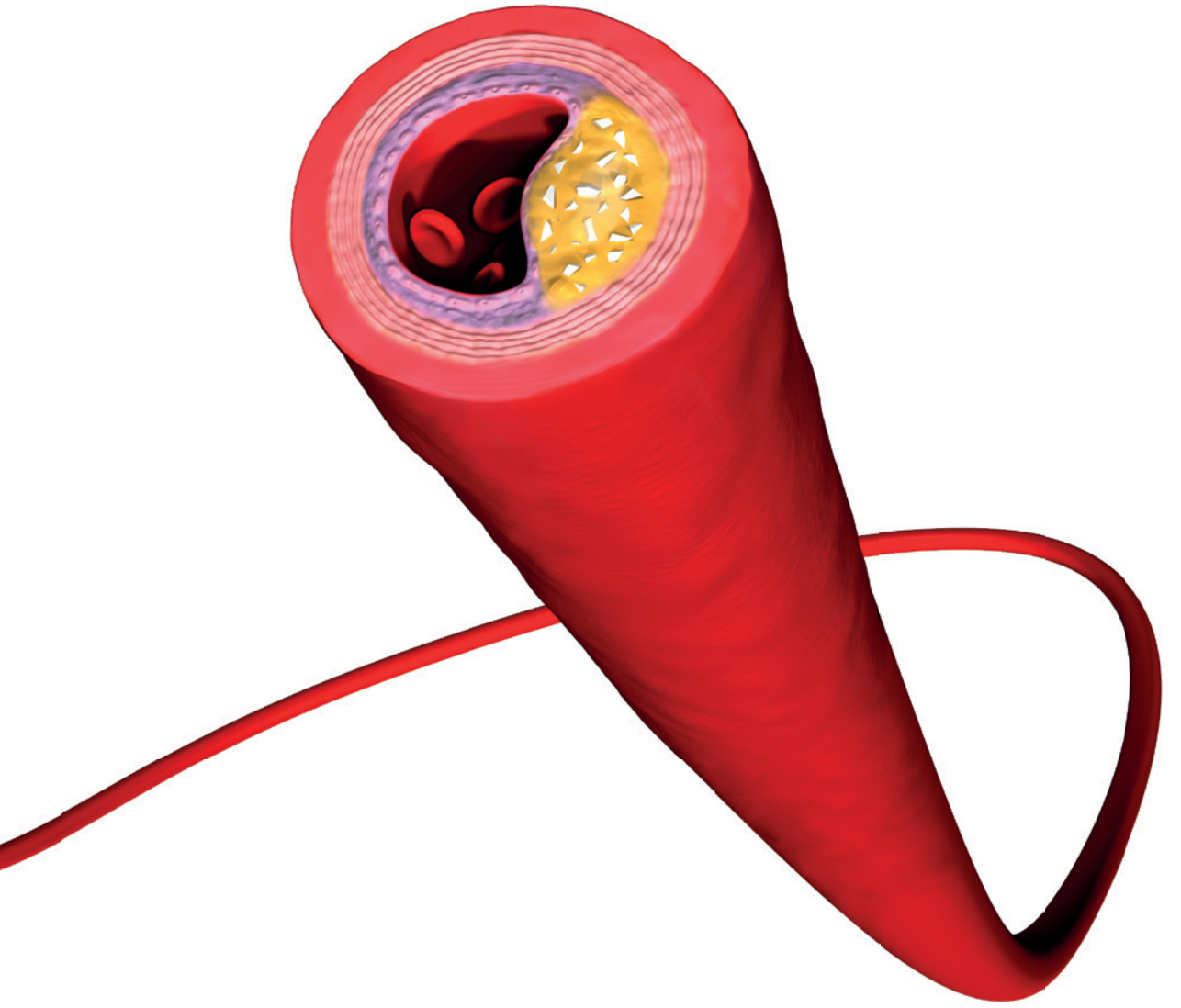
Atherosclerotic Calcification: Determinants and Clinical Neurological Consequences



Daniel Bos

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The work presented in this thesis was conducted at the department of Radiology and the department of Epidemiology of the Erasmus MC, Rotterdam, The Netherlands.

All studies described in this thesis were embedded within the Rotterdam Study, which is supported by the Erasmus MC and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), the Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII) and the Municipality of Rotterdam.

The contributions of the inhabitants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged.

Financial support for the publication of this thesis was kindly provided by the Department of Radiology and the Department of Epidemiology of the Erasmus MC and Alzheimer Nederland.

ISBN: 978-90-5335-756-9
Printed by: Ridderprint B.V.
Cover design: R.D. Voogt
Layout: A.W. Everaers

Atherosclerotic Calcification: Determinants and Clinical Neurological Consequences

Atherosclerotische Verkalking:
Determinanten en Klinische
Neurologische Gevolgen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 11 december 2013 om **11.30** uur

door

Daniel Bos

geboren te Rotterdam

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. A. van der Lugt
	Prof.dr. A. Hofman
Overige leden:	Prof.dr. P.J. Koudstaal
	Prof.dr. J.F. Arenillas
	Prof.dr. M.J.A.P. Daemen
Copromotoren:	Dr. M.W. Vernooij
	Dr. M.A. Ikram

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General Introduction

Atherosclerosis is a highly frequent vascular disease that exerts huge influence on the health care system. Major clinical conditions caused by atherosclerosis are ischemic heart disease (myocardial infarction) and stroke, both top causes of global morbidity and mortality in middle-aged and elderly persons.[1-5] Due to the aging of the population, the global burden of atherosclerosis, and thereby of its clinical consequences, will continue to rise in the coming decades.[6, 7]

In addition to these clinical consequences, increasing evidence suggests that the impact of atherosclerosis may be much larger, especially with regard to the condition of the brain. Various common, pathological alterations in the brain, such as white matter disease, small infarcts or atrophy, may be influenced by atherosclerosis.[8-10] Moreover, atherosclerosis may be an important determinant in the etiology of cognitive impairment, and even dementia.[10-13] Further disentangling the exact role that atherosclerosis plays in the development of these conditions is important, since this knowledge may then serve as a basis to develop opportunities for therapeutic or preventive intervention.

An important topic of interest is the location of atherosclerosis. Although atherosclerosis is a systemic disease that affects the whole arterial system, its burden may vary considerably across different vessel beds.[2, 14, 15] Explanations underlying these location-specific differences may partially lay in a differential susceptibility of vessels to conventional cardiovascular risk factors. On the other hand, genetic factors might also play a determining role in the location-specific atherogenic process.[16, 17] Apart from these location-specific differences in the formation of atherosclerosis, the varying burden of atherosclerosis across vessels could also translate to a differential contribution to subsequent risk of disease. It may for example be argued that the contribution of atherosclerosis to subsequent brain disease is largest in vessels that are in close proximity to the brain. Against this background, the origin of location-specific differences in atherosclerosis burden as well as the potential differential impact on subsequent brain disease needs to be further elucidated.

Advances in imaging techniques have aided considerably in the process of non-invasively visualizing and quantifying atherosclerosis in-vivo.[18] In particular, computed tomography [CT] is an established imaging modality in atherosclerosis research. CT is superior to any other imaging modality to detect calcium, which is a frequent characteristic of the atherosclerotic plaque, and a reliable marker of the total underlying plaque burden.[18-20] Moreover, by using CT it is possible to rapidly assess multiple vessel beds in one examination, and thus to detect and quantify potentially important information on atherosclerosis in various organ systems or vessel beds.

The purpose of my thesis is to expand the knowledge on determinants - or risk factors - and neurological consequences of atherosclerosis, with a specific focus on differences across vessel beds. The research described in my thesis was performed within the framework of the Rotterdam Study;[21] a large prospective, population-based cohort study aimed at investigating determinants of various chronic diseases in the elderly. Using CT-imaging in this population-based setting, I studied atherosclerosis in four major vessel beds: the coronary arteries, aortic arch and the extracranial and intracranial part of the internal carotid arteries.

In the following parts of this thesis I discuss various determinants and neurological consequences of atherosclerosis in the four vessel beds. In **Chapter 2**, the focus is on determinants of atherosclerosis. **Chapter 2.1** starts with the description of the prevalence and risk factors of intracranial carotid artery calcification, as proxy of intracranial atherosclerosis. **Chapters 2.2** and **2.3** are dedicated to genetic determinants of atherosclerotic calcification in the four vessel beds. In these chapters, the relationships between genetic variants of serum lipid levels and systolic blood pressure with atherosclerosis are described. **Chapter 3** depicts relationships of atherosclerosis with neurological endpoints. Part of the studies in this chapter were conducted in the Rotterdam Scan Study,[22] a large-scale prospective population-based magnetic resonance imaging [MRI] study. **Chapter 3.1** addresses the relationship between atherosclerosis in the four vessel beds and three important MRI-markers of subclinical vascular brain disease: white matter lesions, 'silent' lacunar infarcts and microbleeds. **Chapter 3.2** focuses on the relationship of atherosclerosis with preclinical cognitive deficits and cerebral atrophy. Following these chapters, **Chapter 3.3 and 3.4** then address the relationship between atherosclerosis and clinical neurological diseases; dementia and stroke. In **Chapter 4**, emerging imaging markers of vascular risk are highlighted. **Chapter 4.1** focuses on the coexistence of different components of the atherosclerotic plaque [calcification, lipid core and intraplaque hemorrhage], using both CT and MRI. In **Chapter 4.2,** a fully automated quantification-method for epicardial fat, as novel marker of vascular risk, is described. Finally, in **Chapter 5**, I conclude with a review of my main findings in the context of present knowledge and elaborate on future research into atherosclerosis.

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2

Determinants of Atherosclerotic Calcification

2.1

Prevalence and Risk Factors of Intracranial Carotid Artery Calcification

ABSTRACT

Background and Purpose

Intracranial atherosclerosis is one of the leading causes of ischemic stroke worldwide. However, surprisingly little is known about its prevalence and risk factors in a community-dwelling population of white descent. In this study we determined the prevalence and investigated risk factors of intracranial internal carotid artery calcification (ICAC), as a marker of intracranial atherosclerosis.

Methods

To quantify ICAC volume, 2,495 participants from the population-based Rotterdam Study underwent non-enhanced CT of the intracranial internal carotid arteries. We calculated the prevalence of ICAC. Next, we defined sex-specific quartiles and defined the upper quartile as severe ICAC. Risk factors of ICAC were investigated by linear and logistic multivariable modelling, and stratified by sex.

Results

The overall prevalence of ICAC was 82.2%. The median volume of ICAC was 44 mm^3 and was larger in men. Age was independently associated with ICAC in both men and women. In men, excessive alcohol intake and smoking [OR 1.74 (95%CI: 1.28; 2.37) and 1.72 (95%CI: 1.10; 2.70)] were strong risk factors of ICAC, whereas diabetes and hypertension were in women [OR 2.02 (95%CI: 1.29; 3.17) and 1.79 (95%CI: 1.20; 2.68)]. A low HDL-concentration was not associated with ICAC.

Conclusions

ICAC is highly prevalent and occurs in over 80% of middle-aged and elderly white persons. Conventional cardiovascular risk factors are associated with ICAC, but risk factor profiles differ between men and women.

INTRODUCTION

Intracranial atherosclerosis is worldwide one of the leading causes of stroke,[1] and as such leads to considerable morbidity and mortality. Especially in populations from Asian and African descent, atherosclerosis in the intracranial arteries is known to be a strong risk factor for ischemic stroke.[2-3] Recently, concerns were expressed about the possible underestimation of the importance and impact of intracranial atherosclerosis on stroke in white populations.[1,4] Indeed, surprisingly little data are published on the prevalence and risk factors of intracranial atherosclerosis in whites. Hence, it remains unclear what the risk factors are for intracranial atherosclerosis. In contrast, several modifiable risk factors are known for systemic atherosclerosis and coronary atherosclerosis.[5-7] However, due to only moderate correlations between atherosclerosis across different vessel beds,[8-9] the question remains if these associations with risk factors also hold for intracranial atherosclerosis. Given its potential public health impact, identification of modifiable risk factors for intracranial atherosclerosis is essential and may contribute to the prevention of stroke.

Arterial calcification, which can be reliably measured with computed tomography (CT),[10-11] has proven to be a sensitive marker of atherosclerosis.[12-13] In this study, we set out to determine the prevalence and investigate risk factors for intracranial carotid artery calcification (ICAC), as proxy of intracranial atherosclerosis, in a white population consisting of 2,495 persons from the population-based Rotterdam Study.

MATERIALS AND METHODS

Setting and Study Population

This study is based on the population-based Rotterdam Study,[14] an ongoing cohort study that started in 1990, with follow-up every 3-4 years. Over 96% of participants are of white descent. From 2003 until 2006, all participants who completed a regular visit at the research center were invited to undergo multidetector computed tomography (MDCT) of the intracranial carotid arteries (as part of a CT-imaging protocol measuring calcification in various vessel beds). In total, 2,524 participants were scanned. Due to the presence of image artefacts, 29 examinations from the 2,524 were not gradable, leaving a total of 2,495 participants with a complete CT examination for the current study. The Rotterdam Study was approved by the medical ethical committee of the Erasmus MC with a separate, additional approval of the present CT-study. All participants gave informed consent.

Cardiovascular Risk Factors

Information on cardiovascular risk factors was obtained during a home-interview and a visit at the research center.[7,14] Height and weight were measured and the body mass index (BMI) [(weight in kg)/(height in m)^2] was calculated. Obesity was defined as a BMI ≥ 30 kg/m^2. Systolic and diastolic blood pressures were measured twice at the right arm using a random zero-sphygmomanometer. The mean of the two measurements was used for the analyses. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg and/or the use of blood pressure-lowering medication.[7] Fasting blood samples were obtained and serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automatic enzymatic procedure (Hitachi analyzer, Roche Diagnostics). Glucose was determined enzymatically by the Hexokinase method. Diabetes was defined as fasting serum glucose levels ≥ 7.0 mmol/l and/or the use of anti-diabetic therapy.[7] Hypercholesterolemia was defined as a serum total cholesterol ≥ 6.2 mmol/l and/or the use of lipid-lowering medication.[7] We defined low HDL-cholesterol as a HDL-cholesterol < 1.0 mmol/l.[7] Data on alcohol consumption were collected as part of a dietary interview, and expressed in number of glasses per week. A "drink" was defined as 200 ml of beer containing 8.0 g of ethanol, 100 ml of wine containing 10.0 g of ethanol, 50 ml of liquor containing 14.0 g of ethanol, or 75 ml of moderately strong alcohol types containing 10.5 g of ethanol. By adding the amounts of ethanol in the four groups, we calculated the total amount of alcohol in grams per day. The alcohol consumption was considered excessive at >20 g/day.[15] Participants were categorized based on smoking status into "ever smoker" or "never smoker". A history of cardiovascular disease was defined as previous myocardial infarction and/or percutaneous transluminal coronary angioplasty and/or coronary artery bypass graft and/or stroke.[7]

CT Acquisition and Processing

An advanced 16-slice (n=785) or 64-slice (n=1,739) multi-detector CT-scanner (Somatom Sensation, Siemens, Forchheim, Germany) was used to perform non-contrast CT-scanning. The protocol is described elsewhere.[7] The scan parameters and the field-of-view were optimized for the visualization of the intracranial internal carotid arteries. We quantified ICAC in the intracranial internal carotid artery from the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery (Figure 1). Scoring of calcification was done semi-automatically, because automatic software is not available for this region (close relationship between calcification and the skull base).[8] Regions of interest were drawn in consecutive CT-slices by three trained raters in the course of the intracranial internal carotid artery making sure that bony structures were not included. The number of pixels above 130 Hounsfield units[16] was then calculated and the calcification volume (mm^3) was calculated by multiplying the number of

pixels, pixel-size and the increment. Calcification was measured without prior knowledge of risk factors. The inter-rater reliability of this method was very good (intra-class correlation coefficient: 0.99), as described previously.[8]

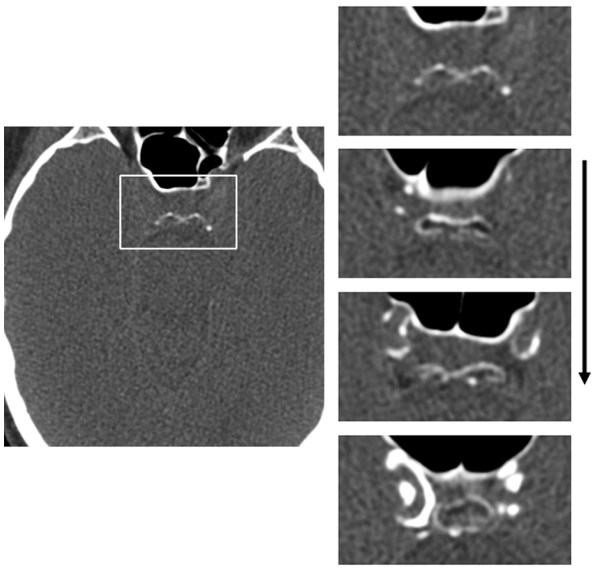

Figure 1. Different degrees of intracranial internal carotid artery calcification
The left side of the figure shows an axial CT-slice without calcification. The four images on the right represent the region of the internal carotid arteries marked with the white rectangle. These four images show increasing degrees of calcification (arrow), representing the four different quartiles of calcification.

Statistical Analysis

We defined the prevalence of ICAC as any amount of calcification in the trajectory of the intracranial internal carotid artery. The prevalence and median calcification volume were calculated for the total group and for men and women separately, according to age category (< 65, 65-70, 70-75, 75-80, and ≥80). Due to the non-normal distribution, calcification volumes were natural-log-transformed, and 1.0 mm^3 was added to the non-transformed values to deal with calcification volumes of zero [Ln(ICAC + 1.0 mm^3)]. We used linear regression to assess the relation between each potential risk factor and ICAC volume. In model 1 we adjusted for age, and in model 2 all risk factors were entered simultaneously. All analyses were stratified by sex. Next, we created sex-specific quartiles for ICAC volume and defined severe ICAC as persons in the upper quartile and non-severe ICAC as persons in the lower three quartiles. This classification into severe versus non-severe ICAC was made because it has been found that especially most severe calcifications are associated with an intracranial carotid artery stenosis.[17-18] Hereafter, we repeated all analyses using logistic regression. Alcohol intake was missing in 21.5%; other covariables were missing in less than 3% of participants. Missing values for alcohol intake were imputed using the Expectation Maximization algorithm based on age and sex.

RESULTS

Table 1 shows the characteristics of the study population. The prevalence of ICAC was 82.2%. In men, the prevalence of calcification was 83.2% and in women 81.2%.

Table 1. Population characteristics

Sample size	2,495
Women	51.7 %
Age, years	69.6 (6.8)
Obesity	23.9 %
Body mass index, kg/m^2	27.7 (4.0)
Hypertension	74.3 %
Systolic blood pressure, mmHg	146.7 (20.3)
Diastolic blood pressure, mmHg	80.1 (10.8)
Use of blood pressure-lowering medication	40.5 %
Diabetes	11.1 %
Serum glucose, mmol/l	5.7 (1.3)
Use of anti-diabetic medication	6.5 %
Hypercholesterolemia	49.6 %
Serum total cholesterol, mmol/l	5.7 (1.0)
Use of lipid-lowering medication	24.4 %
HDL < 1.0 mmol/l	10.7 %
Serum HDL-cholesterol, mmol/l	1.4 (0.4)
Excessive alcohol intake	23.5 %
Past or current smokers	67.9 %
History of cardiovascular disease	11.9 %
Intracranial carotid artery calcification volume*, mm^3	44.3 (7.3 – 144.7)

HDL, high-density lipoprotein
Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables
*Median (interquartile range)

Table 2 shows the sex-specific prevalence and calcification volume of ICAC by age category. We found that age was independently associated with larger ICAC vol-

Table 2. Prevalence and volume of intracranial carotid artery calcification by age

	Women		Men	
	Prevalence	**ICAC volume, mm³**	**Prevalence**	**ICAC volume, mm³**
Age categories	% (n/N)	Median (IQR)	% (n/N)	Median (IQR)
< 65	72.8 (283/389)	12.9 (0.0 - 55.9)	75.3 (256/340)	19.2 (0.6 - 72.2)
65 - 70	80.8 (323/400)	26.1 (4.6 - 84.5)	81.5 (300/368)	44.1 (8.4 - 144.0)
70 - 75	82.6 (185/224)	46.7 (9.2 - 127.3)	89.9 (213/237)	89.3 (27.2 - 273.0)
75 - 80	90.5 (134/148)	88.7 (28.8 - 202.8)	87.8 (137/156)	97.5 (20.4 - 278.7)
≥ 80	94.6 (123/130)	185.0 (86.5 - 320.6)	93.2 (96/103)	199.4 (66.7 - 376.3)

ICAC, intracranial carotid artery calcification; n, number of persons with ICAC; N, all persons in stratum; IQR, interquartile range

umes in both men and women [difference in ln-transformed calcification volume per year increase: 0.04 (95%CI: 0.04; 0.05) and 0.05 (95%CI: 0.04; 0.06), respectively]. Age was also independently associated with severity of ICAC in both sexes [odds ratio (OR) for upper calcification quartile versus lower three: 1.11 (95%CI: 1.08; 1.13) for men and 1.12 (95%CI: 1.09; 1.14) for women].

Age-adjusted associations between cardiovascular risk factors and ICAC volume, stratified by sex, are shown in Table 3, model 1. In men, all cardiovascular risk factors except a low HDL concentration, were associated with larger ICAC volume. In women however, obesity, a low HDL concentration, excessive alcohol intake and smoking status were not associated with ICAC. In the multivariable model (Table 3, model 2) diabetes mellitus and hypercholesterolemia remained significantly associated with larger ICAC volumes in both sexes [difference in men: 0.31 (95%CI: 0.14; 0.47) and 0.15 (95%CI: 0.04; 0.26) | in women: 0.20 (95%CI: 0.04; 0.37) and 0.20 (95%CI: 0.10; 0.30)]. In men only, smoking and excessive alcohol intake were independent risk factors for larger ICAC volumes [difference 0.26 (95%CI: 0.12; 0.40) and 0.18 (95%CI: 0.07; 0.30), respectively].

Table 4 shows the age- and multivariable-adjusted associations between cardiovascular risk factors and severe ICAC. These results are comparable with the results with ICAC as a continuous measure, again showing significant associations between smoking, excessive alcohol intake and severe ICAC in men [OR 1.72 (95%CI: 1.10; 2.70) and 1.74 (95%CI: 1.28; 2.37), respectively], but not in women. In contrast, in women hypertension was independently associated with severe ICAC [OR 1.79 (95%CI: 1.20; 2.68)]. Furthermore, in women, obesity was inversely associated with severe ICAC [OR 0.59 (95%CI: 0.42; 0.84)].

Table 3. Cardiovascular risk factors and intracranial carotid artery calcification

	ICAC*	
	Difference in Z-score (95% CI)	
Men	Model 1	Model 2
Age	0.05 (0.04;0.06)	0.04 (0.04;0.05)
Obesity	0.22 (0.08;0.35)	0.11 (-0.03;0.25)
Hypertension	0.17 (0.04;0.30)	0.04 (-0.09;0.17)
Diabetes mellitus	0.40 (0.24;0.57)	0.31 (0.14;0.47)
Hypercholesterolemia	0.27 (0.16;0.38)	0.15 (0.04;0.27)
HDL < 1 mmol/l	-0.05 (-0.19;0.10)	-0.09 (-0.23;0.06)
Excessive alcohol intake	0.20 (0.08;0.31)	0.18 (0.07;0.30)
Smoking (ever vs. never)	0.34 (0.20;0.49)	0.26 (0.12;0.40)
History of cardiovascular disease	0.48 (0.34;0.62)	0.35 (0.20;0.50)
Women		
Age	0.06 (0.05;0.06)	0.05 (0.04;0.06)
Obesity	0.05 (-0.07;0.16)	-0.03 (-0.15;0.08)
Hypertension	0.29 (0.17;0.41)	0.21 (0.10;0.33)
Diabetes mellitus	0.27 (0.11;0.44)	0.20 (0.04;0.37)
Hypercholesterolemia	0.26 (0.16;0.36)	0.20 (0.10;0.30)
HDL < 1 mmol/l	0.10 (-0.12;0.32)	0.02 (-0.20;0.24)
Excessive alcohol intake	0.01 (-0.14;0.15)	-0.03 (-0.17;0.12)
Smoking (ever vs. never)	0.06 (-0.05;0.16)	0.01 (-0.09;0.11)
History of cardiovascular disease	0.66 (0.48;0.85)	0.61 (0.42;0.80)

ICAC, intracranial carotid artery calcification; CI, confidence interval; HDL, high-density lipoprotein
* Ln(calcification + 1.0 mm^3)
Model 1: Adjusted for age
Model 2: Adjusted for age, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, excessive alcohol intake, smoking status, and history of cardiovascular disease

Table 4. Cardiovascular risk factors and severe intracranial carotid artery calcification

	Severe versus Non-severe ICAC	
	OR (95% CI)	
Men	Model 1	Model 2
Age	1.11 (1.09;1.13)	1.11 (1.08;1.13)
Obesity	1.42 (1.01;1.98)	1.13 (0.78;1.63)
Hypertension	1.37 (0.97;1.95)	1.12 (0.77;1.63)
Diabetes mellitus	1.65 (1.12;2.43)	1.50 (0.98;2.28)
Hypercholesterolemia	1.53 (1.16;2.03)	1.32 (0.97;1.80)
HDL < 1 mmol/l	0.94 (0.65;1.37)	0.86 (0.57;1.30)
Excessive alcohol intake	1.79 (1.34;2.38)	1.74 (1.28;2.37)
Smoking (ever vs. never)	2.06 (1.33;3.19)	1.72 (1.10;2.70)
History of cardiovascular disease	2.04 (1.47;2.83)	1.62 (1.13;2.33)
Women		
Age	1.13 (1.11;1.16)	1.12 (1.09;1.14)
Obesity	0.78 (0.57;1.07)	0.59 (0.42;0.84)
Hypertension	1.99 (1.36;2.90)	1.79 (1.20;2.68)
Diabetes mellitus	2.09 (1.39;3.15)	2.02 (1.29;3.17)
Hypercholesterolemia	1.51 (1.14;2.01)	1.26 (0.93;1.71)
HDL < 1 mmol/l	1.14 (0.63;2.08)	0.92 (0.47;1.81)
Excessive alcohol intake	0.99 (0.66;1.49)	0.83 (0.53;1.31)
Smoking (ever vs. never)	1.38 (1.03;1.83)	1.31 (0.97;1.78)
History of cardiovascular disease	5.17 (3.24;8.25)	5.10 (3.10;8.38)

ICAC, intracranial carotid artery calcification; OR, odds ratio; CI, confidence interval; HDL, high-density lipoprotein
Model 1: Adjusted for age
Model 2: Adjusted for age, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, excessive alcohol intake, smoking status, and history of cardiovascular disease

DISCUSSION

In this large population-based study among elderly white subjects we found that the prevalence of ICAC, as proxy of intracranial atherosclerosis, was higher than 80% in both men and women. We also found that men had larger ICAC volumes than women. In addition, we examined the relation between cardiovascular risk factors and ICAC and showed that conventional cardiovascular risk factors are associated with ICAC, but that the risk profile differs between men and women.

The presence of atherosclerotic calcification, as marker of atherosclerosis, has been studied previously. Coronary calcification has been found in up to 89% of the general elderly population.[5,9,19-20] Furthermore, prevalences of up to 96% have been reported for aortic arch calcification and up to 83% for the extracranial carotid arteries in the general elderly population.[7,19-20] Compared to these prevalences, our overall prevalence of ICAC of 82% seems to be in the same order of magnitude, which is in contrast with earlier claims that intracranial atherosclerosis, occurs infrequently in white populations and is a greater problem in other ethnic groups.[3-4] The overall prevalence of total intracranial atherosclerosis may even be slightly higher, since individuals who may not have ICAC could still have atherosclerosis further downstream in smaller intracranial arteries. Hence, the real impact of intracranial atherosclerosis may be underestimated in whites, which was also recently suggested.[1,4] This is especially important in view of the recently expressed major role of intracranial atherosclerosis as cause of cerebrovascular disease worldwide.[1,4] Moreover, in a previous study, again in a white population, we found that ICAC was a very strong risk factor for subclinical white matter lesions and cerebral infarction.[8]

Associations between cardiovascular risk factors and calcification in the coronary arteries, the aortic arch and the extracranial carotid arteries have been studied in a population-based design.[5-7,19-20] Most of our results are in agreement with the literature dealing with calcification in these other vessel beds. Like we found for ICAC, diabetes, hypercholesterolemia and smoking have been reported to be important risk factors of calcification in the coronary arteries, aortic arch and extracranial carotid arteries.[7,19-20] To our knowledge, our study is the first that examined associations between cardiovascular risk factors and ICAC in an elderly, non-selected white population.

We found that diabetes and hypercholesterolemia were independent risk factors for ICAC for both sexes. Interestingly, this is only partly in agreement with the results of a study in stroke-patients where ICAC was also quantified as a continuous measure.[21] In that study, no independent association between diabetes and ICAC was found.[21] For diabetes, we used another cut-off value; nonetheless,

post-hoc analyses using the same value did not change our results. From our results, we can therefore conclude that diabetes is also independently related to ICAC in the general population. It is worth noting that this strong association between diabetes and ICAC is supported by two recent studies,[22-23] one of which an autopsy report,[23] where the relationship between diabetes and intracranial atherosclerotic disease was also described.

Apart from these shared risk factors for ICAC between men and women, we also found some interesting differences. Most prominent were excessive alcohol intake and smoking, which were very strong risk factors for ICAC in men but not in women. However, there were significantly less women that smoked or drank excessive amounts of alcohol. An important remark here is that our population is relatively old and that at present the number of young women that smoke is considerably larger,[24] which will most likely result in a rise of smoking as risk factor in women, too.[24] On the other hand, hypertension was a strong risk factor for ICAC in women, but less so in men. Similar sex-differences for hypertension have been found for the extracranial carotid arteries.[7] Another interesting difference we found, was an inverse relationship between obesity and severe ICAC in women. This is in line with earlier studies where this paradoxal relationship for calcification in other vessel beds was found.[7,25] However, we further note that in continuous analyses this association was not significant, indicating that some care should be taken when interpreting this association. A low-HDL concentration was not associated with ICAC in our study. This might be due to the small number of participants (only 10%) in our study that had a low HDL-cholesterol concentration.

Strengths of our study include its large sample size and the use of an accurate and reliable CT-based method to assess intracranial carotid artery calcification, as proxy of intracranial atherosclerosis.[26] In addition to previous studies, we analyzed ICAC not only in quartiles but also as a continuous measure. In this way we reduced loss of information, which occurs with categorization into quartiles, and found stronger associations. Our study has also some potential limitations, one of which is that we used two types of CT-scanners. Nonetheless, post-hoc analyses with adjustment for scanner-type did not change the results. Non-participation occurred more frequently in older and more diseased subjects, but this could only have led to an underestimation of the associations. Finally, we measured calcification and not the complete atherosclerotic plaque area. Nevertheless, in autopsy-studies it has been found that arterial calcification measured with CT is a sensitive and reliable marker of atherosclerosis.[11,13] Moreover, a high correlation has also been found between calcification and the presence and amount of plaque in the carotid siphon.[12]

Several other considerations should also be taken into account. First, there are some other causes of calcification in blood vessels (e.g. hyperparathyroidism), but these are rare, especially in the general population. Second, with non-enhanced CT it is not possible to describe any additional characteristics of the plaque, such as shape, stenosis or ulceration, which could be of importance in the prediction of events. Although a high correlation between CT-measured calcification and stenosis has been found in the carotid siphon,[18] recent advances in plaque-imaging with other imaging techniques, for instance magnetic resonance imaging (MRI), may aid to overcome this issue in the future.

To conclude, this study provides novel information on the presence and risk factors of ICAC as a measure of intracranial atherosclerosis. ICAC is highly prevalent and occurs in over 80% of elderly, white persons. Conventional cardiovascular risk factors are associated with ICAC, but risk factor profiles differ between men and women.

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2.2

Genetic Loci of Coronary Calcification and Serum Lipid Levels in Relation to Aortic and Carotid Calcification

ABSTRACT

Background and Purpose

Atherosclerosis in different vessel beds shares lifestyle and environmental risk factors. We investigated whether this also holds for genetic risk factors. Hence, we used genetic loci for coronary artery calcification (CAC) and serum lipid levels -one of the strongest risk factors for atherosclerosis- to assess their relationship with atherosclerosis in different vessel beds.

Methods

In 1,987 persons from the population-based Rotterdam Study we used three SNPs (single nucleotide polymorphisms) for CAC and 132 SNPs for total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides. To quantify atherosclerotic calcification, as marker of atherosclerosis, all participants underwent non-enhanced CT of the aortic arch and carotid arteries. We investigated associations between genetic risk scores of the joint effect of the SNPs and calcification.

Results

The joint effect of CAC-SNPs was associated with larger calcification volumes in all vessel beds [difference in calcification volume per SD increase in genetic risk score 0.15(95%CI: 0.11; 0.20) in aorta, 0.14(95%CI: 0.10; 0.18) in extracranial carotids, 0.11(95%CI: 0.07; 0.16) in intracranial carotids]. The joint effect of total cholesterol-SNPs, LDL-SNPs and of all lipid SNPs together was associated with larger calcification volumes in both the aortic arch and the carotid arteries, but attenuated after adjusting for the lipid fraction and lipid lowering medication.

Conclusions

The genetic basis for aortic arch and carotid artery calcification overlaps with the most important loci of coronary artery calcification. Furthermore, serum lipids share a genetic predisposition with both calcification in the aortic arch and the carotid arteries, providing novel insights into the etiology of atherosclerosis.

INTRODUCTION

Atherosclerosis is a systemic vascular disease[1] with risk factors that overlap across different vessel beds.[2-4] In particular high serum lipid levels are one of the strongest risk factors for atherosclerosis in different vessel beds.[1,4-6] However, the question remains whether this shared etiology also extends to genetic risk factors. This is important because it has been shown that the burden of atherosclerosis differs across vessel beds.[2,7-9] Recently, three single nucleotide polymorphisms (SNPs) were identified that were associated with coronary artery calcification (CAC),[10] a marker of atherosclerosis which can be measured in-vivo with computed tomography (CT).[11] It is unclear to what extent these CAC-SNPs are also associated with calcification in other major vessel beds.

Alternatively, the genetic basis of atherosclerosis can be investigated using the genetics of risk factors of atherosclerosis. Serum lipid levels are amongst the most important risk factors of atherosclerosis1,[5, 12-13] and we hypothesize that SNPs that are associated with lipid levels are also associated with atherosclerosis. Recently, 132 SNPs associated with serum concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglycerides were identified.[14] However, the effect of a single SNP is very small. Therefore, genetic risk scores (joint effect of the SNPs) can be constructed to acquire more power.

Hence, the aim of this study was twofold. Firstly, we determined whether the CAC-SNPs are also associated with calcification in the aortic arch and carotid arteries. Secondly, we explored the relationship between genetics of serum lipid fractions and calcification in the aortic arch and carotid arteries.

MATERIALS AND METHODS

Setting and Study Population

This study was embedded in the Rotterdam Study,[15] a prospective, population-based cohort study that aims to investigate the incidence and determinants of various chronic diseases in the elderly. The original cohort consisted of 7,983 participants aged 55 years or older and was extended with another 3,011 participants in 2000, using the same inclusion criteria. Participants in the Rotterdam Study are virtually all from Caucasian origin (96%). From 2003 onwards, all participants that completed a visit at the research center were invited to undergo CT of the aorta and carotid arteries (as part of a CT-imaging protocol measuring calcification in various vessel beds). In total, 2,524 participants were scanned. Due

to image artefacts, 33 examinations from the 2,524 were not gradable; leaving 2,491 participants with a complete CT examination. Genotype data was present in 1,987 of these participants, encompassing the current study population. This study was approved by the institutional review board and all participants gave informed consent.

Genotyping

Genotyping was performed as part of a large project on complex diseases.[16] We used the Illumina HumanHap550 Duo BeadChip® and the Illumina Infinium II HumanHap 610 Quad Arrays®. All genotyping was done at the Human Genotyping Facility, Genetic Laboratory, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. As described previously,[16] participant-specific quality controls included filters for call rate, heterozygosity, and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate, minor allele frequency, Hardy-Weinberg equilibrium, and differential missingness by outcome or genotype (mishap test in PLINK, http://pngu.mgh.harvard.edu/purcell/plink/). For imputation, we used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15 or 1.0.16 software). For each imputed SNP, quality of imputation was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance. For this study we extracted data on three recently discovered CAC-SNPs[10], and 132 SNPs[14] which are related to serum concentrations of total cholesterol, LDL, HDL, and triglycerides. Imputation quality (Rsq) was > 0.90 (mean 0.95) for the CAC-SNPs and > 0.60 (mean 0.96) for the lipid SNPs. These genetic data have undergone extensive quality checks; including identity-by-state clustering.[16] Persons more than three standard deviations away from the population-mean were excluded. Therefore, the population used in our analyses is ethnically homogenous with all persons from Caucasian origin.

CT Acquisition and Processing

CT-scans were acquired using a 16-slice (n = 785) or 64-slice (n = 1,739) multidetector CT-scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany). No contrast material was administered. To visualize calcification in the aortic arch, the extracranial carotid arteries and the intracranial carotid arteries, we used a scan that reached from the aortic arch to the intracranial circulation (one cm above the sella turcica). Detailed information regarding imaging parameters of the scan is described elsewhere.[4]

Calcification in the aortic arch and the extracranial carotid arteries was quantified with dedicated commercially available software (syngo CalciumScoring, Siemens, Germany), and expressed as calcium volume in cubic millimetres (mm^3). The aortic arch was measured from the origin to the first centimeter of the common ca-

rotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The extracranial carotid arteries were measured at both sides within three cm proximal and distal of the bifurcation.[4] The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Automatic quantification of calcification in this region was not feasible due to the close relationship between arterial calcification and the skull. Therefore, we used a semi-automatic tool, which is described in detail elsewhere.[8] In short, after delineating calcification manually in every consecutive CT-slice, the volume of the intracranial carotid artery calcification was calculated by multiplying the number of pixels above the threshold of 130 Hounsfield units[17] with the pixel size and the slice increment.

Assessment of Serum Lipid Levels and Other Cardiovascular Risk Factors

Serum total cholesterol, HDL and triglyceride concentrations were determined using an automated enzymatic procedure (Hitachi analyzer, Roche Diagnostics).[4] LDL was calculated from these three parameters using the Friedewald formula.[18] Since this formula does not apply when the triglyceride concentration exceeds 4.51 mmol/L, we could not calculate the LDL concentration in twenty-three participants. The use of lipid-lowering drugs was assessed by interview. These measurements were performed prior to the CT-examination (mean interval 4.6 ± 0.6 years). Information on other cardiovascular risk factors was obtained during a home-interview and a visit at the research center around the same time as the CT-examination.[15] These risk factors included: the body mass index (BMI), systolic and diastolic blood pressure, the use of blood pressure-lowering medication, diabetes and smoking status.[4]

Statistical Analysis

We constructed genetic risk scores[19] for the joint effect of the CAC-SNPs and for the joint effect of the lipid SNPs (52 SNPs for total cholesterol, 47 SNPs for HDL, 37 SNPs for LDL, and 32 SNPs for triglycerides) by summing the number of calcium increasing or lipid increasing alleles (lipid decreasing alleles for HDL) weighted by the reported effect estimate of each CAC-SNP or lipid SNP. [10, 14] Next, for the construction of a weighted genetic risk score for the joint effect of all lipid SNPs, we assigned weighting factors to the different components which were derived from the Friedewald equation (total cholesterol=LDL+HDL+0.45*triglyceri des).[18] Following this, total cholesterol got a weighting factor of 2.45, LDL and HDL of 1 and triglycerides of 0.45. We calculated the score and divided by 4.9 (2.45+1.0+1.0+0.45). In a similar way, we calculated a weighted genetic risk score for the joint effect of lipid SNPs, exclusive of total cholesterol (1.0(LDL)+1.0(HDL)+ 0.45(triglycerides) and dividing by 2.45). Additionally, we created quartiles of the weighted compound genetic risk scores. It is important to note that SNPs were

allowed to overlap across the risk scores to take into account their pleiotropic effect.

Since calcification volumes were positively skewed and non-normally distributed, we used natural log-transformed values and added 1 mm^3 to the non-transformed values [Ln(calcification volume +1.0 mm^3)] in order to deal with participants with a calcium score of zero. We used linear regression to assess the association between the joint effect of the CAC-SNPs and calcification volume (model 1). Analyses were additionally adjusted for age, sex, BMI, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, diabetes, total cholesterol, lipid lowering medication and smoking (model 2).

Next, we explored associations of the joint effect of the lipid SNPs per lipid fraction and of the genetic risk scores for all SNPs with calcification volume using linear regression (model 1). These analyses were additionally adjusted for age, sex and the following cardiovascular risk factors: BMI, systolic and diastolic blood pressure, the use of blood pressure-lowering medication, diabetes and smoking status (model 2). Model 3 was additionally adjusted for the respective serum lipid concentration and the use of lipid lowering medication. Next, linear regression was used to test trends over the calcification quartiles. Associations between serum concentrations of total cholesterol, HDL, LDL, triglycerides and calcification volume, adjusted for age, sex and the use of lipid-lowering medication were also assessed with linear regression. IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

RESULTS

Table 1 shows the characteristics of the study population. The mean age at the time of the CT-scan was 69.8 ± 6.8 years and 49.9% of the participants were women.

Associations between the CAC-SNPs, the joint effect of the CAC-SNPs and calcification volume in the different vessel beds are shown in Table 2. The genetic risk score of the CAC-SNPs was significantly related to calcification in all three vessel beds but attenuated after additional adjustment for age, sex and cardiovascular risk factors (Table 2, model 2). When we investigated the associations for each CAC-SNP separately and calcification volume, rs1333049 was strongest associated with calcification in the aortic arch, the extracranial and intracranial carotid arteries [difference in calcification volume per SD increase in risk allele:

0.05 (95%CI: 0.01; 0.09), 0.08 (95%CI: 0.04; 0.13) and 0.12 (95%CI: 0.08; 0.16), respectively] (Table 2). These associations were still present after additional adjustments for age, sex and cardiovascular risk factors (Table 2, model 2). The percent explained variance of the genetic risk score of CAC-SNPs ranged from 1.3 percent for intracranial carotid artery calcification to 2.3 percent for aortic arch calcification.

Table 3 depicts associations between genetic risk scores of the lipid SNPs per fraction and calcification volumes in the different vessel beds. The joint effect of total cholesterol-SNPs was significantly associated with larger calcification volume in all three vessel beds and these associations did not change after additional adjustment for cardiovascular risk factors [difference in calcification volume per SD increase in genetic risk score 0.05 (95%CI: 0.01; 0.09) for the aortic arch, 0.06 (95%CI: 0.02; 0.10) for the extracranial carotid arteries and 0.06 (95%CI:

Table 1. Population characteristics

Sample size	1,987
Women	49.9 %
Age, years	69.8 (6.8)
Body mass index, kg/m^2	27.6 (3.9)
Past or current smokers	68.0 %
Systolic blood pressure, mmHg	146.7 (20.4)
Diastolic blood pressure, mmHg	80.1 (10.9)
Use of blood pressure-lowering medication	39.9 %
Diabetes	10.7 %
Serum total cholesterol, mmol/l	5.8 (1.0)
Serum HDL cholesterol, mmol/l	1.4 (0.4)
Serum LDL cholesterol, mmol/l	3.7 (0.9)
Serum triglycerides, mmol/l	1.5 (0.7)
Use of lipid-lowering medication	13.5 %
Aortic arch calcification volume*, mm^3	265.5 (44.2 - 924.7)
Extracranial carotid artery calcification volume*, mm^3	25.9 (0.0 - 125.9)
Intracranial carotid artery calcification volume*, mm^3	45.9 (8.0 - 148.1)

HDL, high-density lipoprotein; LDL, low-density lipoprotein
Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables
*Median (interquartile range)

Table 2. Associations of SNPs for coronary artery calcium with calcification in other vessel beds

	Aortic arch calcification*	*P*	**Extracranial carotid calcification***	*P*	**Intracranial carotid calcification***	*P*
	Difference in Z-score (95% CI)		Difference in Z-score (95% CI)		Difference in Z-score (95% CI)	
			Model 1			
GRS of CAC-SNPs	0.15(0.11;0.20)	<0.001	0.14(0.10;0.18)	<0.001	0.11(0.07;0.16)	<0.001
rs1333049†	0.05(0.01;0.09)	0.027	0.08(0.04;0.13)	<0.001	0.12(0.08;0.16)	<0.001
rs9349379‡	0.03(-0.01;0.08)	0.172	0.03(-0.02;0.07)	0.201	0.02(-0.02;0.07)	0.331
rs2026458‡	0.02(-0.03;0.06)	0.397	0.02(-0.03;0.06)	0.516	0.01(-0.03;0.06)	0.583
			Model 2			
GRS of CAC-SNPs	0.01(-0.03;0.06)	0.525	0.04(0.00;0.08)	0.068	0.00(-0.04;0.04)	0.968
rs1333049†	0.04(0.00;0.08)	0.059	0.07(0.03;0.11)	<0.001	0.11(0.07;0.15)	<0.001
rs9349379‡	0.03(-0.01;0.07)	0.114	0.03(-0.01;0.07)	0.102	0.03(-0.01;0.07)	0.120
rs2026458‡	0.02(-0.02;0.06)	0.304	0.02(-0.02;0.06)	0.319	0.02(-0.02;0.06)	0.341

GRS, genetic risk score; CAC, coronary artery calcification; SNP, single nucleotide polymorphism
*Standardized calcification volumes [Ln(calcification volume +1.0 mm^3)]
† SNP rs1333049 is located on chromosome 9 near CDKN2B, with coded allele frequency of 0.46 (coded allele: C)
‡ SNPs rs9349379 and rs2026458 are both located on chromosome 6 near PHACTR1, with coded allele frequencies of 0.61 and 0.44 (coded alleles: A&T)
GRS of CAC-SNPs is based on weighted sum of the number of risk alleles of 3 SNPs for coronary artery calcium
Model 1: unadjusted
Model 2: adjusted for age, sex, body mass index, systolic and diastolic blood pressure, blood pressure lowering medication, diabetes mellitus, total cholesterol, lipid lowering medication and smoking status

0.02; 0.10) for the intracranial carotid arteries]. The joint effect of LDL-SNPs was associated with larger calcification volume in the aortic arch and the extracranial carotid arteries, whilst the joint effect of HDL-SNPs was only associated with calcification in the extracranial carotid arteries [difference in calcification volume per SD increase in genetic risk score 0.07 (95%CI: 0.03; 0.11)]. The joint effect of triglyceride-SNPs was solely associated with larger calcification volume in the extracranial carotid arteries.

We additionally adjusted for the respective serum lipid fraction and the use of lipid-lowering medication to investigate whether serum lipid concentrations (and the use of lipid lowering medication) are intermediates in the pathway from genetics to atherosclerosis (Table 3, model 3). Now, all associations between the different genetic risk scores and atherosclerotic calcification in the three vessel beds attenuated. Only the association between the genetic risk score for the HDL-SNPs and extracranial carotid artery calcification was still statistically significant [difference in calcification volume per SD increase in genetic risk score 0.05 (95%CI: 0.01; 0.10)].

The joint effect of LDL-, HDL- and triglyceride-SNPs together was significantly associated with calcification in the three vessel beds [difference in calcification volume per SD increase in genetic risk score 0.07

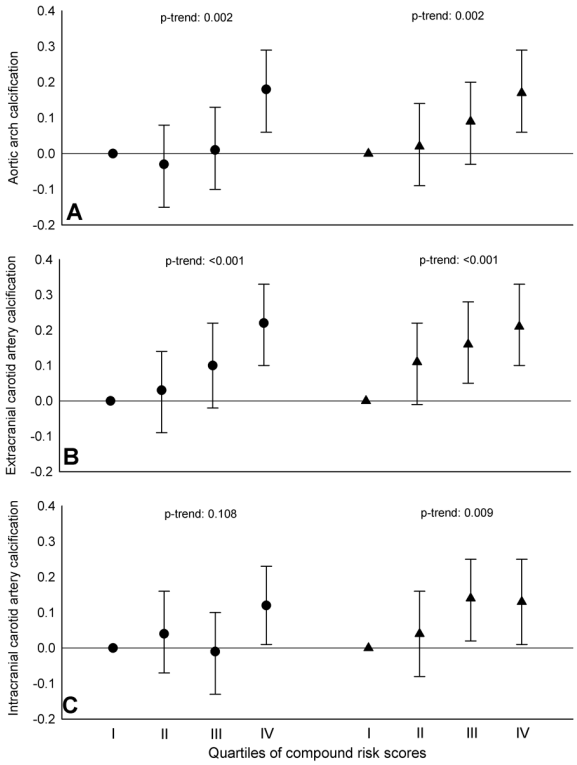

Figure 1. Joint effect of all lipid SNPs and calcification in different vessel beds
SNP, single nucleotide polymorphism
In all three panels (A-C) the quartiles of the weighted genetic risk score of the joint effect of all lipid SNPs are displayed on the x-axis. Values of y represent differences in standardized aortic calcification volume, extracranial carotid calcification volume and intracranial carotid calcification volume. The left column of quartiles (circles) represents the joint effect of HDL-, LDL- and triglyceride-SNPs. The right column (triangles) represents the joint effect of HDL-, LDL-, triglyceride- and total cholesterol-SNPs. Adjusted for age and sex. P-values for linear trends per compound score are displayed.

Table 3. Genetic risk scores for lipid fractions and calcification in different vessel beds

	Aortic arch calcification*		**Extracranial carotid calcification***		**Intracranial carotid calcification***	
	Difference in Z-score (95%CI)	*P*	Difference in Z-score (95%CI)	*P*	Difference in Z-score (95%CI)	*P*
Genetic risk score			Model 1			
Total cholesterol	0.07(0.02;0.11)	0.003	0.07(0.03;0.12)	0.002	0.07(0.02;0.11)	0.004
LDL cholesterol	0.07(0.03;0.12)	0.002	0.08(0.04;0.12)	<0.001	0.05(0.00;0.09)	0.046
HDL cholesterol	0.04(-0.01;0.08)	0.081	0.07(0.03;0.11)	0.002	0.03(-0.01;0.07)	0.183
Triglycerides	0.04(-0.01;0.08)	0.094	0.06(0.01;0.10)	0.010	0.04(-0.01;0.08)	0.121
			Model 2			
Total cholesterol	0.05(0.01;0.09)	0.009	0.06(0.02;0.10)	0.005	0.06(0.02;0.10)	0.008
LDL cholesterol	0.06(0.02;0.10)	0.005	0.07(0.03;0.11)	0.002	0.04(0.00;0.08)	0.080
HDL cholesterol	0.03(-0.01;0.07)	0.109	0.06(0.02;0.10)	0.003	0.03(-0.02;0.07)	0.204
Triglycerides	0.01(-0.03;0.05)	0.531	0.04(0.00;0.08)	0.053	0.02(-0.02;0.06)	0.409
			Model 3			
Total cholesterol	0.02(-0.02;0.06)	0.406	0.01(-0.04;0.05)	0.775	0.02(-0.03;0.06)	0.404
LDL cholesterol	0.02(-0.03;0.06)	0.458	0.02(-0.02;0.06)	0.393	0.00(-0.04;0.05)	0.907
HDL cholesterol	0.01(-0.03;0.05)	0.596	0.05(0.01;0.10)	0.011	0.02(-0.02;0.06)	0.316
Triglycerides	-0.01(-0.05;0.04)	0.791	0.02(-0.03;0.06)	0.426	0.01(-0.03;0.05)	0.686

CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein
*Standardized calcification volumes [Ln(calcification volume +1.0 mm^3)]
GRS is based on weighted sum of the number of risk alleles of 52 SNPs for total cholesterol, 37 SNPs for LDL cholesterol, 47 SNPs for HDL cholesterol and 32 SNPs for triglycerides
Model 1: unadjusted
Model 2: adjusted for age, sex, body mass index, systolic and diastolic blood pressure, blood pressure lowering medication, diabetes mellitus and smoking status
Model 3: adjusted for age, sex, body mass index, systolic and diastolic blood pressure, blood pressure lowering medication, diabetes mellitus and smoking status, the respective serum lipid concentration, and the use of lipid-lowering medication

(95%CI: 0.03; 0.11) for the aortic arch, 0.10 (95%CI: 0.05; 0.14) for the extracranial carotid arteries and 0.05 (95%CI: 0.01; 0.10) for the intracranial carotid arteries]. When total cholesterol-SNPs were added, these associations became slightly stronger. Figure 1A-C demonstrates the relationship between quartiles of the genetic risk score involving all lipid SNPs and calcification volume in all vessel beds.

DISCUSSION

In a large sample of community-dwelling older persons, we found genetic risk factors for atherosclerosis across vessel beds. More specifically, we found that previously discovered SNPs for coronary artery calcification are also jointly associated with calcification in the aortic arch, extracranial and intracranial carotid arteries. Furthermore, genetic loci for serum lipids are related with both calcification in the aortic arch and the carotid arteries.

We found a relationship between the genetic risk score of CAC-SNPs and larger calcification volume in the aortic arch, extracranial and intracranial carotid arteries. However, the strength of the associations differed across the vessel beds and moreover, the percent explained variance of the risk score for calcification also differed. Interestingly, the percent explained variance of the genetic risk score of CAC-SNPs ranged from 1.3 up to 2.3 percent for calcification. When adjusting for cardiovascular risk factors both the association and the amount of variance explained attenuated. Of the three separate CAC-SNPs, only rs1333049 was - significantly and independently of other cardiovascular risk factors - related to atherosclerotic calcification. Again, this association varied in strength across vessel beds. Possibly, differences in pathophysiology of atherosclerosis per vessel bed play a role here.[20] These findings also fit previous findings that correlations for atherosclerotic calcification in different vessel beds are only moderate.[2, 8-9] This could indicate that although atherosclerosis is a systemic disease, various factors play a different role in its development across vessel beds.

We found that the genetic risk scores of total cholesterol-SNPs and LDL-SNPs were most prominently associated with larger calcification volumes in all vessel beds. This is in line with another study,[14] which described that only several SNPs that coded for LDL were associated with coronary artery disease. Furthermore, we found that the joint effect of all LDL-, HDL-, and triglyceride-SNPs was associated with calcification volume in all vessel beds. These associations became stronger when total cholesterol was included in the genetic risk score. Most likely, part of this can be explained by the pleiotropic effect of the SNPs. However, we acknowl-

edge that it remains difficult to discern with full reliability a pleiotropic effect of a SNP from an effect merely driven by the inter-correlation of fractions. Nevertheless, despite strong correlations across lipid fractions, not all SNPs for a certain fraction also relate with other fractions. This indicates that those SNPs that do relate with more than one fraction, have a good likelihood of being pleiotropic.

As with the associations between CAC-SNPs and calcification, we also found differences in the strength of the associations between the genetic risk scores for the lipids and calcification across the vessel beds. This again suggests differences in etiology of atherosclerosis in different vessel beds.

The associations between the genetic risk score of total cholesterol-SNPs, the genetic risk score of LDL-SNPs and calcification did not change after adjustment for other cardiovascular risk factors, but only after additional adjustment for the serum lipid fraction and the use of lipid-lowering medication. This finding strengthens the hypothesis that serum lipid levels are intermediates in the causal pathway between genes and atherosclerosis. In contrast with this, the results for the genetic risk scores of HDL-SNPs and for the triglyceride-SNPs were less unequivocal. Interestingly, we found a prominent association between the genetic risk score of HDL-SNPs (lower serum HDL-level) and calcification in the extracranial carotid artery. This association even remained after additional adjustment for the lipid fraction and the use of lipid-lowering medication. Like for this genetic risk score of HDL-SNPs, we found an association between the genetic risk score of triglyceride-SNPs and extracranial carotid artery calcification only, but this association attenuated after adjustment for age, sex and the serum lipid level. These findings fit the notion that HDL and especially triglycerides are more environmental-/lifestyle-dependent (i.e. alcohol-consumption, obesity, sedentary lifestyle) lipid fractions.[21,22] Data on the relation between HDL, triglycerides and carotid atherosclerosis are scarce and inconclusive. A systematic review showed that lower values of HDL were associated with a higher risk of carotid atherosclerosis.[23] Our finding that the HDL-genetic risk score is associated with extracranial carotid artery calcification supports this observation, but further investigation of this relationship in larger samples is necessary. On the other hand, both the genetic risk scores for triglycerides and HDL were not associated with aortic arch or intracranial carotid artery atherosclerosis, which is in line with a recent finding, that a genetic risk score for HDL was not associated with myocardial infarction.[24] Based on our findings we hypothesize that genetic variants which are associated with higher HDL serum concentrations may not be automatically associated with less atherosclerosis in these vessel beds. This in turn suggests that different lipid fractions could play different roles in the etiology of atherosclerosis in different vessel beds.

Strengths of our study include the assessment of three major vessel beds with the same diagnostic tool (CT) and the fact that we built our genetic risk scores on previous studies. A major advantage of the CT-based calcium scoring is that it provides an accurate measurement of the amount of calcification, and that different vessel beds can be measured in one session. Several considerations should also be discussed, of which the first is the fact that we used only lipid SNPs - one of the strongest determinants for atherosclerosis and calcification[1,4] - and that the effect of other cardiovascular risk factors on calcification is thus not taken into account. Secondly, we have to note that any negative findings can be due to the relatively small sample size. Another consideration is that calcification is only a part of the atherosclerotic plaque. With CT it is not possible to visualize the complete extent of the non-calcified atherosclerotic plaque. Strong evidence nonetheless suggests that calcification volume is an adequate measure for the total underlying plaque burden.[11, 25]

To conclude, we found that genetic risk factors for atherosclerosis overlap across vessel beds. More specifically, the genetic basis for aortic arch and carotid artery calcification overlaps with the most important loci of coronary artery calcification. Furthermore, serum lipids share a genetic predisposition with both calcification in the aortic arch and the carotid arteries, providing novel insights into the etiology of atherosclerosis.

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2.3

Blood Pressure Genes and Atherosclerotic Calcification in the Coronary Arteries, Aortic Arch and Carotid Arteries

ABSTRACT

Background and Purpose

High blood pressure, especially the systolic component, is an important risk factor for atherosclerosis. It is however unclear if and to what extent the genetic basis of high blood pressure overlaps with that of atherosclerosis. We investigated whether genetic loci implicated in systolic blood pressure, identified through GWAS, are also related with atherosclerosis.

Methods

In 1,921 participants from the Rotterdam Study, we constructed genetic risk scores for blood pressure based on single nucleotide polymorphisms (SNPs) derived from a previous GWAS. We extracted all SNPs with MAF > 0.05 and their reported effect estimates using incremental p-value thresholds to create incremental genetic risk scores. For the assessment of atherosclerotic calcification, as proxy of atherosclerosis, all participants underwent computed tomography (CT) of the coronary arteries, aortic arch, extracranial and intracranial carotid arteries. Next, we investigated the relationship of the genetic risk scores with calcification in each of these vessel beds, adjusted for age and sex, and calculated the explained variance (R-square). All analyses were repeated excluding the participants with a low calcification volume (< median volume).

Results

The genetic risk scores were associated with extracranial carotid artery calcification, but not with calcification in the other vessel beds. After excluding participants without or with low calcification volumes, the genetic risk scores were associated with calcification in all vessel beds and explained up to 1.0% of the variance in calcification beyond age and sex. Moreover, there were prominent differences between the different vessel beds.

Conclusions

We found evidence for a shared genetic basis between systolic blood pressure and atherosclerosis. Interestingly, especially in persons with larger volumes of calcification, blood pressure genes contributed considerably to the amount of calcification. Finally, we found prominent differences in these relationships across vessel beds, suggesting a differential genetic etiology.

INTRODUCTION

Atherosclerosis is by far the most important cause of cardiovascular and cerebrovascular disease. Various risk factors for atherosclerosis have been identified,[1] of which high blood pressure, especially high systolic pressure, is amongst the most important.[2-5] Yet, despite the identification of these risk factors, a large part of the variability in total burden of atherosclerosis remains unexplained.[6] During the last decade, heritability studies have shown that genetic factors may also play an important role in the etiology and pathophysiology of atherosclerosis.[7, 8]

Advances in genetic research, such as genome-wide association studies (GWAS), have led to the identification of many common (low-risk) genetic variants [(i.e. single-nucleotide polymorphisms (SNPs)] of various diseases or risk factors of diseases, for example high blood pressure.[9, 10] There may also be overlap between genes for risk factors and for disease.[11, 12] In this light, it would be interesting to take into account not only SNPs that are genome-wide significant, but also SNPs that do not reach this threshold.[11] This could be important because all these common, low-risk SNPs together also contribute to the total burden of disease. Translating this concept to the strong relationship between systolic blood pressure and atherosclerosis, provides the opportunity to investigate whether the genetics of systolic blood pressure overlap with that of atherosclerosis. And if so, it would be interesting to investigate whether the effect of these genes is related to the extent of the atherosclerotic burden of a person, especially because systolic blood pressure is one of the strongest predictors of progression of atherosclerosis.[13] Moreover, there could be differences in this relation across different vessel beds, because only moderate correlations exist between atherosclerosis across vessel beds.[14-16] This knowledge could contribute to insight into the etiology and pathophysiology of atherosclerosis.

Arterial calcification, which can be reliably measured with computed tomography (CT), has proven to be a sensitive marker of atherosclerosis.[17-19] In the present study, we therefore explored the relationship between systolic blood pressure genes and atherosclerosis as assessed through CT-calcification, in 1,921 community-dwelling persons from the Rotterdam Study.

MATERIALS AND METHODS

Setting and Study Population

This study was embedded in the Rotterdam Study,[20] a prospective, population-based cohort study aiming to determine the incidence and determinants of various chronic diseases in the elderly. The original cohort consisted of 7,983 participants aged 55 years or older and was extended with another 3,011 participants in 2000, using the same inclusion criteria. From 2003 onwards, all participants that completed a visit at the research center were invited to undergo CT of the aorta and carotid arteries (as part of a CT-imaging protocol measuring calcification in various vessel beds). In total, 2,524 participants were scanned. After exclusion of scans with image artefacts, 2,413 participants had a complete and usable CT-examination. Genotype data was present in 1,921 of these participants, comprising the current study population. This study was approved by the institutional review board and all participants gave informed consent.

Genotyping

Genotyping was performed as part of a large project on complex diseases.[21] We used the Illumina HumanHap550 Duo BeadChip® and the Illumina Infinium II HumanHap 610 Quad Arrays®. All genotyping was done at the Human Genotyping Facility, Genetic Laboratory, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. As described previously,[21, 22] participant-specific quality controls included filters for call rate, heterozygosity, and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate, minor allele frequency, Hardy-Weinberg equilibrium, and differential missingness by outcome or genotype (mishap test in PLINK, http://pngu.mgh.harvard.edu/purcell/plink/). For imputation, we used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15 or 1.0.16 software). For each imputed SNP, quality of imputation was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance. For the present study we used the SNPs associated with systolic blood pressure identified by a published GWAS on blood pressure from the CHARGE consortium.[10] These genetic data have undergone extensive quality checks, including identity-by-state clustering.[21] Persons more than three standard deviations away from the population-mean were excluded. Therefore, the population used in our analyses is ethnically homogenous with all persons from Caucasian origin.

CT Acquisition and Processing

Imaging was performed using a 16-slice (n = 785) or 64-slice (n = 1739) multidetector CT-scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany). No contrast material was administered. We used a cardiac scan to image

calcification in the coronary arteries. To visualize calcification in the aortic arch, the extracranial carotid arteries and the intracranial carotid arteries, we used a scan that reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Detailed information regarding imaging parameters of the scan is described elsewhere.[2]

We quantified calcification in the coronary arteries, aortic arch and extracranial carotid arteries with dedicated commercially available software (syngo CalciumScoring, Siemens, Germany).[15] For calcification in the intracranial carotid arteries we used a semi-automated scoring method which is described in detail elsewhere.[22,23] Calcification volumes were expressed in cubic millimeters (mm^3).

Blood Pressure Measurement

At baseline, we measured systolic arterial blood pressure (mmHg) twice at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position.[20] We used the average of these two measurements in our analyses. Information on use of blood-pressure lowering medication was obtained by interview.

Statistical Analysis

We constructed genetic risk scores for systolic blood pressure to assess the association between the combined effects of the SNPs within the burden score, with atherosclerotic calcification volume. This method has been applied previously.[11, 12] We categorized the SNPs for blood pressure[10] according to their reported p-value for association with systolic blood pressure using the following thresholds: $p < 5^{-8}$, $p < 10^{-6}$, $p < 10^{-5}$, $p < 10^{-4}$, $p < 10^{-3}$, $p < 0.05$, $p < 0.1$, $p < 0.2$, $p < 0.3$, $p < 0.4$, $p < 0.5$, $p < 0.6$, $p < 0.7$, $p < 0.8$, $p < 0.9$, $p < 1.0$. For each category the effect of every SNP was multiplied by the number of corresponding alleles within each participant. Following this, each participant had 16 different genetic risk scores.

Atherosclerotic calcification volumes were positively skewed and non-normally distributed. Therefore we used natural log-transformed values and added 1 mm^3 to the non-transformed values [Ln(calcification volume +1.0 mm^3)] in order to deal with participants with a calcium score of zero.

We used linear regression to assess the association between the genetic risk scores and calcification volume, adjusted for age and sex. To investigate whether the effect of the risk scores is greater in participants with a larger amount of atherosclerotic calcification, we performed a second analysis excluding the participants with a calcification volume below the median volume for each vessel bed separately. Next, we calculated the R-square of calcification volume per vessel

bed for the systolic blood pressure risk scores. Finally, we adjusted all analyses for the systolic blood pressure at baseline, to investigate whether the risk scores provided more information than a blood pressure measurement.

RESULTS

The population characteristics are shown in Table 1. The mean age at the time of the CT-examination was 69.7 ± 6.8 years and 50.5% of the participants was female.

The number of SNPs within each of the 16 genetic risk scores is shown in Table 2, ranging from 22 SNPs in the smallest to 4,271,042 SNPs in the largest genetic risk score.

For calcification volume in the coronary arteries, age and sex explained 19.4% of the total variance in the whole group (dotted lines Figure 1). For aortic arch, extracranial carotid artery and intracranial carotid artery calcification, this was 17.6%, 13.4% and 13.2%, respectively. The percent explained variance which is attributable to the different genetic risk scores per vessel bed is shown in Figure 1 and ranged from 0.0% to 0.5% across the vessel beds. This figure also shows that only for calcification volume in the extracranial carotid artery, a larger number of SNPs included in the risk score resulted in a significant increase in the explained variance. Beyond the $p < 10^{-3}$-threshold (59,622 SNPs) the explained variance remained stable between 0.4% and 0.5% on top of the variance already

Table 1. Population characteristics

Sample size	1,921
Women	50.5 %
Age, years	69.7 (6.8)
Systolic blood pressure, mmHg	146.8 (20.3)
Diastolic blood pressure, mmHg	80.2 (10.8)
Use of blood pressure-lowering medication	38.5 %
Diabetes	10.8 %
Past or current smokers	67.8 %
Use of lipid-lowering medication	22.1 %

Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables

Table 2. Number of SNPs included in each p-value category

P-value threshold	Systolic blood pressure SNPs, N (%)
$p < 5^{-8}$	22 (0.0)
$p < 10^{-6}$	282 (0.0)
$p < 10^{-5}$	1190 (0.0)
$p < 10^{-4}$	7664 (0.2)
$p < 10^{-3}$	59622 (1.4)
$p < 0.05$	257284 (6.0)
$p < 0.1$	490290 (11.5)
$p < 0.2$	937318 (21.9)
$p < 0.3$	1377780 (32.3)
$p < 0.4$	1811670 (42.4)
$p < 0.5$	2241456 (52.5)
$p < 0.6$	2669458 (62.5)
$p < 0.7$	3096056 (72.5)
$p < 0.8$	3521182 (82.4)
$p < 0.9$	3945106 (92.4)
$p < 1.0$	4271042 (100.0)

SNP, single nucleotide polymorphism
Values are presented as N (%)

explained by age and sex. In the other vessel beds the genetic risk scores did not significantly increase the percent explained variance.

Next, we excluded all participants with a calcification volume below the group-median for each vessel bed. The percent explained variance in calcification volume per vessel bed attributable to the genetic risk scores was substantially higher in the remaining group. The explained variation beyond age and sex ranged from 0.1 – 0.4% for calcification in the coronary arteries, 0.1 – 0.7% in the aortic arch, 0.1 – 1.0% in the extracranial carotid arteries and 0.1 – 0.4% in the intracranial carotid arteries. Moreover, in all four vessel beds, the relationship between the genetic risk scores and calcification volume was significant for the genetic risk scores with the lower p-value thresholds. Figure 2 shows these results. Adjustment for systolic blood pressure at the time of the CT-examination did not change the results.

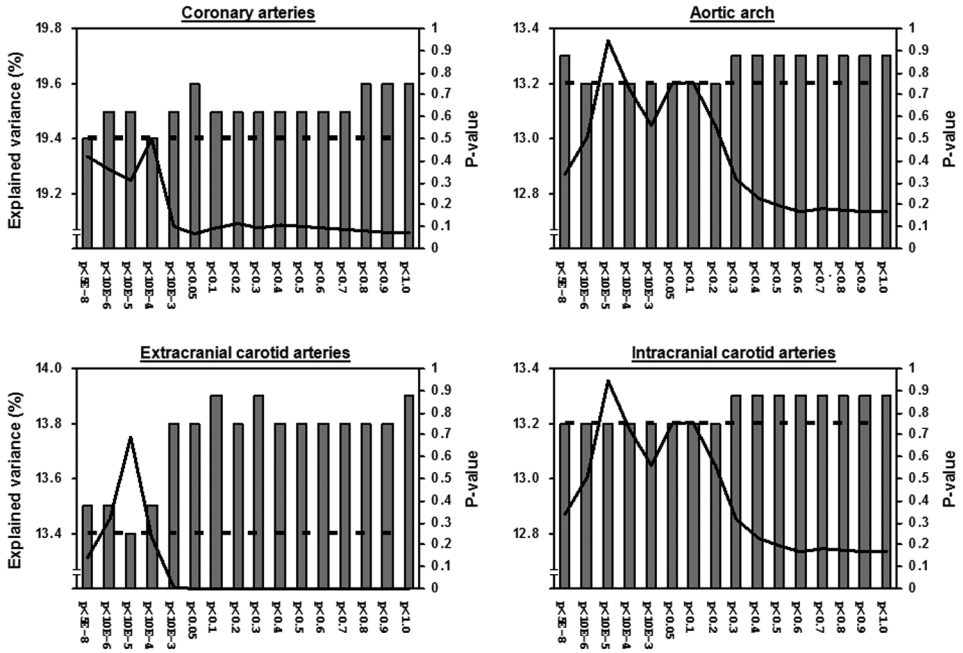

Figure 1. The explained variance of atherosclerotic calcification in four vessel beds by genetic risk scores
The R-square of the genetic risk scores of systolic blood pressure, adjusted for age and sex, for each p-value threshold on calcification volume in the different vessel beds. The y1-axis (left; histogram) shows the explained variance for the different p-value thresholds of the genetic risk scores (x-axis). The y2-axis (right; line) shows the p-values from the association analysis of the risk scores with calcification. The dotted line represents the attribution of age and sex to the explained variance.

DISCUSSION

In this sample of community-dwelling older people we found that genes related to systolic blood pressure are also related with the amount of atherosclerosis. More specifically, we found that the genetic risk scores based on the systolic blood pressure increased the explained variance of atherosclerotic calcification volume, beyond age and sex. This was primarily true for persons with above-median burden of atherosclerotic vessel calcification. As mentioned earlier, our focus was solely on the systolic component of blood pressure. We did so, because several studies have shown that especially the systolic component is an important risk factor for atherosclerosis and cardiovascular disease.[2-5]

Strengths of our study include the assessment of four major vessel beds with the same diagnostic tool (CT) and that we built our genetic risk scores on data from previous GWAS studies. An advantage of the quantification of calcification using

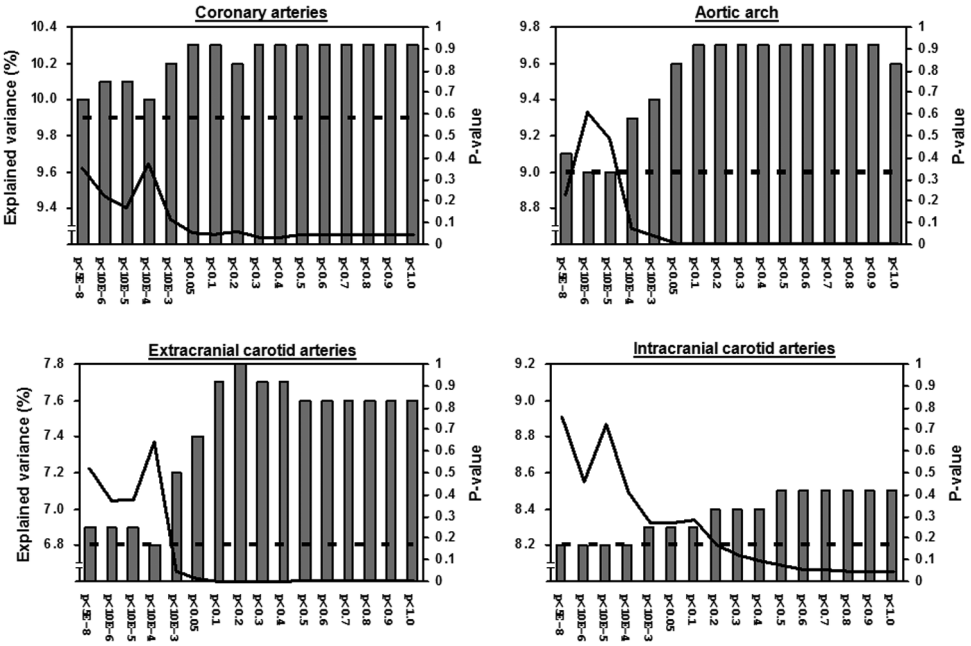

Figure 2. The explained variance of atherosclerotic calcification in four vessel beds by genetic risk scores in participants with calcification
The R-square of the genetic risk scores of systolic blood pressure, adjusted for age and sex, for each p-value threshold on calcification volume in the different vessel beds. The y1-axis (left; histogram) shows the explained variance for the different p-value thresholds of the risk scores (x-axis). The y2-axis (right; line) shows the p-values from the association analysis of the risk scores with calcification. The dotted line represents the attribution of age and sex to the explained variance.

CT is that it provides an accurate measure of the amount of calcification,[17, 23] and that different vessel beds can be measured in one session.

There are also several potential limitations that should be taken into account. First, we have a relatively small sample size, so any negative findings are harder to interpret. Second, calcification is only a part of the atherosclerotic plaque.[1] With CT it is not possible to visualize the total extent of the non-calcified atherosclerotic plaque. However, strong evidence suggests that calcification volume is an adequate measure for the total underlying plaque burden.[18, 19]

In the overall analyses, the relationship between the genetic risk scores for systolic blood pressure and calcification volume was only present in the extracranial carotid artery. This was an unexpected finding, since systolic blood pressure is more prominently associated with coronary or aortic atherosclerosis than with carotid atherosclerosis.[2, 14] Yet, when restricting our analyses to persons with

larger calcification volumes, defined as above the median, we found significant relationships between the genetic risk scores and calcification in all four vessel beds. Interestingly, systolic blood pressure is one of the strongest predictors of progression of atherosclerosis.[13] Our results indeed support that on a genetic level that systolic blood pressure is contributing to the amount of atherosclerosis, particularly if atherosclerosis is already present. Following this, systolic blood pressure may be an important determinant of progression of atherosclerosis.

We found that the statistical significance and strength of the relationship between the genetic risk scores and calcification volume increased with lower p-value thresholds (more SNPs) for the risk scores. Together with the increased percent explained variance, this is suggestive for a polygenic model underlying atherosclerosis, which overlaps with that of systolic blood pressure. Moreover, although low, the amount of variance explained by the genetic risk scores exceeded that of age and gender. Another interesting finding was that, as well as the strength of the associations, the amount of increase in the explained variance attributable to the risk scores varied per vessel bed. From an etiologic perspective this is interesting because previously it has been found that correlations between atherosclerotic disease across different vessel beds are only moderate.[14, 16] Together with our findings, it is therefore conceivable that there are etiological differences in the association between high blood pressure and atherosclerosis in different vessel beds on a genetic level.

Finally, we also added the blood pressure measurement at baseline (time of the CT-examination) to the regression model including age, sex and the genetic risk scores. This did not alter the relationship between the genetic risk scores and calcification volume. This suggests that genetic risk scores contain more information about blood pressure than a single measurement at one time point. Probably this is because information on the lifetime exposure to blood pressure is caught in these scores. A similar result was previously provided for a genetic risk score for glucose levels and its relation to subclinical atherosclerosis.[24]

In conclusion, we found evidence for a shared genetic basis between systolic blood pressure and atherosclerosis. Interestingly, especially in persons with larger volumes of calcification, blood pressure genes contributed considerably to the amount of calcification. Finally, we found prominent differences in these relationships across vessel beds, suggesting a differential genetic etiology.

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3

Neurological Consequences of Atherosclerotic Calcification

3.1

Atherosclerotic Calcification and Subclinical Cerebrovascular Disease

ABSTRACT

Background and Purpose

Calcification in atherosclerotic plaques is a novel marker of atherosclerosis and is related to cardiovascular disease. However, its relationship with cerebrovascular disease has not been investigated extensively. We investigate the relation between calcification in various vessel beds outside the brain and imaging markers of vascular brain disease.

Methods

A total of 885 community-dwelling persons (mean age 66.7 years) underwent CT of the coronary arteries, aortic arch, extracranial and intracranial carotid arteries to assess arterial calcification. Brain MRI scans were performed to assess cerebral infarcts, microbleeds and white matter lesions (WML). We assessed the relationship between atherosclerotic calcification volume and white matter lesion volume, using linear regression. Associations of calcification with the presence of infarcts or microbleeds were assessed with logistic regression. All analyses were adjusted for relevant confounders.

Results

Calcification in each vessel bed was associated with presence of cerebral infarcts and with larger WML volume. Most prominent associations were found between intracranial carotid calcification and WML volume and between extracranial carotid calcification and infarcts. Adjustment for cardiovascular risk factors or ultrasound carotid plaque scores did not change these results. No associations were found between calcification and cerebral microbleeds.

Conclusions

Arterial calcification in major vessel beds is associated with vascular brain disease on MRI. Most notably, we found that larger intracranial carotid calcification load relates to larger WML volumes and larger extracranial carotid calcification load to presence of cerebral infarcts, independent of ultrasound carotid plaque score. This suggests that calcification of atherosclerotic plaque yields additional information than merely presence of plaques, providing novel insights into the etiology of vascular brain disease.

INTRODUCTION

Atherosclerosis is a systemic vascular process which is considered a major cause of cardiovascular and cerebrovascular disease.[1] The more advanced stages consist of calcified plaques,[1] which can be assessed non-invasively with the use of computed tomography (CT).[2-3] Vessel calcification on CT is a predictor of coronary heart disease[4] and a potential marker for clinical stroke.[5]

White matter lesions (WML), cerebral infarcts and cerebral microbleeds are considered important magnetic resonance imaging (MRI) markers of vascular brain disease.[6] Various studies investigating associations between atherosclerosis and MRI-defined markers of vascular brain disease, found that coronary, carotid, and aortic atherosclerosis is associated with WML,[7-11] cerebral infarcts,[10-12] or cerebral microbleeds.[11] Yet, in most of these studies either plain radiographs or ultrasound-based carotid intimal-media-thickness (IMT) and plaque scores were used to measure atherosclerosis.[7,8,9,12] Although assessment of arterial calcification on CT was investigated several times in relation to clinical outcomes,[13] only two studies used CT-assessed arterial calcification in relation to subclinical MRI-markers of vascular brain disease,[10,11] and did so only in the coronary arteries. Finally, quantification of intracranial carotid calcification has hardly been performed.[14]

An important advantage of CT is the fact that multiple vessel beds which may have relevant effects on the brain can be examined at once. Previously, moderate correlations were found between CT-calcification in the coronary arteries, aortic arch and extracranial carotid arteries,[15] suggesting that calcification in different vessel beds may have a different role in disease prediction.

Furthermore, correlations between plaque assessed by ultrasound methods and calcified plaque assessed with CT have found to be only modest,[16] supporting the hypothesis that calcified plaque may carry independent information as well.

In the population-based Rotterdam study, we quantified CT-calcification in four major vessel beds, including the intracranial carotid artery, in almost 900 participants. The purpose of this study was to explore associations between calcification in these vessel beds and MRI-markers of vascular brain disease.

MATERIALS AND METHODS

Setting and Study Population

This study is based on the Rotterdam Study, a prospective, population-based cohort study on determinants of diseases in the elderly.[17] The study started in 1990 when all inhabitants of a suburb of Rotterdam, aged of 55 years or over, were invited to participate.[17] In 2000, the original cohort of 7,983 persons was extended with 3,011 participants with the same inclusion criteria. From 2003 onwards, all participants completing a center visit were invited to undergo CT of the heart, aorta and carotid arteries. A total of 2,524 participants were scanned.

From August 2005 to May 2006, a sample of 1,073 participants from the 2000 cohort extension was randomly selected for participation in the Rotterdam Scan Study, a prospective brain MRI study. Of these participants, 885 also underwent a complete CT-examination, comprising the current study population. The mean interscan interval was eight months (± five months). This study was approved by the Medical Ethics Committee at Erasmus MC, the Netherlands. All participants gave informed consent.

CT Acquisition and Processing

Non-contrast CT was performed using a 16-slice (n=785) or 64-slice (n=1,739) multi-detector CT-scanner (SOMATOM Sensation 16 or 64, Siemens, Germany). Using a cardiac scan and an extra-cardiac scan that reached from the aortic arch to the intracranial circulation (1cm above the sella turcica), four vessel beds were examined: the coronary arteries, the aortic arch, the extracranial and the intracranial carotid arteries. Imaging parameters of both scans are described elsewhere.[15]

Dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany) was used to quantify calcification in the coronary arteries, aortic arch, and extracranial carotid arteries.[15] Automatic quantification of the calcifications in the intracranial internal carotid arteries was not possible due to the close relationship of calcium in the arterial wall with the skull. Therefore, a custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, USA; available at: http://rsb.info.nih.gov/ij) was used.[14] With this plug-in it was possible to manually draw regions of interest (ROI) in axial MDCT-images and to calculate the number of pixels above a predefined Hounsfield value (HU). Thereafter, the volume of the calcification was calculated as the product of the number of pixels above the threshold (130 HU),[2] the pixel size and the increment.

The inter-rater reliability of this method is very good. Based on the ratings of two observers on fifty CT examinations, the intra-class correlation coefficient was 0.99. All calcification volumes are expressed as cubic millimeters (mm^3).

MRI Acquisition and Processing

Brain MRI scans were obtained with a 1.5T-scanner (GE Healthcare, Milwaukee, Wisconsin, USA).[18] The MRI protocol included a T1-weighted (T1w) sequence, a proton density-weighted (PDw) sequence, a fluid-attenuated-inversion-recovery (FLAIR) sequence and a T2*-weighted gradient echo (GRE) sequence. The slice thickness was 1.6mm for all sequences except for the FLAIR sequence (2.5mm). No contrast material was administered.

A previously described validated automated tissue-classification technique was used to classify WML volumes in milliliters (ml).[19] WML were automatically segmented on the FLAIR sequence using an atlas-based k-nearest-neighbor-classifier.[19]

Lacunar and cortical infarcts were rated on the FLAIR, PDw and T1w sequences, and microbleeds on the T2*GRE sequence, blinded for the results of the CT-calcification score.[20] Microbleeds were categorized into one of three locations: lobar, deep or infratentorial.[20]

Assessment of Cardiovascular Risk Factors

Cardiovascular risk factors were assessed by interview, physical examination and laboratory tests as described previously.[20] Ultrasound carotid plaque scores were calculated as follows. The common carotid artery, carotid bifurcation, and internal carotid artery were visualized over a length as large as possible and examined both left and right for the presence of plaques.[21] Briefly, a weighted plaque score ranging from 0 to 6 was computed by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultrasonographic image was available and multiplied by 6 (the maximum number of sites).[21]

Statistical Analysis

Correlations between calcification in the vessel beds were calculated using Spearman's correlation coefficient. In all analyses, calcification in each vessel bed was handled in two ways: using sex-specific quartiles of calcification and using calcification as continuous measure. Sex-specific quartiles were calculated because calcification load significantly differed between men and women, had a non-normal distribution, and to obtain results that were comparable with previous studies.[10-11] In the analyses with calcification as continuous measure (per SD increase) we used natural log-transformed values and added 1.0 mm^3

to the non-transformed values in order to deal with participants with a calcium score of zero. Due to the positive skewness of the WML volume distribution, this measure was natural log-transformed.

Associations between atherosclerotic calcification and WML volume were explored by linear regression; for presence of cerebral infarcts and microbleeds by logistic regression. Analyses with WML volume as outcome were corrected for individual head size. Persons with microbleeds were categorized as "strictly lobar" (restricted to lobar locations) or "deep and/or infratentorial" (deep or infratentorial location, with or without lobar microbleeds present).[20] All analyses were performed per vessel bed. To test whether the associations found per vessel bed were independent of calcification elsewhere, a model was created in which all vessel beds were entered together. All analyses were adjusted for age, sex, and additionally for cardiovascular risk factors. Finally, all analyses were adjusted for carotid plaque scores. SPSS 17.0 (Illinois, USA) was used for statistical analyses.

RESULTS

Table 1 describes the characteristics of the study population. Mean age at the time of the CT-scan was 66.7 (± 5.5) years and 50.8 % of the participants were women. The calcification load differed substantially between men and women (Table 2). The correlations between calcification in various vessel beds ranged from 0.45 to 0.55 (Table 3).

With increasing calcification volume quartile in all vessel beds, persons had larger WML volumes (Table 4). The most prominent association was between intracranial carotid calcification and WML volume, especially for the fourth quartile as compared to the first. Additional adjustment for cardiovascular risk factors or ultrasound carotid plaque scores did not materially change these associations.

Arterial calcification in all vessel beds was also associated with the presence of cerebral infarcts (Table 5). After adjustment for cardiovascular risk factors and ultrasound carotid plaque scores, the association between coronary artery calcification and the presence of cerebral infarcts was no longer significant. Among the other vessel beds, the associations for extra- and intracranial carotid calcification remained the most prominent. Subtyping the infarcts into cortical and lacunar infarcts rendered the number of cortical infarcts per quartile too low to yield meaningful results. However, when analyzed per SD increase in calcification volume, aortic arch calcification was significantly associated with the

Table 1. Population characteristics

Sample size	885
Women	50.8 %
Age, years	66.7 (5.5)
Body mass index, kg/m^2	27.5 (3.7)
Systolic blood pressure, mmHg	143.8 (18.5)
Diastolic blood pressure, mmHg	81.0 (10.2)
Diabetes	10.2
Serum total cholesterol, mmol/l	5.7 (1.0)
Serum HDL cholesterol, mmol/l	1.4 (0.4)
Past or current smokers	68.9 %
Use of blood pressure-lowering medication	36.6 %
Use of lipid-lowering medication	22.5 %
Ultrasound-based carotid plaque score	
0	21.0 %
1	18.8 %
2	19.0 %
≥3	39.0 %
White matter lesion volume*, ml	3.57 (2.14 - 7.01)
Cerebral infarct	9.0 %
Cerebral microbleed	19.9 %

HDL, high density lipoprotein
Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables
* Median (interquartile range)

presence of cortical infarcts and both extra- and intracranial carotid calcification to the presence of lacunar infarcts.

No associations were found between calcifications and the presence of any microbleeds (Table 6). Subtyping the microbleeds according to location did not yield any associations either.

Table 2. Sex-specific quartiles of calcification distribution

		Calcification volumes, mm^3			
		Coronary arteries	Aortic arch	Extracranial carotid arteries	Intracranial carotid arteries
Men	25th percentile	8.8	21.4	0.1	5.9
	50th percentile	80.4	168.1	21.4	40.3
	75th percentile	322.1	666.1	102.0	126.9
Women	25th percentile	0	17.1	0	3.2
	50th percentile	6.0	134.5	4.1	21.8
	75th percentile	63.3	480.1	40.3	78.5

After entering all vessel bed calcifications into one model, the separate associations remained no longer significant except for the above described associations between intracranial carotid calcification and WML volume and extracranial carotid calcification and the presence of brain infarcts.

Table 3. Correlations between calcification in different vessel beds

	Coronary arteries	Aortic arch	Extracranial carotid arteries	Intracranial carotid arteries
Coronary arteries	X	0.45	0.48	0.48
Aortic arch	0.45	X	0.55	0.50
Extracranial carotid arteries	0.48	0.55	X	0.53
Intracranial carotid arteries	0.48	0.50	0.53	X

Values represent Spearman's correlation coefficients ($P < 0.01$ for all correlations)

Table 4. Calcification in different vessel beds and white matter lesion volume

		White matter lesion volume* Difference (95% CI)		
		Model 1	Model 2	Model 3
Coronary calcification	1st	0	0	0
	2nd	0.22 (0.06;0.37)	0.19 (0.04;0.35)	0.20 (0.04;0.35)
	3rd	0.23 (0.08;0.38)	0.21 (0.06;0.37)	0.19 (0.03;0.34)
	4th	0.25 (0.10;0.41)	0.23 (0.07;0.39)	0.19 (0.03;0.35)
	Per SD	0.11 (0.05;0.17)	0.10 (0.04;0.17)	0.09 (0.02;0.15)
Aortic arch calcification	1st	0	0	0
	2nd	0.00 (-0.16;0.15)	0.00 (-0.15;0.15)	-0.01 (-0.17;0.14)
	3rd	0.14 (-0.02;0.29)	0.12 (-0.04;0.28)	0.10 (-0.07;0.26)
	4th	0.28 (0.11;0.44)	0.28 (0.11;0.45)	0.21 (0.03;0.39)
	Per SD	0.11 (0.05;0.17)	0.10 (0.04;0.16)	0.08 (0.02;0.15)
Extracranial carotid calcification	1st	0	0	0
	2nd	0.00 (-0.17;0.16)	0.00 (-0.17;0.16)	-0.03 (-0.19;0.14)
	3rd	0.11 (-0.03;0.26)	0.11 (-0.04;0.25)	0.06 (-0.10;0.22)
	4th	0.31 (0.16;0.46)	0.29 (0.14;0.45)	0.27 (0.09;0.45)
	Per SD	0.13 (0.07;0.19)	0.13 (0.07;0.19)	0.12 (0.05;0.19)
Intracranial carotid calcification	1st	0	0	0
	2nd	0.08 (-0.08;0.23)	0.05 (-0.10;0.21)	0.07 (-0.09;0.22)
	3rd	0.15 (-0.01;0.30)	0.15 (-0.01;0.31)	0.13 (-0.03;0.29)
	4th	0.37 (0.21;0.53)	0.36 (0.19;0.53)	0.32 (0.15;0.49)
	Per SD	0.13 (0.07;0.18)	0.12 (0.06;0.18)	0.11 (0.05;0.17)

CI, confidence interval; SD, standard deviation
*Difference in WML-volume (ln-transformed) for each quartile of vessel calcification compared to the lowest quartile, and per SD increase in standardized calcification volume [ln(calcification + 1.0 mm^3)]
Model 1: Adjusted for age, sex, ICV
Model 2: Adjusted for age, sex, ICV, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking
Model 3: Adjusted for age, sex, ICV, and ultrasound carotid plaque scores

Table 5.Calcification in different vessel beds and presence of cerebral infarcts

		Presence of cerebral infarcts* OR (95% CI)		
		Model 1	Model 2	Model 3
Coronary calcification	1st	1	1	1
	2nd	1.35 (0.61;3.00)	1.17 (0.52;2.64)	1.04 (0.45;2.40)
	3rd	1.45 (0.68;3.12)	1.29 (0.59;2.81)	1.36 (0.63;2.93)
	4th	2.33 (1.13;4.79)	1.93 (0.91;4.09)	2.04 (0.96;4.33)
	Per SD	1.38 (1.05;1.82	1.28 (0.95;1.71)	1.34 (0.99;1.79)
Aortic arch calcification	1st	1	1	1
	2nd	1.32 (0.57;3.08)	1.39 (0.59;3.28)	1.26 (0.54;2.95)
	3rd	1.27 (0.55;2.95)	1.18 (0.49;2.80)	1.04 (0.43;2.49)
	4th	3.14 (1.45;6.82)	2.99 (1.32;6.77)	2.72 (1.19;6.23)
	Per SD	1.49 (1.10;2.01)	1.41 (1.02;1.94)	1.38 (1.00;1.91)
Extracranial carotid calcification	1st	1	1	1
	2nd	2.01 (0.86;4.72)	1.99 (0.83;4.73)	1.93 (0.80;4.65)
	3rd	2.11 (0.94;4.67)	2.11 (0.94;4.75)	2.02 (0.85;4.83)
	4th	3.58 (1.68;7.61)	3.44 (1.57;7.52)	3.47 (1.42;8.52)
	Per SD	1.58 (1.22;2.06)	1.54 (1.18;2.02)	1.61 (1.15;2.25)
Intracranial carotid calcification	1st	1	1	1
	2nd	1.38 (0.58;3.29)	1.44 (0.59;3.48)	1.24 (0.51;3.02)
	3rd	2.17 (0.96;4.89)	2.16 (0.94;4.98)	2.02 (0.88;4.64)
	4th	2.95 (1.32;6.60)	2.79 (1.21;6.43)	2.46 (1.06;5.73)
	Per SD	1.67 (1.25;2.23)	1.63 (1.20;2.21)	1.60 (1.18;2.18)

OR, odds ratio; CI, confidence interval; SD, standard deviation
*OR for each quartile of vessel calcification compared to the lowest quartile, and per SD increase in standardized calcification volume [ln(calcification + 1.0 mm^3)]
Model 1: Adjusted for age, sex
Model 2: Adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking
Model 3: Adjusted for age, sex, and ultrasound carotid plaque scores

Table 6.Calcification in different vessel beds and presence of cerebral microbleeds

		Presence of cerebral microbleeds* OR (95% CI)		
		Model 1	Model 2	Model 3
Coronary calcification	1st	1	1	1
	2nd	1.25 (0.78;2.04)	1.22 (0.75;1.99)	1.17 (0.72;1.91)
	3rd	0.84 (0.51;1.38)	0.83 (0.50;1.38)	0.80 (0.48;1.33)
	4th	1.36 (0.85;2.17)	1.36 (0.83;2.23)	1.26 (0.77;2.07)
	Per SD	1.01 (0.88;1.27)	1.06 (0.87;1.29)	1.03 (0.84;1.25)
Aortic arch calcification	1st	1	1	1
	2nd	0.95 (0.57;1.60)	0.98 (0.58;1.65)	0.97 (0.57;1.63)
	3rd	1.41 (0.87;2.31)	1.46 (0.88;2.43)	1.40 (0.84;2.33)
	4th	1.41 (0.84;2.36)	1.40 (0.81;2.42)	1.42 (0.82;2.47)
	Per SD	1.12 (0.92;1.35)	1.10 (0.90;1.35)	1.10 (0.90;1.35)
Extracranial carotid calcification	1st	1	1	1
	2nd	1.10 (0.65;1.86)	1.09 (0.64;1.85)	1.07 (0.63;1.84)
	3rd	1.05 (0.67;1.67)	1.05 (0.66;1.68)	1.00 (0.60;1.65)
	4th	1.17 (0.74;1.84)	1.12 (0.69;1.81)	1.07 (0.61;1.85)
	Per SD	1.09 (0.91;1.30)	1.07 (0.89;1.29)	1.07 (0.86;1.33)
Intracranial carotid calcification	1st	1	1	1
	2nd	1.04 (0.63;1.70)	1.10 (0.67;1.81)	1.00 (0.61;1.64)
	3rd	0.91 (0.55;1.51)	1.01 (0.60;1.70)	0.86 (0.51;1.44)
	4th	1.45 (0.88;2.37)	1.54 (0.92;2.59)	1.40 (0.83;2.36)
	Per SD	1.12 (0.94;1.35)	1.15 (0.95;1.39)	1.11 (0.91;1.34)

OR, odds ratio; CI, confidence interval; SD, standard deviation
*OR for each quartile of vessel calcification compared to the lowest quartile, and per SD increase in standardized calcification volume [ln(calcification + 1.0 mm^3)]
Model 1: Adjusted for age, sex
Model 2: Adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking
Model 3: Adjusted for age, sex, and ultrasound carotid plaque scores

DISCUSSION

In a large sample from the general population, we found that higher CT-calcification load was associated with larger WML volume and with the presence of cerebral infarcts, but not with presence of cerebral microbleeds. This study is the first in which atherosclerotic calcification, as a measure of atherosclerosis, was quantified in four major vessel beds by the same diagnostic tool (CT).

A possible limitation concerns the fact that the calcified lesion on CT is only a part of the complete atherosclerotic plaque. The "soft" part of the plaque, which is presumed to be most vulnerable and generally does not contain calcium,[1] was therefore not measured. Furthermore, it has been suggested that calcification in carotid or aortic plaques, in patients with atherosclerosis, might actually be a plaque-stabilizing agent and may reduce the risk of stroke.[13, 22-23] An important difference with our study is that we used calcification as an indicator of atherosclerotic burden in a healthy population,[23-24] to study cross-sectionally how this relates to presence of vascular brain disease.

We found that higher CT-calcification load was related to larger WML volumes. This is consistent with the findings of others who studied this relationship only for the coronary arteries.[10-11] Yet, we found that the associations appeared stronger when the investigated vessel bed was closer to the brain, with the most prominent association between intracranial carotid calcification and WML volume. Others also reported that qualitatively-graded CT-calcification of the intracranial carotid artery was associated with white matter intensities.[25] In contrast, others found no association between extracranial or intracranial carotid calcification and WML.[26,27] Both studies assessed WML using a qualitative grading system instead of quantified volumes. This may have led to a loss of information and misclassification resulting in an underestimation of the true associations.

Vidal et al. described a significant association between coronary calcification load and the presence of MRI-defined cerebral infarcts.[11] We found the same results, however after adjustment for cardiovascular risk factors and ultrasound plaque scores, these associations were no longer significant. In our data, it was mainly calcification in the extracranial carotid artery which was associated with the presence of cerebral infarcts. This is in line with a study in stroke patients that showed an association between carotid calcification and acute lacunar infarcts on diffusion imaging,[28] but not with two others who found no association.[14,29] The small number of cortical infarcts did not allow us to analyze infarcts divided into cortical and lacunar infarcts per quartile of CT-calcification. However, when analyzed continuously, aortic arch calcification was significantly associated with cortical infarcts, whereas both extra- and intracranial carotid calcification was

associated with the presence of lacunar infarcts. It may be hypothesized that this contrast reflects the presumed underlying pathophysiology, i.e. that WML and lacunar brain infarcts are primarily an expression of cerebral small vessel disease[6] and that cortical brain infarcts are to a large extent related to embolic or stenotic disease in larger vessels.[30]

Only one study reported associations between vessel calcification (coronary arteries) and cerebral microbleeds.[11] We found no significant associations between calcification in any vessel bed and the presence of microbleeds. Categorizing microbleeds according to location did not alter these findings.

We found that calcification in the different vessel beds correlated and that the associations with vascular brain disease on MRI were partly interrelated. This reflects that atherosclerosis is a generalized process and that calcification in one vessel bed partly reflects the vascular status elsewhere in the body. However, several associations remained when adjusting for atherosclerosis in other vessel beds. This was mainly true for calcification in the carotid arteries; again supporting the notion that atherosclerosis in vessels closer to the brain has a larger impact on vascular brain pathology.[30]

When adjusted for ultrasound plaque scores, the associations between CT-assessed calcifications and vascular brain disease did not change materially. From an etiological point of view, this indicates that the knowledge of whether or not a plaque is calcified provides additional information over plaque load alone. Another benefit of CT is that more vessel beds can be evaluated at the same time.

An important drawback which should be kept in mind though is that the applicability on a large scale of CT-calcification measurement is hampered by radiation exposure, especially in neurologically healthy persons and requires further research. Yet, although outside the scope of the present study, we can speculate about the possibilities for clinical applicability. Nowadays, CT is being used more frequently both for diagnostic as well as screening purposes, for example, for assessment of coronary pathology.[31] Such a CT-scan provides information both on coronary as well as aortic calcification. Our results show that calcification in these vessel beds is not only informative regarding cardiac disease, but also relates to vascular brain disease. Thus, calcium scoring in clinical practice would for example be informative and applicable in persons who receive a CT-examination for other reasons. Another important note is that newer generation CT-scanners, especially dual source and dual energy scanners, will lead to much lower radiation doses in the near future.

In conclusion, this study shows that arterial calcifications in different vessel beds contribute to WML volume and the presence of cerebral infarcts. Compared to ultrasound plaque imaging, quantification of CT-calcification provides additional information with regard to pathophysiology of ischemic brain disease.

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3.2

Atherosclerotic Calcification, Cognition, and Brain Atrophy

ABSTRACT

Background and Purpose

Increasing evidence suggests a role of atherosclerosis in the pathogenesis of cognitive impairment and dementia. Calcification volume measured with computed tomography (CT) is a valid marker of atherosclerosis. This study investigates associations between atherosclerosis (measured using CT) at four locations, cognition and brain changes on MRI.

Methods

To quantify calcification volume, 2414 people from the Rotterdam Study underwent CT of the coronary arteries, aortic arch, extracranial and intracranial carotid arteries. To assess global cognition and performance on memory, executive function, information-processing speed and motor speed, they also underwent neuropsychological tests. In a random subgroup of 844 participants, brain MRI was performed. Automated segmentation and quantification of brain MRI scans yielded brain-tissue volumes in milliliters. Diffusion-tensor imaging (DTI) was used to measure the microstructural integrity of the white matter. Relationships of atherosclerotic calcification with cognition, brain-tissue volumes and DTI measures were assessed with linear regression models and adjusted for relevant confounders.

Results

We found that larger calcification volumes in all vessel beds were associated with lower cognitive scores. Larger calcification volumes were also related with smaller total brain volumes. Specifically, larger extracranial and intracranial carotid calcification volume related to smaller white matter volume. Larger calcification volume in all vessel beds was accompanied by worse microstructural integrity of the white matter.

Conclusions

Larger calcification volume is associated with worse cognitive performance. A larger calcification volume also relates to smaller brain-tissue volumes and worse white matter microstructural integrity, revealing possible mechanisms through which atherosclerosis may lead to poorer cognition.

INTRODUCTION

Increasing evidence suggests a role of atherosclerosis in the development of cognitive impairment,[1, 2] and dementia.[3, 4] Vascular brain injury may underlie this association and can be visualized using magnetic resonance imaging (MRI).[5-7] However, not all studies show consistent results.[8, 9] A potential source of discrepancy between studies could be the use of different measures of atherosclerosis, of which carotid ultrasound is the most frequently used.

Computed tomography (CT) assessed calcification volume is a more novel, observer-independent and sensitive measure to reliably assess atherosclerosis.[10, 11] Two previous studies suggest that CT-assessed calcification is associated with cerebral atrophy on MRI and with poorer cognitive performance.[12, 13] However, these studies only examined calcification in the coronary arteries. Investigating arteries that supply blood directly to the brain, i.e. extracranial and intracranial carotid arteries, may demonstrate clearer associations and also provide direct etiological clues on the relationship between atherosclerosis and brain pathology. Previous studies showing only moderate correlations indeed suggest differences between calcification across vessel beds.[14, 15]

Another consideration is that most previous studies assessed brain pathology using visual rating scales. Advances in MRI and post-processing techniques allow automatic quantification of brain tissue volumes as a more precise measure of brain pathology. Furthermore, novel MRI-sequences such as diffusion-tensor imaging (DTI) yield information on the microstructural integrity of white matter,[16] which is independently associated with cognitive function.[17]

Previously, we reported that CT-assessed calcification volume in various vessel beds is strongly associated with focal brain pathology, i.e. lacunar infarcts and white matter lesions.[18] In the current study we investigated, in the Rotterdam Study, the associations of atherosclerotic calcification on CT (measured in four major vessel beds) with cognitive function, and global brain pathology on MRI. Furthermore, we explored associations with the microstructural brain tissue integrity.

MATERIALS AND METHODS

Setting and Study Population

This study is based on the Rotterdam Study,[19] a prospective, population-based study, aimed at investigating determinants of chronic diseases in the elderly. The original cohort comprised 7,983 participants aged 55 years or older and was extended in 2000-2001 with an additional 3,011 persons. From 2003 onwards, all participants who visited the research center were invited to undergo CT of the heart, aorta and carotid arteries. In total, 2,524 participants were scanned. Due to image artefacts, 110 scans were not gradable, leaving a total of 2,414 complete CT examinations.

From August 2005 to May 2006, a sample of 1,073 participants from the 2000 cohort extension was randomly selected for participation in the Rotterdam Scan Study, a prospective brain MRI study. Of these participants, 885 participants also underwent a complete CT-examination. All participants with cortical infarcts on MRI were excluded (n = 41), because brain segmentation did not yield reliable results in those cases. This leaves a total of 844 participants in the current study with both a CT and MRI examination. The mean inter-scan interval was eight months (± five months). This study was approved by the Medical Ethics Committee at Erasmus MC, the Netherlands. All participants gave informed consent.

CT Acquisition and Processing

A 16-slice (n=785) or 64-slice (n=1,739) multi-detector CT-scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany) was used to perform non-contrast CT-scanning. Using a cardiac scan and a scan which reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica), the following vessels were scanned: the coronary arteries, the aortic arch, the extracranial carotid arteries and the intracranial carotid arteries. Detailed information regarding imaging parameters of both scans is described elsewhere.[20]

Dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany) was used to quantify calcification volume in the coronary arteries, aortic arch, and extracranial carotid arteries. Automatic calcification quantification of calcification in the intracranial internal carotid artery was impossible due to the close relationship between calcium in the arterial wall and the skull. Hence, we used a semi-automatic tool to score calcification in this region.[18, 21] Briefly, this method consisted of delineating calcification manually in each consecutive CT slice, and calculating the amount of calcification by multiplying the number of pixels above the threshold (130 HU)[22] with the pixel size and the slice increment. All calcification volumes are expressed in cubic millimeters (mm^3). The aortic arch was measured from the origin to the first centimeter of the common carotid ar-

teries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The extracranial carotid arteries were measured at both sides within 3 cm proximally and distally of the bifurcation. The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery.

Assessment of Cognitive Function

In the Rotterdam Study, all participants undergo the following neuropsychological tests: a 15-Word Verbal Learning Task, the Stroop test, the Letter-Digit Substitution Task (LDST), the Purdue Pegboard test and a Word-Fluency test. Per participant, we calculated Z-scores for each test. Z-scores for the Stroop test were inverted, because higher scores indicate worse performance whilst higher scores on the other tests indicate better performance. On the basis of the individual test scores, we constructed compound scores for memory, executive function, information-processing speed, global cognition and motor speed in order to obtain more robust outcome measures.[17]

MRI Acquisition and Processing

MRI-scanning was performed on a 1.5T-scanner with an eight-channel head coil (GE Healthcare, Milwaukee, Wisconsin, USA), and included a T1-weighted (T1w) sequence, a proton-density (PDw) weighted sequence, a fluid-attenuated-inversion-recovery (FLAIR) sequence, and a diffusion tensor imaging (DTI) sequence.[23] Automated brain tissue classification based on a k-nearest-neighbor-classifier algorithm extended with white matter lesion segmentation was used to quantify brain volume, grey matter volume, white matter volume, white matter lesion volume and intracranial volume (in milliliters).[24, 25]

Concerning DTI, fractional anisotropy (FA) and mean diffusivity (MD) were measured globally in normal-appearing white matter.[23]

Other Measurements

Detailed information on cardiovascular risk factors was gathered by interview, physical examination and laboratory tests. The risk factors included: body-mass index (BMI), systolic and diastolic blood pressure, diabetes mellitus, cholesterol levels, smoking status and the use of blood-pressure lowering or lipid-lowering medication.[17] Level of education was assessed by self-report. APOE genotyping was performed on coded genomic DNA samples and was coded positive (carrier of one or two ε4-alleles) or negative (non-carrier).[26] With use of ultrasound the common carotid artery, carotid bifurcation, and internal carotid artery were visualized over a length as great as possible and examined both left and right to assess a weighted plaque score ranging from 0 to 6.[27]

Statistical Analysis

Since calcification volume had a highly skewed, non-normal distribution, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values in order to deal with calcium scores of zero [Ln(calcification + 1.0 mm^3)]. Calcification volume was investigated both as a continuous measure and as sex-specific quartiles, due to substantial differences between men and women.

We used linear regression models to investigate the relationship between calcification volume per vessel bed, cognitive function, brain-tissue volumes, and microstructural integrity. In the linear regression models with cognition as outcome, associations were adjusted for age, sex, and educational level (model 1). Additionally, these associations were adjusted for *APOE* ε4-carriership, BMI, systolic and diastolic blood pressure, diabetes mellitus, cholesterol levels, smoking status and the use of blood-pressure lowering or lipid-lowering medication (model 2). The linear regression models with brain-tissue volumes as outcome were identical except that in all models we also adjusted for total head size (total intracranial volume: ICV). Additionally, we constructed a third model in which we adjusted for white matter lesion volume and the presence of lacunar infarcts. Analyses with microstructural integrity as outcome were all additionally adjusted for white matter volume. Finally, we re-analysed all estimates adjusting for ultrasound carotid plaque scores to explore whether atherosclerotic calcification yields additional information over plaque scores. SPSS 17.0 (IBM, Chicago, IL, www.spss.com) was used for the statistical analyses.

RESULTS

Table 1 shows the characteristics of the study population. Mean age at the time of the CT-scan was 69.5 (± 6.7) years and 52.4 % of the participants were women.

We found that larger coronary artery calcification volumes were associated with lower scores in information-processing speed and motor speed (age- and sex-adjusted difference in Z-score per SD increase in calcification volume: -0.04 (95% CI: -0.07;0.00) and -0.06 (95% CI -0.10;-0.02), respectively). For larger aortic arch calcification volumes we found significantly lower cognitive scores in all domains. We also found that calcification in the extracranial or intracranial carotid arteries was associated with lower cognitive scores in all domains, except memory (difference in Z-score per SD increase in calcification volume: -0.03 (95% CI: -0.07;0.01) and -0.04 (95% CI: -0.08;0.00), respectively). We found the most prominent associations with cognitive scores for aortic arch calcification (Table 2, model 1). These differences remained present after adjustment for *APOE* ε4-

Table 1. Population characteristics

Sample size	2,414
Women	52.4 %
Age, years	69.5 (6.7)
Highest education attained:	
Primary education	10.6 %
Low level vocational training	21.3 %
Medium level secondary training	18.1 %
Medium level vocational to university training	49.9 %
Body mass index, kg/m^2	27.7 (4.0)
Systolic blood pressure, mmHg	146.8 (20.1)
Diastolic blood pressure, mmHg	80.2 (10.7)
Diabetes	11.1 %
Serum total cholesterol, mmol/l	5.7 (1.0)
Serum HDL cholesterol, mmol/l	1.5 (0.4)
Past or current smokers	67.2 %
Use of blood pressure-lowering medication	39.3 %
Use of lipid-lowering medication	22.9 %
APOE ε4-carriers	25.8 %
Brain MRI tissue volumes (n=844)	
Total brain volume, ml	923.5 (93.3)
Grey matter volume, ml	522.3 (51.1)
White matter volume, ml	394.8 (56.2)
White matter lesion volume*, ml	1.4 (0.9)

Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables
*ln-transformed

carriership and cardiovascular risk factors, whilst most associations between coronary artery, extracranial and intracranial carotid artery calcification diminished after adjustment (Model 2, table 2). Figure 1A shows the relationship between sex-specific quartiles of calcification and global cognition.

In the subgroup of participants with brain MRI, we found that larger calcification volume in all vessel beds was associated with smaller total brain volume (Table

Table 2. Calcification in different vessel beds and cognition

	Memory	Executive function	Information-processing speed	Global cognition	Motor speed
	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)
Per SD increase in:			Model 1		
Coronary calcification	-0.01 (-0.06;0.03)	-0.03(-0.06;0.00)	-0.04(-0.07;0.00)	-0.03(-0.05;0.00)	-0.06(-0.10;-0.02)
Aortic arch calcification	-0.06(-0.10;-0.01)	-0.06(-0.09;-0.03)	-0.07(-0.11;-0.04)	-0.06(-0.09;-0.03)	-0.06(-0.10;-0.02)
Extracranial carotid calcification	-0.03(-0.07;0.01)	-0.04(-0.07;-0.01)	-0.05(-0.09;-0.02)	-0.04(-0.07;-0.01)	-0.09(-0.13;-0.05)
Intracranial carotid calcification	-0.04(-0.08;0.00)	-0.05(-0.08;-0.02)	-0.04(-0.08;-0.01)	-0.05(-0.08;-0.02)	-0.06(-0.10;-0.02)
			Model 2		
Coronary calcification	0.00(-0.05;0.05)	-0.02(-0.06;0.02)	-0.03(-0.06;0.01)	-0.01(-0.04;0.02)	-0.06(-0.10;-0.01)
Aortic arch calcification	-0.05(-0.10;-0.01)	-0.06(-0.10;-0.03)	-0.07(-0.10;-0.03)	-0.06(-0.09;-0.03)	-0.06(-0.10;-0.01)
Extracranial carotid calcification	-0.02(-0.06;0.03)	-0.03(-0.06;0.00)	-0.04(-0.08;-0.01)	-0.02(-0.05;0.01)	-0.10(-0.14;-0.06)
Intracranial carotid calcification	-0.03(-0.07;0.02)	-0.03(-0.07;0.00)	-0.02(-0.06;0.01)	-0.03(-0.06;0.00)	-0.06(-0.10;-0.02)

CI, confidence interval; SD, standard deviation
Model 1: Adjusted for age, sex and educational level
Model 2: Adjusted for age, sex, educational level, *APOE* ε4-carriership, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking

Table 3. Calcification in different vessel beds and brain-tissue volumes

	Total brain volume	**Grey matter volume**	**White matter volume**
	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)
Per SD increase in:		Model 1	
Coronary calcification	-0.04(-0.07;-0.01)	-0.06(-0.10;-0.01)	-0.03(-0.08;0.02)
Aortic arch calcification	-0.06(-0.08;-0.03)	-0.05(-0.09;0.00)	-0.07(-0.11;-0.02)
Extracranial carotid calcification	-0.05(-0.08;-0.02)	-0.02(-0.06;0.03)	-0.09(-0.13;-0.04)
Intracranial carotid calcification	-0.05(-0.08;-0.02)	-0.02(-0.07;0.02)	-0.08(-0.12;-0.04)
		Model 2	
Coronary calcification	-0.03(-0.06;0.00)	-0.06(-0.11;-0.01)	-0.02(-0.07;0.03)
Aortic arch calcification	-0.05(-0.08;-0.02)	-0.05(-0.09;0.00)	-0.04(-0.09;0.01)
Extracranial carotid calcification	-0.04(-0.07;-0.02)	-0.02(-0.07;0.02)	-0.07(-0.12;-0.03)
Intracranial carotid calcification	-0.04(-0.07;-0.01)	-0.02(-0.06;0.03)	-0.07(-0.12;-0.02)
		Model 3	
Coronary calcification	-0.03(-0.06;0.00)	-0.05(-0.09;0.00)	-0.01(-0.06;0.03)
Aortic arch calcification	-0.05(-0.08;-0.03)	-0.04(-0.08;0.01)	-0.05(-0.10;-0.01)
Extracranial carotid calcification	-0.05(-0.07;-0.02)	-0.01(-0.05;0.04)	-0.07(-0.11;-0.02)
Intracranial carotid calcification	-0.05(-0.07;-0.02)	-0.01(-0.06;0.03)	-0.06(-0.11;-0.02)

CI, confidence interval; SD, standard deviation
Model 1: Adjusted for age, gender and total intracranial volume
Model 2: Adjusted for age, gender, total intracranial volume, ApoE4-carriership, body mass index, systolic blood pressure, diastolic blood pressure, blood pressure-lowering medication, diabetes, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking
Model 3: Adjusted for age, gender, total intracranial volume, lacunar infarcts and white matter lesion volume

3). Specified for separate brain tissues, we found that larger coronary artery calcification volume related to smaller grey matter volumes (difference in Z-score per SD increase in calcification volume: -0.06 (95% CI: -0.10;-0.01)). On the other

Table 4. Calcification in different vessel beds and white matter microstructural integrity

	Mean FA in NAWM	**Mean MD in NAWM**
	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)
Per SD increase in:	Model 1	
Coronary calcification	-0.05(-0.12;0.03)	0.08(0.01;0.15)
Aortic arch calcification	-0.09(-0.16;-0.02)	0.11(0.04;0.18)
Extracranial carotid calcification	-0.09(-0.16;-0.02)	0.10(0.03;0.17)
Intracranial carotid calcification	-0.09(-0.16;-0.02)	0.14(0.08;0.21)
	Model 2	
Coronary calcification	-0.02(-0.09;0.05)	0.04(-0.03;0.10)
Aortic arch calcification	-0.06(-0.13;0.00)	0.05(-0.01;0.11)
Extracranial carotid calcification	-0.06(-0.16;0.01)	0.03(-0.03;0.09)
Intracranial carotid calcification	-0.06(-0.13;0.01)	0.08(0.02;0.14)

FA, fractional anisotropy; NAWM, normal appearing white matter; MD, mean diffusivity, CI, confidence interval; SD, standard deviation
Model 1: Adjusted for age, sex, and total intracranial volume
Model 2: Adjusted for age, sex, total intracranial volume, lacunar infarcts, white matter lesion volume and white matter volume

hand we found that larger extracranial and intracranial carotid artery calcification volumes were related to smaller white matter volumes (difference in Z-score per SD increase in calcification volume: -0.09 (95% CI: -0.13;-0.04) and -0.08 (95% CI: -0.12;-0.04)). Additional adjustment for either cardiovascular risk factors or white matter lesion volume and the presence of lacunar infarcts did not influence these associations materially (Table 3, model 2 & 3). Figure 1B depicts the relationship between the sex-specific calcification quartiles in each vessel bed and total brain volume.

Regarding white matter microstructure, we found that larger calcification volume in each vessel bed was associated with lower mean FA values and higher mean MD values (Table 4). Adjustment for *APOE* ε4-carriership and cardiovascular risk

factors did not alter these results. However, adjustment for the presence of lacunar infarcts, white matter volume and white matter lesion volume rendered all associations statistically non-significant, except the relationship between intracranial carotid artery calcification and mean MD (change in Z-score per SD increase in calcification volume of 0.08 (95%CI : 0.02;0.14)) (Table 4, model 2). The relationship between calcification-quartiles in each vessel bed and mean FA are shown in figure 1C. Re-analysing with adjustment for the ultrasound carotid plaque scores did not change any of the results.

DISCUSSION

In a large sample of community-dwelling persons aged 60 years and older, we found that larger atherosclerotic calcification volume was associated with worse cognitive performance. We also found clear associations between larger calcification volumes, smaller brain-tissue volumes and worse white matter microstructural integrity.

In most vessel beds, larger calcification volume was associated with worse cognitive performance, especially executive function, information-processing speed and motor speed. This is consistent with results from other studies that investigated the relationship between coronary calcification only and cognition,[12,13] and with observations that cardiovascular risk factors are associated with worse cognitive performance.[1,28] We found that the associations across vessel beds were different. This supports the hypothesis that the presence and amount of calcification in vessels which are anatomically closer to the brain may also have more effect on the brain. We extended our analyses to brain volumes and found that larger calcification volumes were primarily related to smaller white matter volumes. This is especially interesting, since executive function, information-processing speed and motor speed are cognitive domains which are known to be mostly affected by white matter atrophy.[17,29] Moreover, this supports the hypothesis that mainly white matter is affected by vascular disease.[30-32]

In addition to the associations found with white matter volume, we found that larger volumes of calcification in the coronary arteries were associated with smaller grey matter volumes. In view of the established relation between vascular disease and white matter loss, this was an interesting but unexpected finding. Although grey matter atrophy in relation to vascular risk has been described before,[13] this requires further investigation.

Finally, we extended previous research by also assessing the microstructural in-

tegrity of the normal appearing white matter, which is considered a more subtle and earlier marker of brain disease. It has been suggested that cardiovascular risk factors are associated with worse microstructural integrity of the white matter.[33] Though several studies have investigated the relation between atherosclerosis and white matter lesions, none assessed the quality of the surrounding normal appearing white matter.[12,13,34,35] We found that calcification volume in all vessel beds was associated with a worse microstructural integrity of the normal appearing white matter. After additional adjustment for white matter lesion volume, most of these associations were no longer statistically significant. This attenuation could be due to the fact that the normal appearing white matter of people with a larger white matter lesion volume is generally of poorer quality.[36] Nevertheless, the association between intracranial carotid artery calcification and mean diffusivity of the normal appearing white matter was present even after adjustment for white matter lesion volume.

The strengths of our study include the large study population, our assessment of four major vessel beds with the same diagnostic tool (CT), and automated quantification not only of brain-tissue volumes but also microstructural white matter integrity. Most studies investigating the relationship between atherosclerosis and cognition or brain-tissue volumes used ultrasound-based methods for the assessment of atherosclerosis.[5,7,37] A major advantage of CT is that it can provide an accurate assessment of calcification volume in different vessel beds with one examination.[10,11] When we adjusted all analyses for ultrasound-based carotid plaque scores, the relationship between CT-calcification, cognition, brain tissue volumes and white matter microstructural integrity remained present. This indicates that quantification of arterial calcification using CT provides additional information with respect to the relationship between atherosclerosis, cognition and structural brain pathology. A potential limitation of our study is that we used two types of MDCT-scanners (16-slice and 64-slice) to assess calcification. However, post-hoc analyses with adjustment for scanner type did not change the results. Another consideration is that calcification is only part of the atherosclerotic plaque. With CT it is not possible to visualize the complete atherosclerotic plaque area. Strong evidence, nonetheless suggests that calcification volume is a suitable measure for the total underlying plaque burden.[10]

Additional prospective studies are required to examine whether measuring the amount of atherosclerotic calcification may eventually help improve the prediction of cognitive decline or neurodegeneration. One thing which should be kept in mind is that the clinical feasibility of the assessment of calcification with CT is affected by radiation exposure and costs, especially in neurologically healthy persons. It is important, however, to note that newer generation CT-scanners, especially dual source and dual energy scanners, will lead to much lower radiation

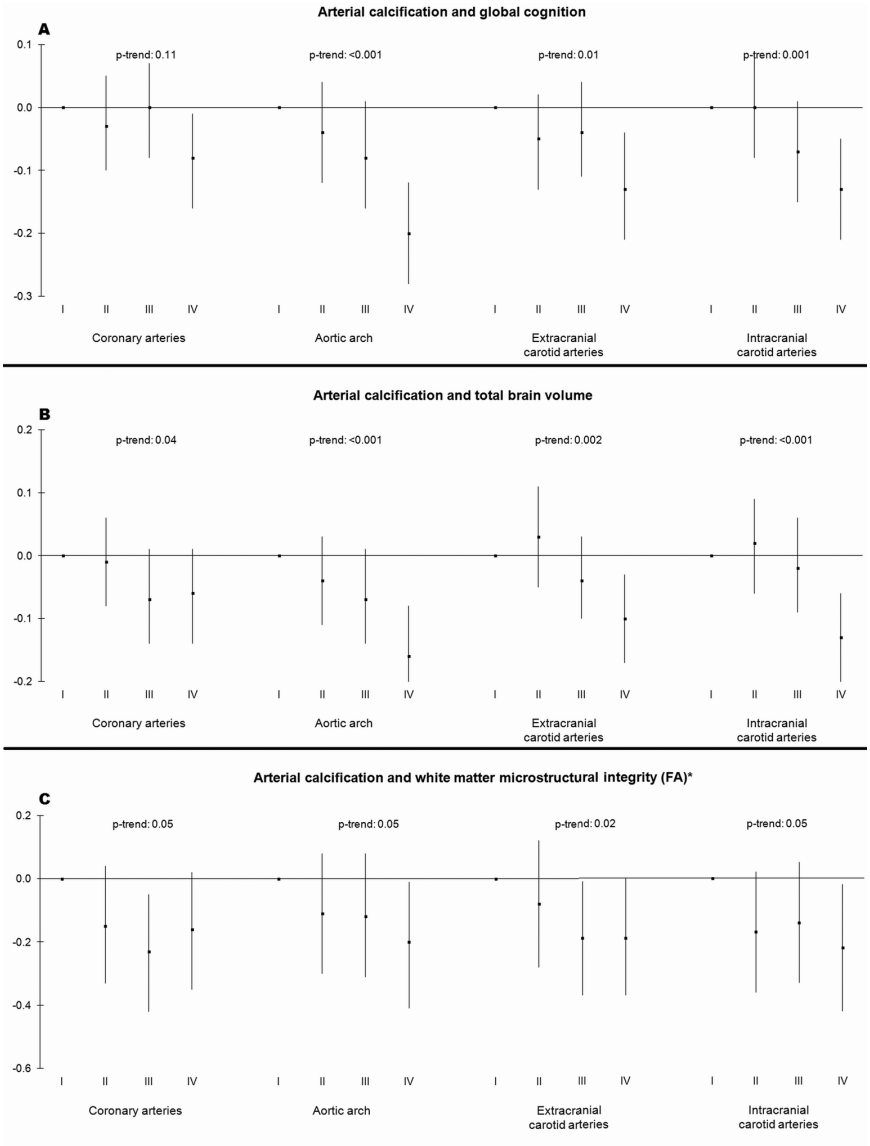

Figure 1A-C. Gender-specific quartiles of calcification and measures of cognition and cerebral atrophy

FA, fractional anisotropy

In all three panels (A-C) the sex-specific quartiles of calcification in each vessel bed are displayed on the *x*-axis. Values of *y* represent differences in Z-score (of A: global cognitive performance, B: total brain volume and C: microstructural integrity of the white matter) per calcification quartile.

Adjusted for age, gender and total intracranial volume. Linear trends per vessel bed are displayed.

* For white matter microstructural integrity values of the mean of the fractional anisotropy are presented. A decrease in these values reflects worse microstructural integrity of the white matter.

doses. Therefore, cost-effectiveness studies will be needed, carefully balancing the disadvantages and advantages of using CT-calcification to this end.

In summary, in our study we have shown that larger CT-assessed calcification volume is related to worse cognitive performance. We also found that a larger calcification volume is associated with smaller brain-tissue volumes and with worse microstructural integrity of the white matter, revealing possible mechanisms through which atherosclerosis leads to poorer cognition.

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3.3

Atherosclerotic Calcification, Cognitive Decline, and the Risk of Dementia

ABSTRACT

Background and Purpose

Increasing evidence suggests that atherosclerosis is a potentially modifiable risk factor for cognitive impairment and dementia, including Alzheimer's disease. Longitudinal data are nevertheless scarce, and it remains unclear whether atherosclerosis in different vessel beds differentially affects cognition and dementia. Hence, we investigated relationships of atherosclerosis in four vessel beds with risk of dementia and cognitive decline.

Methods

At baseline (2003-2006), a random sample of 2,364 participants from the population-based Rotterdam Study underwent computed tomography of the coronary arteries, aortic arch, extracranial, and intracranial carotid arteries to quantify atherosclerotic calcification. Participants were followed for onset of dementia until April 2012. Both at baseline and at a follow-up examination (2008-2012), participants underwent the Mini Mental-State Examination and a neuropsychological test battery to assess cognition.

Results

During 13,397 person years of follow-up, 90 participants were diagnosed with dementia, of which 73 had Alzheimer's disease. Larger calcification volume in all vessels, except in coronary arteries, was associated with a higher risk of dementia [hazard ratio per SD increase in calcification volume: 1.38 (95%C.I.:1.02;1.86), 1.39 (95%C.I.:1.09;1.77), and 1.31 (95%C.I.:1.01;1.70), for aortic arch, extracranial, and intracranial carotid arteries]. This was similar for Alzheimer's disease. Results only slightly attenuated after adjustment for cardiovascular risk factors. Censoring for stroke (n=152) did not change these associations. Larger calcification volume in all four vessel beds was associated with global cognitive decline, even after exclusion of persons who developed dementia during follow-up.

Conclusions

Atherosclerosis is related to a higher risk of dementia and cognitive decline. This seems to be driven by systemic atherosclerosis rather than atherosclerosis restricted to a specific vessel bed. These findings suggest that adequate interventions targeted at reducing or stabilizing atherosclerosis may have a beneficial effect on the occurrence of dementia.

INTRODUCTION

Dementia, including Alzheimer's disease, is a devastating condition with a huge societal impact, both in terms of patient suffering and financial costs.[1,2] An important feature of dementia is the long preclinical phase, during which subtle cognitive deficits develop that can only be measured using dedicated neuropsychological tests.[3] The underlying etiology of dementia and cognitive decline is multi-factorial and involves different pathologies which interact and accumulate over the course of years.[4] In addition to beta-amyloid and tau pathology, the role of vascular pathology in the etiology of dementia and Alzheimer's disease is increasingly being recognized.[5,6]

Atherosclerosis is highly frequent in the aging population and is considered the most important hallmark of vascular pathology.[7] Thus far, most studies have focused on atherosclerosis in the carotid bifurcation in relation to dementia.[8-10] Indeed, both carotid intima-media thickness and carotid plaques have been associated with dementia, including Alzheimer's disease.[8-10]

However, several important questions remain unanswered. First, atherosclerosis is a systemic disease, but its burden differs considerably across vessel beds.[7,11,12] It is therefore conceivable that the contribution of atherosclerosis to dementia may vary depending on the vessel bed. Such differential contribution of atherosclerosis in various vessel beds to disease risk has already been demonstrated for stroke, and even for mortality.[13,14] Second, the study of vascular factors in dementia is often complicated by stroke, which can act as an intermediate factor.[9,10] It is therefore important to also investigate any causal role of atherosclerosis in dementia independent of stroke. Finally, given that both atherosclerosis and dementia develop over the course of years, it is important to study how atherosclerosis affects the preclinical phase of dementia, namely the period of cognitive decline without overt clinical disease.

Disentangling the exact role of atherosclerosis in dementia is important, because this knowledge may then serve as a basis to develop opportunities for therapeutic or preventive intervention. Against this background, we aimed to study the relationship of atherosclerosis in the coronary arteries, aortic arch, extracranial and intracranial internal carotid arteries with incident dementia, including Alzheimer's disease and the potential influence of clinical stroke on these associations. Finally, we focused on the relationship of atherosclerosis with cognitive decline.

MATERIALS AND METHODS

Setting and Study Population

This study is based on the Rotterdam Study; a prospective, population-based study aimed at investigating determinants of chronic diseases in the elderly.[15] The original cohort comprised 7,983 participants aged 55 years or older and was extended in 2000-2001 with 3,011 persons. At study entry and every 3 to 4 years, all participants are re-examined in a dedicated research center.

Between 2003 and 2006, all participants visiting the research center were invited to undergo non-enhanced computed tomography (CT). Therefore, 2003-2006 is taken as baseline for the current study. In total, we scanned 2,524 participants (response rate 78%). Both in 2003-2006 and the following visit in 2008-2012 persons underwent cognitive testing. The follow-up for dementia took place continuously from baseline until April 2012. This study was approved by the Medical Ethics Committee at Erasmus Medical Center, the Netherlands. All participants gave informed consent.

CT Acquisition and Processing

We used a 16-slice (n = 785) or 64-slice (n = 1,739) multidetector computed tomography (CT) scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany) to perform non-contrast CT-scanning. Using a cardiac scan and a scan that reached from the aortic arch to the intracranial vasculature (1 cm above the sella turcica), we scanned the following vessel beds: the coronary arteries, the aortic arch, the extracranial carotid arteries, and the intracranial carotid arteries (Figure 1). Detailed information regarding imaging parameters of both scans is described elsewhere.[12]

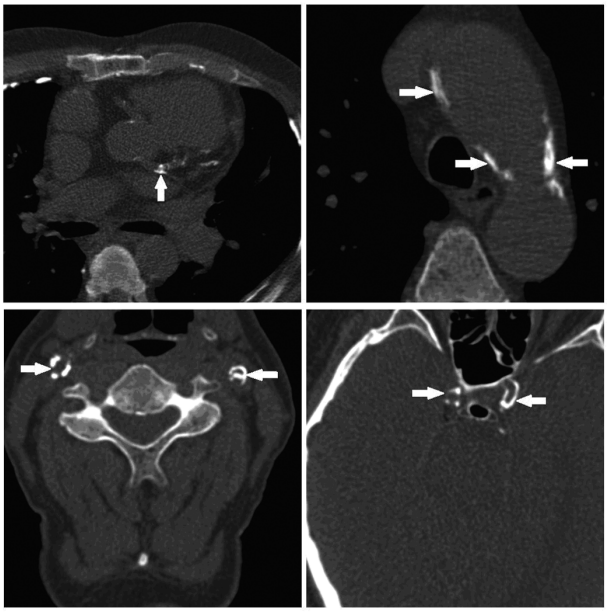

Figure 1. Examples of calcification in the four examined vessel beds
Arrows indicate atherosclerotic calcified lesions in: the left coronary artery (upper left), the aortic arch (upper right), the left and the right extracranial internal carotid arteries (lower left), and the left and right intracranial internal carotid arteries (lower right).

We used dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany) to quantify calcification volume in the coronary arteries, aortic arch, and extracranial carotid arteries.[12] For calcification in the intracranial carotid arteries we used a semi-automated scoring method which is described in detail elsewhere.[16] The interrater reliability of this method is very good (intraclass correlation coefficient, 0.99).[16] Calcification volumes in each vessel bed are expressed in cubic millimeters. Correlations between calcification across the four vessel beds ranged from 0.5 to 0.6.[12,17]

Ascertainment of Dementia

We screened participants for dementia at baseline and follow-up using a three-step protocol.[18,19] Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Screen-positives (MMSE < 26 or GMS organic level > 0) subsequently underwent an examination and an informant interview with the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Participants who were suspected of having dementia, underwent, if necessary, further neuropsychological testing. Additionally, the total cohort was continuously monitored for dementia through computerized linkage between the study database and medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. When information on neuro-imaging was required and available, it was used for decision making on the diagnosis. In the end, a consensus panel consisting of research physicians led by an experienced neurologist, decided on the final diagnosis in accordance with standard criteria using the DSM-III-R criteria for dementia and the NINCDS-ADRDA for Alzheimer's disease.[18] Participants were followed-up until April, 2012.

Assessment of Cognitive Function and Cognitive Decline

Both at baseline and at a follow-up examination in 2008-2012, participants underwent MMSE.[20] In addition, each participant underwent a more extensive neuropsychological test battery which consisted of the following tests: the Stroop test (reading, colour-naming, and interference subtask), the Letter-Digit Substitution Task (LDST), a Word-Fluency test (WFT) and a 15-Word verbal Learning Task (15-WLT).[21,22] We calculated decline for MMSE and each cognitive test by subtracting the test-score at follow-up from the test-score at baseline for each individual. The differences for cognitive tests were subsequently standardized (i.e. Z-scores) to aid comparisons across tests. Z-scores for each subtask of the Stroop test were inverted, because higher scores for these indicate worse performance whilst higher scores on the other tests indicate better performance. Finally, we averaged the Z-scores to yield a measure of global cognition.

Other Measurements in the Rotterdam Study

We collected detailed information on cardiovascular risk factors by interview, physical examination and blood sampling.[15] The following cardiovascular risk factors were measured: obesity, hypertension, diabetes, hypercholesterolemia, low HDL-cholesterol and smoking status. Obesity was defined as a body-mass index (BMI) of ≥ 30 kg/m^2. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of blood pressure lowering medication.[23] Diabetes was defined as fasting serum glucose levels ≥ 7.0 mmol/l and/or the use of anti-diabetic therapy.[24] Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/l and/or the use of lipid-lowering medication.[25] Low HDL-cholesterol was defined as HDL-cholesterol < 1mmol/l.[25] Smoking was categorized into never or ever smoked. Level of education was assessed by self-report.[22] We performed *APOE*-genotyping on coded genomic DNA samples and coded it positive (carrier of one or two ε4-alleles) or negative (non-carrier). Finally, information on history of stroke was collected through interview. Information on incident stroke was collected through continuous monitoring of the participants, as described in detail elsewhere.[26]

Population for Analysis

Due to the presence of a pacemaker, coronary stent implantations or image artefacts, 111 out of 2,524 examinations were not gradable, leaving a total of 2,413 participants with a complete CT examination. From these, 2,364 participants were at risk for developing dementia (incomplete dementia-screening or prevalent dementia excluded), encompassing the study population at baseline.

From these 2,364 participants, 437 refused a second cognitive examination or had died during follow-up, 38 were incapable of follow-up visit (e.g. physical limitations), 16 had been institutionalized or moved, 10 could not be reached, for 7 participants the appointment was postponed for logistical reasons and in 9 participants the cognitive assessment was incomplete and could not be used. This left 1,847 participants with data on cognitive change (MMSE or at least one cognitive test).

Statistical Analysis

Since calcification volume had a skewed, non-normal distribution, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values in order to deal with calcium scores of zero [Ln(calcification + 1.0 mm^3)].

We assessed the relationship between calcification volume in each vessel bed and the risk of dementia using Cox regression models. In the first model we adjusted for age, sex and educational level (model 1). In a second model, we adjusted for cardiovascular risk factors (obesity, hypertension, diabetes mellitus, hypercho-

lesterolemia, low HDL-cholesterol and smoking status) and *APOE* ε4-carriership status (model 2). Next, associations between calcification and dementia were re-analysed after exclusion of participants with prevalent stroke at baseline (n = 78) or incident stroke during the dementia follow-up (n = 74).

We used linear regression to study the relationship of calcification volume with decline in MMSE and global cognition. The adjustments we performed were identical to those in models 1 and 2 used for dementia, with the addition of time interval between baseline and follow-up as covariate. Subsequent analyses were performed after exclusion of persons with stroke (n = 90) and dementia (n = 42). IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

RESULTS

The baseline characteristics of our study participants are summarised in Table 1. Mean age at baseline was 69.4 years and 52.3% of the participants was female. During 13,397 person years of follow-up, 90 participants developed dementia. Of these participants, 73 were diagnosed with Alzheimer's disease, 3 with vascular dementia and 14 with other/undetermined types of dementia. In total, 152 participants suffered a stroke of whom 13 later developed dementia. Hence, 77 participants developed dementia without previous stroke (63 with Alzheimer's disease). On average, people declined 0.27 ± 2.00 points on the MMSE during a mean follow-up period of 6.0 ± 0.5 years.

We found that larger calcification volumes in the aortic arch, extracranial carotid arteries and intracranial carotid arteries, but not in the coronary arteries, were related to a higher risk of dementia (Table 2, model 1). Additional adjustment for cardiovascular risk factors and *APOE* ε4-status slightly attenuated these associations (Table 2, model 2). We found similar associations for Alzheimer's disease (Table 2).

After censoring for stroke, both extracranial and intracranial carotid artery calcification remained statistically significantly associated with dementia [hazard ratio (HR) per standard deviation (SD) increase in calcification volume: 1.32 (95%C.I.: 1.02; 1.71) and 1.34 (95%C.I.: 1.01; 1.78), respectively]. Effect sizes for the remaining associations also remained similar, though statistically non-significant (Table 3).

Table 1. Population characteristics

Sample size	2,364
Women	52.3%
Age, years	69.4 (6.7)
Highest education attained:	
Primary education	10.3%
Low level vocational training	20.8%
Medium level secondary training	17.9%
Medium level vocational to university training	49.1%
Obesity	23.9%
Hypertension	73.7%
Systolic blood pressure, mmHg	146.7 (20.1)
Diastolic blood pressure, mmHg	80.3 (10.7)
Diabetes	11.2%
Serum glucose, mmol/l	5.7 (1.3)
Hypercholesterolemia	48.6%
Serum total cholesterol, mmol/l	5.7 (1.0)
Low HDL-cholesterol	10.6%
Smoking, ever	67.5%
APOE ε4-carriers	25.6%

HDL, high density lipoprotein; APOE, apolipoprotein E
Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables

For cognitive decline, we found that calcification in all vessel beds, including the coronary arteries, was associated with decline in global cognition and, apart from extracranial carotid artery calcification, with decline in MMSE (Table 4, model 1). Additional adjustment for cardiovascular risk factors and *APOE* ε4 status attenuated most associations, but the associations of coronary artery calcification with MMSE and the association of intracranial carotid artery calcification with MMSE and global cognition remained statistically significant [decline on MMSE per SD increase in: coronary artery calcification 0.15 (95% C.I.:0.25; 0.05), intracranial carotid artery calcification: 0.12 (95% C.I.:0.22; 0.01), decline in global cognition per SD increase in intracranial carotid artery calcification: 0.03 (95%C.I.:0.06; 0.00)] (Table 4, model 2).

Table 2. Atherosclerotic calcification and the risk of dementia and Alzheimer's disease

	Dementia	Alzheimer's disease
	n/N = 90/2364 HR (95% CI)	n/N = 73/2364 HR (95% CI)
Per SD increase in:	Model 1	
Coronary calcification	1.10 (0.86;1.41)	1.16 (0.88;1.53)
Aortic arch calcification	1.38 (1.02;1.86)	1.46 (1.04;2.06)
Extracranial carotid calcification	1.39 (1.09;1.77)	1.31 (1.01;1.72)
Intracranial carotid calcification	1.31 (1.01;1.70)	1.29 (0.96;1.74)
	Model 2	
Coronary calcification	1.12 (0.85;1.48)	1.17 (0.85;1.60)
Aortic arch calcification	1.27 (0.93;1.73)	1.32 (0.93;1.89)
Extracranial carotid calcification	1.37 (1.05;1.79)	1.26 (0.94;1.68)
Intracranial carotid calcification	1.32 (0.98;1.77)	1.22 (0.88;1.70)

n, number of cases; N, number of persons at risk; HR, hazard ratio; CI, confidence interval; SD, standard deviation
Model 1: Adjusted for age, sex, and level of education
Model 2: Adjusted for age, sex, level of education, obesity, hypertension, diabetes, hypercholesterolemia, low HDL-cholesterol levels, smoking status and *APOE* ε4-carrier status

Table 3. Atherosclerotic calcification and the risk of dementia and Alzheimer's disease, censored for stroke

	Dementia	Alzheimer's disease
	n/N = 77/2212 HR (95% CI)	n/N = 63/2212 HR (95% CI)
Per SD increase in:		
Coronary calcification	1.05 (0.80;1.36)	1.09 (0.81;1.46)
Aortic arch calcification	1.34 (0.97;1.83)	1.32 (0.92;1.88)
Extracranial carotid calcification	1.32 (1.02;1.71)	1.24 (0.94;1.65)
Intracranial carotid calcification	1.34 (1.01;1.78)	1.28 (0.93;1.76)

n, number of cases; N, number of persons at risk; HR, hazard ratio; CI, confidence interval; SD, standard deviation
Adjusted for age, sex, and level of education

Table 4. Calcification in different vessel beds and global cognitive decline

	MMSE	Global cognition
	Difference in points (95% CI)	Difference in Z-score (95% CI)
Per SD increase in:	Model 1	
Coronary calcification	-0.15 (-0.25;-0.05)	-0.03 (-0.06;0.00)
Aortic arch calcification	-0.11 (-0.21;-0.01)	-0.03 (-0.05;0.00)
Extracranial carotid calcification	-0.07 (-0.16;0.03)	-0.03 (-0.06;0.00)
Intracranial carotid calcification	-0.09 (-0.19;0.00)	-0.04 (-0.07;-0.02)
	Model 2	
Coronary calcification	-0.16 (-0.27;-0.05)	-0.02 (-0.05;0.01)
Aortic arch calcification	-0.11 (-0.22;-0.01)	-0.01 (-0.04;0.02)
Extracranial carotid calcification	-0.07 (-0.17;0.04)	-0.02 (-0.05;0.01)
Intracranial carotid calcification	-0.12 (-0.22;-0.01)	-0.03 (-0.06;0.00)

CI, confidence interval; SD, standard deviation
Model 1: Adjusted for age, sex, time interval and level of education
Model 2: Adjusted for age, sex, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, smoking status and *APOE* ε4-carrier status

After censoring for stroke, coronary artery calcification remained statistically significantly associated with MMSE [decline on MMSE per SD increase in coronary artery calcification 0.11 (95% C.I.:0.21; 0.01)]. Effect sizes for the remaining associations rendered statistically non-significant. After we excluded all persons who developed dementia during follow-up, we still found similar associations of calcification with cognitive decline. Associations of calcification with the separate cognitive tests are shown in Table 5. We found most prominent associations between calcification volume in all vessel beds and decline in scores on the LDST.

DISCUSSION

In this large sample of community-dwelling middle-aged and elderly persons, we found that atherosclerotic calcification in multiple vessel beds is related to a higher risk of dementia, including Alzheimer's disease. These associations were similar across various vessel beds and were independent of clinical stroke. Furthermore, we found atherosclerotic calcification to be associated with cognitive decline in non-demented persons.

Table 5. Calcification in different vessel beds and cognitive decline per single test

	Stroop Reading*	Stroop Naming*	Stroop CWI*	LDST	WFT	15-WLT DR	15-WLT IR
	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)	Difference in Z-score (95% CI)
Per SD increase in:							
Coronary calcification	0.01 (-0.05;0.07)	0.02 (-0.03;0.08)	-0.01 (-0.06;0.05)	-0.06 (-0.12;-0.01)	-0.03 (-0.08;0.03)	-0.04 (-0.09;0.02)	-0.04 (-0.10;0.02)
Aortic arch calcification	-0.02 (-0.08;0.03)	-0.00 (-0.05;0.05)	-0.01 (-0.07;0.04)	-0.06 (-0.11;0.00)	-0.01 (-0.06;0.04)	-0.02 (-0.08;0.04)	-0.03 (-0.08;0.03)
Extracranial carotid calcification	-0.01 (-0.06;0.05)	-0.02 (-0.07;0.03)	0.01 (-0.04;0.07)	-0.06 (-0.11;-0.01)	0.00 (-0.05;0.05)	-0.07 (-0.13;-0.02)	-0.01 (-0.06;0.05)
Intracranial carotid calcification	-0.02 (-0.07;0.04)	-0.02 (-0.07;0.04)	-0.02 (-0.07;0.03)	-0.05 (-0.10;0.00)	-0.06 (-0.11;-0.01)	-0.04 (-0.09;0.02)	-0.02 (-0.08;0.03)

CI, confidence interval; SD, standard deviation; CWI, Colour Word Interference; LDST, Letter–Digit Substitution Task; WFT, Word Fluency Task; WLT DR, 15-word learning test delayed recall; WLT IR, 15-word verbal learning test immediate recall
Adjusted for age, sex, time interval and level of education
*Inverted scores; lower scores indicate worse performance

Strengths of our study include the longitudinal population-based setting, the image-based quantification of atherosclerosis, and the focus on both cognitive decline and dementia.

Moreover, our close collaboration with general practitioners in the study area, in combination with the structure of the Dutch health care system allowed us to accomplish virtually complete follow-up with regard to development of dementia.

Some potential limitations should also be addressed. First, calcification is only a part of the atherosclerotic plaque. Using non-enhanced CT, it is not possible to visualize the complete atherosclerotic plaque area and thus potentially interesting information on additional plaque characteristics, such as shape, stenosis or ulceration could not be measured. Nonetheless, strong evidence suggests that CT-based calcification volume provides an adequate reflection of the total underlying plaque burden.[27] Second, for the assessment of calcification we used two types of multidetector CT-scanners (16 slice and 64 slice CT), which could have influenced the measurements. However, post-hoc analyses with adjustment for scanner type did not change the results.

We found that atherosclerotic calcification in multiple vessel beds was related to a higher risk of dementia. Several explanations may underlie this association. First, our findings could be explained by an intermediary role for clinical stroke. Atherosclerosis is a powerful risk factor for stroke,[28] and in turn stroke-patients have an almost two-fold increased risk of dementia.[6,29] However, censoring at time of stroke changed little in our associations of calcification and incident dementia. A similar absence of effect of clinical stroke has been shown for the relationship between carotid intima-media thickness and dementia.[9,10] Another point of consideration is that atherosclerosis in the carotid artery represents the strongest location of atherosclerosis related to stroke.[30,31] In contrast, we found associations with dementia for other vessel beds as well. This also points towards our findings being independent from clinical stroke. Actually, this suggests that generalized atherosclerosis, which probably is a better reflection of one's vascular status rather than localized atherosclerosis, associates with dementia. In line with this, we found that adjustment for systemic cardiovascular risk factors slightly attenuated our results.

A second explanation linking atherosclerosis to dementia is subclinical small vessel disease.[6,32] Autopsy studies provide strong evidence that the majority of patients with Alheimer's disease show small vessel disease in their brain.[6] This includes infarcts, micro-infarcts, microbleeds, demyelinization and axonal damage.[6] In-vivo, imaging studies have shown that MRI-markers of small vessel disease, e.g. white matter lesions and lacunar infarcts, are associated with the risk

of dementia.[6,33,34] We have previously demonstrated that atherosclerotic calcification in all four vessel beds is strongly associated with these MRI-markers of subclinical vascular brain disease.[17] Together, this points towards a role for small vessel disease in the association of atherosclerosis with dementia.

Finally, chronic hypoperfusion may explain the association between atherosclerosis and dementia, especially Alzheimer's disease.[6] As a consequence of slowly-progressing structural changes of cerebral vessels due to atherosclerosis, cerebral perfusion is impaired which could lead to subclinical vascular brain disease on the one hand, but but may on the other hand cause loss of functionality of the blood-brain barrier. This in turn might allow increased parenchymal deposition of beta-amyloid protein and thereby the formation of amyloid plaques, an important characteristic of Alzheimer's disease.[5,6]

Research on dementia is very often challenging due to the possibility of competing risks.[9] In our study competing risk due to mortality might explain the lack of association between coronary artery calcification and dementia. The coronary arteries are the single most important location of atherosclerosis for risk of cardiac events, including cardiac death.[13] It is therefore conceivable that persons in our study with the largest load of coronary calcification died of competing risks and therefore did not remain at risk of developing dementia. Indeed, post-hoc analyses revealed that especially coronary artery calcification was associated with mortality.

By investigating cognitive decline in non-demented persons in addition to the endpoint of dementia, we managed to circumvent competing risks to a certain extent. We found that atherosclerotic calcification in all locations, including the coronary arteries, was associated with cognitive decline. Moreover, when we repeated our analyses after excluding persons who converted to dementia during follow-up, we found that the results remained unchanged. This implies that our results were unlikely to be driven by preclinical dementia, but that atherosclerosis already plays an important role in the early stages of cognitive deterioration, presumably already years before the possible conversion to clinical dementia. Whereas our study had a follow-up period of 6 years, others found carotid atherosclerosis to be associated with cognitive decline over a 10-year period.[35,36] These findings indicate a potential window of opportunity for treatment of atherosclerosis which could possibly delay or even stop the cognitive decline and ultimately aid in the prevention of dementia.

In conclusion, our findings further establish the role of atherosclerosis in the etiology of dementia and cognitive decline. This calls for studies evaluating whether interventions targeted at reducing or stabilizing atherosclerosis would have a beneficial effect on the occurrence of dementia.

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3.4

Intracranial Carotid Artery Calcification and the Risk of Stroke

ABSTRACT

Background and Purpose

Intracranial atherosclerosis represents a relatively unexplored, but potentially important cause of stroke in whites. We investigated the relationship between intracranial carotid artery calcification (ICAC), as marker of intracranial atherosclerosis, and the risk of stroke in a white population.

Methods

Between 2003 and 2006, 2,323 stroke-free persons from the general population (mean age: 69.5 years) underwent computed tomography (CT) to quantify ICAC volume. All participants were continuously followed for the occurrence of stroke until January 1, 2012. We constructed Cox regression-models to relate ICAC with the risk of stroke, adjusting for age and sex. Additionally, we adjusted for cardiovascular risk factors, ultrasound carotid plaque score, and CT-assessed calcification volume in the coronary arteries, aortic arch and carotid artery bifurcation. Finally, we calculated the proportion of strokes attributable to ICAC, using the population attributable risk (PAR).

Results

During 14,055 person years of follow-up, 91 participants developed a stroke, of which 74 were ischemic. Larger ICAC volume was related to a higher risk of stroke, independent of cardiovascular risk factors, ultrasound carotid plaque score, and calcification volume in other vessels [fully adjusted hazard ratio (HR) per SD increase in ICAC volume: 1.43 (95%CI:1.04;1.96)]. ICAC contributed to 75% of all strokes, whilst for aortic arch and extracranial carotid artery calcification this was only 45% and 25% respectively.

Conclusions

Our findings establish intracranial atherosclerosis as a major risk factor for stroke in the general white population and suggest that its contribution to the proportion of all strokes may be greater than that of large-artery atherosclerosis in more proximal located vessel beds.

INTRODUCTION

Stroke is the most frequent neurological disease, and the second most important cause of global mortality.[1-3] The lifetime risk of stroke is estimated to be at least 1 in 6 and in the coming decades its burden is expected to increase even further.[4,5]

Of all strokes, approximately 80% to 90% are of ischemic origin.[2] Ischemic stroke has a multifactorial etiology involving various overlapping and interacting pathways, such as vascular disease, inflammation, hemostasis, metabolism, and genetic factors.[2] Among these, vascular disease is by far the most important and has been the target of many preventive and therapeutic interventions for stroke.[1, 2] When studying vascular disease, it is important to consider that its burden may vary substantially across different vessel beds.[6,7] As such, several locations of vascular disease are considered relevant in stroke etiology. First, cardiac diseases, such as coronary heart disease and atrial fibrillation, increase the risk of stroke.[1,2,8] Second, large-artery atherosclerosis is recognized as a major risk factor of stroke.[2,9,10] Thirdly, strokes may occur after occlusion of the small penetrating intracerebral arteries, in so-called cerebral small vessel disease.[1,2,9] It is important to note that these do not represent mutually exclusive categories, but that in most patients vascular pathology is present in multiple sites.

Within this etiologic framework, large-artery atherosclerosis is considered to include all vessels from the aortic arch to the major cerebral arteries. Atherosclerosis of the intracranial vasculature is globally considered the most important risk factor for stroke. However, current numbers are solely driven by populations from Asian and African origin, which make up the largest proportion of strokes worldwide.[11-13] In contrast, there are no data on the burden of stroke attributable to intracranial atherosclerosis in white populations. Yet surprisingly, most clinical trials targeting intracranial atherosclerosis include a majority of whites in their samples.[14,15] There is now an emerging awareness that robust data from white populations on the role of intracranial atherosclerosis in ischemic stroke are urgently needed.[16-18] These can then serve as basis for designing future intervention studies.

Recently, using intracranial carotid artery calcification (ICAC) as proxy, we demonstrated a prevalence of intracranial atherosclerosis exceeding 80% in a general white population.[19] In this report, we set out to investigate in a white population the longitudinal association of ICAC with incident stroke over a six year follow-up period. We were specifically interested in estimating the total burden of stroke attributable to ICAC in whites.

MATERIALS AND METHODS

Setting and Study Population

This study is based on the Rotterdam Study, a prospective, population-based study investigating determinants of chronic diseases in the elderly.[20] The original cohort consisted of 7983 participants aged 55 years or older and was extended in 2000-2001 with 3,011 persons. At study entry and every 3 to 4 years, all participants are re-examined in a dedicated research center. The Rotterdam Study represents a homogeneous middle-class population, largely of white descent (>96%).

For the current study, we used the visit between 2003 and 2006 as baseline, because only in this period we invited participants who visited the research center to undergo non-enhanced computed tomography (CT) of the intracranial carotid arteries.[6] We scanned 2,524 participants (response rate 78%). This study was approved by the institutional review board of Erasmus Medical Center, the Netherlands. All participants gave informed consent.

Assessment of Intracranial Atherosclerosis

We used a 16-slice (n = 785) or 64-slice (n = 1,739) multidetector CT-scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany) to perform non-enhanced CT-scanning. Using a cardiac scan and a scan that reached from the aortic arch to the intracranial vasculature (1 cm above the sella turcica), we scanned the following vessel beds: the coronary arteries, the aortic arch, the extracranial carotid arteries, and the intracranial carotid arteries. Detailed information regarding imaging parameters of both scans is provided elsewhere.[7]

As proxy for intracranial atherosclerosis, we measured ICAC bilaterally in the intracranial internal carotid artery from its horizontal petrous segment to its top (until the circle of Willis) (Figure 1). For quantification of ICAC, we used a semi-automated scoring method which is described in detail elsewhere.[19] Briefly, we manually drew regions of interest around calcification in the course of the intracranial internal carotid arteries in consecutive CT-slices. Next, we calculated calcification volumes by multiplying the number of pixels above 130 Hounsfield Units with the pixel-size and the increment.

Calcification volumes in the coronary arteries, aortic arch and extracranial internal carotid arteries were quantified using dedicated commercially available software (Syngo CalciumScoring, Siemens, Germany).[7] All calcification volumes are expressed in mm^3. Correlations between calcification across the four vessel beds ranged from 0.5 to 0.6.[7,21]

Follow-up for Stroke

The definition of stroke was based on the WHO criteria including a syndrome of rapidly developing symptoms of focal or global cerebral dysfunction lasting 24 hours or longer or leading to death, with apparent vascular cause.[22,23] We assessed prevalent stroke at baseline during interview and verified these data with medical records.[22] After enrolment, we continuously monitored participants for incident stroke through linkage of the study database with files from general practitioners. Nursing home physicians' files and files from general practitioners of participants who moved out of the district were also checked.[22] Additional information was obtained from hospital records.

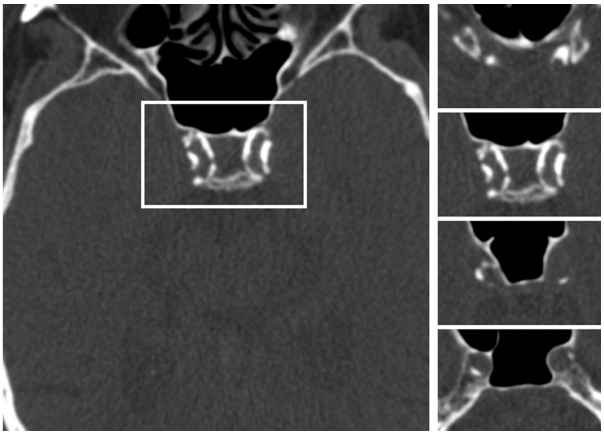

Figure 1. Example of calcification of the intracranial internal carotid artery
The left image shows an axial CT slice in which the white rectangle marks the region of the internal carotid arteries. The four images on the right represent the course of the intracranial carotid artery from the petrous bone (lower image) to the top (upper image) with calcifications (indicated by white arrowheads).

Potential strokes were reviewed by research physicians and verified by an experienced stroke neurologist. We categorized strokes into ischemic or haemorrhagic on the basis of neuroimaging-reports. If neuroimaging was unavailable, the stroke was classified as unspecified.[22] Subarachnoid haemorrhages due to ruptured aneurysms were not considered stroke events.[22] Follow-up for incident stroke was complete until January 1, 2012.

Other Measurements in the Rotterdam Study

We obtained information on cardiovascular risk factors by interview, physical examination and blood sampling.[20] Obesity was defined as a body-mass index (BMI) ≥ 30 kg/m^2. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg and/or use of blood pressure-lowering medication.[24] Diabetes was defined as fasting serum glucose levels ≥ 7.0 mmol/l and/or use of antidiabetic therapy.[25] Hypercholesterolemia was defined as a serum total cholesterol ≥ 6.2 mmol/l and/or use of lipid-lowering medication.[26] We defined low high-density lipoprotein (HDL)-cholesterol as HDL-cholesterol < 1.0 mmol/l.[26] Smoking was categorized into never or ever smoked.

Besides these cardiovascular risk factors, we also assessed other imaging markers of atherosclerosis. These were atherosclerotic calcification volumes in the coronary arteries, aortic arch and extracranial carotid arteries (as described above), and ultrasound carotid plaquescore. Using ultrasound, we visualized the common carotid artery, carotid artery bifurcation, and internal carotid artery and examined both left and right to assess a weighted plaque score ranging from 0 to 6.[27]

Population for Analysis

We restricted our population to persons from white descent, as assessed by self-report, which were 2,452 from the 2,524 persons that underwent CT. Due to image artefacts, 27 examinations from these 2,452 were not gradable for ICAC, leaving a total of 2,425 participants with complete data on ICAC volume. Persons with prevalent stroke (n = 98) and persons that did not participate in the stroke follow-up (n = 4) were excluded, leaving 2,323 participants at risk for stroke in the population for analysis.

Statistical Analysis

Due to the non-normal distribution of ICAC volume, we performed a natural log-transformation and added 1.0 mm^3 to the non-transformed values to deal with calcium volumes of zero [Ln(ICAC + 1.0 mm^3)]. We calculated hazard ratios (HR) for stroke per standard deviation (SD) increase in ICAC volume, using Cox regression models. The proportional hazards assumption was met. In model 1, we adjusted for age, sex, and scanner type. In model 2, we additionally adjusted for the following cardiovascular risk factors: obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, and smoking. In model 3, we additionally adjusted for the ultrasound carotid plaquescore and CT-assessed calcification volumes in the coronary arteries, aortic arch, and extracranial carotid artery.

Next, we dichotomized ICAC into presence versus absence of ICAC and related that to the risk of stroke. In contrast to established categories for absolute coronary calcification scores, i.e. Agatston score,[28] there are no such categories for ICAC. Therefore, we categorized calcification into present versus absent, because this is most readily identified in a clinical situation. We used the same three Cox-regression models as above, with the only difference that in model 3, calcification in other vessel beds was also dichotomized into present/absent. Stroke-free survival in the absence and presence of ICAC was estimated and compared using the Kaplan-Meier method and log rank test.

Finally, we calculated the population attributable risk (PAR) for stroke of calcification in each of the four vessel beds using the following formula:[29]

$$PAR = PD\left(\frac{RR-1}{RR}\right)x100\%$$

In this formula, RR represents the relative risk, i.e. HR, which we calculated for the presence of calcification in each vessel bed. *Pd* is the proportion of cases exposed to the risk factor (the presence of calcification). PAR provides a measure between 0 and 100%, which can be interpreted as the fraction of strokes that is due to calcification.[29] IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

RESULTS

Table 1 shows the baseline characteristics of the study population. The mean age was 69.5 years and 52.2% of the participants were female. During 14,055 person years of follow-up (mean 6.1 years), 91 participants suffered a stroke, of which 74 were ischemic, 10 were haemorrhagic, and 7 unspecified.

Table 2 shows the associations between ICAC and the risk of stroke. We found that larger ICAC volumes were associated with a higher risk of stroke. These results were similar for ischemic stroke. Additional adjustment for cardiovascular risk factors did not change any of these results [HR per SD increase in ICAC volume: 1.52 (95%C.I.: 1.17; 1.98) for stroke, and 1.53 (95%C.I.: 1.14; 2.04) for ischemic stroke]. Also after additional adjustment for ultrasound carotid plaquescore and calcification volumes in the other vessel beds, we found that larger ICAC volumes remained significantly associated with a higher risk of stroke [HR per SD increase in ICAC volume: 1.43 (95%C.I.: 1.04; 1.96)]. The effect size for the association with ischemic stroke was similar, although statistically non-significant (Table 2, model 3, upper panel).

We found that the presence of ICAC was associated with a higher risk of any stroke and ischemic stroke, even after adjustment for cardiovascular risk factors [HR for presence versus absence of ICAC: 4.15 (95%C.I.: 1.51; 11.42) for stroke, and 3.43 (95%C.I.: 1.24; 9.51) for ischemic stroke]. Additional adjustment for ultrasound carotid plaquescore and the presence of calcification in the other vessel beds also did not change these associations (Table 2, model 3, lower panel). Kaplan-Meier curves revealed that the stroke-free survival in persons with ICAC was significantly shorter than persons without ICAC (Figure 2).

Table 3 shows the proportion of strokes that is attributable to calcification in each of the four vessel beds. We found that ICAC played a role in up to 75% of all strokes, whilst for aortic arch calcification and calcification in the extracranial

carotid artery, this was 45% and 25%, respectively. For coronary artery calcification we did not calculate the PAR for stroke, because the HR was below one.

Table 1. Baseline characteristics of study participants

Sample size	2,323
Women	52.2%
Age, years	69.5 (6.7)
Obesity	23.7%
Body mass index, kg/m^2	27.7 (4.0)
Hypertension	73.9%
Systolic blood pressure, mmHg	146.7 (20.1)
Diastolic blood pressure, mmHg	80.2 (10.8)
Diabetes	10.7%
Serum glucose, mmol/l	5.7 (1.2)
Hypercholesterolemia	48.9%
Serum total cholesterol, mmol/l	5.7 (1.0)
Low HDL	10.6%
Serum HDL-cholesterol, mmol/l	1.4 (0.4)
Past or current smokers	67.5%
Presence of intracranial carotid artery calcification	81.4%
Intracranial carotid artery calcification volume*, mm^3	41.1 (6.2 – 135.1)

HDL, high-density lipoprotein
Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables
*Median (interquartile range)

Table 2. Intracranial carotid artery calcification and the risk of stroke

		Any stroke	Ischemic stroke
		n/N = 91/2323 HR (95%CI)	n/N = 74/2323 HR (95% CI)
ICAC, continuous Per SD	Model 1	1.53 (1.18;1.97)	1.51 (1.14;2.01)
	Model 2	1.52 (1.17;1.98)	1.53 (1.14;2.04)
	Model 3	1.43 (1.04;1.96)	1.39 (0.98;1.99)
ICAC, dichotomous Presence versus absence	Model 1	4.25 (1.55;11.66)	3.49 (1.27;9.64)
	Model 2	4.15 (1.51;11.42)	3.43 (1.24;9.51)
	Model 3	4.64 (1.44;14.95)	3.52 (1.08;11.47)

n, number of cases; N, number of persons at risk; HR, hazard ratio; CI, confidence interval; ICAC, intracranial carotid artery calcification; SD, standard deviation
Model 1: Adjusted for age, sex, and scanner type
Model 2: Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, and smoking
Model 3: Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, smoking, ultrasound carotid plaquescore, and calcification in the coronary arteries, the aortic arch and the carotid bifurcation (as appropriate; volume for continuous analyses, presence for dichotomous analyses)

Table 3. Population attributable risks for stroke per vessel bed

	PD	Any stroke HR (95% CI)	PAR
Presence of calcification in:			
Coronary arteries	0.87	0.80 (0.40;1.57)	N.A.*
Aortic arch	0.98	1.84 (0.44;7.77)	45%
Extracranial carotid arteries	0.86	1.41 (0.72;2.83)	25%
Intracranial carotid arteries	0.96	4.64 (1.44;14.95)	75%

PD, Proportion of stroke-cases exposed to calcification; HR, hazard ratio; CI, confidence interval; PAR, population attributable risk
Adjusted for age, sex, scanner type, obesity, hypertension, diabetes mellitus, hypercholesterolemia, low HDL-cholesterol, smoking, presence of calcification in the other vessel beds, and for ultrasound carotid plaque score
* The PAR for coronary artery calcification was not calculated because the HR is below one

DISCUSSION

In this large population-based cohort study among middle-aged and elderly white persons we found that both presence and volume of ICAC, as marker of intracranial atherosclerosis, were associated with a higher risk of stroke. This was independent of both conventional cardiovascular risk factors and atherosclerosis in other extracranial vessel beds. We also found that ICAC contributed to 75% of all strokes, whereas for aortic arch calcification and extracranial carotid artery calcification this was only 45% and 25%, respectively.

With the current study we demonstrated that intracranial atherosclerosis is a major risk factor for stroke in a white population. Thus far, evidence for a role of intracranial atherosclerosis in the etiology of stroke mainly comes from research in populations from Asian and African descent.[13,16,17] In these populations, intracranial atherosclerosis is an established major risk factor for ischemic stroke, accounting for up to 50% of all strokes.[13,16] We acknowledge that the total burden of intracranial atherosclerosis comprises atherosclerotic disease in more arteries than only the intracranial carotid arteries that we measured.[13,17] However, atherosclerotic plaques in those other arteries are typically non-calcified,[30,31] and can therefore not be measured using non-enhanced CT. Consequently, the total amount of intracranial atherosclerosis might be larger than we measured, which directly implies that its impact on stroke is likely to be even higher.

Using PAR, we estimated that intracranial atherosclerosis contributes to the occurrence of 75% of all strokes. The PAR for stroke of intracranial atherosclerosis was notably larger than that of atherosclerosis in the aortic arch or carotid bifurcation. Theoretically, a PAR of 75% indicates that the incidence of stroke may be reduced by 75% if intracranial atherosclerosis could be completely eradicated. However, a few considerations should be noted for better interpretation of a PAR. First, this figure of 75% does not mean that only 25% of strokes remain to be explained by other causes than intracranial atherosclerosis.[29] In fact, the sum of PARs for all possible risk factors of stroke exceeds 100%, reflecting interaction between risk factors. This also signifies that other unknown causes may still contribute considerably to the development of stroke. Nevertheless, the high PAR illustrates the large potential gain in public health that could be achieved by further developing therapeutic and preventive strategies aimed at reducing the amount of intracranial atherosclerosis. Possibly, earlier and more aggressive treatment of modifiable risk factors for intracranial atherosclerosis could stave off its formation and may thereby contribute to the primary prevention of stroke. A beneficial effect of aggressive medical management on the occurrence of stroke has already been demonstrated in patients with symptomatic intracranial atherosclerotic stenosis.[14] Second, although we adjusted for conventional cardio-

vascular risk factors, as well as for calcification in other vessel beds, the HR that we used may still be subject to residual confounding. If so, a more unbiased HR would have yielded an attenuation of the PAR. Still, the PAR of ICAC was highest among all studied vessel beds.

In the current literature, a major focus is on coronary artery calcification as an emerging determinant of various cardiovascular events. Some studies have shown coronary artery calcification to even improve the risk prediction of stroke,[8] but this remains debatable.[32,33] In our dataset we found no association between coronary artery calcification and stroke when taking into account calcification in all vessel beds. In a post-hoc analysis, we found that coronary artery calcification was associated with stroke in a crude age and sex-adjusted model only. This further corroborates that ICAC is a more important determinant of stroke than coronary artery calcification. Future studies should therefore also investigate the predictive value of ICAC for stroke.

Strengths of our study include the population-based setting and the longitudinal design. Moreover, we used an accurate image-based method to quantify ICAC. We tried to minimize the number of persons that were lost to follow-up by using thorough stroke monitoring procedures. These allowed us to identify nearly all stroke events, including fatal strokes and strokes in participants living in nursing homes, who were not referred to a hospital. There are also several potential limitations that should be taken into account. First, by using non-enhanced CT we were only able to measure calcification and not the complete atherosclerotic plaque. Although strong evidence from autopsy studies shows that CT-based calcification is a sensitive and reliable marker of the total underlying atherosclerotic burden,[34,35] this might have led to misclassification in certain instances. Second, it was not possible to describe any additional plaque characteristics, for example shape, stenosis, or ulceration, which may all be of importance with regard to future events. Although a high correlation between CT-measured calcification and stenosis has been found in the carotid siphon,[36,37] advances in plaque imaging with other imaging techniques, e.g. MRI, may aid to overcome this issue in the future.

In conclusion, our findings suggest that intracranial atherosclerosis is a major risk factor for stroke in the general white population. Moreover its contribution to the proportion of all strokes may be greater than that of large-artery atherosclerosis in other vessel beds.

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4

Emerging Imaging Markers of Vascular Risk

4.1

Coexistence of Calcification, Intraplaque Hemorrhage, and Lipid Core in the Carotid Atherosclerotic Plaque

ABSTRACT

Background and Purpose

There is growing evidence that the composition of carotid atherosclerotic plaques may be of clinical relevance. Yet, little is known on coexistence of potentially vulnerable and stabilizing components within asymptomatic plaques. Using multimodal imaging of atherosclerotic plaques in community-dwelling persons, we investigated the relationship between intraplaque calcification, hemorrhage and lipid core.

Methods

In 329 subjects from the population-based Rotterdam Study, all with ultrasound-confirmed carotid wall thickening, we performed CT and high-resolution MRI of carotid artery bifurcations to assess plaque characteristics (n=611 carotid arteries with plaques). With logistic regression models we investigated within each carotid plaque the relationship of calcification volume – as a potential stabilizing component -with presence of potential vulnerable components, intraplaque hemorrhage and lipid core, adjusting for age, sex and maximal plaque thickness. Next, we stratified on degree of stenosis (≤ or > 30%) to evaluate effect modification by plaque severity.

Results

Larger calcification volume was associated with a higher prevalence of intraplaque hemorrhage, and a lower prevalence of lipid core [fully-adjusted odds ratio (OR) per standard deviation (SD) increase in calcification volume: 2.02(95%C.I.:1.53;2.66) and 0.72(95%C.I.:0.58;0.89), respectively]. Stratification on degree of stenosis showed no difference in the association between calcification volume and hemorrhage over strata, whilst the relationship between larger calcification volume and lower prevalence of lipid seemed more pronounced in persons with high degree of stenosis.

Conclusions

In this population-based setting, we found that plaques with a higher load of calcification contain more often hemorrhagic components, but less often lipid core. Our results suggest that both in small and large plaques, intraplaque calcification may not be a stabilizing factor per se.

INTRODUCTION

Atherosclerosis is a systemic vascular disease, which is highly frequent in middle-aged and elderly persons.[1, 2] Atherosclerosis is characterised by thickening of the arterial vessel wall which can be due to accumulation of lipid material, calcification, fibrous elements and/or intraplaque hemorrhage.

Atherosclerosis in the carotid artery is well established as a causal factor in clinical stroke.[3] Initial studies on carotid atherosclerosis primarily focused on atherosclerotic burden, as assessed by carotid intima-media thickness, degree of stenosis, or plaque size.[4, 5] More recent evidence shows that the composition of the atherosclerotic plaque may also provide essential information for cardiovascular disease prevention and management.[6-8] Clinical studies have convincingly shown that certain plaque components, i.e. intraplaque hemorrhage and lipid core, are associated with plaque instability and plaque rupture, and as such with an increased risk of stroke.[7-9] Contrary to these markers of plaque instability, there is emerging evidence that other plaque components, in particular calcification, may actually have a stabilizing effect on the plaque, which might lead to a decreased risk of stroke.[10-12]

Whereas clinical studies primarily represent symptomatic atherosclerosis, it is increasingly recognized that studying atherosclerosis in preclinical, asymptomatic individuals (for example in a population-based setting) can provide important insights into the etiology and prognosis of atherosclerosis. Although risk factors for specific plaque components have been described, it remains unknown how different plaque components associate with each other within these asymptomatic plaques. Advances in non-invasive imaging techniques, specifically computed tomography (CT) and magnetic resonance imaging (MRI), have led to the possibility to non-invasively study atherosclerosis in large samples from the general population.[4] Using these imaging techniques, important information on the prevalence of different plaque components in asymptomatic persons has been obtained during the last decades.[13-15] In particular, it was found that intraplaque hemorrhage and lipid core are also highly frequent plaque components in the general population.[16]

Thus far, studies have used a single imaging modality (either CT or MRI) to investigate atherosclerotic plaques. This precludes the concomitant study of intraplaque hemorrhage and lipid core, which are best seen on MRI, as well as calcification, which is optimally detected with CT and possibly reflects plaque stability.[4]

Therefore, in the present study we set out to investigate the coexistence of various atherosclerotic plaque components within the carotid artery using a multimodality imaging approach in a sample of 329 community-dwelling persons from the Rotterdam Study. We hypothesized that a high load of calcification would be related to less frequent occurrence of vulnerable components (i.e., hemorrhage and lipid) within the same plaque.

MATERIALS AND METHODS

Setting and Study Population

This study is embedded in the Rotterdam Study,[17] a prospective, population-based cohort study among persons aged 55 years or older. The original cohort consisted of 7,983 participants and was extended in 2000-2001 with 3,011 persons. At study entry and every 3 to 4 years, all participants are re-examined in a dedicated research center. From 2003 until 2006, all participants that completed a visit at the research center in this period were invited to undergo CT of the extracranial internal carotid arteries.[14] In total, 2,524 participants were scanned. From October 2007 onwards, carotid MRI was incorporated in the study in all persons with carotid wall thickening in the left, right or both carotid arteries on ultrasound.[16] Invitation for carotid MRI was independent from previous participation in the carotid CT study. Until September 2010, 829 persons from the original and extended cohort underwent carotid MRI, of whom 349 had undergone CT in the period 2003-2006. The present study focuses on these participants who had both a complete CT- and MRI-examination until September 2010 (n = 349). Moreover, we excluded all participants with a history of stroke (n = 20) to restrict our analyses to a completely asymptomatic population, leaving a study population of 329 persons. The mean time interval between CT and MRI was 4.6 ± 1.1 years, with the carotid CT scan preceding the MRI in all subjects.

CT Acquisition and Processing

CT imaging was performed using a 16-slice (n = 122) or 64-slice (n = 207) multidetector computed tomography (CT) scanner (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany). No contrast material was administered. To visualize calcification in the extracranial carotid arteries we used a scan that reached from the aortic arch to the intracranial vasculature (one cm above the sella turcica). Detailed information regarding imaging parameters of the scan is described elsewhere.[14]

Calcification in the extracranial carotid artery was measured bilaterally within three centimeters proximal and distal of the bifurcation, and quantified with dedicated commercially available software (syngo CalciumScoring, Siemens, Germany).[14] Calcification volumes in both left and right carotid arteries were expressed as cubic millimeters (mm^3).

MRI Acquisition and Processing

Imaging of the carotid arteries was performed on a 1.5-T scanner (GE Healthcare, Milwaukee, WI, USA) with a bilateral phased-array surface coil (Machnet, Eelde, The Netherlands). We planned the high-resolution MRI sequences so that the carotid plaques were imaged completely on both sides, using a previously described standardized protocol.[16] First, both carotid bifurcations were identified by means of 2D Time of Flight MR-Angiography. Thereafter, high-resolution MRI sequences were planned to image the carotid bifurcations on both sides: a PDw Fast Spin Echo (FSE) Black-blood (BB) sequence; a PDw-FSE-BB with an increased in-plane resolution; a PDw- Echo Planar Imaging (EPI) sequence; a T2w-EPI sequence; a 3D-T1w-Gradient Echo (GRE) sequence; and, finally, a 3D phased-contrast MR-Angiography.

Carotid plaques were analysed by a single observer with more than three years experience in rating MRI carotid, as previously described.[16] Analysis involved examination of both carotid arteries. First, presence of atherosclerotic plaque in each carotid artery was assessed. Next, carotid plaque thickness was quantified by measuring maximum carotid wall thickness in the PDw-FSE images and by calculation of luminal stenosis using the NASCET criteria.[16] In carotid arteries with maximum wall thickness of ≥ 2.0 mm on MRI (n= 611 carotids in 329 participants), presence or absence of intraplaque hemorrhage and lipid core was assessed, using criteria as described elsewhere.[16]

Statistical Analysis

Since calcification volume had a highly skewed, non-normal distribution, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values in order to deal with calcium scores of zero [Ln(calcification + 1.0 mm^3)].

We used logistic regression models to investigate the relationship of CT-assessed calcification volume in each extracranial internal carotid artery with degree of stenosis (dichotomized into 0-30% and >30%) presence of intraplaque hemorrhage and lipid core on MRI. In model 1, we adjusted for age and sex. In model 2,

we additionally adjusted for maximum carotid wall thickness as proxy of plaque size, measured on MRI. Next, we stratified on degree of carotid stenosis (0-30% versus > 30%) and repeated above analyses, to assess whether the relationships between calcification volume and the other plaque components were modified by severity of atherosclerosis. IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, New York) was used for statistical analyses.

RESULTS

The characteristics of the study population are described in Table 1. The mean age at the time of the CT-examination was 70.0 ± 6.2 years and 43.0% were women.

As expected, larger calcification volume was associated with presence of carotid stenosis [odds ratio (OR) per standard deviation (SD) increase in calcification volume: 1.54 (95%C.I.:1.15;2.05)]. Furthermore, higher calcification load was related to presence of intraplaque hemorrhage [odds ratio OR per SD increase in calcification volume: 2.61 (95%C.I.:1.99;3.41)], but not to lipid (Table 2, model 1). Additional adjustment for maximum plaque thickness rendered the association with carotid stenosis insignificant, but the association with intraplaque hemorrhage remained [OR per SD increase in calcification volume: 2.02 (95%C.I.:1.53;2.66)]. Moreover, in this model, larger calcification volume also associated with lower prevalence of lipid core in the plaque [OR per SD increase in calcification volume: 0.72 (95%C.I.:0.58;0.89)] (Table 2, model 2).

After stratification for the degree of stenosis, we found that larger calcification volume was significantly associated with the presence of intraplaque hemorrhage in both strata [OR per SD increase in calcification volume: 2.03 (95%C.I.:1.20;3.46) and 2.69 (95%C.I.:1.95;3.71)] (Table 3, model 1). After additional adjustment for the maximum plaque thickness, this association did not change. In persons with a high degree of stenosis (>30%), larger calcification volume was additionally associated with lower prevalence of lipid core in the plaque [OR per SD increase in calcification volume: 0.56 (95%C.I.:0.34;0.92)] (Table 3, model 1). Additional adjustment for maximum plaque thickness rendered the association between larger calcification and a lower prevalence of lipid core in persons with low degree of stenosis (0-30%) to be also statistically significant [OR per SD increase in calcification volume: 0.75 (95%C.I.:0.58;0.96)] (Table 3, model 2). The P-value for interaction was 0.07 for lipid core, and 0.34 for intraplaque hemorrhage.

Table 1. Population characteristics

Sample size	329
Carotids	611
Women	43.0 %
Age*, years	70.0 (6.2)
Body mass index, kg/m^2	27.3 (3.4)
Systolic blood pressure, mmHg	147.5 (20.1)
Diastolic blood pressure, mmHg	79.6 (11.5)
Past or current smokers	72.5 %
Diabetes	12.8 %
Total cholesterol, mmol/l	5.5 (1.0)
Calcification volume†, mm^3	59.0 (16.6 - 144.1)
Presence of intraplaque hemorrhage	26.5 %
Presence of lipid core	23.7 %
Carotid stenosis >30%	13.1 %
Maximum carotid wall thickness, mm	3.3 (1.0)

Values are means (standard deviation) for continuous variables and percentages for dichotomous variables
* Age at time of CT examination
† Median with interquartile range

Table 2. Association between calcification (CT) and other plaque characteristics (MRI)

		Stenosis*	**Lipid core**	**Intraplaque hemorrhage**
		OR (95%CI)	OR (95%CI)	OR (95%CI)
Calcification volume, per SD	Model 1	1.54(1.15;2.05)	0.91(0.75;1.11)	2.61(1.99;3.41)
	Model 2	1.04(0.76;1.41)	0.72(0.58;0.89)	2.02(1.53;2.66)

SD, standard deviation; OR, odds ratio; CI, confidence
*Stenosis is defined as > 30%
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex and maximum plaque thickness

Table 3. Association between calcification (CT) and other plaque characteristics (MRI) stratified by degree of stenosis

		Stenosis ≤ 30%		Stenosis >30%	
		Lipid core	Intraplaque hemorrhage	Lipid core	Intraplaque hemorrhage
		OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Calcification volume, Per SD	Model 1	0.95(0.76;1.18)	2.69(1.95;3.71)	0.56(0.34;0.92)	2.03(1.20;3.46)
	Model 2	0.75(0.58;0.96)	2.15(1.54;3.00)	0.53(0.32;0.88)	2.10(1.18;3.73)

SD, standard deviation; OR, odds ratio; CI, confidence
P for interaction was 0.07 for lipid core, and 0.34 for intraplaque hemorrhage
Model 1: adjusted for age and sex
Model 2: adjusted for age, sex and maximum plaque thickness

In persons with a high degree of stenosis (>30%), larger calcification volume was additionally associated with lower prevalence of lipid core in the plaque [OR per SD increase in calcification volume: 0.55 (95%C.I.:0.34;0.91)] (Table 3, model 1). Additional adjustment for maximum plaque thickness rendered the association between larger calcification and a lower prevalence of lipid core in persons with low degree of stenosis (0-30%) to be also statistically significant [OR per SD increase in calcification volume: 0.76 (95%C.I.:0.60;0.97)] (Table 3, model 2). P for interaction was 0.06 for lipid core, and 0.37 for intraplaque hemorrhage. Excluding persons with a history of stroke did not change these associations.

DISCUSSION

In a sample of 329 persons with asymptomatic carotid wall thickening from the general population, we found that larger calcification volume was associated with a higher prevalence of intraplaque hemorrhage, but conversely also with a lower prevalence of lipid core. These associations remained after adjustment for markers of atherosclerotic load, i.e. degree of stenosis and maximum plaque thickness.

The major strengths of our study include the population-based setting and the use of multiple imaging modalities within study subjects. We used both CT and MRI to assess the different plaque components in each carotid artery. MRI is very well suitable to accurately visualize soft

plaque components, i.e. intraplaque hemorrhage and lipid. On the other hand, CT is superior in detecting calcification. To our knowledge, combining the strengths of multiple imaging modalities for the study of carotid atherosclerotic plaque in a population-based setting has not been applied before. Furthermore, we assessed plaque components per carotid artery and analyzed these on the level of the carotid artery instead of on a person-level, enabling to study the coexistence of components within the plaque. Several other potential limitations should also be considered. First, we did not use contrast-enhanced MRI and where thus potentially less sensitive in our detection of lipid core.[18] However, the non-contrast enhanced sequences we used were repeatedly shown to have high accuracy in validation studies.[19-21] Second, we could not image other characteristics of plaque vulnerability, for example fibrous cap inflammation or instability, thereby lacking the possibility to evaluate the association between calcification load and these signs of vulnerability. Furthermore, we assessed solely the presence or absence of hemorrhage and lipid core and not the volume of these components. Third, due to logistical reasons, only 349 of all persons that underwent CT, also underwent an MRI examination, and the time interval between the CT- and MRI-examinations was relatively long (4.6 years on average). Yet, post-hoc analyses with adjustment for scan interval did not yield different results. Moreover, because atherosclerosis is a chronic, slowly progressing disease, this time interval might indeed not influence our results.

We found that larger carotid calcification volume at the level of the bifurcation related to presence of intraplaque hemorrhage. This is an interesting finding because intraplaque hemorrhage is considered an important, if not, the most important vulnerable plaque component.[22] On the other hand, plaque calcification is often considered a stabilizing plaque characteristic.[8] The association we found did not seem to be driven simply by plaque size or atherosclerotic burden, as it did not alter after adjustment for wall thickness and was even more pronounced in persons with low degree of carotid stenosis. This raises the question whether plaque calcification is really a plaque stabilizer.[10]

Contrary to intraplaque hemorrhage, we found that larger calcification volumes were associated with a lower prevalence of lipid core, which was more pronounced at higher degree of stenosis (> 30%). This may indeed suggest that the amount of calcification within a plaque leaves less room for lipid, which may be suggestive for stabilization of the plaque to some extent, nevertheless the exact details of how this process unravels remains unclear. Furthermore, we should consider that at higher degrees of stenosis selective non-participation may have occurred for persons with vulnerable plaque, which may also have influenced this association.

Our findings thus suggest that a larger amount of atherosclerotic calcification in the carotid plaque does not automatically indicate that the plaque is more stable. More evidence for this comes from studies in which the location (superficial, deep) and type of calcification (patchy, coalesced) were suggested to be important with regard to the plaque instability.[23, 24] Especially those plaques with multiple patchy locations of calcification might be at higher risk of rupture due to the mechanical instability caused at the interface of 'hard' calcification with 'soft', noncalcified plaque.[23] Unfortunately, we could not investigate this because contrast-enhanced CT would be necessary to detect certain of these characteristics. Our findings underline that more research into the characteristics of plaque calcification, e.g. morphology and location, and into the relationship with vulnerable plaque components and future cardiovascular events (in a prospective design) is necessary.

In conclusion, we found that there is a complex relationship between calcification, intraplaque hemorrhage and lipid core within the carotid atherosclerotic plaque. Plaques with a higher load of calcification contain more often hemorrhagic components, but less often lipid core. These findings urge for prospective studies investigating the interrelation of these different plaque components with regard to future cerebrovascular events.

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4.2

Epicardial Fat Volume: an Automated Quantification Method

ABSTRACT

Background and Purpose

There is increasing evidence that epicardial fat plays an important role in the development of cardiovascular disease. Obtaining the epicardial fat volume from routinely performed non-enhanced cardiac CT scans is therefore of clinical interest. The purpose of this work is to investigate the feasibility of automatic pericardium segmentation and subsequent quantification of epicardial fat on non-enhanced cardiac CT scans.

Methods

Imaging data of 98 randomly selected subjects belonging to a larger cohort of subjects who underwent a cardiac CT scan at our medical center were retrieved. The data were acquired on two different scanners. Automatic multi-atlas based method for segmenting the pericardium and calculating the epicardial fat volume has been developed. The performance of the method was assessed by 1) comparing the automatically segmented pericardium to a manually annotated reference standard, 2) comparing the automatically obtained epicardial fat volumes to those obtained manually, and 3) comparing the accuracy of the automatic results to the interobserver variability.

Results

Automatic segmentation of the pericardium was achieved with a Dice similarity index of 89.1 ± 2.6% with respect to Observer 1 and 89.2 ± 1.9% with respect to Observer 2. The correlation between the automatic method and the manual observers with respect to the epicardial fat volume computed as the Pearson's correlation coefficient (R) was 0.91 ($P < 0.001$) for both observers. The inter-observer study resulted in a Dice similarity index of 89.0 ± 2.4% for segmenting the pericardium and a Pearson's correlation coefficient of 0.92 ($P<0.001$) for computation of the epicardial fat volume.

Conclusions

We developed a fully automatic method that is capable of segmenting the pericardium and quantifying epicardial fat on non-enhanced cardiac CT scans. We demonstrated the feasibility of using this method to replace manual annotations by showing that the automatic method performs as good as manual annotation on a large dataset.

I. INTRODUCTION

Cardiovascular disease is one of the leading causes of death worldwide.[1] Epicardial fat is the adipose tissue which is found between the myocardium and the visceral layer of the pericardium, and thus directly surrounds the entire heart as well as the coronary arteries. Increasing evidence implicates epicardial fat in the etiology of cardiovascular disease.[2–4] It is thought that through local production of inflammatory factors it may directly contribute to the formation of coronary atherosclerosis.[5–7] Few studies have found that epicardial fat is associated with cardiovascular risk factors.[8, 9] Other studies have shown that epicardial fat is a dominant factor in case of coronary artery disease.[10–12] A few population based studies have also been performed. Ding et al. investigated whether epicardial fat is an independent predictor of future heart disease events as compared to conventional risk factors on 998 individuals from the MESA study.[13] Mahabadi et al. quantified epicardial fat volume on 4093 subjects and found that epicardial fat predicts coronary events in the general population.[14]

Several methods for epicardial fat quantification have recently been developed. However, most of the methods described previously require manual delineation of the pericardium, which is subsequently used to quantify the volume of fat. Taguchi et al. traced the epicardial, subcutaneous and visceral fat.[15] Wheeler et al. used landmark points to initialize the heart segmentation.[16] Rosito et al. traced the pericardium to delineate the heart from the surrounding structures.[8] Ding et al. used a few landmark points around the heart to enclose it in an envelope,[13] similar to the method proposed by Wheeler et al.[16] More recently, a semi-automatic method was proposed by Dey et al.[18,19] Their method needs two manual inputs. First, the user needs to select the top and the bottom slice in which the heart is contained. Once this is done, the method uses region growing and anatomical information to segment the heart. Second, the user needs to select 5 to 7 control points on the axial slices to pinpoint the location of the pericardium. The degree of interaction of this method is still substantial.

Population and clinical studies, as well as clinical workflow, would potentially greatly benefit from a precise and fully automatic method for epicardial fat quantification. Ultimately, findings from such studies could further establish the role of epicardial fat in the development of cardiovascular disease. In this paper, we present a method which is able to automatically segment the pericardium on non-enhanced cardiac CT scans and subsequently quantify the epicardial fat volume within the pericardium. Our method uses an atlas-based segmentation approach in order to segment the pericardium. The atlas-based segmentation approach is an adaptation of our previous work, where atlas-based segmentation was evaluated with respect to segmenting the heart and its chambers in a

multi-center, multi-vendor contrast-enhanced CT (CTA) study.[20] We evaluated our method on 98 CT scans with respect to 1) the accuracy of pericardium segmentation 2) the accuracy of epicardial fat quantification and 3) accuracy of the results with respect to the interobserver variability. The evaluation was conducted by comparing the performance of our method to two independent manual observers.

The remainder of the paper is organized as follows. The datasets and the imaging protocol used to obtain the scans are explained in Section II. Section III provides an overview of our method, and explains the atlas building, multi-atlas based segmentation, quantification of epicardial fat, reference standard and statistical analysis. Section IV describes the experiments we performed. We present our results in Section V, discussion and future work in Section VI and finally the conclusion is provided in Section VII.

II. STUDY POPULATION AND IMAGING PROTOCOL

For this study, we randomly selected 98 subjects from the population-based Rotterdam Study,[21] who underwent a multi-detector computed tomography (MDCT) scan of the heart. These persons were scanned within the context of a larger MDCT-project involving calcium-scoring in multiple vessel beds.[22] The Rotterdam Study was approved by the Institutional Review Board with additional specific approval of the CT study. All participants gave written informed consent.

Participants were scanned on two different generations of Siemens scanners (Sensation 16 (n = 55) or Sensation 64 (n = 43), Siemens Medical Solutions, Forchheim, Germany). The cardiac scan ranged from the apex of the heart to the tracheal bifurcation.

Scan parameter settings were as follows. A tube voltage of 120 kV was used for all scans. The images from both scanners were reconstructed with a slice thickness of 3.0 mm, an increment of 3.0 mm and an average field of view (FOV) of 180 mm. The images were reconstructed with a matrix of 512 × 512 using a b35f (medium/smooth) kernel, and contained on average 52 slices.

III. METHODS

A. Method overview

The quantification method consists of two steps: 1) pericardium segmentation, and 2) epicardial fat volume quantification. For pericardium segmentation, we used a multi atlas-based segmentation approach, as described in the work of Kirişli et al.[20] In this approach, manually segmented CTA scans (atlases) are registered to the subject's CT scan.

Subsequently, the segmentations of these atlases are mapped onto the subject's scan to be analyzed. The mapped segmentations from each of the atlases are fused to obtain the final pericardium segmentation. This whole procedure is fully automatic. The epicardial fat is subsequently quantified by applying a threshold of -200 to -30 HU[23] to the segmented pericardium, followed by connected component analysis. Details with respect to the atlases used, the registration approach, and

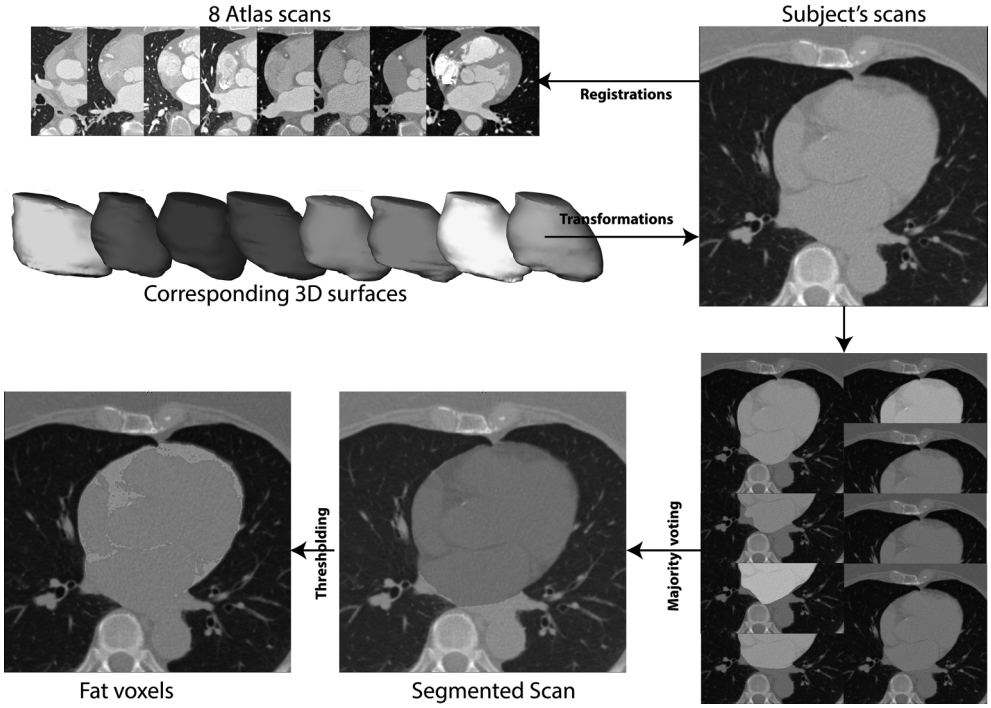

Figure 1. Overview of the segmentation process
Top left: Eight CTA atlas scans and the corresponding manually segmented 3D surfaces. Top right: CT scan to be segmented. The atlas scans are registered and the 3D surfaces are transformed to match the subject scan. Bottom right: The 3D surfaces are combined using majority voting. Bottom left: Resulting fat voxels obtained after thresholding and connected component analysis.

the fat quantification are presented in the subsequent sections. Figure 1 shows an overview of all the steps involved in the automatic method.

B. Atlas selection and surface computation

CTA scans were used as atlas images because of their higher resolution and the better visibility of the cardiac chambers (due to the presence of contrast material). This enables the observers to accurately delineate the pericardium manually. Eight previously acquired CTA scans from different subjects were included in as the atlas scans. Detailed information on atlas selection and image reconstruction can be found elswhere.[20] The manually obtained contours of the atlas scans were converted to 3D surfaces.

C. Multi-atlas based segmentation

Multi-atlas segmentation is a process in which multiple atlas images with corresponding manually annotated label images are individually registered to the subject scans.[24] The segmentation of the subject scans is then obtained by fusing all the resulting transformed label images.[25] In this work we use majority voting to fuse the label images. Image registration is used to spatially align the atlas scans and the subjects' scan.[26] Detailed information on registration is provided elsewhere.[27, 28]

The resulting transformations from the registration steps are used to map each of the eight atlas surfaces to the subject's scan. The points on the 3D surface of the pericardium are transformed according to the generated transformation parameters. Once all the eight 3D atlas surfaces are transformed on to the subject's scan, they are binarised and majority voting is applied to obtain the final pericardium segmentation (See Figure 1 for a visual representation).

D. Epicardial fat quantification

The automatically obtained pericardium segmentation is used as a region of interest (ROI) to quantify the adipose tissue voxels. A threshold window of -200 to -30 HU is applied to obtain the adipose tissue. A connected-component analysis[29] is subsequently applied to all adipose tissue voxels using an 18-neighbourhood rule, in order to remove regions smaller than 10 voxels (2.8 mm^3) in size, which we consider to be noise.

E. Reference Standard

Two experienced observers (D.B. and A.R.), blinded to the patient information as well as to the results of each other, manually traced the pericardium in each of the CT scans, as shown in Figure 2. A dedicated tool implemented in MeVisLab (http://www.mevislab.de) was used by the observers for manual annotations. Once the pericardium was delineated, a threshold window of -200 to -30 HU was

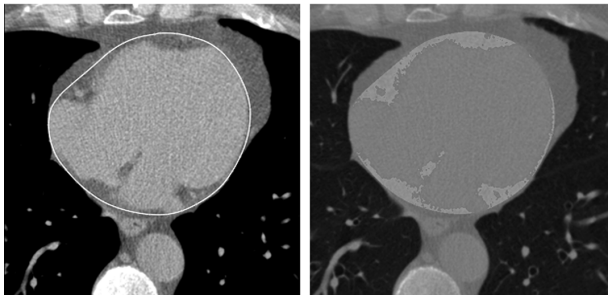

Figure 2. Manual heart segementation
Left: a random axial slice showing the result of a manually obtained whole heart segmentation (with adjusted windowing level, for better visibility). Right: corresponding slice showing the voxels containing epicardial fat.

applied to the segmented region.[23]

Adipose tissue voxels were then automatically extracted using connected-component analysis and the volume of fat in milliliters (ml) was computed. The reference standard obtained this way contains both pericardium segmentations and epicardial fat volume quantifications (Figure 2).

F. Statistical Analysis

We report the Dice similarity index and the mean surface distance error between the pericardial heart segmentation by the automatic method and each of the manually obtained segmentations. To evaluate the performance of the fat quantification method per patient, Pearson's correlation coefficient (R) was calculated, linear regression was performed, and Bland-Altman plots were created. Furthermore, the accuracy of the method was compared to the inter-observer variability. The analyses were performed using MATLAB version 7.9.0 (The MathWorks, Natick, MA) and IBM SPSS Statistics version 20 (IBM Corp, Armonk, NY).

4.2

IV. EXPERIMENTS

Our method consists of segmenting non-enhanced CT scans with the help of contrast enhanced CT atlas scans. The registration problem we face here is that the fixed image and moving image are asymmetric, having different characteristics. The CT scans in which we aim to quantify the epicardial fat has an average in-plane resolution of 0.35×0.35 mm^2 and a slice thickness of 3.0 mm, whereas the CTA atlases have an average in-plane resolution of 0.32×0.32 mm^2 and a slice thickness of 0.4 mm. In order to obtain the optimal parameters to register the CTA atlases and the CT subjects, we performed pilot experiments on a subset of 35 randomly selected CT datasets. Two registration strategies were investigated and the segmentation results were compared to the results of one of the observers. In both strategies, the CTA atlas was used as the fixed image and the subjects' CT scan was used as the moving image.

Strategy one: The similarity metric was computed by randomly sampling intensity values from the whole image.

Strategy two: The similarity metric was computed by randomly sampling intensity values with the heart region only (by using a fixed heart mask).

As mentioned previously, a two-stage registration approach was used. In the first stage an affine transformation was used. In the second stage, a non-rigid registration using a B-spline transformation[30] was employed while using the results of the affine transformation to initialize the registration. Mutual Information[31] was used as similarity measure for the cost function.[27,28] Optimization was performed using Adaptive Stochastic Gradient Descent and, the number of voxels sampled in each iteration was set to 2048 and the number of iterations were set to 512 for the affine transformation and 2048 for the B-spline transformation.[32] Further details about parameter selection and optimizations have been described previously.[20] All registrations were performed using Elastix[33], a publicly available software package (http://elastix.isi.uu.nl).

The heart mask was used in both stages of the registration approach. The main purpose of using the fixed mask was to prevent the registration to be affected by tissues surrounding the heart, such as the lungs, rib cage and the vertebra. The mask was created by dilating the original manually annotated pericardium by 1 cm. The masks were created once, and were created for the atlas scans and not the subject scans.

We found that the average accuracy of both strategies was very similar in terms of the mean, but using the mask resulted in a smaller standard deviation. It was also confirmed visually that the accuracy of finding the pericardium using strategy two was better than when using strategy one. Further experiments on the entire dataset were thus performed using the registration with the mask.

V. RESULTS

The baseline characteristics of the study population are shown in Table 1. The mean age was 69.4 (± 5.6) years and 46.9 % were women.

Table 1. Population characteristics

Sample size	98
Women	46.9 %
Age, years	69.4 (5.6)
Body mass index, kg/m^2	27.4 (3.9)
Systolic blood pressure, mmHg	147.8 (22.3)
Diastolic blood pressure, mmHg	82.2 (11.1)
Past or current smokers	70.4 %
Diabetes	7.1 %
Total cholesterol, mmol/l	5.8 (0.9)

Values are mean (standard deviation) for continuous variables, percentages for dichotomous variables

A. Agreement between the automatic method and the observers

A visual check showed that 95 out of 98 segmentations were successful. Three segmentations failed due to registration errors caused by anatomical and FOV variations. These scans were excluded from further analysis. The subjects had an average fat volume of 101 ± 38 ml according to Observer 1 and 113 ± 43 ml according to Observer 2. The automatic method found the average fat volume to be 102 ± 34 ml. A Dice similarity index of 89.1% and 89.2% was obtained between the automatic segmentation and each of the manual segmentations, respectively. The mean surface distance between the automatically derived cardiac surface and the observer segmentations was 3.8 ± 1.1 mm and 3.5 ± 0.7 mm, respectively. A Pearson correlation R of 0.91 ($P < 0.001$) was obtained for fat quantification results between the automatic segmentation and each of the manual segmentations. The mean absolute difference between the automatic method and each of the manual segmentations with respect to the amount of quantified fat was 11.6 ml and 16.6 ml, respectively. The linear regression plots are shown in Figure 3. The numbers obtained from the Bland-Altman analysis and the confidence intervals of the linear regression are shown in Table III. It can be noted that the bias from the Bland-Altman analysis with respect to observer 1 is almost zero and the automatic method slightly overestimates the volume of epicardial fat as compared to observer 2.

B. Inter-Observer agreement

An average Dice similarity coefficient of 88.9% was found between the segmentations of the observers. The mean surface distance between the two observers was 4.3 ± 1.0 mm over all datasets. With respect to the amount of quantified epicardial fat, the mean absolute difference between the two observers was 15.6 ml, and

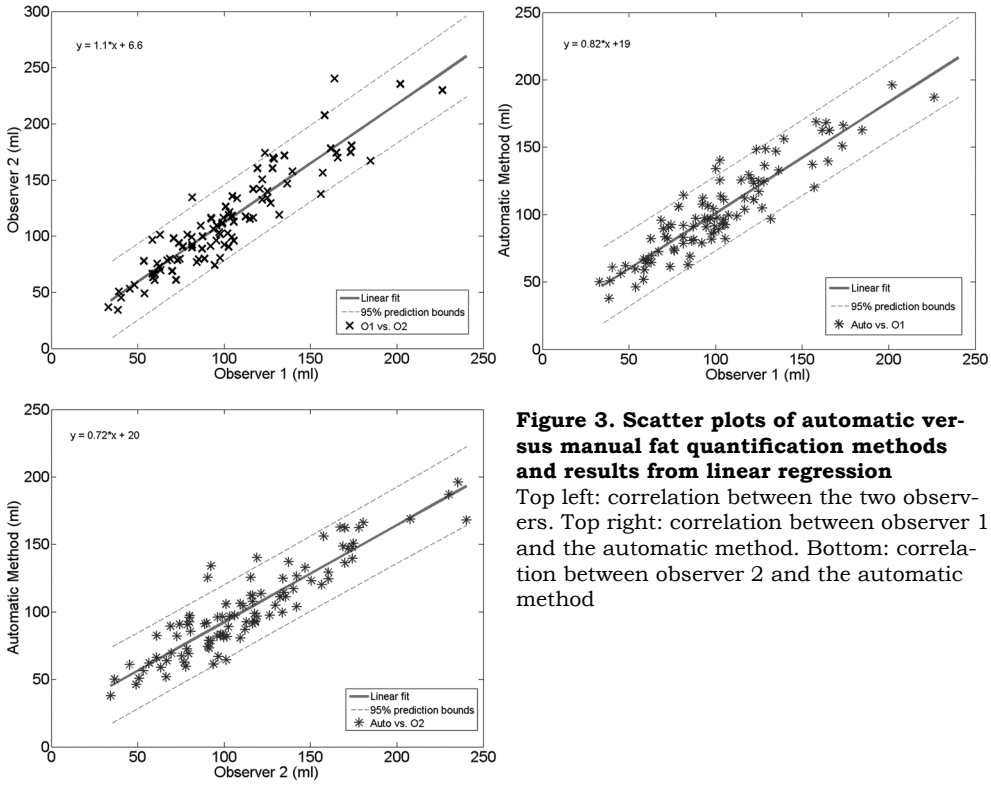

Figure 3. Scatter plots of automatic versus manual fat quantification methods and results from linear regression
Top left: correlation between the two observers. Top right: correlation between observer 1 and the automatic method. Bottom: correlation between observer 2 and the automatic method

Table 3. Performance of the whole heart segmentation; comparing the automatic method to each of the observers, and the observers to each other

	Automatic versus Observer 1	**Automatic versus Observer 2**	**Observer 1 versus Observer 2**
Segmentation measures			
Dice Similarity Index (mean ± SD), %	89.1 ± 2.6	89.2 ± 1.9	88.9 ± 2.5
Mean Surface Distance (mean ± SD), mm	3.8 ± 1.1	3.5 ± 0.7	4.3 ± 1.0
Quantification Measures			
Correlation R	0.91	0.91	0.92
Linear Regression (CI for regressioncoefficient)	0.75 to 0.90	0.65 to 0.79	0.96 to 1.15
Bland-Altman Bias (95% CI)	-0.8 (-31;29)	11.3 (-25;47)	12.1 (-21;45)

SD, standard deviation; mm, millimeter; CI, confidence interval

the Pearson correlation coefficient was 0.92 ($P < 0.001$). A Bland-Altman analysis of the data showed that the limits of agreement were between -45.3 and 21.3 ml and a bias of 12.1.

VI. DISCUSSION

In this study, we presented a fully automatic method for epicardial fat quantification. The method is based on automatic pericardium segmentation. A good correlation with manual quantification was observed, with differences very similar to the inter-observer variability.

The Dice similarity index (overlap area) between the automatic pericardium segmentation and each of the manual annotations was slightly better than the inter-observer Dice similarity coefficient. The mean surface distance error between the automatic and manual segmentations corresponds to 1.5 voxels in the slice direction, which can be considered small. When the actual amount of fat volume quantified using our method was compared to each of the observers, it resulted in a mean absolute difference of 11.6 ml and 16.6 ml respectively. This difference in volume is very close to the inter-observer agreement, which was 15.6 ml. The same conclusion can be drawn from the correlation coefficient R obtained with respect to the quantified fat volume of the automatic method and the manual observers. Compared to the existing quantification methods, our method is the first that is fully automatic. Other methods either use manual tracings of the different tissue types, or a manual approach to delineate the pericardium, before quantifying the adipose tissue voxels. [8, 13–17] This is a tedious and time-consuming task to perform. Other, semi-automatic methods need two interactions,[18, 19] which could limit the use of the method in processing a large number of datasets in an epidemiologic setting.

We had to exclude three segmentations from further analyses because of the following issues. The first subject appeared to have undergone pneumonectomy causing a very unusual position of the heart. The second had a heart shape anatomically quite different from the others, and the third had a different field of view compared to the atlas scans used. The large difference between the atlas scan and the subject scan caused the registration to fail, which resulted in erroneous segmentation of the pericardium.

There has been some confusion in the literature between the nomenclatures of the adipose tissue contained within the pericardium;[34] some studies call it epicardial fat tissue, whereas others call it pericardial fat tissue. Based on the

definition provided by Iacobellis et al.,[35] we decided to denote the adipose tissue contained within the pericardium as epicardial fat. In short, in this definition epicardial fat is the adipose tissue between the myocardium and the visceral layer of the pericardium.

We did not investigate to what extent the method can be used on multiple scanner types; in this study, we only demonstrated the feasibility of using the method on two generations of Siemens scanners. However, as our method is based on multi-atlas segmentation, we are confident that the same approach would work on other scanner types, as long as the subject scans and the atlas scans have a similar field of view. It has been demonstrated in our previous study,[20] that atlas-based segmentation of the pericardium was performed with a similar accuracy with respect to multi-vendor/multi-center CTA datasets. If required, the method could utilize atlas scans from the same scanner.

In the current setup, visual inspection was still required to check the accuracy of the pericardium segmentation, which resulted in discarding the three scans on which the segmentation failed. Instead of discarding these scans, or in case of small failures, manual correction before fat quantification is an option. We did not integrate this in our protocol, as the results were sufficiently accurate without adaptation.

VII. CONCLUSION

We developed and evaluated an automatic method for pericardium segmentation and subsequent epicardial fat quantification. We demonstrated that our automatic approach achieved good correlation to manual quantifications. The automatic method described in this paper could potentially be used on large clinical or population studies in order to investigate the relationship between epicardial fat volume and clinical cardiovascular disease.

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5

General Discussion

Over the past decades, advances in non-invasive imaging techniques have aided considerably in the process of visualizing and quantifying atherosclerosis.[1] Apart from studies in patients, it is now also possible to non-invasively image atherosclerosis in large samples of persons without clinically overt disease. This concept of population-imaging is particularly useful for the study on atherosclerosis, because atherosclerosis often remains asymptomatic for years before clinical events occur.[2] This provides unique opportunities to study the etiology of atherosclerosis, and to detect early vessel changes which may indicate an increased risk of subsequent disease.

The objective of this thesis was to gain new insights into the etiology and clinical neurological consequences of atherosclerosis, using population-imaging. A specific focus was on the atherosclerosis burden in different vessel beds. Specifically, I addressed the potential implications that may accompany these differences; on the one hand with regard to the etiology of atherosclerosis and on the other hand with respect to the subsequent risk of overt disease. All studies described in this thesis were embedded within the prospective, population-based Rotterdam Study.[3]

In the current chapter I will first summarize and review the main findings. Next, I discuss methodological issues which should be considered when interpreting the findings. Finally, I will conclude with potential implications of my research with regard to clinical practice and future research.

MAIN FINDINGS

Risk Factors for Atherosclerosis

Lifestyle- and environmental risk factors

Currently, several modifiable lifestyle- and environmental cardiovascular risk factors are known that contribute considerably to the formation of atherosclerosis. Several of the most important include hypercholesterolemia, hypertension, diabetes mellitus and smoking, which are all targets of intervention in current clinical practice.[4] However, as extensively discussed in this thesis, it has come to light that the burden of atherosclerosis may vary considerably across different vessel beds. The origin of these differences may - at least partly - lay in location-specific differences in susceptibility to these cardiovascular risk factors. Over the past decades, associations between cardiovascular risk factors and atherosclerosis at several locations have been extensively studied.[5-9] In addition, studies have been performed that specifically addressed potential distinct cardiovascular risk factor profiles for the presence of atherosclerosis in different arteries.[6, 9, 10] Indeed,

for coronary artery atherosclerosis, aortic arch atherosclerosis and carotid artery atherosclerosis, such differences were clearly present,[6, 10] strengthening the hypothesis of a varying susceptibility to cardiovascular risk factors in different vessels.

Within this wide range of research into risk factors of atherosclerosis in various vessels, it is surprising that atherosclerosis in the intracranial vasculature was yet relatively unexplored. From a clinical perspective, knowledge on whether or not intracranial atherosclerosis is caused by the same risk factors as atherosclerosis in other vessels is extremely valuable, specifically because intracranial atherosclerosis accounts for a huge number of strokes worldwide.[11, 12] Indeed, I found that most established risk factors for atherosclerosis at other locations were also associated with intracranial atherosclerosis (chapter 2.1). Nonetheless the contribution of the various risk factors appeared to be markedly different from their contribution to atherosclerosis in other vessel beds. Diabetes was by far the strongest risk factor for intracranial atherosclerosis in the general aging population within my study, whereas in other vessel beds hypertension and hypercholesterolemia have been described to exert more effect on atherogenesis.[5, 9] Interestingly, an autopsy study in patients with fatal stroke also described diabetes as one of the strongest risk factors of intracranial atherosclerosis,[13] which was further confirmed by another group investigating risk factors for intracranial atherosclerosis.[14] Moreover they described distinct biological risk factor profiles between intracranial and extracranial atherosclerosis, which again fits the concept of differences in the etiology of atherosclerosis across different vessel beds.[14]

Another issue which is important to keep in mind is the presence of sex-related differences in cardiovascular risk factor profile of atherosclerosis. It has been repeatedly postulated that in women, circulating hormone levels, especially estrogens, may considerably contribute to these differences. With regard to intracranial atherosclerosis we also found sex-related differences in risk factor profile, which are extensively discussed in chapter 2.1. In this discussion, I highlight the role of hypertension in a bit more detail. In my study, the contribution of hypertension to intracranial atherosclerosis varied considerably between men and women. After adjustment for the presence of other cardiovascular risk factors, hypertension was still a very strong contributor to intracranial atherosclerosis in women, whereas it was not in men. There may be various explanations - which are also applicable to atherosclerosis in general - underlying this contrast, of which two seem most likely. First, at older age (>75 years) the prevalence of systolic hypertension is about 15% higher in women than in men.[15] This could thus indicate that it contributes more to the formation of atherosclerosis, simply because the prevalence is higher. Second, hypertension has been found to cause more vascular endothelial damage in women compared to men, possibly due to

various complex changes induced by menopause.[15, 16] In turn, endothelial damage is important for the initiation of atherosclerosis. Together, this may imply that more aggressive treatment of hypertension in women could translate into a relatively large beneficial effect on the occurrence and amount of specifically intracranial atherosclerosis (and its neurological consequences, to be further discussed below).

Genetic risk factors

Despite extensive research that has been performed on the identification of the abovementioned lifestyle- and environmental cardiovascular risk factors, a large part of the variability in the total burden of atherosclerosis remains yet unexplained.[17] This inevitably indicates that other, unknown factors also considerably contribute to the development of atherosclerosis. During the last years, it has become clear that genetic factors may play an important role in the development of atherosclerosis.[18, 19] Genome-wide association studies have become a popular tool to identify common genetic variants of diseases and of risk factors for diseases.

Translating this specifically to atherosclerosis, several important loci have been identified that are associated with coronary artery calcification.[20] Against the background of the previously demonstrated location-specific etiology of atherosclerosis, we determined whether these genetic loci were also associated with calcification in other locations than the coronary arteries (chapter 2.2). Indeed, we found that the genetic basis for aortic arch and carotid artery calcification largely overlaps with that of coronary artery calcification. However, we found that the genetic variants contributed differentially to the burden of atherosclerotic calcification in these vessel beds. This suggests that also on the genetic level, there are profound differences in the etiology of atherosclerosis across vessel beds. Additionally, we also investigated the genetic basis of atherosclerosis using the genetics of strong risk factors of atherosclerosis, i.e. serum cholesterol levels and blood pressure (chapter 2.2 and 2.3).[21, 22] Using genetic risk scores based on the identified genetic variants for serum lipid fractions and blood pressure, we demonstrated relationships of these genetic risk scores with atherosclerosis in multiple vessel beds.

In summary, these results suggest that there are both shared and unique genetic factors implicated in the formation of atherosclerosis in various vessel beds. Future studies are warranted and may further unravel the exact etiology of atherosclerosis, through the identification of new genetic variants.

Atherosclerosis and neurological consequences

From the previous paragraph it follows that the differential atherosclerotic burden across various vessel beds likely reflects the location-specific etiology of ath-

erosclerosis. Also from the perspective of atherosclerosis being a risk factor or even a cause of cerebrovascular disease, it is conceivable that the contribution of atherosclerosis to cerebrovascular disease may vary depending on the location. With this concept in mind we investigated the role of atherosclerosis in various preclinical and clinical cerebral conditions.

Cerebrovascular disease

Stroke is the most frequent neurological disease, and the second most important cause of cardiovascular mortality.[23] The lifetime risk of stroke is estimated to be at least 1 in 6 and the burden of stroke is expected to increase even further.[24, 25] Yet, it should be kept in mind that clinical stroke represents only the tip of the iceberg of the total burden of cerebrovascular disease. Especially subclinical cerebrovascular disease, consisting of 'silent' infarcts, microbleeds and white matter pathology, occurs much more frequent and is associated with a variety of clinical conditions later in life. Improvements in imaging techniques, specifically magnetic resonance imaging (MRI), have led to the possibility of detection and quantification of several of these subclinical markers of cerebrovascular disease, which in turn allows the possibility to study their etiology. The identification of risk factors subclinical cerebrovascular disease may aid in the development of therapeutic or preventive strategies that are aimed at improving brain health.

Against this background, we investigated whether atherosclerosis is associated with the presence and amount of MRI-markers of early cerebrovascular damage in the general population. In summary, we found that atherosclerosis was associated with white matter lesion-burden and the presence of lacunar infarcts. This suggests that atherosclerosis indeed might be involved in the etiology of subclinical cerebrovascular disease (chapter 3.1). Two other studies that investigated this relationship for coronary artery calcification found similar results.[26, 27] However, investigating and comparing the various vessels beds we found most prominent associations for atherosclerosis located in vessels that were closest to the brain, i.e. the extracranial and intracranial internal carotid arteries.

Additionally we investigated the role of intracranial atherosclerosis in clinical stroke. The importance of intracranial atherosclerosis is evident from research in populations of Asian and African descent. In these populations intracranial atherosclerosis has been established as the major cause of stroke, contributing to up to 50% of all stroke cases,[12] whereas in whites the impact of intracranial atherosclerosis remains unexplored. We were the first to demonstrate, in a general population-setting, that intracranial atherosclerosis is a major cause of stroke in whites (chapter 3.4). Even after adjustments for large-artery atherosclerosis in the aortic arch and the carotid artery bifurcation, this relationship was firmly present. Moreover, we found that intracranial atherosclerosis contributed to 75%

of all strokes, whilst for aortic arch atherosclerosis or atherosclerosis in the carotid artery bifurcation this was 45% and 25%, respectively. Altogether, these findings emphasize that existing location-specific differences in atherosclerotic burden indeed translate into differences in risk of subsequent cerebrovascular disease.

Cognitive impairment and dementia

The second major devastating age-related brain disease is dementia, with Alzheimer's disease as its most prevalent subtype.[28-30] Its global burden is expected to increase rapidly with an expected number of at least 80 million people with dementia in 2040.[28, 30] The underlying etiology of dementia and cognitive decline is multi-factorial and involves different pathologies which interact and accumulate over the course of years.[31] In addition to beta-amyloid and tau pathology, the role of vascular pathology, including atherosclerosis is increasingly being recognized in the etiology of dementia and Alzheimer's disease.[32, 33]

With our study on atherosclerosis and the risk of dementia, we further established the role of atherosclerosis in the etiology of dementia. Most importantly, we found that systemic atherosclerosis was associated with a higher risk of dementia. Actually, this suggests that generalized atherosclerosis, which probably is a better reflection of one's vascular status than localized atherosclerosis in a single vessel bed, associates with dementia. Moreover, we studied the role of atherosclerosis in cognitive impairment, to determine whether atherosclerosis is already implicated in the long preclinical phase of dementia. During this period subtle cognitive deficits develop that can already be measured using dedicated neuropsychological tests.[34] This phase provides a potential window of opportunity for the treatment of factors that are causative for dementia.

In this light, we indeed found that atherosclerosis was already associated with worse cognitive performance, both cross-sectionally and longitudinally (chapter 3.2 and 3.3). According to what we found for dementia, the relationships of atherosclerosis and cognitive performance were approximately similar over all vessel beds. A possible explanation for this may lay in the fact that both atherosclerosis and dementia develop over the course of years. The longstanding deterioration of the arterial system may lead to cognitive decline and dementia through hypoperfusion.[33] This may result in preclinical vascular brain damage, as discussed above, but this may also cause atrophic brain changes which are frequently found in dementia. Indeed, we found that atherosclerosis was related to smaller brain tissue volumes, which fits this hypothesis (chapter 3.2). Lastly, hypoperfusion may cause loss of functionality of the blood-brain barrier. This might allow increased parenchymal deposition of beta-amyloid protein and thereby the formation of amyloid plaques, which is an important hallmark of Alzheimer's disease.[32, 33]

Our finding that atherosclerotic calcification was associated with a higher risk of Alzheimer's disease is in line with this.

Emerging imaging markers of vascular risk

Rapid technological advances in imaging modalities are accompanied by a continuous search for newer and better markers of disease. Also, for the assessment of vascular risk several potentially important new markers have come to light. It has for example been shown that not only the size of the atherosclerotic plaque matters, but that plaque composition is also important with regard to future clinical events. Another emerging marker of vascular risk is the amount of epicardial fat, for which we were the first to develop a fully automated quantification method which can be applied on non-enhanced CT-scans.

The relationship between multiple atherosclerotic plaque components

Relatively new in the field of imaging of atherosclerosis, is the use of magnetic resonance imaging [MRI]. Especially for the identification of different components within the atherosclerotic plaque, MRI is a very promising method.[1] As already pointed out above, this is particularly interesting because increasing evidence suggests that besides the total atherosclerosis burden, the composition of the plaque also provides valuable information[33, 34] on the risk of subsequent clinical events.[35, 36]

To gain more insight into the etiology of atherosclerosis, we investigated the interrelation between the most prominent plaque components in a sample of the general population with asymptomatic carotid wall-thickening. An important strength of this study was that we used a multi-modality imaging approach, to optimally study these three components, which were: calcification as assessed with CT, and lipid core and intraplaque hemorrhage as assessed with MRI. Interestingly, we found that larger calcification volumes within carotid plaques were associated with the presence of intraplaque haemorrhage, but also with a lower prevalence of lipid core. Even though more research is needed to replicate these findings, this was an interesting finding. Especially because the current way of thinking about plaque composition positions intraplaque hemorrhage as the most important marker of plaque vulnerability,[37, 38] i.e. highest risk of rupturing and causing a clinical event, whereas calcification is rather thought to protect the plaque from rupturing.[35]

Epicardial fat as novel marker of vascular risk

Before proceeding to the role of epicardial fat as novel marker of vascular risk, it is important to define it clearly. Various studies interchangeably use pericardial fat and epicardial fat, suggesting that these are the same, whilst they are definitely not.[39, 40] Epicardial fat is located between the outer wall of the myocardium

and the visceral layer of pericardium. Pericardial fat is anterior to the epicardial fat and therefore located between visceral and parietal pericardium.[39]

The importance of the epicardial fat as new marker of vascular risk, lies in its anatomical closeness to the myocardium and that both tissues share the same microcirculation.[39] Increasing evidence indeed demonstrates that the amount of epicardial fat plays an important role in the development of cardiovascular disease.[40-42] Local production of inflammatory factors may be an important underlying mechanism through which epicardial fat directly contributes to the formation of coronary atherosclerosis, and ultimately to the development of acute cardiac events.[43] Further studies are needed on both the etiology of epicardial fat as on its relationship with risk of cardiovascular events. Specifically data from large general populations and clinical databases could bring research on epicardial fat to a next level. As a consequence, several methods for epicardial fat quantification have recently been developed. However, these quantification methods all require substantial manual input.[43-45] Yet in order to be applied on a large scale, fully automated methods are necessary for speed, accuracy and reliability. Therefore, we developed a fully automated method that is capable of segmenting and quantifying epicardial fat volume. We demonstrated the feasibility of using this method on a large dataset and showed that the automatic method performs as good as manual annotation.

Methodological Considerations

Study Design

All studies described in this thesis were part of The Rotterdam Study; a prospective population-based cohort study.[3] Population-based cohort studies provide a unique opportunity to accurately study the incidence, but also the etiology of a large variety of diseases. Another specific advantage of population-based studies is that the findings may be generalized to a large portion of the population.[46] Despite these advantages, there are also several potential limitations that should be taken into account. First, like all other studies, population-based studies may be subject to the specific types of bias, i.e. selection bias, information bias and confounding. Despite the best efforts to minimize these biases through random sampling from the general population, through high response rates, blinded measurements and adjustment for known confounders, the results may still to some extent be subject to bias.

Several studies in this thesis are of a cross-sectional design. A known limitation of cross-sectional research is the lack of the ability to establish a temporal effect; i.e. determine whether the determinant actually precedes the outcome. This specifically applies to chapters 3.1 and 3.2 in which I investigated relationships of atherosclerosis with preclinical vascular brain disease and cognitive impairment.

Here, it can only be speculated that atherosclerosis reflects a causal explanation in these processes. Yet, we found strong temporal associations of atherosclerosis with clinical stroke and dementia (chapter 3.3 and 3.4), which are the recognized end-stages of these preclinical conditions. Against this background, we may postulate that a temporal and possibly causal relationship might thus also exist between atherosclerosis and subclinical vascular brain disease and cognitive deficits.

Atherosclerotic Calcification as Marker of Atherosclerosis

Throughout this thesis, I used atherosclerotic calcification as marker of atherosclerosis. However, several important issues need to be addressed to well-appreciate the value of its use in the current setting. First, it should be kept in mind that the non-calcified part of the atherosclerotic plaque cannot be measured using non-enhanced CT. This inevitably means that we could not measure other potentially interesting plaque characteristics, such as shape, or total size. Nonetheless, it has repeatedly been reported that the amount of calcification is well reflective of the total underlying atherosclerosis burden.[47-49] Second, in all studies in this thesis calcification was handled as indicator of atherosclerosis in otherwise 'healthy' community-dwelling persons. The latter is especially important, because the value of assessing the amount of atherosclerotic calcification in patients with known cardiovascular disease might be different. As already discussed above, especially in these patients, assessing the plaque composition may yield more information with regard to the risk of a cardiovascular event, than the amount of calcification per se.

A 'compound calcification score'

The majority of our studies are specifically focused on atherosclerotic calcification in multiple vessel beds. The thought behind this lies in the fact that correlations between atherosclerosis across vessel beds are only moderate,[5, 50] and thus may hold valuable different or additional information. As already extensively discussed, we found that this is indeed the case, both with regard to etiology as with respect to risk of subsequent disease.

An important topic in the current era of risk assessment and prevention of disease, is the combination of various clinical or imaging markers into a combined score for disease risk.[4] In this light, assessing the value of the 'total calcification load', i.e. the volume of calcification in all vessel beds, might also be valuable. However, whether or not the combination of calcification volumes in multiple vessels really improves prediction of clinical events should be thoroughly investigated. Here, I discuss several aspects which should be kept in mind while considering such a strategy.

The first involves the radiation dose to which persons are exposed when assessing calcification in multiple vessel beds. Although these dosages were low in our study (Chapter 3.1) and will only become lower with newer generation scanners, it is an important point to consider. Specifically in the context of using such a score for risk prediction or as screening tool, it should be kept in mind that persons undergoing the CT are 'healthy' and should in principle not be exposed to radiation. Second, given the results described in my thesis, in combination with studies on clinical cardiac events, it might not be necessary to image such a large area for obtaining an accurate risk estimate for consecutive clinical disease. For example; when one wants to assess the risk of clinical cardiac disease, coronary calcification should be measured,[51-53] whereas calcification in the intracranial carotid artery would probably predict best one's risk of stroke. On the other hand, for chronic conditions such as cognitive decline or dementia, the 'overall' deterioration of the arterial system probably plays a more important role than the atherosclerotic burden at one particular location. Given that there are only moderate correlations between calcification across different vessel beds, one could argue that assessing calcification in only one vessel bed is not enough for a good reflection of the status of the complete arterial system. Hence, in the case of these diseases, a compound score might indeed provide additional information. A third consideration is how to calculate a 'compound calcification score'. A simple, straightforward method would be to add the calcification scores in the various vessels and divide by the total number of vessels. However, we know that amounts of atherosclerotic calcification differ substantially over the various vessel beds.[5, 50] Therefore, some sort of weighing should probably be applied to calculate such a score.

Clinical implications and future research

Over the years, epidemiologic research has aided considerably to the development of effective treatment and, in certain instances, even to preventive strategies for several diseases. However, specifically with regard to atherosclerosis, stroke and dementia we still have a long way to go before such strategies will be operational. Worldwide, the number of persons developing atherosclerosis, suffering a stroke, or developing dementia is still increasing, and will likely continue to do so, given the aging of the population.[23, 24, 30] Findings described in this thesis may contribute to the development of both therapeutic and preventive strategies for these devastating diseases. Yet, several aspects with regard to the understanding of atherosclerosis, but also with respect to its role in the development of stroke or dementia still remain unclear. In this final part of my thesis, I concentrate on two topics that may be important in the near future.

Medical treatment of cardiovascular risk factors

The first topic involves current standards of the treatment of atherosclerosis, or more general, cardiovascular risk factors that lead to atherosclerosis. The first important issue which should be kept in mind is that atherosclerosis at different locations in the arterial tree is influenced differentially by various cardiovascular risk factors. More specifically, it should be kept in mind that when treating one cardiovascular risk factor, the formation of atherosclerosis at one or two particular locations might be stabilized more than atherosclerosis at a different location. A second important topic to consider is that individuals with few conventional cardiovascular risk factors are the least likely to be targeted for preventive therapies, but as a group they experience the largest number of cardiovascular events.[54] This might be a direct result from the fact that the currently known conventional cardiovascular risk factors only explain a part of the total variability of atherosclerosis, and possibly thus also of total cardiovascular disease. Likely, genetic predisposition also plays an important role. Yet, for coronary artery calcification several genetic variants have already been identified, and more will likely follow.[2, 20] In summary, further research on disentangling the etiology of atherosclerosis is greatly needed, specifically focusing on the genetic basis of atherosclerosis.

Another topic of interest is the effect of treatment of cardiovascular risk factors on the development of dementia. In our study on atherosclerosis and the risk of dementia, we demonstrated that not atherosclerosis located in one specific vessel bed, but the 'systemic' component of atherosclerosis, i.e. the overall condition of the vascular system, is more important with respect to the risk of dementia. Possibly, earlier and more aggressive medical treatment of cardiovascular risk factors might help to reduce dementia incidence, through staving off the formation of atherosclerosis. Actually, early evidence suggests that this might already be occurring to some extent. It was for example found that the age-specific dementia incidence has decreased between 1990 and 2005.[55] A possible explanation underlying this may be the widespread implementation of preventive medical strategies for cardiovascular risk factors. More research, preferably in the shape of clinical trials, is needed to further establish the relation of treatment of cardiovascular risk factors, regression of atherosclerosis, and dementia.

Predicting clinical stroke: the potential role of intracranial atherosclerosis

One of the most prominent findings described in my thesis was the strong association between the amount of intracranial carotid artery calcification and the risk of stroke. As already mentioned, it was surprising that this has not been investigated before, even more so because in populations from Asian or African descent intracranial atherosclerosis is already long established as the most im-

portant risk factor for ischemic stroke. Within our current way of thinking about the etiology of stroke, our findings may have important implications. However, before actually translating these findings to the clinical setting, several questions remain to be answered. First, it is important to keep in mind that although a determinant or risk factor may be significantly associated to a certain disease, its value in individual risk prediction of that disease must still be established. Therefore, an essential first step of further research should address the actual predictive value of intracranial atherosclerosis with regard to stroke. To illustrate this further, I compare the relationship of intracranial atherosclerosis and stroke to some extent with the relationship of coronary atherosclerosis with myocardial infarction. The coronary calcium score for example has proven additional value in the prediction of acute cardiac events beyond the Framingham risk score profile.[51, 52] As a consequence, clinically relevant risk categories have been developed, and the coronary calcium score is increasingly being used in current clinical practice.[23] For now, I can only speculate that such a strategy would also become reality for intracranial atherosclerosis and the prediction of stroke, but there is no disputing that intracranial carotid artery atherosclerosis has huge potential to become an accurate and reliable predictor of stroke. Especially, because neither coronary artery calcification, aortic arch calcification, nor extracranial carotid artery calcification were found to improve the risk prediction of stroke.[52]

The second issue are possibilities for treating intracranial atherosclerosis. According to our results, by completely eradicating intracranial carotid artery atherosclerosis, we could theoretically reduce the incidence of stroke with 75% at most. Yet, treatment options for intracranial atherosclerosis have already been studied in patients with symptomatic intracranial atherosclerotic disease.[56, 57] Interestingly, from these studies it was concluded that most benefit with regard to recurrent stroke could be obtained by aggressive medical treatment, as compared to stenting. These promising treatment opportunities call for further research on interventions targeted at reducing or stabilizing intracranial atherosclerosis to evaluate its effect on the occurrence of stroke.

In conclusion, atherosclerosis impacts the public health care enormously, by increasing the risk of major cardiovascular as well as neurological diseases. Given the aging of the population and the accompanying rise of these diseases, huge amounts of work are still needed to ultimately all benefit of healthier aging.

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6

Summary / Samenvatting

SUMMARY

Chapter 1, the general introduction, provides the rationale and aim of this thesis. Atherosclerosis is a highly frequent vascular disease in middle-aged and elderly persons, and may lead to serious clinical conditions of which myocardial infarctions and strokes are the most prominent. Beside these clinical consequences, increasing evidence suggests that the impact of atherosclerosis may be much larger, especially with regard to brain disease. Several conditions, ranging from white matter damage to dementia, may be influenced by atherosclerosis. Additionally, an emerging topic of interest is the location of atherosclerosis. Although atherosclerosis may occur systemically across the arterial system, its burden may vary considerably across different vessel beds. This may directly influence the extent of the contribution to these subsequent diseases. Moreover, the origin of the differences in burden of atherosclerosis across vessel beds needs further elucidation.

Using computed tomography (CT) examinations in approximately 2500 participants from the population-based Rotterdam Study, we examined atherosclerotic calcification, as proxy of atherosclerosis, in the coronary arteries, aortic arch, extracranial carotid arteries, and intracranial carotid arteries.

In **Chapter 2**, the focus is on determinants - or risk factors - of atherosclerotic calcification in the four examined vessel beds. To date, several modifiable lifestyle- and environmental cardiovascular risk factors are known to contribute considerably to the formation of atherosclerosis. These include hypercholesterolemia, hypertension, diabetes mellitus and smoking. However, as depicted above, the burden of atherosclerosis may vary considerably across different vessel beds. One explanation for this may lie in location-specific differences in susceptibility to these cardiovascular risk factors.

Chapter 2.1 focuses on atherosclerosis in the intracranial carotid artery, a potentially very important vessel bed with regard to brain disease. Globally, intracranial atherosclerosis is the most important risk factor for stroke. Yet, data on its prevalence, risk factors or impact in white populations lacks. Interestingly, we found a strikingly high prevalence of intracranial carotid artery atherosclerosis of over 80%. Regarding risk factors, we found that most established risk factors for atherosclerosis at other locations were also associated with intracranial atherosclerosis. However, the contribution of the various risk factors was different from their contribution to atherosclerosis in other vessel beds. We found that diabetes was by far the strongest risk factor for intracranial atherosclerosis in the general aging population, whereas in other vessel beds hypertension and hypercholesterolemia have been described to exert more effect on atherogenesis.

Another important source of the location-specific differences in the formation of atherosclerosis may lie in genetic differences. In **Chapter 2.2 and 2.3** the focus is on several genetic factors that are implicated in the development of atherosclerosis. Previously, several important genetic loci have been identified which were associated with coronary artery calcification. We determined whether these genetic loci were also associated with calcification in other locations than the coronary arteries. Indeed, we found that the genetic basis for aortic arch and carotid artery calcification largely overlaps with that of coronary artery calcification. But again we found differences in contribution to the burden of atherosclerotic calcification in these vessel beds. This suggests that also on the genetic level, there are profound differences in the etiology of atherosclerosis across vessel beds. We also investigated the genetic basis of atherosclerosis using the genetics of strong risk factors of atherosclerosis, i.e. serum cholesterol levels and blood pressure. Using genetic risk scores based on the identified genetic variants for serum lipid fractions and blood pressure, we demonstrated that higher genetic risk scores were related to a larger burden of atherosclerosis in multiple vessel beds.

Chapter 3 is dedicated to neurological consequences of atherosclerosis. The first two chapters focus on subclinical changes in brain health. **Chapter 3.1** is dedicated to subclinical cerebrovascular disease, consisting of 'silent' infarcts, microbleeds and white matter pathology. These pathologic alterations occur frequently and are associated with a variety of clinical conditions later in life. In this light, in **Chapter 3.1**, we explored whether atherosclerosis is associated with the presence and amount of these alterations, which we measured using brain magnetic resonance imaging (MRI), in the general population. We found that atherosclerosis was associated with white matter lesion-burden and the presence of lacunar infarcts. This suggests that atherosclerosis might indeed fulfil an important role in the development of subclinical cerebrovascular disease. In **Chapter 3.2**, the focus is on the relationship between atherosclerosis, cognitive function and brain atrophy. We found that a larger amount of atherosclerotic calcification was associated with worse cognitive performance. Moreover, it also related to smaller brain tissue volumes and worse white matter microstructural integrity, revealing possible mechanisms through which atherosclerosis may lead to poorer cognition.

Besides these subclinical neurological outcomes we also investigated the role of atherosclerosis in two major clinical age-related brain diseases; dementia and stroke. In **Chapter 3.3**, we investigated the role of atherosclerosis in the etiology of dementia. We found that systemic atherosclerosis was associated with a higher risk of dementia. This suggests that generalized atherosclerosis, which probably is a better reflection of one's vascular status rather than localized atherosclerosis in a single vessel bed, associates with dementia. Besides the relationship with

dementia, we also studied the role of atherosclerosis in cognitive decline to determine whether atherosclerosis is already implicated in the long preclinical phase of dementia. Following the results from the cross-sectional study described above (**Chapter 3.2**), we confirmed that atherosclerosis is associated with cognitive decline. In **Chapter 3.4** we assessed the relationship of atherosclerosis and clinical stroke, primarily focusing on intracranial atherosclerosis. In addition to the high prevalence we found (**Chapter 2.1**), we now also demonstrated that intracranial atherosclerosis is a major cause of stroke in whites. We found that it contributes to 75% of all strokes, whilst for aortic arch atherosclerosis or atherosclerosis in the carotid artery bifurcation this was only 45% and 25%.

In **Chapter 4** novel imaging markers of vascular risk are discussed. As a result of the rapid technological advances in imaging modalities, the search for newer and better markers of disease ever continues. For the assessment of vascular risk several potentially important new markers have come to light, such as plaque composition and the amount of epicardial fat. **Chapter 4.1** describes the relationship between the three most prominent plaque components, i.e. calcification, lipid core and intraplaque hemorrhage. Important to note is that we used a multi-modality imaging approach using both CT and MRI, to optimally study these three components. We found that there is a complex relationship between calcification, intraplaque hemorrhage and lipid core within the carotid atherosclerotic plaque. Plaques with a higher load of calcification contain more often hemorrhagic components, but less often lipid core. In **Chapter 4.2** focuses on the quantification of epicardial fat, a potentially very important marker of cardiovascular disease. Specifically data from large general populations and clinical databases are necessary to investigate the exact value of epicardial fat in cardiovascular disease. Yet, in order to be applied on a large scale, fully automated methods are necessary for speed, accuracy and reliability. Therefore, we developed a fully automated method that is capable of segmenting and quantifying epicardial fat volume. We demonstrated the feasibility of using this method on a large dataset and showed that the automatic method performs as good as manual annotation.

In **Chapter 5**, the general discussion, the main findings are discussed in the light of current knowledge. Methodological considerations, clinical implications of the findings, and directions for future research are also discussed in this chapter.

SAMENVATTING

Atherosclerose is een veelvoorkomende vasculaire aandoening in mensen van middelbare en oudere leeftijd. Veelvoorkomende klinische gevolgen van atherosclerose zijn het hartinfarct en het herseninfarct, wereldwijd twee van de meestvoorkomende oorzaken van morbiditeit en mortaliteit. In het bijzonder met betrekking tot de conditie van de hersenen zijn er aanwijzingen dat de invloed van atherosclerose nog groter is. Naast het herseninfarct, komt er meer en meer bewijs voor een rol van atherosclerose in subklinische hersenschade en zelfs dementie. Daarnaast is de lokatie waar atherosclerose voorkomt in toenemende mate een belangrijk onderwerp van onderzoek. Hoewel atherosclerose in het gehele arteriële vaatbed kan voorkomen, kan de hoeveelheid per bloedvat aanzienlijk verschillen. Vooralsnog is het onduidelijk wat deze verschillen veroorzaakt. Daarnaast is het onduidelijk of deze verschillen ook een weerslag hebben op het ontstaan van daaropvolgende ziekte van een orgaan.

Het doel van dit proefschrift is het onderzoeken van determinanten en neurologische gevolgen van atherosclerose, met de nadruk op atherosclerose in verschillende bloedvaten. Met behulp van computer tomografie (CT) -onderzoeken van bijna 2500 deelnemers uit de Rotterdam Studie (populatie onderzoek) hebben we hoeveelheid atherosclerotische verkalking, als maat voor atherosclerose, in de kransslagaders, aortaboog, en in het extracraniële en intracraniële deel van de halsslagaders onderzocht.

In **hoofdstuk 2** worden verschillende determinanten - risicofactoren – van atherosclerotische verkalking beschreven. Vandaag de dag zijn er vele risicofactoren voor cardiovasculaire ziekte bekend, waaronder hypercholesterolemie, hypertensie, diabetes mellitus en roken. Van deze risicofactoren is het bekend dat ze sterk bijdragen aan de vorming van atherosclerose, maar aangezien de hoeveelheid atherosclerose sterk kan verchillen per bloedvat kan het zo zijn dat de invloed van deze factoren op de vorming van atherosclerose verschilt per bloedvat. In **hoofdstuk 2.1** onderzochten we atherosclerotische verkalking in het intracraniële deel van de halsslagaders. Wereldwijd is intracraniële atherosclerose de belangrijkste oorzaak voor een herseninfarct, maar prevalentiecijfers en risicofactoren zijn grotendeels onbekend in de blanke populatie. Wij vonden dat de prevalentie van intracraniële atherosclerose in de bevolking erg hoog (> 80%) is. Zoals verwacht vonden we dat risicofactoren die atherosclerose in andere vaten veroorzaken, ook gerelateerd zijn aan intracraniële atherosclerose. We vonden echter wel dat de bijdrage van de verschillende risicofactoren anders was dan hun bijdrage aan atherosclerose in andere bloedvaten. Een goed voorbeeld is diabetes mellitus; wij vonden dat dit verreweg de sterkste risicofactor was voor intracraniële atherosclerose, terwijl hypertensie en hypercholesterolemie belangrijker zijn voor de vorming van atherosclerose in andere vaten.

Een andere belangrijke oorzaak die ten grondslag kan liggen aan de variërende hoeveelheid atherosclerose tussen bloedvaten is genetische aanleg. De **hoofdstukken 2.2 en 2.3** zijn gewijd aan enkele genetische factoren die invloed hebben op de vorming van atherosclerose. Eerder onderzoek heeft laten zien dat er enkele zeer belangrijke genetische varianten zijn die sterk bijdragen aan de hoeveelheid verkalking in de kransslagaders. Wij onderzochten of deze genetische varianten ook gerelateerd waren aan verkalking in de aortaboog en de halsslagaders. Er was inderdaad een relatie tussen deze genetische factoren en verkalking in de aortaboog en de halsslagaders, maar ook hier waren er duidelijke lokatie-specifieke verschillen zichtbaar met betrekking tot de bijdrage aan de hoeveelheid atherosclerose.

Daarnaast onderzochten we of genetische varianten waarvan bekend is dat ze leiden tot hypercholesterolemie en hypertensie ook een direct effect hebben op de vorming van atherosclerose. Met behulp van genetische risicoscores vonden we inderdaad dat deze genetische factoren ook gerelateerd waren aan de hoeveelheid atherosclerose in de verschillende bloedvaten.

Hoofdstuk 3 van dit proefschrift is gewijd aan neurologische gevolgen van atherosclerose. **Hoofdstuk 3.1** gaat over de rol van atherosclerotische verkalking in de ontwikkeling van subklinische hersenschade. Subklinische hersenschade omvat pathologische veranderingen in de structuur van de hersenen welke geen klinische symptomen geven en kan bestaan uit wittestofschade, kleine 'stille' infarcten en microbloedingen. Met behulp van magnetische resonantie beeldvorming (MRI) van de hersenen hebben wij deze veranderingen in beeld gebracht en hebben we onderzocht of atherosclerose gerelateerd is aan het voorkomen van deze veranderingen. We vonden een verband tussen atherosclerose in de verschillende vaten en meer wittestofschade en de aanwezigheid van kleine 'stille' infarcten. Daarnaast vonden we dat de invloed van atherosclerotische verkalking groter was naarmate de verkalking zich dichter bij het brein bevond. In **hoofdstuk 3.2** onderzochten we het verband tussen atherosclerose, cognitie en hersenatrofie. We vonden een relatie tussen meer verkalking en een slechtere cognitieve prestatie, maar ook kleinere hersenvolumina. Mogelijk beïnvloedt atherosclerose de cognitieve prestatie dus via de relatie met kleinere hersenvolumes.

Naast deze subklinische veranderingen hebben we ook onderzocht wat het verband is tussen atherosclerose in de verschillende bloedvaten en het risico op dementie en een herseninfarct. **Hoofdstuk 3.3** richt zich op dementie. We vonden dat atherosclerose in alle bloedvaten geassocieerd was met een hoger risico op dementie. De sterkte van de relaties was vrijwel gelijk voor alle vaten; met andere woorden lijkt voor dementie, een chronische ziekte, dus niet zozeer de lokatie van atherosclerose, maar juist het gegeven of iemand gegeneraliseerde vaatschade

heeft, van belang. Ook vonden we dat atherosclerose al in een vroeg stadium, namelijk het stadium van cognitieve achteruitgang, betrokken is in het ziekteproces. In navolging op **hoofdstuk 2.1** onderzochten we in **hoofdstuk 3.4** wat de ivloed van intracraniële atherosclerose is op het risico op een herseninfarct. We vonden dat ook in de blanke populatie intracraniële atherosclerose een zeer belangrijke oorzaak is voor het herseninfarct. We zagen zelfs dat intracraniële atherosclerose bijdraagt aan 75% van alle herseninfarcten, in tegenstelling to 45% voor atherosclerose in de aortaboog en 25% voor atherosclerose in de bifurcatie van de halsslagaders.

In **hoofdstuk 4** worden nieuwe markers voor cardiovasculair risico besproken. **Hoofdstuk 4.1** beschrijft de relatie tussen drie prominente karakteristieken van de atherosclerotische plaque, namelijk verkalking, de aanwezigheid van een vetkern en de aanwezigheid van intraplaque bloedingen. Vernieuwend aan deze studie was dat we gebruik hebben gemaakt van twee beeldvormingstechnieken, namelijk CT en MRI, om deze karakteristieken optimaal te bestuderen. We vonden een complexe relatie tussen de drie componenten; verkalking was gerelateerd aan de aanwezigheid van intraplaque bloeding, maar ook aan een lagere prevalentie van een vetkern. In **hoofdstuk 4.2** beschrijven we een nieuwe methode voor het kwantificeren van de hoeveelheid epicardiaal vet, een potentiele belangrijke marker voor cardiovasculaire ziekte. Om de exacte waarde van epicardiaal vet met betrekking tot cardiovasculaire ziekte te bestuderen zijn grote hoeveelheden onderzoeksgegevens uit populatiestudies en klinische studies nodig. Dit benadrukt het belang van een accurate, automatische methode voor de kwantificatie van epicardiaal vet. In **hoofdstuk 4.2** beschrijven we een volledig automatische methode voor de kwantificatie van epicardiaal vet.

Tot slot bediscussieer ik in **hoofdstuk 5** de belangrijkste bevindingen, enkele methodologische overwegingen, de implicaties van mijn onderzoek en doe ik suggesties voor toekomstig onderzoek.

7

Dankwoord
About the Author
PhD Portfolio
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Dankwoord

Gedurende mijn promotie heb ik het voorrecht gehad om met vele mensen intensief samen te werken. Het proefschrift dat nu voor u ligt is dan ook zeker niet het werk van mij alleen en op deze plaats wil ik iedereen bedanken voor de goede samenwerking. Een aantal mensen wil ik in het bijzonder noemen.

Allereerst mijn promotoren, prof. dr. Aad van der Lugt en prof. dr. Albert Hofman.

Beste Aad, mede dankzij jou heb ik mijn promotie als een geweldige tijd ervaren. Jouw tekenende enthousiasme en begeleiding waren erg inspirerend. Daarnaast wil ik je bedanken voor de laagdrempeligheid om met jou een afspraak te kunnen maken. Ik hoop dat we onze samenwerking kunnen voortzetten.

Beste Bert, Ik heb veel geleerd van je scherpe, wetenschappelijke inzicht. Veel dank voor de mogelijkheid om binnen de wereldwijd bekende Rotterdam Studie mijn promotieonderzoek te kunnen doen.

Mijn copromotoren, dr. Meike Vernooij en dr. Arfan Ikram.

Beste Meike en Arfan, halverwege het eerste jaar van mijn promotie werden jullie mijn copromotoren. Het was een voorrecht om met jullie samen te werken en ik bewonder jullie intelligentie, werklust en efficiëntie. De tweewekelijkse afspraken met zijn drieën waren altijd erg zinvol, inspirerend (en gezellig).

Meike, dank je dat je altijd tijd had voor een 'korte' afspraak en het beantwoorden van mijn emails (die regelmatig meerdere vragen bevatten ;)). Inmiddels ben je ook mijn mentor voor de opleiding radiologie, dank dat je deze taak op je wil nemen.

Arfan, dank voor je scherpe en sterke wetenschappelijke commentaar op mijn manuscripten. Ons doel om reviewer commentaar te ontvangen waarin "..very well-written.." genoemd werd, hebben we denk ik wel bereikt. Daarnaast wil ik je ook bedanken voor onze niet werk-gerelateerde gesprekken over het Spaanse voetbal en de Champions League.

Tevens wil ik mijn promotie commissie hartelijk bedanken voor het willen uitwisselen van gedachten over mijn proefschrift. Speciale dank gaat uit naar de leden van de leescommissie; prof. dr. Peter Koudstaal, prof. dr. Mat Daemen en prof. dr. Juan Arenillas.

Beste prof. dr. Gabriel Krestin, bedankt voor de mogelijkheid om aan deze afdeling te kunnen promoveren en vervolgens mijn opleiding tot radioloog te kunnen volgen.

Ik wil ook alle collega's van de BIGR-groep bedanken voor hun hulp. Henri, jou wil ik in het bijzonder bedanken voor de hulp met het organiseren van de CT-database en de hulp met mijn eindeloze dataverzoeken (CT, fMRI, MRI scans).

Veel dank gaat uit naar de collega's op het ERGO centrum. Jullie zijn een onmisbare schakel in de dataverzameling van de Rotterdam Studie. Mijn speciale dank gaat uit naar Lydia en Pauli voor de vloeiende samenwerking met betrekking tot de implementatie van het RS-fMRI protocol.

Tevens wil ik de collega's van de automatisering bedanken. Beste Eric, Frank, Jolande, Nano en beide René's, dank voor de ondersteuning, alle computerhulp en het management van alle data van de duizenden ERGO deelnemers.

Ook wil ik mijn al mijn collega's van de de BIGR, de afdeling Radiologie en Epidemiologie bedanken voor de samenwerking en de gezelligheid.

Van de afdeling Radiologie wil ik in het bijzonder de volgende mensen bedanken: Anouk, Carolina, Janne, Jasper, Joost (voor de squash-lessen), Maartje, Mariana, Marion (een belangrijk deel van de basis voor mijn interesse in wetenschappelijk onderzoek is gelegd tijdens mijn keuze-onderzoek bij jou), Marjon, Philip, Quirijn, Rahil, Rebecca, Rozanne en Sjel.

Carolina, you have been my roommate since we moved to HE-125. It was great working with you, we had a great time and I am sure that you will finish your PhD in time, too! Thank you that you want to be my paranimf!

Rebecca, Rozanne en Sjel, ook jullie bedankt voor de gezelligheid. Met name de bezoeken aan de ECR blijven mooie herinneringen aan mijn PhD tijd (ik hoop voor jullie ook ;)) Veel succes met het afronden van jullie proefschriften!

Quirijn, bedankt voor de fijne samenwerking. Ik denk dat we er een mooi artikel van hebben gemaakt!

Rahil, thanks for the fast and efficient cooperation on the epicardial fat project! I hope we can continue working on this and produce some more nice papers. Good luck with your new job.

Van de afdeling epidemiologie wil ik in het bijzonder mijn (ex) medepromovendi van de "neuro-groep" bedanken: Ben, Elisabeth, Hazel, Hieab, Jory, Liz, Marielle, Marileen, Renée, Rens, Renske, Saira, Saloua, Vincent en Vincent. Het grootste deel van mijn promotietijd was ik alleen op dinsdagen bij jullie. Het was een leuke tijd, zelfs de 'grote scan dagen' en de 'grote invoerdagen', waren uiteindelijk erg

gezellig. Ik hoop dat voor jullie de 'grote segmentatie *maanden* (!)' ook meevielen. Hieab heeft er in ieder geval nog een prijs mee gewonnen. Ik hoop jullie nog regelmatig te zien in de toekomst!

Lieve vrienden, Frank, Frank L, Jan, Marielle, Jeff, Sanne, Rogier, Stefan, Faber, Hester en Roel. Bedankt voor de welkome afleiding tijdens mijn promotie! De vakanties, weekendjes weg en feestjes waren - en zijn nog steeds - een groot succes. Roel, de game- en filmavonden met z'n drieen zijn altijd gezellig en ik hoop dat we dat nog lang blijven voortzetten.

Lieve familie en schoonfamilie, jullie wil ik bedanken voor de steun en het vertrouwen. Lieve pa en ma, bedankt voor jullie oneindige steun, motiverende houding en interesse in mijn onderzoek. Jullie zijn geweldig.

Frank, je staat hierboven ook al genoemd, maar hier wil ik je nogmaals in het bijzonder bedanken voor de eindeloze avonden samen voetbal kijken en de zondagen in de Kuip. Het was (en is nog steeds) de perfecte afleiding van mijn dagelijkse werkzaamheden.

Lieve schoonfamilie, bedankt voor jullie interesse in mijn onderzoek en voor de afleiding in de vorm van gezellige avonden, etentjes en weekendjes weg.

Robert, bedankt voor het ontwerpen van de voorkant en de muzieksessies die ermee gepaard gingen.

Liefste Kim, het is geweldig om met jou samen te leven. Iedere dag opnieuw besef ik hoe gelukkig ik met jou ben. Dank je voor alles!

PhD Portfolio

SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

	Year	ECTS
General research skills		
Master's degree in Health Sciences, spec. Clinical Epidemiology	2010-2012	70.0
• Introduction to clinical research	2010	
• Working with SPSS for Windows	2010	
• Clinical decision analysis	2010	
• Methods of public health research	2010	
• Markers and prognostic research	2010	
• Study design	2010	
• Classical methods for data analysis	2010	
• Modern statistical methods	2010	
• Diagnostic research	2011	
• Prognosis research	2011	
• Principles of epidemiologic data-analysis	2011	
• Quality of life measurement	2011	
• Maternal and child health	2011	
• Principles of genetic epidemiology	2011	
• Clinical epidemiology	2011	
• Methodologic topics in epidemiologic research	2011	
• Courses for the quantitative researcher	2012	
• Causal inference	2012	
• Introduction to global public health	2012	
• Case-control studies	2012	
• History of epidemiologic ideas	2012	
• Practice of epidemiologic analysis	2012	
• Pharmaco-epidemiology	2012	
In-depth courses		
• Cardiovascular imaging and diagnostics (COEUR)	2010	1.5
• Methodology of patient orientated research and preparation of application for grants (CPO)	2011	1.0
• Biomedical English writing and communication	2011	4.0
• Study Design: Beyond Simple Randomization (CPO/CQM)	2012	0.5
• Resting State fMRI - Analysis and Interpretation (ESMRMB), Magdeburg, Germany.	2012	1.5

Invited lectures

• Cardiovascular Research School Erasmus MC (COEUR), Rotterdam, the Netherlands; seminar: Imaging the carotid arteries: structure & function. Lecture: Atherosclerotic calcification in the carotid arteries: the impact on the brain.	2011	1.0
• Neuroradiology section meeting, Dutch Society of Radiology (NVvR), Utrecht, the Netherlands. Lecture Atherosclerotic calcification: determinants and clinical neurological outcomes.	2012	1.0

(Inter)national conferences and presentations

• European Congress of Radiology, Vienna, Austria. *Poster presentation:* Arterial calcifications as a marker of vascular brain disease.	2011	1.0
• Alzheimer's Imaging Consortium, Paris, France. *Poster presentation:* Calcification in major vessel beds relates to vascular brain disease.	2011	1.0
• Alzheimer's Association International Conference on Alzheimer's Disease, Paris, France. *Oral presentation:* Arterial calcification in relation to cognition and structural brain changes. *Poster presentation:* Calcification in major vessel beds relates to vascular brain disease.	2011	2.0
• Radiologendagen, Maastricht, the Netherlands. *Oral presentation:* Atherosclerotische verkalking is gerelateerd aan cognitieve prestatie en structurele hersen-veranderingen op MRI. (Winner of NvVR 2011 Best scientific paper award)	2011	2.0
• Annual meeting of the Radiological Society of North America, Chicago, USA. *Oral presentation:* Atherosclerotic calcification relates to cognition and brain changes on MRI.	2011	2.0
• European Congress of Radiology, Vienna, Austria. *Poster presentation:* Prevalence and determinants of intracranial carotid artery calcification; the Rotterdam Study.	2012	1.0
• Radiologendagen, 's-Hertogenbosch, the Netherlands. *Oral presentation:* Intracranial carotid artery atherosclerosis: Increased risk of stroke.	2012	2.0

• European Congress of Radiology, Vienna, Austria. *Oral presentation:* Atherosclerotic calcification is related to cognitive decline: the Rotterdam study. *Oral presentation:* Genetic loci for coronary calcification and serum lipids relate to aortic and carotid calcification. *Poster presentation:* Automatic method for quantification of pericardial fat on non-enhanced cardiac CT	2013	4.0

Reviewing papers

• Referee activities for various international scientific Journals (e.g. *Plos One, Circulation: Cardiovascular Genetics*)	2012 - current	

Teaching

• Supervising Master thesis		
- Supervisor of M.J.M. van der Rijk, Medicine, Erasmus University Rotterdam. Topic: Prevalence and determinants of intracranial carotid artery calcification.	2011	3.0
• Other teaching activities		
- Teaching practicals in epidemiology to 4th year medical students at Erasmus University, Faculty of Medicine.	2010 - 2012	3.0
- Supervising/teaching research-students.	2011 - 2013	3.0

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Bos D, Ikram MA, Verhaaren BF, Hofman A, Van Duijn CM, CHARGE Blood Pressure Working Group, Van der Lugt A, Vernooij MW. Blood pressure genes relate to atherosclerotic calcification in major vessel beds. *Submitted.*

Bos D, Portegies ML, Van der Lugt A, Koudstaal PJ, Hofman A, Krestin GP, Franco OH, Vernooij MW, Ikram MA. Intracranial carotid artery atherosclerosis is an important cause of stroke in whites: the Rotterdam Study. *Submitted.*

Shahzad R, **Bos D**, Metz CT, Rossi A, Kirişli HA, Van der Lugt A, Klein S, Witteman JC, De Feyter PJ, Niessen WJ, Van Vliet LJ, Van Walsum T. Automatic quantification of epicardial fat volume on non-enhanced cardiac CT scans using a multi-atlas segmentation approach. Medical Physics 2013;40:091910.

Van den Bouwhuijsen QJ*, **Bos D***, Ikram MA, Hofman A, Krestin GP, Franco OH, Van der Lugt A, Vernooij MW. Coexistence of calcification, intraplaque hemorrhage and lipid core within the asymptomatic atherosclerotic carotid plaque: The Rotterdam Study. *Submitted.*
* joint first-authorship

Bos D, Shahzad R, Van Walsum T, Van Vliet LJ, Franco OH, Hofman A, Vernooij MW, Niessen WJ, Van der Lugt A. Epicardial fat volume is related to atherosclerotic calcification in multiple vessel beds. *Submitted.*

De Kruif M, **Bos D**, Huijgen FJ, Hofman A, Vernooij MW, Uitterlinden AG, Ikram MA, Van Meurs JB. Structural MRI alterations in community dwelling individuals with chronic pain. *Submitted.*

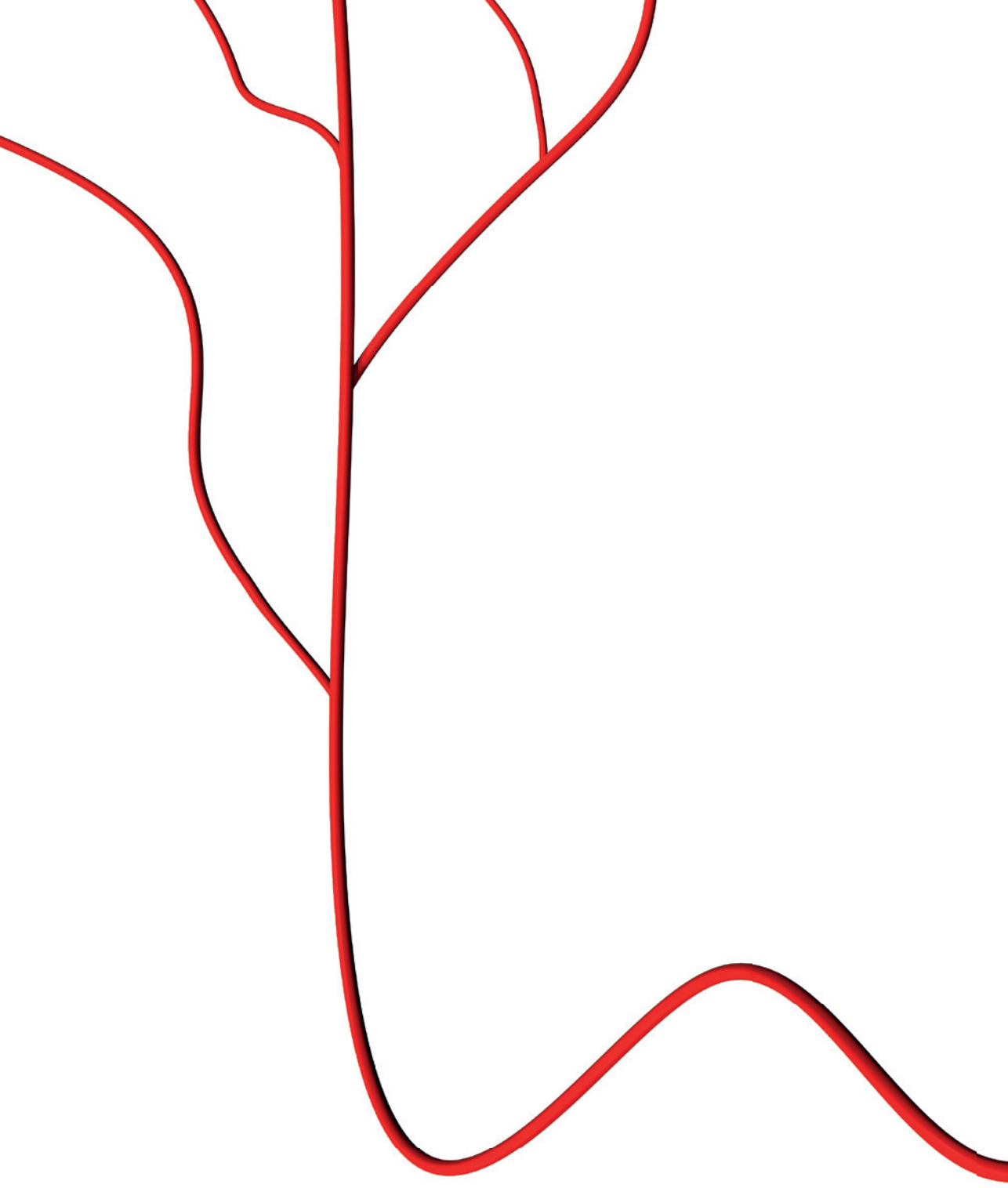
About the Author

Daniel Bos was born on January 26, 1985 in Rotterdam (the Netherlands), and grew up in Ridderkerk. After graduating in 2003 at the 'Farel College' in Ridderkerk, he started medical school at the Erasmus University Medical Center, Rotterdam. In 2007, he obtained his Master degree in Medicine. His Master thesis was on the optimisation of hypercapnia paradigms for functional magnetic resonance imaging (fMRI), and was conducted at the Department of Radiology, Erasmus Medical Center (supervisors: Dr. M. Smits and Prof. Dr. A. van der Lugt). In 2009, he obtained his medical degree cum laude.

From 2010 onwards, he conducted his PhD-project as described in this thesis at the Department of Radiology (head: Prof. Dr. G.P. Krestin) and Epidemiology (head: Prof. Dr. A. Hofman) of Erasmus MC, under the supervision of Prof. Dr. A. van der Lugt, Prof. Dr. A. Hofman, Dr. M.W. Vernooij, and Dr. M.A. Ikram.

In 2011, he won the 'Best Scientific Paper Award' for his presentation at the annual meeting of the Radiological Society of the Netherlands (Radiologendagen, Nederlandse Vereniging voor Radiologie). In 2012, he obtained his Master's degree in Health Sciences (specialisation: Clinical Epidemiology) at the Netherlands Institute for Health Sciences (NIHES).

In July 2013, Daniel started his residency training in Radiology at Erasmus MC.



Stellingen behorende bij het proefschrift:

"Atherosclerotic calcification:
Determinants and Clinical Neurological Consequences"

1.De etiologie van atherosclerose verschilt per vaatbed. Dit impliceert dat de effectiviteit van systemische behandeling van risicofactoren voor atherosclerose kan variëren tussen vaatbedden.
(dit proefschrift)

2. Atherosclerose veroorzaakt niet uitsluitend klinische hersenschade. Subklinische hersenschade, variërend van witte stof laesies tot subtiele cognitieve achteruitgang, is tevens een belangrijke consequentie van atherosclerose. *(dit proefschrift)*

3. Systemische arteriële vaatschade door atherosclerose draagt bij aan het ontstaan van dementie, waaronder de ziekte van Alzheimer.
(dit proefschrift)

4. Intracraniële atherosclerose is een onderschatte oorzaak van het herseninfarct in de blanke populatie en verdient aanzienlijk meer aandacht in de klinische praktijk. *(dit proefschrift)*

5. Zowel vanuit klinisch als etiologisch oogpunt moet er meer nadruk worden gelegd op de locatie van atherosclerose in het vaatstelsel.
(dit proefschrift)

6.Absoluut gezien krijgen er aanzienlijk meer mensen met weinig of licht verhoogde risicofactoren een hartinfarct of beroerte, dan mensen met veel of sterk verhoogde risicofactoren *(Circulation 2011;123;551-565)*. Beeldvorming van atherosclerose op populatieniveau kan een belangrijke bijdrage leveren aan een substantiële reductie in cardiovasculaire ziekte.

7. Het willen ontvangen van een donororgaan moet gepaard gaan met de bereidheid tot donatie.

8. Publicatiebias belemmert de vooruitgang van de wetenschap; kennis dat bepaalde verbanden niet bestaan is even waardevol als kennis dat deze wel bestaan.

9.Verhoging van de pensioengerechtigde leeftijd zal leiden tot meer arbeidsongeschiktheid.

10. De fysiologie van het menselijk oog laat het in veel van de gevallen niet toe om een 'buitenspelpositie' in het voetbal juist te beoordelen *(BMJ 2004; 329:1470-1472)*. Gezien de grote belangen in het huidige profvoetbal, dienen videobeelden te worden gebruikt om de scheidsrechter hierbij te assisteren.

11. Pessimisme is een win-win situatie: of je hebt gelijk of het valt mee. *(Theo Maassen)*

Daniel Bos, Rotterdam, 11-12-2013