lower eGFR, indicating worse kidney function, is independently associated with lower cerebral blood flow. Our findings extended previous literature by providing further evidence for the vascular origin of the link between impaired kidney function and brain disorders. Understanding of these concomitant pathologies can be of importance for early detection of subjects who are at risk for developing structural and functional brain abnormalities.

Online supplement
http://jasn.asnjournals.org/content/early/2015/08/05/ASN.2014111118/suppl/DCSupplemental

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CHAPTER 5

Discussion

Chronic kidney disease (CKD) and age-related brain disorders such as stroke and dementia are leading causes of morbidity and mortality worldwide.[1,2] Co-existence of kidney and brain disorders in old age is common and it is associated with poor prognosis.[3,4] Patients with CKD experience more severe cerebrovascular events and, due to interferences of kidney impairment with stroke management, post-stroke mortality and morbidity are more frequently observed in these patients.[5,6] Consequences of this situation are immense both for patients and the healthcare system and calls for early identification of subjects at risk.[7] Shared traditional cardiovascular risk factors can partly explain the coexistence of these two clinical entities,[8] but further pathophysiological pathways that play a role in their co-existence need to be elucidated. Kidney and brain are highly vascularized and share several physiologic and hemodynamic features. Furthermore, the kidneys are key regulators of systemic vascular tone and total body volume which might further highlight the importance of intact kidney function on prevention of vascular dysfunction. Vascular dysfunction can manifest as stiffness in large arteries and systemic small vessel disease and can affect various vascular beds.[9] Findings of this thesis support a role for vascular dysfunction in pathogenesis of both kidney impairment and brain disorders and stimulate further research on the interaction between the kidney and brain.

Key findings

Vascular stiffness and end-organ damage

Brachial systolic and diastolic blood pressure change with advancing age, and have been related to several cardiovascular outcomes. However, over the past decade, numerous studies showed that pulse pressure (the difference between systolic and diastolic blood pressure) is a better predictor for cardiovascular outcomes in older subjects as

compared to systolic or diastolic blood pressure alone.[10] Pulse pressure reflects the cushioning capability of arteries which itself is dependent on elastic properties of the arterial wall. Stiffening of arterial walls results in an increase in systolic and a decrease in diastolic blood pressures which can lead to deleterious effects in various organs.[11] Regional flow, local pulse pressure, and tissue-specific differences in microvascular structure are the factors that mediate the potential harmful effects of arterial stiffening on the structure and function of various organs.[11,12] In high-flow and low-impedance organs, like the brain and kidneys, pressure pulsatility penetrates further into the microcirculation.[13] Several studies have demonstrated that increased aortic stiffness, measured with pulse wave velocity, is associated with reduced kidney function and brain outcomes.[14-17] However, since arterial stiffness is not uniform along the arterial tree it is possible that stiffness in different arteries can be differentially related to kidney function and brain disorders.[18] In **chapter 3.1** of this thesis, in an individual participant meta-analysis, we observed that stiffness in the carotid artery is a better predictor for stroke compared with aortic stiffness. In addition, we showed that carotid stiffness modestly improves stroke risk prediction beyond the Framingham Stroke risk score. Likewise, in **chapter 2.4** we found that carotid stiffness is more prominently associated with decline in kidney function as compared with aortic stiffness. These findings might suggest that arterial stiffness, in particular carotid stiffness, can be a target for CKD and stroke prevention.

Serum uric acid: still a culprit?

For long, serum uric acid was acknowledged for its antioxidant properties and its protective role against aging, oxidative stress and oxidative cell injury.[19] However, over the last decade, epidemiological studies and clinical trials suggested that uric acid can be a risk factor for cardiovascular disorders.[20] Whether serum uric acid is an independent risk factor or simply a marker for cardiovascular disorders is still controversial.[21] Several studies investigated the independent role of serum uric acid in the pathogenesis of hypertension and CKD.[22,23] In **chapter 2.1**, combining the findings from the Rotterdam Study with several published population-based studies, we showed that serum uric acid is an independent risk factor for CKD. Given the potential causal effect of serum uric acid on hypertension, we further investigated the role of hypertension in this link. We observed a stronger association in hypertensive individuals indicating that hypertension might mediate this association.

The complex interrelationship between uric acid and cardiovascular risk factors makes it difficult to assess the causality of this link.[21] In **chapter 2.2**, we studied the association of uric acid genetic variants from genome wide association studies with systolic and diastolic blood pressure. In contrast to other studies,[24,25] we found that a higher uric acid genetic risk score is associated with lower systolic and diastolic blood pressure. Further explorations suggested that diuretics treatment may moderate the association of uric acid generic risk score and systolic blood pressure. Variability in serum uric acid is multifactorial and influenced by both genetic and environmental factors such as dietary intake and medications.[19] Hence, to unravel the role of uric and the mechanisms behind these associations, further studies with larger sample size and selected populations are needed.

Thrombosis and kidney health

As discussed previously, intact kidney function is dependent on adequate blood flow to the glomerulus.[3] Therefore, both macro- and microvascular health are crucial for kidney function. In fact, kidney is one of the most susceptible organs to thrombotic events in the microcirculation.[26] This is supported by the evidence from patients with thrombotic thrombocytopenic purpura (TTP), which is caused by a complete deficiency of ADAMTS13.[27] In TTP patients, ADAMTS13, a VWF protease, deficiency results in microthrombi formation in the circulation as well as the small vessels of the kidney, contributing to renal insufficiency.[27,28] In **chapter 2.5** we observed that this link can be extended to relatively healthy general population. We showed that markers of prothrombotic state, VWF-to-ADAMTS13 ratio, VWF:Ag, and ADAMTS13 activity, are associated with decline in kidney function. The associations were independent of cardiovascular risk factors, medications, and blood group. Although the effect estimates indicated a slight increase in kidney disease risk, previous studies showed that even trivial declines in glomerular filtration rate are associated with a considerable increase in the risk of end stage renal disease.[29] Therefore, future research on potential predictive value of prothrombotic factors in addition to traditional risk factors is of great importance. In addition, in this thesis we introduced ADAMTS13 as a novel prothrombotic biomarker for decline in kidney function which might open new opportunities for early identification of community dwelling middle-aged and older subjects at risk for accelerated kidney impairment.

Brain white matter integrity: Contributors and consequences

White matter is a key component of the brain consisting of glial cells and myelinated axons that transmit signals between various regions of the brain and the spinal cord.[30] White matter constructs around 50% of the brain volume and consumes 43.8% of the

brain's total energy.[30] White matter lesions are common findings in brain MRI scans of older subjects; however, it was not long before it rose to recognition for its important role in the brain disorders.[31] Several studies reported a link between presence of white matter lesions and adverse health outcomes, as well as shorter survival.[32,33] However, it seems that white matter lesions are only the "tip of the iceberg" of white matter pathology.[34] Early changes in the microstructure of cerebral white matter, quantified with diffusion-MRI, can demonstrate the whole extent of the cerebral pathology and hence will be of great importance for preventive strategies.[35] In **chapter 4.1**, we have shown that early stage changes of cerebral white matter are associated with kidney function. In **chapter 3.2** we investigated whether these subtle changes can predict mortality in individuals free of stroke and dementia. We showed that individuals with loss of microstructural organization of the cerebral white matter are at increased risk of mortality, independent of cardiovascular risk factors and macrostructural MRI changes. The association was more prominent with cardiovascular mortality rather than non-cardiovascular mortality indicating that white matter is more prone to vascular insults. Collectively, diffusion-MRI parameters may be markers reflecting the overall health status and may be used as clinical tools to assess the prognosis of individuals.

Kidney and brain: vascular cross-talk

Neurodegenerative and cerebrovascular disorders are major causes of morbidity and mortality in patients with kidney disease.[6,36] In patients with end stage renal disease, prevalence of cognitive impairment is between 30 to 70 percent.[36] While previous studies reported similar prevalence of Alzheimer disease in patients with impaired kidney function and their matched controls,[37] incidence and prevalence of subclinical and clinical cerebrovascular disorders are reported to be higher in all stages of CKD.[6,38] Such findings suggest that the link between decline in kidney function and cognitive impairment can be mediated through vascular mechanisms. The association

between kidney function and brain outcomes can be due to common traditional cardiovascular risk factors such as hypertension and diabetes which can lead to a vascular injuries in both kidney and brain.[39] Additionally, impaired kidney function with alterations in water and electrolytes balance, vascular resistance and promoting chronic inflammation and sympathetic nerve overreactivity can contribute to vascular injury and endothelial dysfunction in the brain (Figure 1).[4,8] In **chapter 4.1**, **4.2**, and **4.3** of this thesis, we investigated the link between kidney function and subclinical cerebrovascular disorders such as cerebral small vessel disease on MRI, white matter microstructure, and levels of cerebral perfusion. These studies benefit from wide range of early stage cerebrovascular abnormalities and application of different and robust markers of kidney function. In general, we observed that worse kidney function, in particular high albumin-to-creatinine ratio, is associated with higher loads of white matter lesions, lacunes, cerebral microbleeds, and worse white matter microstructure integrity, and lower cerebral blood flow. In addition, we found a more prominent association between glomerular filtration rate and prevalence of stroke or dementia in participants with lower cerebral blood flow, suggesting a role for cerebral blood flow levels in the association of kidney function with brain outcomes. Altogether, these findings recommend that clinicians should consider concomitant pathologies for early detection of patients with kidney impairments who are at risk for developing structural and functional brain abnormalities.

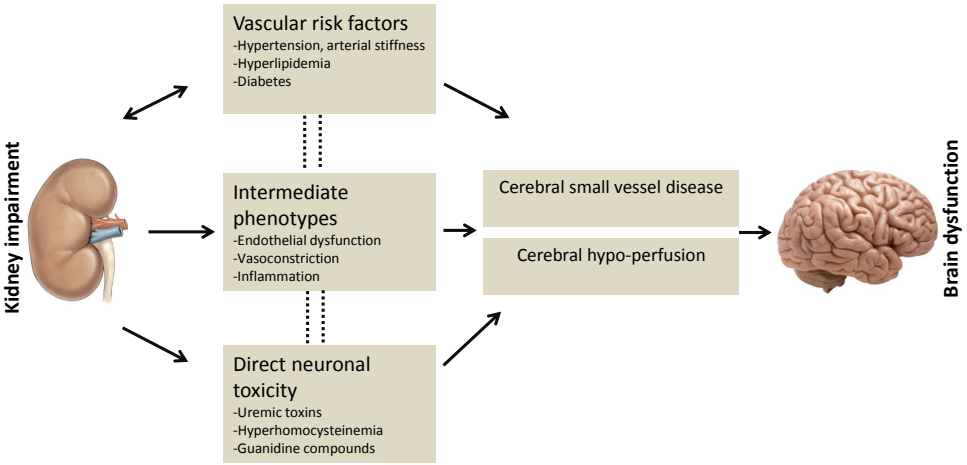

Figure 1. Schematic presentation of potential kidney-brain association

Methodological considerations

Markers of kidney function

Kidney disease is often diagnosed based on symptoms such as high blood pressure, peripheral edema and fatigue.[40] However, many patients are asymptomatic especially at earlier stages.[40] Glomerular filtration rate (GFR) and albuminuria are two main markers of kidney function. They can be used both for diagnosis and evaluation of the degree of kidney disease.[41] The normal value for GFR depends on sex and body mass index and it decreases with age.[42] Measurement of direct GFR is complex, and

time consuming to perform in large number of individuals. If people have relatively constant body mass index and diet, estimated GFR (eGFR) from serum markers such as creatinine or cystatin C would be an efficient way of assessing kidney function in population-based research setting.[43,44]

eGFR equations- The two most commonly applied equations to calculate eGFR in the population-based setting are Modification of Diet in Renal Disease (MDRD)[45] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.[46] The CKD-EPI equation and the MDRD equation may perform differently based on stage of kidney function and patient characteristics.[47] The CKD-EPI equation performs better in normal or mildly reduced GFR. In addition, the CKD-EPI equation is superior in elderly, certain races, diabetics, and at higher levels of body mass index.[47,48] However, the MDRD study equation performs better at lower levels of GFR and body mass index.[48] Given that participants in the Rotterdam Study are relatively old and have mild decrease in GFR, in this thesis for the assessment of kidney function we used CKD-EPI equation.

Creatinine or Cystatin C?- Both creatinine and cystatin C measurements are subject to variations due to laboratory essays and subject's certain characteristics.[49,50] Differences in methods and equipment can lead to variation in reported serum creatinine values and therefore, variations in the eGFR based on creatinine.[50] In this thesis, to reduce the variation in creatinine levels, we first used the IDMS traceable measurements and then calibrated creatinine measurements based on age and sex specific mean creatinine values of NHANES participants. Creatinine values vary not only due to laboratory differences, but also based on dietary intake and muscle mass. Vegetarian diet or reduction in muscle mass due to malnutrition and muscle wasting can lead to different amounts of creatinine.[46] However, cystatin C has been claimed to be unaffected by

gender, age or muscle mass. Nevertheless, several studies showed that cystatin C is affected by thyroid hormone, and it is correlated with markers of inflammation, fat mass, and diabetes.[49] Therefore, it is proposed that the combined CKD-EPI creatinine-cystatin C equation may be preferable to estimate GFR rather than either creatinine or cystatin C alone.[46] As it is shown in Figure 2, although mean values of different eGFR measurements in the Rotterdam Study are similar, eGFR based on cystatin C values marked more CKD cases compared with eGFR based on creatinine and eGFR based on both creatinine and cystatin C measurement.

Albumin-to-creatinine ratio- Spot urine albumin-to-creatinine ratio can be applied for not only assessing kidney function but also for staging it.[51] Application of both urine albumin and creatinine helps to avoid the confounding effect of urine volume on levels of urine albumin.[52] In addition, dividing albumin levels (less variable measure in a population-based setting) by creatinine, with more variability, makes the distribution closer to normal. In this thesis, when available we used spot urine albumin-to-creatinine ratio besides eGFR measurements to define CKD.

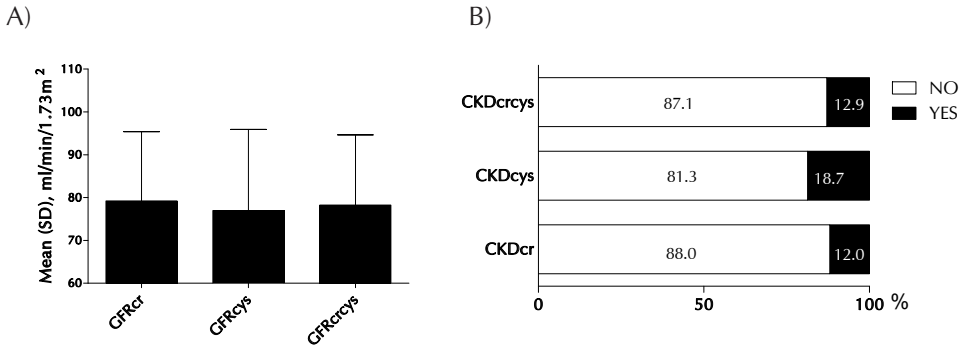

Figure 2. A) Mean values of eGFR based on creatinine, cystatin C and both measurements in Rotterdam Study, **B)** Prevalence of CKD based on eGFRcreat, eGFRcytatin C, and eGFRcreatinine-cystatin

Definition of CKD in population-based settings

In the clinical setting, CKD is defined based on persistent albuminuria and/or low eGFR for 90 days or more.[51] However, in the large population-based settings this definition might not be plausible. In the research settings, frequent measurements of eGFR and albuminuria aside being expensive, requires regular center visits.[44] Therefore, participants who are lost to follow up due to death and other co-morbidities will be excluded from the study. And in fact participants who died or are not capable of visiting the research center due to comorbidities are more prone to have impaired kidney function which can always underestimate the number of cases. Another approach for defining CKD would be to define cases based on hospitalization or general practitioners records.

However, since CKD symptoms appear only in later stages, individuals in early stages of the disease will be neglected.[51,53] In this thesis, we defined prevalent cases of CKD based on eGFR < 60 ml/min per 1.73 m^2, and when urine albumin and urine creatinine data were available based on the combination of eGFR<60 ml/min per 1.73 m^2 and albumin-to-creatinine ratio < 30mg. Incident cases were defined among the individuals free of CKD at baseline (eGFR>60 ml/min per 1.73 m^2), who had a decline in eGFR to less than 60 ml/min at the next visit. Single time point measurement of creatinine can be prone to measurement error, and regress to the mean, so rather than a stringent cut off (60 ml/min per 1.73 m^2), we also used the continuous decline in eGFR per year. When event date was required, to estimate the censoring date of the cases, we assumed a linear decrease in eGFR. Given this assumption, the date that each case had passed the eGFR threshold of 60 ml/min per 1.73 m^2 was taken as the censoring date and it was used to calculate the follow up time for incident cases. For controls, the time spent between the two examinations was used as the follow up time.

Indices of arterial stiffness

In **chapter 2.3**, **2.4** and **3.1** of this thesis we used three indices of the arterial stiffness: brachial pulse pressure, carotid femoral pulse wave velocity, and carotid distensibility. Arterial stiffness is not uniform along the arterial tree; therefore, stiffness in different arteries can have differential predictive values for various outcomes.[54,55] In this thesis, carotid stiffness seemed to be a better predictive tool for kidney function decline and stroke incidence compared with carotid femoral pulse wave velocity. This association can be explained by both physiological and methodological aspects of the arterial stiffness. Carotid is an elastic artery; this type of vessels showed to transfer higher load of pressure specifically to peripheral organs such as the kidney and brain.[18] In addition, previous studies showed that aorta stiffens further than carotid with aging and exposure to cardiovascular risk factors.[54] Therefore, it is possible that aortic stiffness measured with carotid femoral pulse wave velocity would predict cerebrovascular outcomes and kidney decline progression better in patient populations with higher loads of cardiovascular risk factors. Another explanation is methodological differences in measuring stiffness indices. Manual estimation (rather than direct measurement) of the distance between carotid and femoral artery is necessary for measuring carotid femoral pulse wave velocity.[55] Previous studies showed that arteries stretch and become tortuous with ageing and exposure to cardiovascular risk factors, which can lead to non-random measurement errors.[1,54] In contrast, due to small length of carotid arteries, measurement of this artery is done cross-sectionally without using any model of circulation.[55] In the Rotterdam Study, for computing the carotid distensibility coefficient, we used the brachial pulse pressure rather than the carotid pulse pressure. Substantial differences have been reported between carotid and brachial pulse pressures which can lead to an underestimation of the distensibility measurements and subsequently an underestimation of the association with the disease.

Role of genetic information in establishing causality

Epidemiology uses different methods to address "causes" of disorders. One of the recent approaches is application of genetic information.[56] Since alleles are allocated randomly during gamete formation, genetic variants are believed to be independent of potential confounders. In the past decade, genome wide association studies identified several genetic variants for many complex disorders. However, not all the genetic variants are specific to a disease. The so called "pleiotropy" phenomenon refers to the situation where the genetic variant has either independent effect on two distinct phenotypes or has an effect on a phenotype through another.[57] In genome wide association studies, genetic variants that are associated with occurrence of disorder or high level of a serum marker will be identified. However, coexistence of two phenotypes or high serum level of a marker secondary to a medication or a disorder cannot be recognized. This can lead to tendentious or unforeseen results. In **chapter 2.2** of this thesis, due to the pleiotropic effect of a gene, we observed a reverse association between uric acid genetic risk score and blood pressure. A genetic variant in SLC2A9 gene showed an interaction with diuretic use in relation to systolic blood pressure. Given that uric acid is an adverse effect of diuretics, we can conclude that the genetic variant has a pleiotropic effect. It is very important to be aware of the pleiotropic effect of genes, especially in Mendelian Randomization approach which can lead to invalidate results.

Clinical implications and future directions

Despite shorter survival of patients with CKD, these patients are at a greater risk for developing cerebrovascular events and dementia. In this thesis, we evaluated the relationship between kidney disease and brain outcomes in different stages and we showed that this link extends to earlier stage in pathogenesis of both the brain and

kidney disorders. We have further highlighted the role of vascular health in the link between kidney function and neurological features.

The long-distance interorgan cross-talk between kidney impairment and brain pathologies is commonly seen in clinic and thus it is important to get further attention in the research settings. Simultaneous damage in both organs may begin much earlier than we think and therefore, recognition and interventions preventing the progression of early kidney disease can potentially reduce the burden of neurological disorders. As the search for dementia biomarkers increases in coming years, it is sensible to design studies to assess the predictive capability of kidney function markers for neurovascular and neurodegenerative disorders. For instance, dementia risk indicators that have been so far developed are based on genetic predisposition or brain MRI assessments of microvascular disease, such as white matter lesions. It could be worth developing dementia risk prediction tools which incorporates different kidney function markers in addition to a number of vascular risk factors such as hypertension, and diabetes. In fact evaluation of kidney function markers is considerably easier and cheaper as compared with cerebral microvascular diseases with brain MRI. In addition, cost effectiveness studies will be useful to assess whether screening for mild kidney dysfunction would help preventing neurological outcomes such as stroke and dementia.

Further steps are needed to be taken to obtain robust evidence and further unravel the mechanisms behind the link between kidney function and cerebrovascular and neurodegenerative disorders. For example, vasoactive species like inhibitors of nitric oxide synthase such as asymmetric dimethyl arginine (ADMA) accumulate in CKD and lead to vasoconstrictive effects which can lead to cerebral hypo-perfusion.[58] Furthermore, impaired kidney function is associated with high levels of inflammation and previous literature showed that at least part of the occurrence of cerebrovascular and neurodegenerative disorders are explained with inflammation.[59,60] Other potential mechanisms, such as the role of direct neuronal toxicity of the uremic state,[8] could

also be a target as an intermediary factor. Recently developed mediation analysis methods have the potential to estimate the contribution of intermediate variables that lie in the pathway between two traits. It offers both identification and estimation of the mediatory effect. In addition, this statistical method has the ability to dealing with multiple mediators, which will be useful for covering multiple pathways that might play a role in the complex interrelationship between the kidney and brain.

In this thesis we showed that a vascular connection between brain and kidney exists. Another important step is to evaluate whether medications with protective vascular effects can be operational on apparent or silent damaged organs. Based on shared vulnerability of both brain and kidney to vascular injury from central aortic pressure, a reasonable approach would be the use of medications with capability of reducing central pulse pressure. Antihypertensive medications such as renin-angiotensin system blockers and calcium-channel antagonists could be logical candidates. So far effectiveness of these medications on both kidney and brain is controversial;[61,62] therefore, clinical trials with long follow-up time can be of great importance.

In conclusion, as the population is aging, occurrence of multi-morbidities also increases. It is time to consider multi-organ approaches and think out of the dimensions of one organ.

"The sons of Adam are limbs of each other, having been created of one essence. When the calamity of time affects one limb, the other limbs cannot remain at rest."

Saadi Shirazi (1184, 1283)

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CHAPTER 6

Summary/Samenvatting

The kidney and brain are vascular organs which share various physiologic features. Intact structural integrity of kidney and brain arteries is essential for their functioning and impairment in these arteries can lead to hypo-perfusion in both organs. In this thesis, we aimed to expand the current knowledge on the role of vascular dysfunction in progression of kidney impairment and occurrence of neurological outcomes. We also aimed to highlight the vascular link between the kidney and brain and accentuate that even slight decline in kidney function is associated with adverse brain outcomes.

Chapter 2 of this thesis provides evidence on the association between vascular dysfunction and decline in kidney function. In **chapter 2.1**, we showed that hyperuricemia is independently associated with a decline in kidney function and this association is stronger in hypertensive individuals which may indicate that hypertension mediates this association. This has brought us to the next step (**chapter 2.2**), where we examined whether the uric acid gene variants are associated with blood pressure. We found that higher uric acid genetic risk score is associated with lower systolic and diastolic blood pressure. However, we observed that diuretics treatment may modify this association. We suggest that genetic variants from genome wide association study can be associated with an intermediate factor or have a pleiotropic role and therefore should be applied for Mendelian Randomization with caution. In **chapter 2.3** and **2.4**, we investigated the bidirectional association between kidney function and arterial stiffness. In **chapter 2.3**, the cross-sectional association between kidney function and arterial stiffness was evaluated. Kidney function was associated with stiffer arteries independent of cardiovascular risk factors. In **chapter 2.4**, we investigated whether arterial stiffness can contribute to a decline in kidney function. Combining the Rotterdam Study data with previous published studies, we showed that markers of arterial stiffness are independently associated with future decline in kidney function. In addition, we provided further evidence for the association between pulse pressure and decline in kidney function, using genetic variability in pulse pressure.

Kidney function is dependent on adequate and constant blood flow to the glomerulus. Thus kidney is a susceptible organ to thrombotic events in the microcirculation. In **chapter 2.5**, we showed that higher levels of prothrombotic factors are associated with steeper decline in kidney function in the general population with normal baseline kidney function.

In **chapter 3**, we sought to investigate the association between vascular dysfunction and the brain. In **chapter 3.1**, in a meta-analysis of aggregate and individual participant data, we showed that greater carotid stiffness was associated with a higher stroke incidence. This association was independent of aortic stiffness (measured as carotid-femoral pulse wave velocity). In addition, estimation of carotid stiffness modestly improved stroke risk prediction beyond Framingham stroke risk score factors. In **chapter 3.2**, we showed that stroke and dementia free individuals with loss of microstructural organization of cerebral white matter are at increased risk for mortality. The association was more prominent with cardiovascular mortality rather than non-cardiovascular mortality, indicating that cerebral white matter is more prone to vascular insults.

In **chapter 4**, we investigated whether different markers of kidney function are associated with brain features. **Chapter 4.1** provides evidence that even slight reduction in kidney function is associated with worse white matter microstructural integrity. The association was not confined to any specific tracts and rather diffused in all regions of the white matter. In **chapter 4.2** we presented that markers of kidney function in particular albumin-to-creatinine ratio are associated with cerebral small vessel disease (white matter lesions, lacunes, and microbleeds).

The brain integrity is dependent on cerebral perfusion, and intact kidney function is crucial for the regulation of total blood volume and vascular tone. Therefore, impairments in kidney function can lead to disturbances in regulation of blood flow in organs that are critically dependent on adequate blood flow such as the brain. In **chapter 4.3** we showed that lower glomerular filtration rate is associated with lower

cerebral blood flow. In addition, subjects with both impaired kidney function and low cerebral blood flow had lowest cognitive function and more prevalence of stroke and dementia.

In **chapter 5** the main findings of this thesis are reviewed and several relevant methodological issues are discussed.

De nieren en het brein zijn vasculaire organen die verscheidene fysiologische kenmerken delen. Intacte structurele integriteit van de renale en cerebrale arteriën zijn essentieel voor het functioneren en disfunctioneren van deze arteriën, wat kan leiden tot hypoperfusie van beide organen. In dit proefschrift, beogen wij de huidige kennis uit te breiden betreffende de rol van vasculaire disfunctie in de progressie van nierschade en het optreden van neurologische uitkomsten. We trachten ook de vasculaire link tussen de nier en het brein te belichten en te benadrukken dat zelfs geringe achteruitgang in nierfunctie geassocieerd is met ongunstige brein uitkomsten.

Hoofdstuk 2 van dit proefschrift geeft inzicht in de associatie tussen vasculaire disfunctie en nierfunctie achteruitgang. In **hoofdstuk 2.1** tonen wij aan dat hyperurikemie onafhankelijk met achteruitgang van de nierfunctie is geassocieerd en dat deze associatie sterker is in individuen met hypertensie, wat impliceert dat hypertensie deze associatie medieert. Dit bracht ons bij de volgende stap (**hoofdstuk 2.2**), waar we onderzochten of varianten in urinezuur genen geassocieerd zijn met bloeddruk. Wij vonden dat een hogere genetische score voor urinezuur geassocieerd is met een lagere systolische en diastolische bloeddruk. Wij observeerden echter dat diuretica gebruik mogelijkerwijs deze associatie modificeert. Wij stellen dan ook dat genetische variaties van genoom wijde associatie studies geassocieerd zouden kunnen zijn met een intermediaire factor of een pleiotrope rol zouden kunnen hebben en derhalve met voorzichtigheid in Mendeliaanse Randomisatie toegepast moeten worden. In **hoofdstuk 2.3 en 2.4** onderzochten wij de bi-directionele relatie tussen nierfunctie en arteriële stijfheid. In **hoofdstuk 2.3** evalueerden we de cross-sectionele relatie tussen arteriële stijfheid en nierfunctie waarbij wij vonden dat nierfunctie geassocieerd is met stijvere vaten, onafhankelijk van cardiovasculaire risicofactoren. In **hoofdstuk 2.4** onderzochten we of arteriële stijfheid kan bijdragen aan nierfunctie verlies. Wij laten zien dat, als wij de resultaten van de Rotterdam Studie combineren met eerder gepubliceerde data, markers van arteriële stijfheid onafhankelijk geassocieerd zijn

met nierfunctie achteruitgang. Verder leveren wij, door het gebruik van genetische variabiliteit in polsdruk, bewijs voor een associatie tussen polsdruk en achteruitgang van nierfunctie.

Nierfunctie is afhankelijk van een adequate en constante bloedvoorziening naar de glomeruli. Zodoende is de nier een orgaan dat kwetsbaar is voor trombotische events in de microcirculatie. In **hoofdstuk 2.5** laten wij zien dat hogere bloedwaardes van pro-trombotische factoren geassocieerd zijn met een steilere nierfunctie achteruitgang in individuen uit de algemene populatie met een normale nierfunctie op baseline.

In **hoofdstuk 3** hebben wij ons onderzoek toegespitst op de associatie tussen vasculaire disfunctie en het brein. In **hoofdstuk 3.1** voerden wij een meta-analyse uit van zowel gecombineerde als individuele participanten data uit, waarin we laten zien dat een grotere carotide stijfheid geassocieerd is met een hogere incidentie van beroertes. Deze associatie is onafhankelijk van aorta stijfheid (gemeten als carotide-femoralis bloedpulssnelheid). Bovendien, de geschatte carotide stijfheid verbetert bescheiden het voorspelde risico op beroerte bovenop de risico factoren van de Framingham beroerte risico score. In **hoofdstuk 3.2** tonen wij aan dat individuen zonder beroerte en dementie met verlies van de micro-structurele organisatie van de cerebrale witte stof een hoger risico hebben op mortaliteit. De associatie is sterker voor cardiovasculaire mortaliteit vergeleken met non-cardiovasculaire mortaliteit, wat indiceert dat cerebrale witte stof meer geneigd is tot vasculaire insulten. In **hoofdstuk 4** onderzochten wij of verscheidene markers van nierfunctie geassocieerd zijn met bepaalde aspecten van het brein. **Hoofdstuk 4.1** levert bewijs dat zelfs een lichte reductie in nierfunctie geassocieerd kan zijn met een slechtere micro-structurele integriteit van de witte stof. Deze associatie was niet beperkt tot een specifieke systeem maar meer diffuus in alle gebieden van de witte stof. In **hoofdstuk 4.2** presenteren wij markers van de nierfunctie, met name albumine-creatinine ratio, die geassocieerd zijn met cerebrale ziekten van de kleine vaten(witte stof laesies, lacunes en microbloedingen).

De integriteit van het brein is afhankelijk van cerebrale perfusie en intacte nierfunctie is cruciaal voor de regulatie van de totale bloedvolume en vasculaire tonus. Daarom kan nierfunctieverlies lijden tot verstoring van de regulatie van de bloeddoorstroming in organen waarbij een adequate bloeddoorstroming van cruciaal belang is, zoals het brein. In **hoofdstuk 4.3** tonen wij aan dat een lagere glomerulaire filtratiesnelheid geassocieerd is met een lagere cerebrale bloeddoorstroming. Tevens hebben individuen met zowel een verminderde nierfunctie als een lagere cerebrale bloeddoorstroming de laagste cognitieve functie en vaker beroertes en dementie.

In **hoofdstuk 5** bespreken wij de belangrijkste bevinden van dit proefschrift en bediscussiëren wij de belangrijkste methodologische vraagstukken.

CHAPTER 7

Acknowledgements
PhD portfolio
List of publications
About the author

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The PhD path was an adventure for me, with all its ups and downs. But whenever I look back at it, it always brings a smile on my face. Statistically speaking, this means that ups were significantly more than downs. And if I look at the size of the smile I would say the effect size is also considerably large!

These years were not only about learning scientific matters but were also about how to thrive as a person and earn invaluable experiences. Here, I would like to express my sincere gratitude to all those who helped me in this journey.

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PhD Portfolio summary

Name:	Sanaz Sedaghat
Erasmus MC department:	Epidemiology
PhD period:	September 2012 - September 2015
Promotor:	Prof.dr. O.H. Franco Duran

Training	Year	Workload Hours/ECTs
Courses and workshops		
Doctor of Science, Genetic Epidemiology, NIHES	2012-2013	70
SNPs and Human Diseases	2012	2.0
Meta-analysis Workshop	2013	0.5
Linux For Scientists	2013	0.6
Biomedical English Writing	2013	1.4
An Introduction to the Analysis of the Next-generation Sequencing Data	2013	1.4
Basic Course on R	2014	1.0
Integrity in Scientific Research, Erasmus MC	2015	2.0
Meetings and conferences		
Consortium meeting 'CHARGE, Houston, United States	2012	0.5
European Renal Association Conference, Paris, France *(Oral presentation)*	2012	1.0
Developmental Origins of Health and Disease, Rotterdam, the Netherlands	2013	1.0
Consortium Meeting 'CHARGE, Rotterdam, the Netherlands	2013	0.5
Dutch Annual Epidemiology Conference (WEON), Leiden, the Netherlands *(Oral presentation)*	2014	1.0
Joint meeting ISH-ESH Hypertension, Athens, Greece *(Two oral presentations)*	2014	2.0
European Renal Association conference, Amsterdam, the Netherlands *(Oral presentation)*	2014	1.0
Student Delegate at the 64th Annual Meeting of Nobel Prize Laureates, Lindau, Germany	2014	2.0
Alzheimer's Association International Conference, Washington D.C, United States *(Poster presentation)*	2015	1.0
Research Visit to the National Institute of Health (NIH), Washington D.C USA	2015	8.0

Training	Year	Workload Hours/ECTs
Teaching activities- Supervising master students		
Teaching assistant, Principles of Research in Medicine and Epidemiology, NIHES	2013	1.0
Teaching assistant, The Practice of Epidemiologic Analysis, NIHES	2013-2014	1.0
Mateus Luvizotto, Serum uric and arterial stiffness: a systematic review	2013	2.0
David Imo, Fluid intake and decline in kidney function	2014	2.0
Other		
Peer review for scientific journals	2012-2015	2.0

List of publications

1. Serum uric acid and chronic kidney disease: the role of hypertension. **Sanaz Sedaghat**, Ewout Hoorn, Frank van Rooij, Albert Hofman, Oscar H. Franco, Jacqueline Witteman, Abbas Dehghan. PLoS One. 2013.

2. Association of uric acid genetic risk score with blood pressure components: The Rotterdam Study**. Sanaz Sedaghat**, Raha Pazoki, Andre G Uitterlinden, Albert Hofman, Bruno Striker, M. Arfan Ikram, Oscar H Franco, Abbas Dehghan. Hypertension. 2014.

3. EN-RAGE: A Novel Inflammatory Marker for Incident Coronary Heart Disease. Ligthart S, **Sanaz Sedaghat**, Ikram MA, Hofman A, Franco OH, Dehghan A. Arterioscler Thromb Vasc Biol. 2014.

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6. Effects of lifestyle-related interventions on blood pressure in low and middle-income countries: systematic review and meta-analysis. Baena CP, Olandoski M, Younge JO, Buitrago-Lopez A, Darweesh SK, Campos N, **Sanaz Sedaghat**, Sajjad A, van Herpt TT, Freak-Poli R, van den Hooven E, Felix JF, Faria-Neto JR, Chowdhury R, Franco OH. J Hypertens. 2014.

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Abbas Dehghan. Obesity. 2015.

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10. LDL cholesterol still a problem at old age? A Mendelian randomization study. Iris Postmus, Joris Deelen*, **Sanaz Sedaghat***, Stella Trompet, Anton JM de Craen, Bastiaan T Heijmans, Oscar H Franco, Albert Hofman, Abbas Dehghan, P Eline Slagboom, Rudi GJ Westendorp, J Wouter Jukema. International Journal of Epidemiology.2015.

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14. Arterial stiffness and decline in kidney function. **Sanaz Sedaghat,** Francesco U.S. Mattace-Raso, Ewout J. Hoorn, Andre G Uitterlinden, Albert Hofman, M. Arfan Ikram, Oscar H Franco, Abbas Dehghan. Clinical Journal of American Society of Nephrology, 2015.

About the author

Sanaz Sedaghat was born on September 18, 1985 in Shiraz, Iran. She completed her high school education on 2003 and started her Bachelor in Nursing Sciences at Shiraz Medical University of Sciences in the same year. Between 2007 and 2009, she was trained as a specialized nurse in the Coronary Care Unit, Shahid Faghihi Hospital. From 2009 to 2010, she participated in several research projects and gained training and experience in medical journalism.

In 2010, she received a scholarship from the Erasmus University Medical Center to study a master of science in Clinical Epidemiology at Netherlands Institute for Health Sciences (NIHES). She graduated in 2012 and her research project was awarded as the NIHES best master research thesis. In the same year, she started her PhD at the department of Epidemiology under the supervision of Dr. Abbas Dehghan, Dr. M Arfan IKram, and Prof. Oscar H Franco, as described in this thesis. Finding Genetic Epidemiology as an emerging field in the population sciences, during her first PhD year she participated in the Doctor of Science program in Genetic Epidemiology and received her degree in 2013. In summer 2015, she was awarded a visiting scholarship and served as visiting researcher at National Institute of Health/Laboratory of Epidemiology, Demography, and Biometry,USA. She will continue working at the department of Epidemiology as a post-doctoral fellow, under the supervision of Dr. M Arfan Ikram.