Subclinical Measures of Atherosclerosis

Genetics and Cardiovascular Risk Prediction

Maryam Kavousi

Acknowledgments

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

Most of the studies described in this thesis were performed within the Rotterdam Study, which is supported by Erasmus MC and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Dutch Heart Foundation, the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the municipality of Rotterdam. Support for genotyping was provided by the Netherlands Organization for Scientific Research (NWO) (175.010.2005.011, 911.03.012), and the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project number 050-060-810.

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 Epidemiology, Netherlands Consortium for Healthy Ageing (NCHA), and by the Metagenics.

Front cover image: wragg@istockphoto.com
Back cover image: Juan Gaertner | www.shutterstock.com
Cover and interior design: Anna Bosselaar | Zoiets Communicatie (anna@zoiets.com)
Printed by Optima Grafische Communicatie BV (www.ogc.nl)

ISBN 978-94-6169-424-9

Subclinical Measures of Atherosclerosis

Genetics and Cardiovascular Risk Prediction

Subklinische maten van atherosclerose;
genetica en cardiovasculaire risicovoorspellingen

Proefschrift

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

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
dinsdag 8 oktober 2013 om 13.30 uur

door

Maryam Kavousi

geboren te Shiraz, Iran

Promotiecommissie

Promotoren:	Prof.dr. A. Hofman
	Prof.dr. O.H. Franco Duran
Overige leden:	Prof.dr. M.L. Bots
	Prof.dr. E.W. Steyerberg
	Prof.dr. F. Zijlstra

The research described in this thesis was supported by the Netherlands Organization for Scientific Research (NWO) (Vici 918.76.619) and the Netherlands Consortium for Healthy Ageing/Netherlands Genomics Initiative (NCHA/NGI) (project number 050-060-810).

Financial support by the Metagenics for the publication of this thesis is gratefully acknowledged.

To Payam and Sam

And for my parents

MANUSCRIPTS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

Chapter 2.1
O'Donnell CJ *, Kavousi M *, Smith AV *, Kardia SLR *, Feitosa MF, Hwang SJ, Sun YV, Province MA, Aspelund T, Dehghan A, Hoffmann U, Bielak LF, Zhang Q, Eiriksdottir G, van Duijn CM, Fox CS, de Andrade M, Kraja AT, Sigurdsson S, Elias-Smale SE, Murabito JM, Launer L, van der Lugt A, Kathiresan S, the CARDIoGRAM Consortium, Krestin GP, Herrington DM, Howard TD, Liu Y, Post W, Mitchell BD, O'Connell JR, Shen H, Shuldiner AR, Altshuler D, Elosua R, Salomaa V, Schwartz SM, Siscovick DS, Voight BF, Bis JC, Glazer NL, Psaty BM, Boerwinkle E, Heiss G, Blankenberg S, Zeller T, Wild P, Schnabel RB, Schillert A, Ziegler A, Münzel TF, White C, Rotter JI, Nalls M, Oudkerk M, Johnson AD, Newman AB, Uitterlinden AG, Massaro JM, Cunningham J, Harris TB, Hofman A, Peyser PA, Borecki IB, Cupples LA, Gudnason W, Witteman JCM. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction.
Circulation. 2011;124:2855-2864.

Chapter 2.2
Bis JC *, Kavousi M *, Franceschini N *, Isaacs A *, Abecasis GR *, Schminke U *, Post WS *, Smith AV *, Cupples LA *, Markus HS, Schmidt R, Huffman JE, Lehtimäki T, Baumert J, Münzel T, Heckbert SR, Dehghan A, North K, Oostra B, Bevan S, Stoegerer EM, Hayward C, Raitakari O, Meisinger C, Schillert A, Sanna S, Völzke H, Cheng YC, Thorsson B, Fox CS, Rice K, Rivadeneira F, Nambi V, Halperin E, Petrovic KE, Peltonen L, Wichmann HE, Schnabel RB, Dörr M, Parsa A, Aspelund T, Demissie S, Kathiresan S, Reilly MP; the CARDIoGRAM Consortium, Taylor K, Uitterlinden A, Couper DJ, Sitzer M, Kähönen M, Illig T, Wild PS, Orru M, Lüdemann J, Shuldiner AR, Eiriksdottir G, White CC, Rotter JI, Hofman A, Seissler J, Zeller T, Usala G, Ernst F, Launer LJ, D'Agostino RB Sr, O'Leary DH, Ballantyne C, Thiery J, Ziegler A, Lakatta EG, Chilukoti RK, Harris TB, Wolf PA, Psaty BM, Polak JF, Li X, Rathmann W, Uda M, Boerwinkle E, Klopp N, Schmidt H, Wilson JF, Viikari J, Koenig W, Blankenberg S, Newman AB, Witteman J, Heiss G, Duijn CV, Scuteri A, Homuth G, Mitchell BD, Gudnason V, O'Donnell CJ. Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque. Nat Genet. 2011;43:940-947.

Chapter 2.3
Murabito JM *, White CC *, Kavousi M *, Sun YY *, Feitosa MF *, Nambi V *, Lamina C *, Schillert A *, Coassin S, Bis JC, Broer L, Crawford DC, Franceschini N, Frikke-Schmidt R, Haun M, Holewijn S, Huffman JE, Hwang SJ, Kiechl S, Kollerits B, Montasser ME, Nolte IM, Rudock ME, Senft A, Teumer A, van der Harst P, Vitart V, Waite LL, Wood AR, Wassel CL, Absher DM, Allison MA, Amin N, Arnold A, Asselbergs FW, Aulchenko Y, Bandinelli S, Barbalic M, Boban M, Brown-Gentry K, Couper DJ, Criqui MH, Dehghan A, den Heijer M, Dieplinger B, Ding J, Dörr M, Espinola-Klein C, Felix SB, Ferrucci L, Folsom AR, Fraedrich G, Gibson Q, Goodloe R, Gunjaca G, Haltmayer M, Heiss G, Hofman A, Kieback A, Kiemeney LA, Kolcic I, Kullo IJ, Kritchevsky SB, Lackner KJ, Li X, Lieb W, Lohman K, Meisinger C, Melzer D, Mohler ER 3rd, Mudnic I, Mueller T, Navis G, Oberhollenzer F, Olin JW, O'Connell J, O'Donnell CJ, Palmas W, Penninx BW, Petersmann A, Polasek O, Psaty BM, Rantner B, Rice K, Rivadeneira F, Rotter JI, Seldenrijk A, Stadler M, Summerer M, Tanaka T, Tybjærg-Hansen A, Uitterlinden AG, van Gilst WH, Vermeulen SH, Wild SH, Wild PS, Willeit J, Zeller T, Zemunik T, Zgaga L, Assimes TL, Blankenberg S, Boerwinkle E, Campbell H, Cooke JP, de Graaf J, Herrington D, Kardia SL, Mitchell BD, Murray A, Münzel T, Newman A, Oostra BA, Rudan I, Shuldiner AR, Snieder H, van Duijn CM, Völker U, Wright AF, Wichmann HE, Wilson JF, Witteman JC, Liu Y, Hayward C, Borecki IB, Ziegler A, North KE, Cupples LA, Kronenberg F. Association between chromosome 9p21 variants and the ankle-brachial index identified by a meta-analysis of 21 genome-wide association studies.
Circ Cardiovasc Genet. 2012;5:100-112.

Chapter 3.1
Elias-Smale SE *, Kavousi M *, Verwoert GC, Koller MT, Steyerberg EW, Mattace-Raso FUS, Hofman A, Hoeks APG, Reneman RS, Witteman JCM. Common Carotid intima-media thickness in cardiovascular risk stratification of older people; The Rotterdam Study.
Eur J Prev Cardiol. 2012;19:698-705.

Chapter 3.2.1
Kavousi M, Elias-Smale S, Rutten JWH, Leening MJG, Vliegenthart R, Verwoert GC, Krestin GP, Oudkerk M, de Maat MPM, Leebeek FWG, Mattace-Raso FUS, Lindemans J, Hofman A, Steyerberg EW, van der Lugt A, van den Meiracker AH, Witteman JCM. Evaluation of newer risk markers for coronary heart disease risk classification: A cohort study.
Ann Intern Med. 2012;156:438-444.

Chapter 3.2.2
Hlatky MA. Editorial: Framework for evaluating novel risk markers.
Ann Intern Med. 2012;156:468-469. (Reproduced with permission)

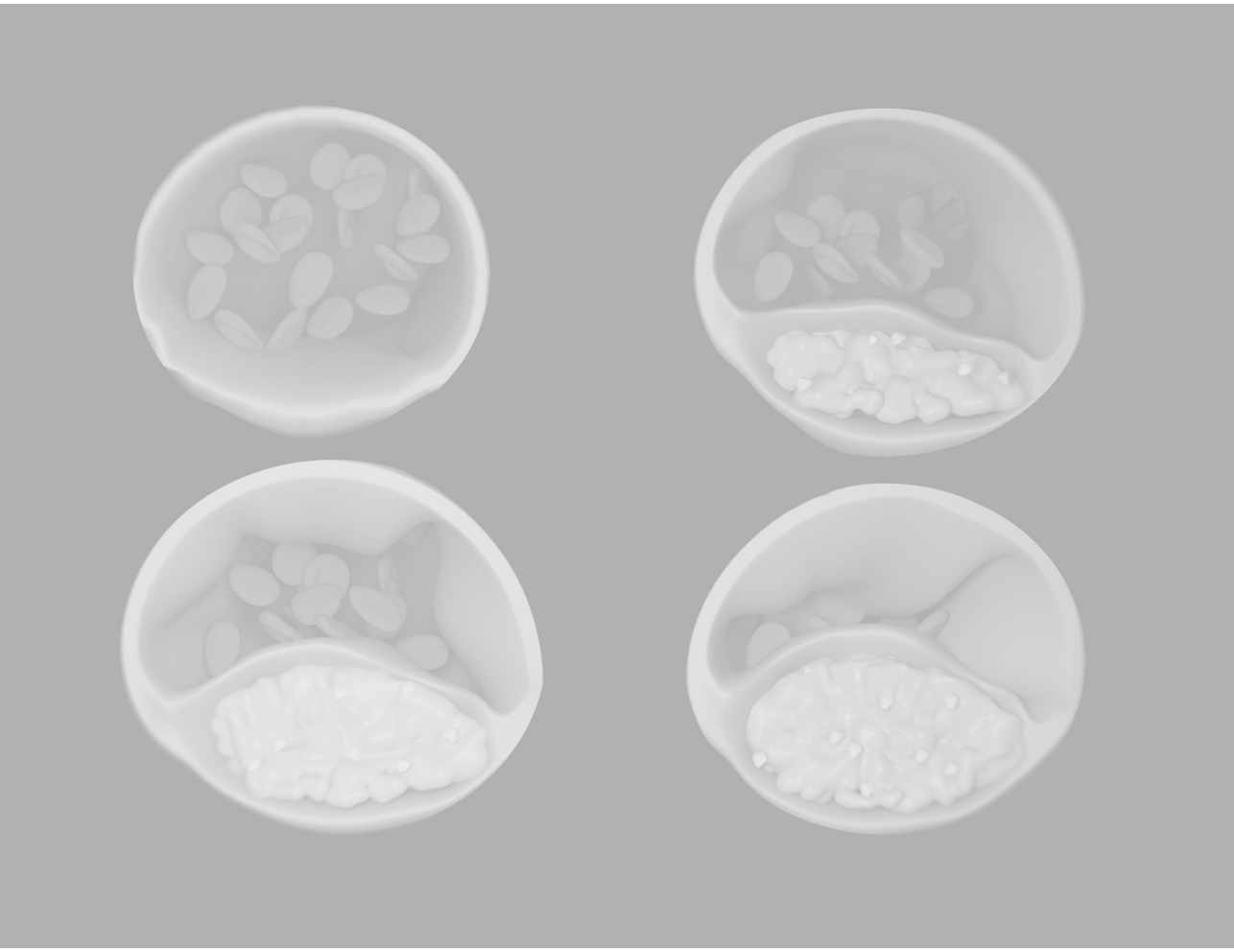
Chapter 3.3
Kavousi M, Leening MJG, Ikram MA, Heeringa J, Krestin GP, Oudkerk M, de Maat MPM, Rutten JWH, Leebeek FWG, Mattace-Raso FUS, Lindemans J, Koudstaal PJ, Hofman A, van den Meiracker AH, Stricker BH, Franco OH, Steyerberg EW, van der Lugt A, Witteman JCM. Contribution of emerging risk markers to global cardiovascular risk assessment. Submitted.

Chapter 3.4
Kavousi M, Leening MJG, Witteman JCM. Markers for prediction of cardiovascular disease risk. JAMA. 2012;308:2561.

Chapter 4.1
Kavousi M, Leening MJG, Ikram MA, Heeringa J, Koudstaal PJ, Hofman A, Stricker BH, Steyerberg EW, Franco OH, Witteman JCM. Cardiovascular risk assessment in women: Prediction of short-term and long-term risk; The Rotterdam Study. Submitted.

* Equal shared contribution.

CONTENTS



CHAPTER 1

Introduction

Atherosclerosis is a chronic, progressive, systematic condition with a long asymptomatic phase. Atherosclerosis develops gradually as a subclinical condition over the life course and eventually becomes clinically apparent as ischemic heart disease, cerebrovascular disease, or peripheral arterial disease.

Subclinical atherosclerosis, or preclinical atherosclerosis, refers to the early stage of the atherosclerosis process when within the vascular walls "something has started to change", yet the cardiovascular disease is not clinically evident. Detecting the forthcoming disease at this stage, before the clinical manifestations, has gained interest over the past decade. Coronary artery calcification, carotid intima-media thickness, and ankle-brachial index are three measures of subclinical atherosclerosis burden that can be detected and quantified non-invasively.

GENETICS OF SUBCLINICAL MEASURES OF ATHEROSCLEROSIS

Exploring the genetic loci associated with subclinical atherosclerosis burden in different vascular beds provides new insights into the biological processes involved in atherosclerosis initiation, progression, and complications and may ultimately suggest new strategies for prediction, prevention, and treatment of cardiovascular disease. Prior to the introduction of genome-wide association studies, the candidate gene or linkage studies were not successfully consistent in identifying novel genetic variants related to these subclinical measures of atherosclerosis; coronary artery calcification, carotid intima-media thickness, and ankle-brachial index. However, familial aggregation and heritability estimates suggested a significant genetic component for these measures of subclinical atherosclerosis burden [1-3]. The advent of genome-wide association studies has allowed for considerable progress in identification of common genetic variants underlying common complex disorders. Likewise, application of this approach to the subclinical measures of atherosclerosis reveals novel gene discoveries which open new windows into understanding the complex nature of the atherosclerosis process.

SUBCLINICAL MEASURES OF ATHEROSCLEROSIS AND CARDIOVASCULAR RISK PREDICTION

One of the most common features of atherosclerotic disease is its clinical manifestation as unheralded cardiovascular events. Approximately 40% to 60% of major occlusive atherosclerotic cardiovascular events (myocardial infarction, sudden death) occur as the first manifestation [4]. Accurate identification of individuals at risk of such events is therefore highly desirable. This recognition formed the basis for development of different cardiovascular risk scoring algorithms [5-7]. The principle underlying all of the risk prediction tools is to estimate the risk of developing cardiovascular disease within a certain time frame, usually a 10-year window, for each individual. Assessment of the apparently healthy individuals for developing cardiovascular event provides the foundation for targeted preventive efforts.

The utility of the existing risk scoring algorithms, based on the traditional risk factors, to predict cardiovascular events is limited [8]. Over the past few years, substantial effort has been devoted to examining the addition of newer risk markers to established risk scoring systems. Detection of subclinical atherosclerosis by non-invasive measures; coronary artery calcium score, carotid intima-media thickness, carotid plaque burden, and ankle-brachial index, has shown to be predictive of future cardiovascular events. Similarly, a large number of emerging risk markers are continuously developed and proposed as representative measures of atherosclerosis burden [9]. All of these markers, proposed as indicators of cardiovascular disease risk, are frequently evaluated as potential additions to standard risk assessment strategies. Due to the increasing number of such efforts, recent guidelines recommend that several measures be used for assessing the increment in risk prediction accuracy offered by newer risk markers [10,11]. However, data on direct comparisons of the subclinical measures of atherosclerosis and other newer risk markers in cardiovascular risk prediction within the same cohort of individuals, implementing the new recommendations, is limited. Use of only some of the recommended assessment methods may provide misleading impressions of the clinical utility of novel markers in cardiovascular risk prediction.

Most of the risk scoring algorithms have traditionally focused on one specific component of cardiovascular disease, generally on coronary heart disease. Recently, there has been an increasing recognition that the focus of risk assessment tools should be directed towards a broader definition of cardiovascular disease instead of targeting coronary heart disease only [12]. However, when addressing the utility of newer risk markers in prediction of the broader cardiovascular outcome, it should be acknowledged that the added value of the risk marker is an aggregation of its different contributions to various cardiovascular components. This concept has not yet been addressed in large population-based settings.

CARDIOVASCULAR DISEASE RISK PREDICTION IN WOMEN

Appreciation of the influence of gender on cardiovascular risk assessment and management is increasingly gaining attention. New modifications to the general approach for cardiovascular risk prediction and stratification in women have recently been proposed [13]. It is now suggested to focus the women's guidelines on the risk estimation for the broad cardiovascular outcome rather than solely for coronary heart disease. Moreover, lowering the threshold for defining the "high cardiovascular risk category" in women is recommended [13]. Based on the observations regarding the high "lifetime" risk for cardiovascular disease in women [14], complementing cardiovascular risk prediction in women with risk estimations for a longer term horizon is advocated [13]. However, data on application of the new recommendations for cardiovascular risk prediction in women to the population-based settings is sparse.

OUTLINE OF THIS THESIS

This thesis focuses on coronary artery calcification, carotid intima-media thickness, and ankle-brachial index as three measures of subclinical atherosclerosis burden.

In the second chapter of this thesis, the aim is to unravel the genetic determinants of these three subclinical measures of atherosclerosis; coronary artery calcification **(chapter 2.1)**, carotid intima-media thickness **(chapter 2.2)**, and ankle-brachial index **(chapter 2.3)**. To investigate the association of DNA variants with these three subclinical measures of atherosclerosis, we use the powerful approach of genome-wide association (GWA) studies. The GWA studies are performed within the framework of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium [15].

The third chapter of this thesis focuses on the predictive ability of subclinical measures of atherosclerosis in cardiovascular disease risk prediction. In **chapter 3.1**, the predictive performance of carotid intima-media thickness in prediction of coronary heart disease and stroke is examined. We expand the current knowledge on cardiovascular risk prediction with comparing the predictive performance of newer risk markers in coronary heart disease risk prediction. **Chapter 3.2** compares the added value of the three subclinical measures of atherosclerosis in coronary heart disease risk prediction with that of some emerging biomarkers. **Chapter 3.3** goes one step further and addresses the differential ability of the three subclinical measures in prediction of various components of cardiovascular disease; coronary heart disease, heart failure, and stroke, and compares them with some other newer biomarkers and markers of cardiac function that are presumed to be prognostic indicators for the cardiovascular disease. Following this topic on the differential ability of subclinical measures in prediction of various components of cardiovascular disease, **chapter 3.4** is dedicated to coronary artery calcification as a

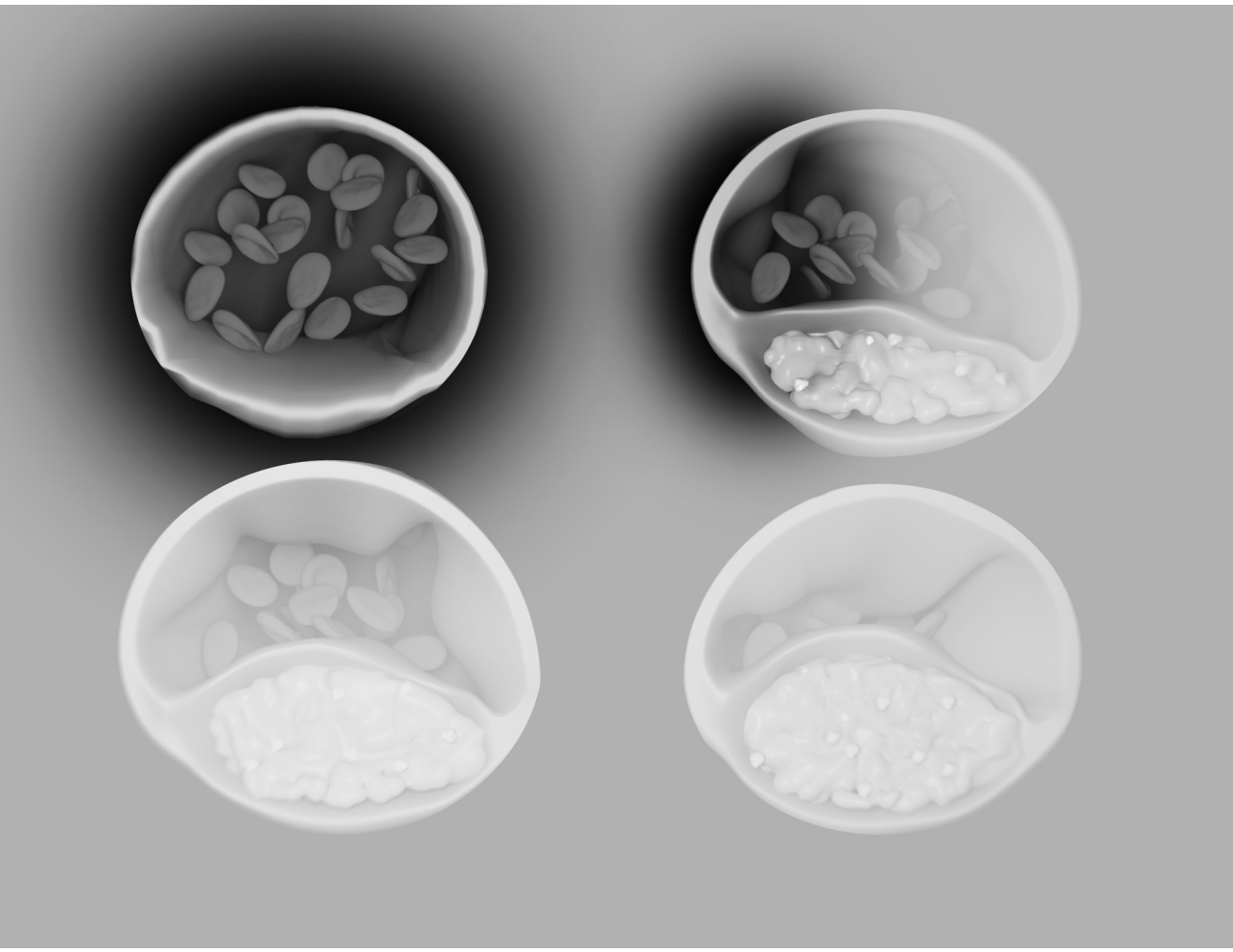
screening tool for cardiovascular disease. For the cardiovascular risk prediction studies described in this chapter, we use data from the population-based Rotterdam Study [16].

In the fourth chapter of this thesis, the recently modified area of cardiovascular risk prediction in women is discussed. In **chapter 4.1**, we apply the newly introduced recommendations for improving cardiovascular risk prediction in women to the population-based Rotterdam Study [16].

Finally, the general discussion **(chapter 5)**, summarizes the main findings of the studies included in this thesis and places them in a broader perspective, addresses the methodological considerations, elaborates on potential clinical implications, and discusses the directions for future research.

REFERENCES

1. Murabito JM, Guo CY, Fox CS, D'Agostino RB. Heritability of the ankle-brachial index: the Framingham Offspring study. Am J Epidemiol. 2006;164:963-968.
2. Peyser PA, Bielak LF, Chu JS, Turner ST, Ellsworth DL, Boerwinkle E, et al. Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. Circulation. 2002;106:304-308.
3. Fox CS, Polak JF, Chazaro I, Cupples A, Wolf PA, D'Agostino RA, et al. Genetic and environmental contributions to atherosclerosis phenotypes in men and women: heritability of carotid intima-media thickness in the Framingham Heart Study. Stroke. 2003;34:397-401.
4. Shah PK. Screening asymptomatic subjects for subclinical atherosclerosis: can we, does it matter, and should we? J Am Coll Cardiol. 2010;56:98-105.
5. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837-1847.
6. Butler J, Kalogeropoulos A, Georgiopoulou V, Belue R, Rodondi N, Garcia M, et al. Incident heart failure prediction in the elderly: the health ABC heart failure score. Circ Heart Fail. 2008;1:125-133.
7. D'Agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. Stroke. 1994;25:40-43.
8. Lloyd-Jones DM. Cardiovascular risk prediction: basic concepts, current status, and future directions. Circulation. 2010;121:1768-1777.
9. Helfand M, Buckley DI, Freeman M, Fu R, Rogers K, Fleming C, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med. 2009;151:496-507.
10. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, et al. 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2010;122:e584-636.
11. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119:2408-2416.
12. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117:743-753.
13. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. Circulation. 2011;123:1243-1262.
14. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PW, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. Circulation. 2006;113:791-798.
15. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. Circ Cardiovasc Genet. 2009;2:73-80.
16. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011;26:657-686.



CHAPTER 2

Genome-Wide Association Studies of Subclinical Measures of Atherosclerosis

Manuscript based on this chapter

Bis JC *, Kavousi M *, Franceschini N *, Isaacs A *, Abecasis GR *, Schminke U *, Post WS *, Smith AV *, Cupples LA *, Markus HS, Schmidt R, Huffman JE, Lehtimäki T, Baumert J, Münzel T, Heckbert SR, Dehghan A, North K, Oostra B, Bevan S, Stoegerer EM, Hayward C, Raitakari O, Meisinger C, Schillert A, Sanna S, Völzke H, Cheng YC, Thorsson B, Fox CS, Rice K, Rivadeneira F, Nambi V, Halperin E, Petrovic KE, Peltonen L, Wichmann HE, Schnabel RB, Dörr M, Parsa A, Aspelund T, Demissie S, Kathiresan S, Reilly MP; the CARDIoGRAM Consortium, Taylor K, Uitterlinden A, Couper DJ, Sitzer M, Kähönen M, Illig T, Wild PS, Orru M, Lüdemann J, Shuldiner AR, Eiriksdottir G, White CC, Rotter JI, Hofman A, Seissler J, Zeller T, Usala G, Ernst F, Launer LJ, D'Agostino RB Sr, O'Leary DH, Ballantyne C, Thiery J, Ziegler A, Lakatta EG, Chilukoti RK, Harris TB, Wolf PA, Psaty BM, Polak JF, Li X, Rathmann W, Uda M, Boerwinkle E, Klopp N, Schmidt H, Wilson JF, Viikari J, Koenig W, Blankenberg S, Newman AB, Witteman J, Heiss G, Duijn CV, Scuteri A, Homuth G, Mitchell BD, Gudnason V, O'Donnell CJ. Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque.

Nat Genet. 2011;43:940-947.

2.2 Genome-Wide Association Study for Carotid Intima-Media Thickness and Carotid Plaque

SUMMARY

Background: Carotid intima-media thickness (cIMT) and plaque determined by ultrasonography are established measures of subclinical atherosclerosis that each predict future cardiovascular disease events.

Methods: We conducted a meta-analysis of genome-wide association data in 31,211 participants of European ancestry from nine large studies in the setting of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. We then sought additional evidence to support our findings among 11,273 individuals using data from 7 additional studies.

Results: In the combined meta-analysis, we identified three genomic regions associated with common cIMT and two different regions associated with the presence of carotid plaque ($P<5\times10^{-8}$). The associated SNPs mapped in, or near, genes related to cellular-signaling, lipid metabolism, and blood pressure homeostasis and two of the regions were associated with coronary artery disease ($P<0.006$) in the CARDIoGRAM Consortium.

Conclusions: Our findings may provide new insight into pathways leading to subclinical atherosclerosis and subsequent cardiovascular events.

INTRODUCTION

Coronary heart disease (CHD) and stroke rank among the leading causes of death in the industrialized world [1] and a significant genetic component underlies both outcomes. These clinical events are often preceded by the development of subclinical atherosclerosis, typically a thickening of the artery wall due to deposition of cholesterol rich material in the arteries that supply blood to major organs [2]. Generalized atherosclerosis results from endothelial dysfunction, inflammation, abnormalities in lipoprotein metabolism [3], coagulation and fibrinolysis [4].

Measures of subclinical atherosclerosis, disease that occurs before symptoms are noted, are predictive of incident clinical events and can be detected non-invasively and with reasonable precision in population samples using high resolution ultrasound techniques. Both cIMT and plaque, reflecting a thickening of the carotid artery wall or the presence of large irregular arterial wall deposits, respectively, are established measures of subclinical atherosclerotic disease. While there may be variation in carotid ultrasound measurement techniques, multiple independent studies have established consistent association of carotid phenotypes with coronary events and stroke in prospective studies of young, middle-aged, and older adults [5,6] and recent consensus prevention guidelines cite cIMT as a potentially useful measure for prediction [7]. While there is a correlation between common cIMT and carotid plaque, common cIMT reflects carotid artery wall thickening that may result from multiple vascular etiologies including hypertension and atherosclerosis, whereas carotid plaque is an indicator of the discrete occurrence of carotid atherosclerosis. Several recent studies provide evidence that carotid plaque is a better predictor of future cardiovascular disease risk than common cIMT [8-10].

Numerous family studies established consistent evidence for moderate heritabilities for common cIMT, internal cIMT and carotid plaque. However, candidate gene studies have not found consistent associations between single nucleotide polymorphisms (SNPs) and cIMT [11], and genome-wide linkage scans completed to date have revealed only suggestive regions for common cIMT [12,13]. We performed a genome-wide association study (GWAS) of three measures of subclinical carotid atherosclerosis; common cIMT, internal cIMT, and plaque, in a sample of up to 31,211 participants from nine population-based studies. In addition, we followed-up our discovery findings in a second stage that included 11,273 participants from 7 independent studies.

METHODS

Our analyses were performed within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium [22], which includes five large population-based prospective cohort studies; the Aging Gene-Environment Susceptibility-Reykjavik

Study (AGES), the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Rotterdam Study I (RS-I). Four additional community-based studies - the Old Order Amish (Amish) Study, the Erasmus Rucphen Family (ERF) Study, the SardiNIA Study, and the Study of Health in Pomerania (SHIP) - collaborated with CHARGE for these analyses. For all studies participating in the meta-analyses, each participant provided written informed consent and the Institutional Review Board at the parent institution for each respective study approved the study protocols.

Different cohorts used different ultrasound devices to assess cIMT or plaque. Most studies, calculated the 'mean of the maximum IMT' by averaging the maximum IMT of both the right and left CCA, near and far walls, or far wall only in some other studies. Carotid plaque definition included the presence of any plaque in most studies and stenosis greater than 25% in others.

We performed a GWAS of three measures of subclinical carotid atherosclerosis; common cIMT, internal cIMT, and plaque, in a sample of up to 31,211 participants from the nine population-based studies that performed genome-wide genotyping with commercial SNP arrays and imputed to the approximately 2.5 million autosomal SNPs in the Phase II HapMap CEU reference panel. In addition, we followed-up our discovery findings in a second stage that included 11,273 participants from 7 independent studies.

RESULTS

The cross-sectional discovery genome-wide analysis of carotid artery phenotypes included 31,211 participants from nine community-based studies whose mean age ranged from 44 to 76 years. Characteristics of the samples are presented in Table 1. In the studies in which all three carotid measures were available, the correlations between common cIMT and plaque ranged from 0.27 to 0.39, and between common cIMT and internal cIMT, from 0.36 to 0.67.

The *a priori* threshold for genome-wide significance was 5×10^{-8}, and a p-value > 5×10^{-8} but <4×10^{-7}, corresponding to not more than one expected false positive finding over 2.5 million tests, was considered suggestive evidence for association in our analyses.

Figure 1A provides a plot of -log10 (p-values) for the associations of the approximately 2.5 million SNPs with common cIMT by chromosome and position for the meta-analysis of the nine discovery studies. P-values from the meta-analysis of plaque (n=25,179 participants) are presented according to their genomic positions in Figure 1B. Overall, from the discovery meta-analysis of common cIMT and plaque, we carried forward 3 genome-wide significant SNPs and 5 suggestive SNPs to the second stage. Our second stage included 11,273 participants from seven community-based studies, six of which

Table 1. Participant characteristics, discovery phase

Characteristic	AGES N=3,073	AMISH N=1,054	ARIC N=7,767	CHS N=3,261	ERF N=1,809	FHS N=3,004	RS-I N=4,699	SardiNIA N=4,235	SHIP N=2,309
Age, years	76.4 (5.4)	48.1 (15.9)	54.3 (5.7)	72.3 (5.4)	48.5 (14.5)	58.5 (9.7)	68.9 (8.7)	43.5 (17.5)	61.8 (9.5)
Women, %	57.7%	49.4%	53.0%	61.0%	56.5%	53.3%	59.3%	56.2%	48.6%
Hypertension, %	80.6%	9.3%	27.0%	51.0%	51.4%	40.5%	55.9%	29.1%	72.4%
Diabetes, %	11.6%	2.1%	8.0%	12.0%	6.1%	8.6%	10.0%	4.8%	10.1%
Current smoker, %	12.6%	9.4%	25.0%	11.0%	39.4%	15.6%	23.4%	20.2%	19.2%
Total cholesterol, mg/dL	217.9 (44.5)	211.3 (48.1)	214.7 (40.5)	213.0 (38.9)	214.4 (42.6)	205.9 (39.7)	256.0 (46.8)	208.6 (42.1)	234.3 (47.9)
HDL cholesterol, mg/dL	61.0 (17.1)	55.7 (14.8)	50.7 (16.8)	55.3 (15.8)	49.5 (14.1)	51.1 (16.1)	51.8 (13.9)	64.4 (14.9)	55.3 (17.8)
Triglyceride, mg/dL	107.0 (59.0)	74.9 (47.1)	136.0 (89.5)	140.4 (76.4)	118.6 (68.1)	142.3 (138.6)	N/A	87.2 (61.4)	177.6 (134.8)
BMI, kg/m2	27.1 (4.5)	26.9 (4.7)	26.9 (4.7)	26.3 (4.5)	26.8 (4.7)	27.9 (5.1)	26.3 (3.7)	25.3 (4.7)	28.5 (4.6)
Prevalent CVD, %	21.9%	6.9%	5.0%	0.0%	3.1%	10.4%	30.8%	1.7%	8.4%
IMT common carotid	0.97 (0.1)	0.74 (0.2)	0.77 (0.2)	1.03 (0.2)	0.82 (0.2)	0.74 (0.2)	1.02 (0.2)	0.54 (0.1)	0.93 (0.2)
IMT internal carotid	N/A	N/A	0.86 (0.5)	1.23 (0.5)	N/A	0.79 (0.5)	N/A	N/A	N/A
Plaque/stenosis present, %	66.9%	N/A	18.0%	66.0%	66.4%	17.7%	61.0%	N/A	70.1%

Numbers in table are Mean (SD) or percentage. N in the column headers indicates the number of participants with common carotid IMT available.
Abbreviations: BMI=body mass index; HDL=high density lipoprotein. Diabetes was defined as fasting blood glucose > 125 mg/dL, a random blood glucose of > 200 mg/dL, or use of insulin or oral hypoglycemic agents; hypertension was defined as blood pressure >140/90 mmHg or on anti-hypertensive medication; current cigarette smoking was defined as self-reported cigarette smoking of at least 1 cigarette per day for a year at any attended exam; cardiovascular disease was defined as coronary heart disease, stroke or transient ischemic attack, or congestive heart failure.

Figure 1A. Genome-wide Manhattan plot for common carotid IMT

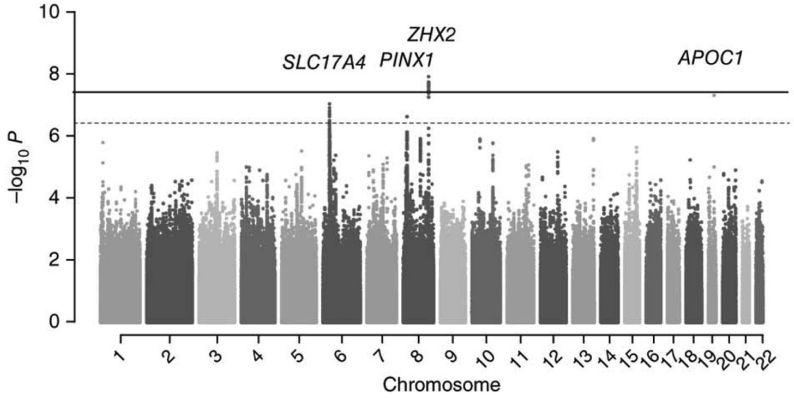


Figure 1B. Genome-wide Manhattan plot for presence of plaque

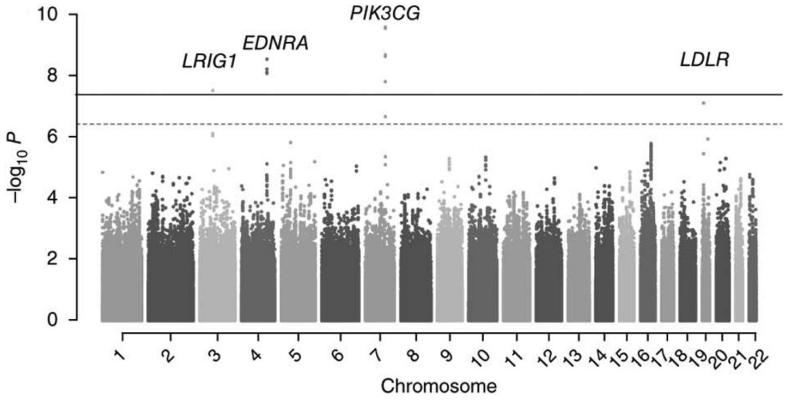


Figure 1. A–B: Genome-wide Manhattan plots for common cIMT and plaque

Plots show the individual p-values (based on discovery meta-analysis) against their genomic position for common carotid IMT (Figure 1A) and for the presence of plaque (Figure 1B). The dashed line indicates the threshold for follow-up, $p<4 \times 10^{-7}$ and the solid line indicates the threshold for genome-wide significance, $p<5 \times 10^{-8}$. The nearest genes are indicated above points that surpassed our genome-wide significance threshold.

provided results for common cIMT (total N=10,403) and three of which provided results for plaque (N=6,013).

Table 2A presents the genome-wide significant association results for the discovery meta-analysis for common cIMT and plaque and Table 2B presents the results for the second stage and combined meta-analyses for common cIMT and plaque.

We show the discovery GWAS results for the 100 kb region surrounding the signal SNPs for common cIMT and plaque along with the recombination rates and the known genes in that region in Figures 2 and 3.

Table 2A. Discovery meta-analysis for common cIMT and plaque

SNP	Chr	Nearest gene	Alleles	Discovery GWAS (cIMT)				
				AF	ß	SE	N	p-value
rs11781551	8	*ZHX2*	A/G	0.48	-0.0081	0.0014	30,894	1.3×10^{-8}
rs445925	19	*APOC1*	A/G	0.11	-0.0179	0.0033	12,395	5.2×10^{-8}
rs6601530	8	*PINX1*	G/A	0.45	0.0078	0.0015	28,124	2.5×10^{-7}
SNP	**Chr**	**Nearest gene**	**Alleles**	**Discovery GWAS (plaque)**				
				AF	**OR (95% CI)**		**N**	**p-value**
rs17398575	7	*PIK3CG*	A/G	0.25	1.17 (1.12 - 1.23)		23,520	2.8×10^{-10}
rs1878406	4	*EDNRA*	T/C	0.13	1.21 (1.13 - 1.28)		24,089	3.1×10^{-9}

Table 2B. Second stage and combined meta-analysis for common cIMT and plaque

SNP	Chr	Nearest gene	Alleles	Second stage meta-analysis (cIMT)					Combined meta-analysis (cIMT)		
				AF	ß	SE	N	p-value	ß	SE	p-value
rs11781551	8	*ZHX2*	A/G	0.47	-0.0072	0.0020	10,401	0.0004	-0.0078	0.0012	2.4×10^{-11}
rs445925	19	*APOC1*	A/G	0.10	-0.0116	0.0047	4,790	0.01	-0.0156	0.0028	1.7×10^{-8}
rs6601530	8	*PINX1*	G/A	0.46	0.0073	0.0029	4,507	0.01	0.0078	0.0014	1.7×10^{-8}
SNP	**Chr**	**Nearest gene**	**Alleles**	**Second stage meta-analysis (plaque)**					**Combined meta-analysis (plaque)**		
				AF	**OR (95% CI)**		**N**	**p-value**	**OR (95% CI)**		**p-value**
rs17398575	7	*PIK3CG*	A/G	0.25	1.20 (1.07 - 1.35)		5,735	0.002	1.18 (1.12 - 1.23)		2.3×10^{-12}
rs1878406	4	*EDNRA*	T/C	0.13	1.31 (1.13 - 1.52)		5,738	0.0003	1.22 (1.15 - 1.29)		6.9×10^{-12}

Alleles indicate the coded (named first) & non-coded allele; AF indicates allele frequency for the coded allele, an average weighted by study size; OR indicates odds ratio, CI, confidence interval; N indicates effective sample size, calculated by taking the sum of each study's sample size multiplied by the SNP's imputation quality. When more than one SNP at a locus surpassed our p-value threshold, we presented the SNP with the lowest p-value.

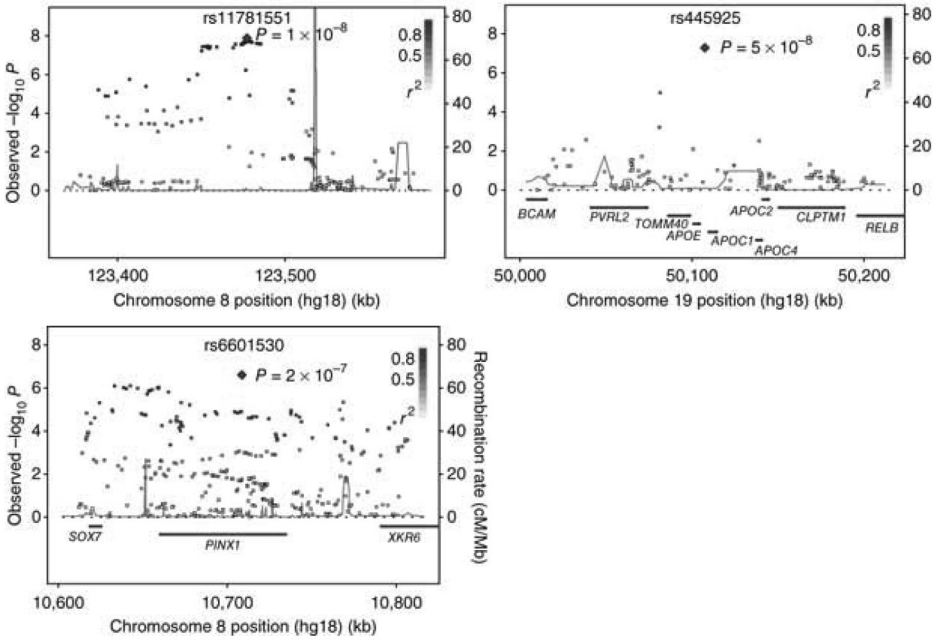


Figure 2. Regional plots for common carotid IMT SNPs

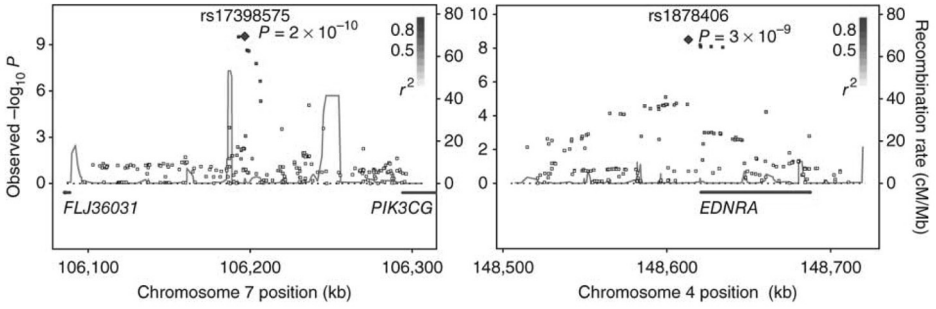


Figure 3. Regional plots for carotid plaque SNPs

Plots are centered on the most significant SNP at each locus along with the meta-analysis results for SNPs in the 100kb region surrounding it. All SNPs are plotted with their discovery meta-analysis p-values against their genomic position, with the most significant SNP in the region indicated as a diamond and other SNPs shaded according to their pairwise correlation (r^2) with the signal SNP.

Figures 4 and 5 show the study-specific findings from the combined meta analyses of common cIMT and plaque, respectively.

Common cIMT

For common cIMT, 3 independent loci achieved our genome-wide significance threshold ($p<5\times10^{-8}$) in the combined meta-analysis.

The strongest association was for rs11781551, found on 8q24 approximately 385 kb from *ZHX2*, where the A allele (allele frequency [AF]=0.48), was associated with lower common cIMT (ß=-0.0078, $p= 2.4\times10^{-11}$), i.e. a 0.8% lower mean common cIMT per copy of the A allele. The second association was for rs445925, located 2.3 kb from *APOC1* on 19q13, a region that also includes *APOE, APOC2,* and *APOC4*. The G allele (AF=0.11) was associated with lower common cIMT (ß=-0.0156, $p=1.7\times 10^{-8}$). The third association was for rs6601530, located within the *PINX1* gene on 8q23.1. Each copy of the G allele (AF=0.45) was associated with higher common cIMT (ß=0.0078, $p=1.7\times10^{-8}$). We also identified a suggestive locus, marked by rs4712972 near the *SLC17A4* gene on 6p22, where the A allele was associated with higher common cIMT (ß=0.0099, $p= 7.8\times10^{-8}$).

While our genome-wide significant and suggestive SNPs from combined meta-analyses for common cIMT explained a small proportion of the trait variance (up to 1.1%), we further constructed an additive genetic risk score (0–8 alleles) comprised of the number of common cIMT risk alleles at the four loci. In the discovery samples, the additive risk score showed graded increasing association with common cIMT across all studies with an average increase of 9.5% in common cIMT from the lowest (0–2) to the highest (6–8) risk category.

Plaque

In analysis of carotid artery plaque, 2 independent loci achieved the genome-wide significance threshold ($p<5\times10^{-8}$) in the combined meta-analysis.

The most significant signal was observed for rs17398575, situated 96.5 kb from the *PIK3CG* gene on 7q22. Per copy of the T allele (AF=0.25), we observed an 18% increased odds of presence of plaque ($p=2.3\times10^{-12}$). The second signal was centered at rs1878406, located 8.5 kb from *EDNRA* on 4q31. Each copy of the T allele (AF=0.13) was associated with a 22% increased odds of the presence of plaque ($p=6.9\times10^{-12}$). Furthermore, two SNPs showed suggestive evidence for association in our combined meta-analysis. The first suggestive locus was rs17045031 on 3p13 where each copy of the A allele was associated with decreased odds of the presence of plaque ($p=1.0\times10^{-7}$). Our second suggestive locus was rs6511720, near *LDLR* on 19p13. Per copy of the T allele we observed a decreased odds of the presence of plaque ($P=3.8\times10^{-7}$).

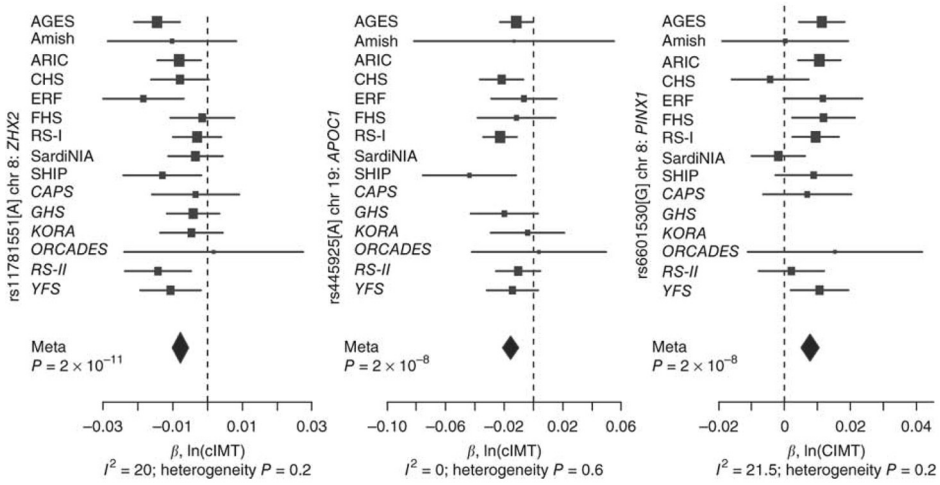


Figure 4. Forest plots for common carotid IMT SNP associations

Plots show the study-specific association estimates (ß) and 95% confidence intervals for the nine discovery and second stage studies, presented as bars. The scale is ln(cIMT). The association estimate and confidence interval for the meta-analysis combining discovery and second stage results is presented as a diamond. Blank spaces indicate occasions in which a particular study was not able to provide results for a given SNP.

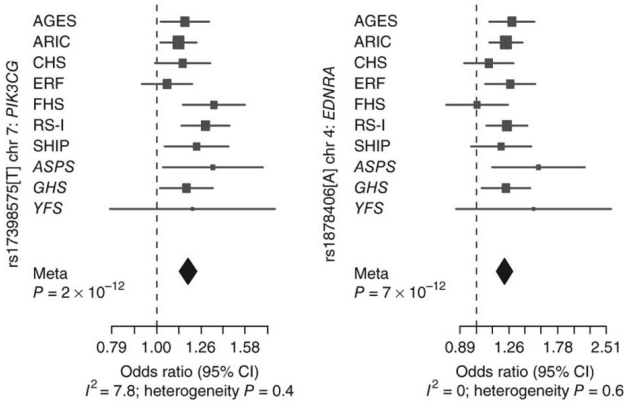


Figure 5. Forest plots for carotid plaque SNP associations

Plots show the study-specific association estimates (OR) and 95% confidence intervals for the nine discovery and second stage studies, presented as bars. The association estimate and confidence interval for the meta-analysis combining discovery and second stage results is presented as a diamond. Blank spaces indicate occasions in which a particular study was not able to provide results for a given SNP.

For both cIMT and plaque, secondary discovery genome-wide meta-analyses conditioned on the genome-wide significant and suggestive SNPs from the combined meta-analyses did not reveal any additional associations.

Internal cIMT

No SNP achieved our significance threshold for follow up in the discovery analyses of internal cIMT. Results for internal cIMT SNPs are not shown.

Cross-Phenotype Comparisons

We also examined the genome-wide significant and suggestive SNPs from our combined meta-analyses for common cIMT and plaque across the three carotid phenotypes. The directions of association were generally consistent and three SNPs, rs445925 (*APOC1*) from the common cIMT analysis and rs17398575 (*PIK3CG*) and rsrs1878406 (*EDNRA*) from the plaque analysis, were associated with all three phenotypes ($p<0.05/8/2=0.003$) in cross-phenotype comparisons.

Associations with Coronary Artery Disease

We investigated the genome-wide significant and suggestive SNPs from our combined meta-analyses for common cIMT and plaque for their potential associations with coronary artery disease (CAD) in the CARDIoGRAM Consortium (Table 3). Two SNPs from our plaque analysis had a p-value for association with CAD less than 0.006 (0.05/8 tests). The first, rs6511720, near *LDLR*, where the G allele was associated with both higher plaque risk in our study and higher CAD risk (p=0.0002); and rs1878406, near *EDNRA* where the C allele was associated with lower risk of plaque and lower risk of CAD ($p=2\times10^{-6}$). One SNP from common cIMT analysis, rs445925 near *APOC1*, showed a suggestive association with CAD with the same allele (A) being associated with higher common cIMT and higher CAD risk (p=0.02). Another SNP identified in the plaque analysis, rs17045031 near *LRIG1*, showed a suggestive association with CAD, with the G allele associated with both lower odds of plaque and lower risk of CAD (p=0.04).

Conversely, none of SNPs reported to be associated with coronary artery disease in the CARDIoGRAM Consortium [14] had a significant association (i.e., a p-value less than 0.00072, a conservative Bonferroni correction for 23 tests across three phenotypes) in our discovery meta-analyses of common cIMT, internal cIMT, or plaque (data not shown).

Table 3. Association of genome-wide significant and suggestive common cIMT and plaque SNPs with CAD in the CARDIoGRAM Consortium

source	SNP	Chr	Nearest Gene	Allele	AF	OR (95% CI)	N	p-value
Common cIMT	rs11781551	8	*ZHX2*	G	0.53	1.02 (0.99 – 1.05)	83,379	0.2
	rs445925	19	*APOC1*	G	0.91	1.11 (1.02 – 1.20)	34,216	0.02
	rs6601530	8	*PINX1*	G	0.40	1.02 (0.99 – 1.05)	79,512	0.1
	rs4712972	6	*SLC17A4*	G	0.86	1.02 (0.97 – 1.06)	84,001	0.5
Plaque	rs17398575	7	*PIK3CG*	G	0.73	0.98 (0.95 – 1.01)	83,028	0.2
	rs1878406	4	*EDNRA*	C	0.86	0.91 (0.87 – 0.95)	81,804	2×10^{-6}
	rs6511720	19	*LDLR*	G	0.90	1.13 (1.06 – 1.21)	56,420	0.0002
	rs17045031	3	*LRIG1*	G	0.94	1.09 (1.00 – 1.18)	80,655	0.04

Allele indicates the coded allele in the CARDIoGRAM Consortium meta-analysis; AF indicates allele frequency for the coded allele; OR indicates odds ratio, CI, confidence interval; N indicates sample size.

DISCUSSION

In this meta-analysis of GWAS data from nine studies of common cIMT and seven studies of plaque, we identified genome-wide significant associations between 3 regions and common cIMT and between 2 regions and the presence of carotid plaque in over 40,000 participants of European ancestry. Interestingly, *EDNRA* one of our genome-wide significant regions in the combined meta-analysis of plaque was related to multiple carotid phenotypes and was also associated with coronary artery diseases in the recent large meta-analysis by the CARDIoGRAM Consortium.

Three SNPs emerged as genome-wide significant from our combined meta-analysis of common cIMT. The strongest association, on chromosome 8 (rs11781551), is an intergenic SNP located 385 kb from the *ZHX2* gene. Members of this gene family are nuclear homodimeric transcriptional repressors that interact with the A subunit of nuclear factor-Y (NF-YA) and contain two C2H2-type zinc fingers and five homeobox DNA-binding domains. Little information about these proteins exists regarding cardiovascular disease or population studies.

A second association, on 19q13 (rs445925), fell upstream of the *APOC1* gene. While this region has been of interest for its role in neurological genetics because of the *APOE* gene, it is also been frequent candidate gene for cardiovascular disease traits [15]. Although some previous studies have found associations of variation at the *APOE* locus and common cIMT [16], among 4 of our discovery studies that had independently measured the *APOE* epsilon variants, the correlation between rs445925 and the e4 allele was less than 0.05. Further, models that included both the *APOE* e4 and the *APOC1* variant indicated that the *APOE* gene was not associated with common cIMT in these 4 studies (data not shown), while the *APOC1* variant still showed a significant association with common cIMT. While *APOE* variants have been implicated in cases of familial dyslipidemia and premature atherosclerosis and in recent genome-wide association studies with variation in multiple lipoprotein measures [17], our results suggest that *APOC1* is the primary variant of interest for carotid traits.

The third association (rs6601530) was located in an intron of the Pin2-interacting protein 1 (*PINX1*) gene. The protein, a telomerase inhibitor [18] that plays a role in chromosomal segregation in mitosis [19], has been investigated in relation to cancers, but was not considered a candidate gene for cardiovascular phenotypes.

The region on chromosome 6 marked by rs4712972, which includes the *SLC17A4, SLC17A1,* and *SLC17A3* genes showed suggestive evidence for association with common cIMT in our combined meta-analysis. This region may merit further investigation as recent genome-wide association studies have implicated this region with uric acid levels [20,21]. Although high uric acid levels have been associated with cardiovascular disease and all cause mortality [22], the contribution to atherosclerotic vascular disease remains controversial [23].

Plaque Associations

For plaque, two regions were genome-wide significant in our combined meta-analysis. The first region was within 100kb of the *PIK3CG* gene, which encodes one of the pi3/pi4-kinase family of proteins. These proteins are important modulators of extracellular signals, including those elicited by E-cadherin-mediated cell-cell adhesion, which plays an important role of endothelin in maintenance of the structural and functional integrity of epithelia. The fact that this region was reported as a top hit in a recent GWAS of both platelet volume [24] and aggregation [25] suggests pleiotropy and highlights the interconnectedness of multiple cardiometabolic traits.

The second genome-wide significant region was near the *EDNRA* gene. Because of the role of endothelin as a potent vasoconstrictor, the endothelin receptor, type A is a target for pharmacologic treatments to reduce blood pressure [26]. In addition, variation in the gene was associated with blood pressure [27], atherosclerosis [28], and cardiovascular disease endpoints [29] in candidate gene studies.

Two more regions showed suggestive evidence for association in our combined meta-analysis for plaque. The first region, near the *LDLR* gene is a particularly interesting candidate for subclinical atherosclerosis because of its role in familial hypercholesterolemia and its appearance in recent genome-wide association studies for lipid traits [30-33] and myocardial infarction [14,34]. Notably, the *LDLR* SNP recently reported to be associated with MI (rs1122608) is located 38 kb away and is in modest LD (r^2=0.2 in HapMap CEU) with the signal SNP (rs6511720) in our analysis that also showed an association with CAD in the CARDIoGRAM Consortium. The second was in the vicinity of *LRIG1*, which negatively regulates growth factor signaling and is involved in the regulation of epidermal stem cell quiescence.

Interestingly, we found three loci (*APOC1, PIK3CG,* and *EDNRA*) that were associated with all three carotid phenotypes. Among these, the *EDNRA* locus was also significantly associated with coronary artery disease in the recent large meta-analysis by the CARDIoGRAM Consortium. These associations may provide important insights into the pathophysiological mechanisms relating the genes to atherosclerosis and subsequent coronary artery disease. In particular, the concordance of association with SNPs in *EDNRA* with both carotid plaque and CHD suggests a common etiology for subclinical and clinically apparent disease that warrants further investigation.

The strengths of the current study include the large sample size, the population-based designs, the collaboratively designed pre-specified analysis plan, and the high quality of both genotyping and phenotyping. Further, our ability to relate our findings to the outcome of CAD in a large independent meta-analysis provides important additional context to our results. These associations are unlikely to be due to population stratification since the discovery sample was restricted to whites of European origin and was also investigated for global latent population substructure.

The study also has limitations. A single cross-sectional IMT assessment was used in all studies and ultrasound protocols varied across participating studies. For example, plaque definition included the presence of any plaque in most studies and stenosis greater than 25% in others. The heterogeneity of measurement techniques may have compromised our ability to detect small associations. Despite this heterogeneity, the ability to detect consistent genetic associations for several carotid measures suggests that additional signals may be discovered in future studies utilizing a larger sample size or a higher resolution technique such as magnetic resonance imaging. Further, few studies had internal cIMT measures since these are more difficult to obtain than common cIMT measurements and thus limited our ability to discover associations with this phenotype. Although our sample size was reasonably large, we still had limited power to detect associations with small effect sizes. Genome-wide association studies are known for revealing associations with common variants and may miss rare variants not covered by the commercial genotyping arrays. For instance, the sparse coverage of the *APOC1* and *LDLR* gene regions resulted in varying imputation quality and a lower effective sample size for the analysis of these two regions.

Because we did not conduct follow-up fine mapping of the results, and because some SNPs were distant from known genes, it is likely that the identified SNPs are not causal variants, but, instead, may be in linkage disequilibrium with variants that were not analyzed. Because some of our associations attained genome-wide significant p-values only in the combined meta-analysis, confirmation of our findings in other populations and further exploration of these genomic regions with dense genotyping, expression, and translational studies will be required to better understand the role of these genes in subclinical atherosclerotic disease.

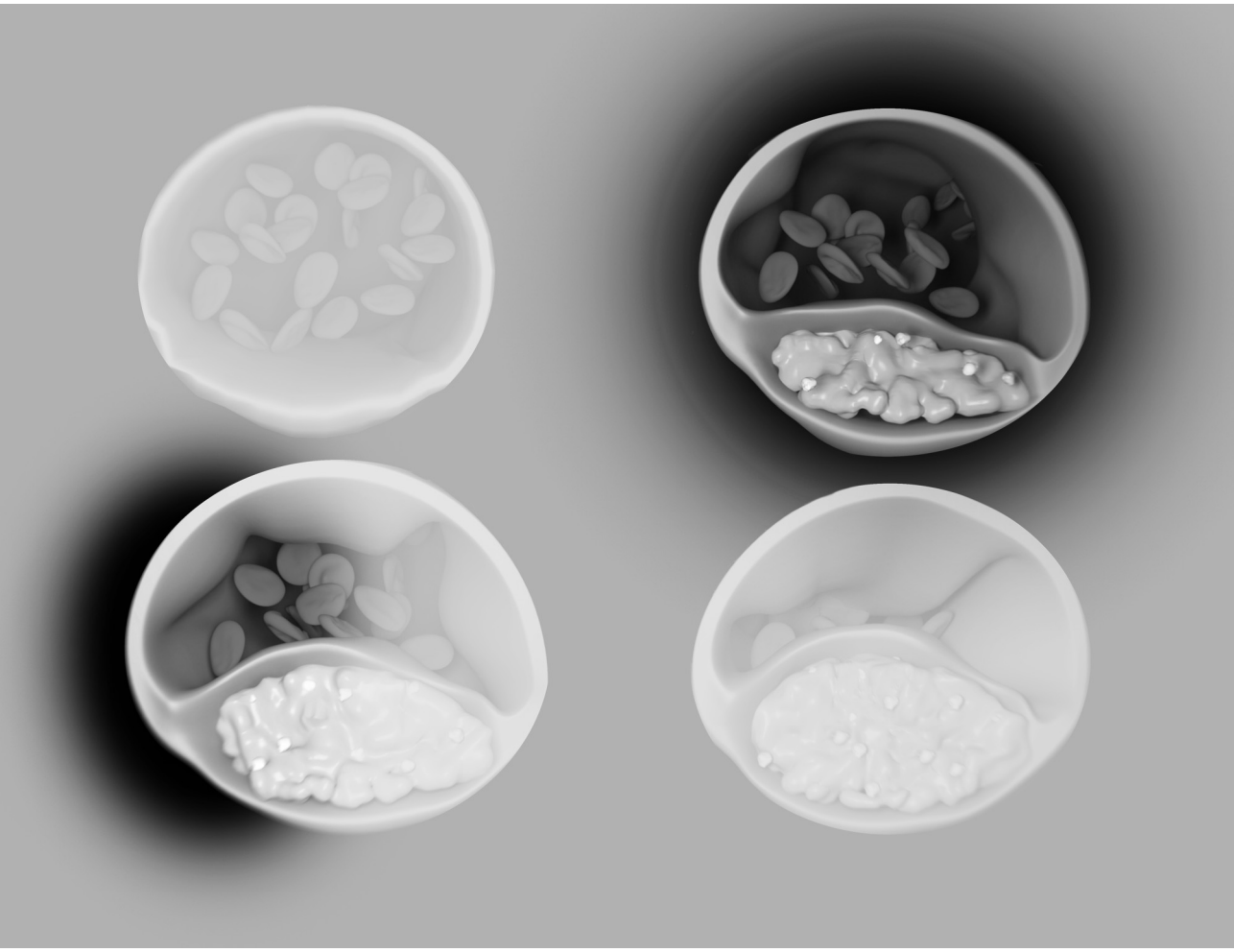
In summary, our meta-analysis of GWAS data from nine community-based studies has revealed 5 new loci for common cIMT and plaque. These loci implicate LDL metabolism (*APOC1*), endothelial dysfunction (*EDNRA*), platelet biology (*PIK3CG*), and telomere maintenance (*PINX1*). Two of our identified loci are also associated with coronary artery disease in the recent large meta-analysis by the CARDIoGRAM Consortium. Exploring the molecular, cellular and clinical consequences of genetic variation at these loci may yield novel insights into the pathophysiology of clinical and subclinical cardiovascular disease.

REFERENCES

1. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Executive summary: heart disease and stroke statistics-- 2010 update: a report from the American Heart Association. Circulation. 2010;121:948-954.
2. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol. 2006; 47 :C7-C12.
3. Insull W Jr. The Pathology of Atherosclerosis: Plaque Development and Plaque Responses to Medical Treatment. Am J Med. 2009;122:S3-S14.
4. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999; 340:115-126.
5. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115:459-467.
6. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21:93-111.
7. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2010;122:e584-e636.
8. Johnsen SH, Mathiesen EB, Joakimsen O, Stensland E, Wilsgaard T, Løchen ML, et al. Carotid atherosclerosis is a stronger predictor of myocardial infarction in women than in men: a 6-year follow-up study of 6226 persons: the Tromso Study. Stroke. 2007;38:2873-2880.
9. Mathiesen EB, Johnsen SH, Wilsgaard T, Bønaa KH, Løchen ML, Njølstad I, et al. Carotid plaque area and intima-media thickness in prediction of first-ever ischemic stroke: a 10-year follow-up of 6584 men and women: the tromso study. Stroke. 2011;42:972-978.
10. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J Am Coll Cardiol. 2010;55:1600-1607.
11. Manolio TA, Boerwinkle E, O'Donnell CJ, Wilson AF. Genetics of ultrasonographic carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:1567-1577.
12. Wang D, Yang H, Quiñones MJ, Bulnes-Enriquez I, Jimenez X, De La Rosa R, et al. A genome-wide scan for carotid artery intima-media thickness: the Mexican- American Coronary Artery Disease family study. Stroke. 2005;36:540-545.
13. Fox CS, Cupples LA, Chazaro I, Polak JF, Wolf PA, D'Agostino RB, et al. Genomewide linkage analysis for internal carotid artery intimal medial thickness: evidence for linkage to chromosome 12. Am J Hum Genet. 2004;74:253-261.
14. Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011;43:333–338.
15. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007;298:1300-1311.
16. Paternoster L, Martinez-Gonzalez NA, Charleton R, Chung M, Lewis S, Sudlow CL. Genetic effects on carotid intima-media thickness: systematic assessment and meta-analyses of can-

didate gene polymorphisms studied in more than 5000 subjects. Circ Cardiovasc Genet. 2010;3:15-21.
17. Chasman DI, Paré G, Mora S, Hopewell JC, Peloso G, Clarke R, et al. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet. 2009;5:e1000730.
18. Zhou XZ, Lu KP. The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. Cell. 2001;107:347-359.
19. Yuan K, Li N, Jiang K, Zhu T, Huo Y, Wang C, et al. PinX1 is a novel microtubule-binding protein essential for accurate chromosome segregation. J Biol Chem. 2009;284:23072-23082.
20. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, et al. Meta- analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009;5:e1000504.
21. Dehghan A, Köttgen A, Yang Q, Hwang SJ, Kao WL, Rivadeneira F, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet. 2008;372:1953-1961.
22. Meisinger C, Koenig W, Baumert J, Doring A. Uric acid levels are associated with all-cause and cardiovascular disease mortality independent of systemic inflammation in men from the general population: the MONICA/KORA cohort study. Arterioscler Thromb Vasc Biol. 2008;28:1186-1192.
23. Stark K, Reinhard W, Grassl M, Erdmann J, Schunkert H, Illig T, et al. Common polymorphisms influencing serum uric acid levels contribute to susceptibility to gout, but not to coronary artery disease. PLoS One. 2009;4:e7729.
24. Soranzo N, et al. A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function. Blood. 2009; 113:3831-3837.
25. Johnson AD, Yanek LR, Chen MH, Faraday N, Larson MG, Tofler G, et al. Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. Nat Genet. 2010;42:608- 613.
26. Nakov R, Pfarr E, Eberle S. Darusentan: an effective endothelinA receptor antagonist for treatment of hypertension. Am J Hypertens. 2002;15:583-589.
27. Rahman T, Baker M, Hall DH, Avery PJ, Keavney B. Common genetic variation in the type A endothelin-1 receptor is associated with ambulatory blood pressure: a family study. J Hum Hypertens. 2008; 22:282-288.
28. Yasuda H, Kamide K, Takiuchi S, Matayoshi T, Hanada H, Kada A, et al. Association of single nucleotide polymorphisms in endothelin family genes with the progression of atherosclerosis in patients with essential hypertension. J Hum Hypertens. 2007;21:883-892.
29. Oguri M, Kato K, Yokoi K, Itoh T, Yoshida T, Watanabe S, et al. Association of genetic variants with myocardial infarction in Japanese individuals with metabolic syndrome. Atherosclerosis. 2009;206:486- 493.
30. Chasman DI, Paré G, Zee RY, Parker AN, Cook NR, Buring JE, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and Apolipoprotein B among 6382 white women in genome-wide analysis with replication. Circ Cardiovasc Genet. 2008;1:21-30.
31. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, et al. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet. 2009;41:35-46.

32. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet. 2009;41:56-65.
33. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet. 2009;41:47-55.
34. Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009;41:334-341.
35. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of Prospective Meta- Analyses of Genome-Wide Association Studies From 5 Cohorts. Circ Cardiovasc Genet. 2009;2:73-80.
36. Johnson AD, O'Donnell CJ. An open access database of genome-wide association results. BMC Med Genet. 2009;10:6.



CHAPTER 3

Subclinical Measures of Atherosclerosis in Cardiovascular Risk Prediction

Manuscript based on this chapter

Elias-Smale SE *, Kavousi M *, Verwoert GC, Koller MT, Steyerberg EW, Mattace-Raso FUS, Hofman A, Hoeks APG, Reneman RS, Witteman JCM. Common carotid intima-media thickness in cardiovascular risk stratification of older people; The Rotterdam Study.
Eur J Prev Cardiol. 2012;19:698-705.

3.1 Carotid Intima-Media Thickness in Cardiovascular Risk Stratification

SUMMARY

Background: Non-invasive measures of atherosclerosis, such as carotid intima-media thickness (cIMT), may improve global cardiovascular risk prediction. The aim of this study was to determine whether common cIMT in addition to traditional risk factors improves risk classification in a general population of older people.

Methods: A group of 3,580 non-diabetic people aged 55-75 years and free of cardiovascular disease at baseline were followed for a median time of 12.2 years. Compared to models based on Framingham risk factors, we studied the ability of common cIMT measurement to better classify people into categories of low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of hard coronary heart disease (CHD) and stroke.

Results: In older men, addition of cIMT to Framingham risk factors did not improve prediction of hard CHD or stroke. In older women, addition of cIMT to Framingham risk factors significantly improved risk classification. cIMT improved the C-statistic of the model for hard CHD from 0.711 to 0.719 and for stroke from 0.712 to 0.721, at good calibration. Reclassification was least in the majority of women classified as low risk for hard CHD (4%, n=76) and for stroke (3%, n=62) and most substantial in women at intermediate risk for hard CHD (43%, n=70) and for stroke (28%, n=76). The net reclassification improvement in women was 8.2% (*P*=0.03) for hard CHD and 8.0% (*P*=0.06) for stroke.

Conclusions: cIMT had some additional value beyond traditional risk factors in the cardiovascular risk stratification of older women, but not of older men.

INTRODUCTION

Cardiovascular disease (CVD) is the major cause of morbidity and premature death in the Western world. The underlying atherosclerosis develops over many years and symptoms usually do not occur until the disease is already at an advanced stage. The majority of CVD is related to modifiable risk factors and modification of these factors has been shown to reduce CVD morbidity and mortality [1].

The current, clinically accepted concept of matching treatment intensity to the degree of cardiovascular risk suggests that it is important to identify the most accurate approach to risk stratification as a solid base for the best treatment strategies in people at risk of CVD. In accordance with current guidelines, global risk factor assessment using algorithms such as the SCORE risk chart [2] or Framingham Risk Score [3] are increasingly used to stratify people in categories of low, intermediate, and high risk, based on 10-year absolute risk of CVD or coronary heart disease (CHD). However, these risk factor algorithms are of limited accuracy, especially in women and the elderly [4,5]. To improve risk stratification, expert panels have proposed non-invasive measures predicting atherosclerotic disease, such as carotid intima-media measurement (cIMT), to complement the risk stratification based on traditional risk factors [1,3,6,7]. Previous studies have shown that the addition of a cIMT measurement to established risk factors leads to small and sometimes significant improvements in cardiovascular risk prediction by means of increases in the C-statistic [8]. However, in the evaluation of the clinical value of a new test, it is important to assess the reclassification of individuals into different risk categories when the new test is added to traditional risk factors [9]; to date limited research has addressed this issue [10,11].

In the population-based Rotterdam study, we stratified non-diabetic men and women of 55–75 years who were free of CVD at baseline into three widely used clinical risk categories of low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of hard CHD and stroke based on Framingham risk factors and studied the ability of common cIMT to reclassify people to a more accurate risk category.

METHODS

Study Population

The study was embedded in the Rotterdam Study, which is a prospective, population-based study among subjects aged 55 years and older in a municipality of Rotterdam that started in 1990. The rationale and design of the Rotterdam Study has been described elsewhere [12,13]. The baseline examination took place from 1990 to 1993. Of the 7,983 participants (response rate 78%), 5,643 underwent a common cIMT measurement.

Missing cIMT measurements were due to restricted availability of ultrasonographers. In line with current guidelines for cardiovascular screening [3], we excluded people of 75 years and older (n=1,302) and people already defined as being at high cardiovascular risk based on having diabetes (N=431) or established cardiovascular disease (CVD) at baseline (N=330).

Prevalent CVD was defined as a history of clinically manifest myocardial infarction, coronary artery bypass grafting, percutaneous coronary intervention, stroke, symptomatic peripheral artery disease (PAD), aortic or carotid surgery. Hence, the current analysis was carried out in 3,580 asymptomatic individuals. The Medical Ethics Committee of Erasmus Medical Center approved the study, and all participants gave informed consent.

Common Carotid IMT Measurement

Ultrasonography of both carotid arteries was performed with a 7.5MHz linear array transducer and a duplex scanner (ATL UltraMark IV; Advanced Technology Laboratories). Measurements of the common carotid artery (CCA) intima-media thickness involved a length of 10mm distal of the bulb. cIMT was determined as the average of mean, near- and far-wall IMT, providing the average of left and right arteries. The procedure has been described in detail previously [14].

Cardiovascular Risk Factors

Information on cardiovascular risk factors was acquired during the baseline examination as described previously [15]. Diabetes was defined as a non fasting glucose> 11 mmol/l and/or use of anti-diabetic medication.

Clinical Outcomes

The follow-up procedures of the Rotterdam Study have been reported previously [15]. None of the people considered for analysis was lost to follow up. As outcome, we used hard CHD; consisting of incident myocardial infarction (MI) and coronary heart disease mortality, and stroke; which included ischaemic stroke (mortality). If a person died within 4 weeks of an MI or ischaemic stroke, events were coded as fatal. Follow up was completed by January 2005.

Statistical Analysis

Common cIMT and Risk of CVD

We used Cox proportional hazard regression analysis to estimate the hazard ratio (HR) of a 1-standard deviation increase of cIMT to predict hard CHD and stroke for the total population and as well as stratified by gender. In the analysis, we corrected for age and, if appropriate, gender. We additionally adjusted for current smoking, total cholesterol,

HDL cholesterol, systolic blood pressure and use of antihypertensive medication, and for atrial fibrillation for the stroke outcome. We used log minus log plots to check for proportionality of hazards over time and found no indications of violation of the proportionality assumption.

Risk Prediction Models

We used Cox proportional hazard regression analysis to derive estimates of individual 10-year risk of hard CHD and stroke. Analyses were performed by gender. For the outcome hard CHD, we used the same predictors as defined in the Framingham risk score, in accordance with the ATP III guidelines [3]. The model thus included age, gender, systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL cholesterol and current smoking (model 1). For the outcome stroke, model 1 also included atrial fibrillation, in line with the Framingham stroke risk score [16]. In a second step, we extended model 1 with common cIMT (model 2). Both models were fitted to the gender-specific subsets of the Rotterdam Study data. We used restricted cubic spline transformations with four knots for continuous variables in the models, four knots being a good compromise between flexibility and loss of precision caused by over-fitting of the model [17]. We used the likelihood ratio test to study the null-hypothesis of no effect of common cIMT to predict hard CHD and stroke beyond Framingham risk predictors. To examine the discriminative ability of the two models, we calculated the bootstrap corrected C-statistic using 150 repetitions for both gender-specific models. The purpose of using the bootstrap method is to correct for over-optimism of the fitted models [18]. Next, we computed reclassification percentages to study the incremental ability of common cIMT to classify subjects in risk categories according to commonly used categories of 10-year hard CHD and stroke risk: low (<10%), intermediate (10–20%), and high risk (>20%) [3]. Observed 10-year risk of CVD was estimated by Kaplan-Meier survival analysis for each cell of the reclassification table to show calibration of reclassified people with observed risk. To evaluate true improvement in classification by addition of common cIMT to the Framingham model we calculated the net reclassification improvement (NRI) [19].

In secondary analyses, we repeated the prediction analyses using the mean of the maximum of IMT values of near and far walls of both common carotid arteries as a determinant instead of the mean of the mean IMT values.

Covariables were missing in less than 2% of people. We used single imputation by the Expectation Maximization method. All analyses were performed using SPSS for Windows (SPSS, Inc., Chicago, Illinois, USA) and R version 2.7.2. (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics of men and women are shown in Table 1. The total population of 3,580 people consisted of 1,398 men and 2,182 women (61%). Median follow-up duration (inter-quartile range) was 12.2 (11.7–13.1) years. During this period, 530 events occurred of which 267 were in men and 263 in women. One hundred and six men and 68 women had a myocardial infarction, 89 men and 118 women had an ischaemic stroke whereas 60 men and 59 women died of CHD and 12 men and 18 women had a fatal ischaemic stroke.

For the risk of hard CHD, the age- and gender adjusted hazard ratios (95% CI) for a 1-standard deviation increase in common cIMT in the total population was 1.26 (1.13–1.41) and decreased to 1.16 (1.03–1.30) after additional adjustment for the remaining cardiovascular risk factors. Corresponding risks of stroke were: 1.33 (1.18–1.50) and 1.25 (1.10–1.42). In gender specific subsets, the analogous hazard ratios for the outcome hard CHD were 1.08 (0.92–1.27) and 1.02 (0.86–1.20) in men and 1.56 (1.34–1.81) and 1.40 (1.19–1.65) in women. For stroke outcome, corresponding hazard ratios were 1.28 (1.07–1.54) and 1.18 (0.97–1.43) in men and 1.38 (1.18–1.62) and 1.32 (1.12–1.56) in women.

In hard CHD risk prediction in men, addition of common cIMT did not improve model performance of the refitted Framingham risk model. The likelihood chi-square of model 2 did not significantly increase compared to model 1 (P=0.84). Hence, the

Table 1. Baseline characteristics of the population, by gender

Variable	Men N = 1,398	Women N = 2,182	*P* Value
Age (years)	64.5±5.3	64.8±5.6	0.068
Body mass index (kg/m^2)	25.7±2.9	26.6±3.9	<0.001
Current smoking (%)	30.3	22.5	<0.001
Systolic blood pressure (mmHg)	137±22	136±22	0.072
Diastolic blood pressure (mmHg)	76±12	73±11	<0.001
Antihypertensive treatment (%)	10.2	15.7	<0.001
Total cholesterol (mmol/L)	6.4±1.1	6.9±1.2	<0.001
HDL cholesterol (mmol/L)	1.2±0.3	1.5±0.4	<0.001
Lipid lowering medication (%)	1.5	2.2	0.137
Antithrombotic medication (%)	3.6	1.4	<0.001
Atrial fibrillation (%)	2.9	2.0	0.113
Common cIMT (mm)	0.82±0.14	0.77±0.12	<0.001

HDL, high-density lipoprotein; IMT, intima-media thickness. Values are mean (±standard deviation) for continuous variables and percentages for dichotomous variables.

C-statistic did not improve by addition of cIMT: 0.611 (model 1) and 0.610 (model 2). In contrast, addition of common cIMT significantly improved model performance in women. The likelihood chi-square increased by 15.2 points (P<0.001). The C-statistic improved from 0.711 to 0.719, indicating slightly better average discriminative ability of the model including cIMT.

In stroke risk prediction in men, addition of common cIMT did not improve model performance of the refitted Framingham risk model. The likelihood chi-square did not significantly increase (*P*=0.11), the C-statistic slightly improved by addition of cIMT from 0.692 (model 1) to 0.698 (model 2). In women, the likelihood chi-square increased by 10.0 points (*P*=0.002). The C-statistic improved from 0.712 to 0.721.

Table 2 displays categories of estimated 10-year hard CHD risk based on the Framingham risk model before and after adding common cIMT in men and women. In men, percentages in the low, intermediate and high-risk categories were 53%, 43% and 4%, respectively. For women these percentages were 92%, 7% and 1%. In accordance with the finding that addition of cIMT did not improve the C-statistic of the Framingham risk model, additional cIMT measurements led to hardly any reclassification among men. Correspondingly, the net reclassification improvement (NRI) was 0.21% (*P*=0.50) for men. In women, additional cIMT measurement led to more reclassification. Of women at low risk, 4% (n=73) reclassified to the intermediate risk category and three women to high risk. Of women at intermediate risk, 35% (n=57) were downgraded to low risk and 8% (n=13) upgraded to high risk. Of women at high risk, 29% (n=4) were downgraded to the intermediate risk category. Addition of common cIMT in women led to a net gain in reclassification of 9.1% in people with an event and a net decline in reclassification of 0.9% in people without event, resulting in a NRI of 9.1–0.9=8.2% (*P*=0.03).

Table 3 displays categories of estimated 10-year stroke risk based on the Framingham risk model before and after adding common cIMT in men and women for the outcome of stroke. In men, percentages in the low, intermediate and high-risk categories were 85%, 13% and 2%, respectively. For women corresponding percentages were 87%, 12% and 1%. In men, more reclassification was observed compared with the outcome of stroke. However, this was not clinically significant, by means of the NRI (3.9%; *P*=0.16). Of women at low risk, 3% (n=59) of people reclassified to the intermediate risk category and three people to high risk. Of women at intermediate risk, 19% (n=51) were downgraded to low risk and 9% (n=25) upgraded to high risk. Of women at high risk, 37% (n=10) were downgraded to the intermediate risk category. Addition of common cIMT in women led to a net gain in reclassification of 9% in people with an event and a net decline in reclassification of 1% in people without event, resulting in a NRI of 9.0–1.0=8% (*P*=0.06). Repeating the analyses using the mean of the maximal cIMT values of near and far walls did not lead to substantial changes in reclassification percentages and NRI values (data not shown).

Table 2. Risk reclassification comparing the Framingham risk model and Framingham risk model plus common cIMT by gender in the prediction of hard CHD

Framingham 10-year risk categories (%)		10-year risk categories for Framingham risk covariates + cIMT			N (%) reclassified
		<10%	10-20%	>20%	
Men					
<10%	N = 742 (53%)	740 (100%)	2 (0%)	0 (0%)	2 (0%)
	Observed risk (95%CI)	0.07 (0.06, 0.09)	NA	NA	
10-20%	N = 598 (43%)	7(1%)	589 (99%)	2 (0%)	9 (1%)
	Observed risk (95%CI)	NA	0.13 (0.11, 0.16)	NA	
≥20%	N = 58 (4%)	0 (0%)	0 (0%)	58 (100%)	0 (0%)
	Observed risk (95%CI)	NA	NA	0.24 (0.16, 0.37)	
NRI: 0.21%, *P*=NS					
Women					
<10%	N = 2005 (92%)	1929 (96%)	73 (4%)	3 (0%)	76 (4%)
	Observed risk (95%CI)	0.04 (0.03, 0.05)	0.11 (0.06, 0.20)	0.58 (0.19, 0.97)	
10-20%	N = 163 (7%)	57 (35%)	93 (57%)	13 (8%)	70 (43%)
	Observed risk (95%CI)	0.05 (0.02, 0.13)	0.13 (0.08, 0.21)	0.28 (0.12, 0.59)	
≥20%	N = 14 (1%)	0 (0%)	4 (29%)	10 (71%)	4 (29%)
	Observed risk (95%CI)	NA	0.18 (0.03, 0.76)	0.22 (0.06, 0.62)	
NRI: 8.2%, *P*=0.03					

CI, confidence interval; NRI, net reclassification improvement.

Table 3. Risk reclassification comparing the Framingham risk model and Framingham risk model plus common cIMT by gender in the prediction of stroke

Framingham 10-year risk categories (%)		10-year risk categories for Framingham risk covariates + cIMT			N (%) reclassified
		<10%	10-20%	>20%	
Men					
<10%	N = 1188 (85%)	1152 (97%)	36 (3%)	0 (0%)	36 (3%)
	Observed risk (95%CI)	0.09 (0.08, 0.11)	0.19 (0.10, 0.36)	NA	
10-20%	N = 181 (13%)	29 (16%)	145 (80%)	7 (4%)	36 (20%)
	Observed risk (95%CI)	0.12 (0.05, 0.29)	0.12 (0.08, 0.18)	0.55 (0.26, 0.88)	
≥20%	N = 29 (2%)	0 (0%)	4 (14%)	25 (86%)	4 (14%)
	Observed risk (95%CI)	NA	NA	0.22 (0.10, 0.45)	
NRI: 3.9%, *P*=0.16					
Women					
<10%	N = 1886 (87%)	1824 (97%)	59 (3%)	3 (0%)	62 (3%)
	Observed risk (95%CI)	0.03 (0.02, 0.04)	0.11 (0.06, 0.21)	0.29 (0.15, 0.51)	
10-20%	N = 269 (12%)	51 (19%)	193 (72%)	25 (9%)	76 (28%)
	Observed risk (95%CI)	0.08 (0.05, 0.13)	0.12 (0.06, 0.23)	0.29 (0.15, 0.51)	
≥20%	N = 27 (1%)	0 (0%)	10 (37%)	17 (63%)	10 (37%)
	Observed risk (95%CI)	NA	0.09 (0.01, 0.49)	0.10 (0.03, 0.36)	
NRI: 8.0%, *P*=0.06					

CI, confidence interval; NRI, net reclassification improvement.

Generally, point estimates of the observed risks agreed with the corresponding categories of predicted risk indicating good calibration, except for cells containing small numbers.

DISCUSSION

This population-based study shows that the addition of common cIMT measurement to the Framingham risk model does not significantly improve the risk prediction of hard CHD and stroke in older men and only modestly improves risk classification for these outcomes in older women. Most substantial reclassification was observed in women classified as intermediate risk based on traditional risk factors. Of the women classified as being at intermediate risk for hard CHD (7% of all women), cIMT measurement reclassified 35% of people to low and 8% to high-risk categories. The net reclassification improvement was 8.2% (P=0.03) for the total population of women. Of the women classified as being at intermediate risk for stroke (12% of all women), cIMT measurement reclassified 19% of people to low and 9% to high-risk categories. The net reclassification improvement for the total population of women was 8% (P=0.06).

CIMT and Future Cardiovascular Disease

An essential prerequisite for the use of cIMT in cardiovascular risk stratification is its ability to predict future cardiovascular events. A meta analysis by Lorenz et al. [20] comprising eight large, longitudinal population- based cohort studies showed that common cIMT is a strong predictor of MI and stroke in the total population. The overall age- and gender-adjusted estimates of relative risk per 1-standard deviation increase of cIMT reported in this meta-analysis were 1.26 (1.21–1.30) for MI and 1.18 (1.16–1.21) for stroke. In line with these results, the corresponding estimates we found for hard CHD and stroke risk in the total population were 1.26 (1.13–1.41) and 1.33 (1.18–1.50). We found that associations were stronger in women than in men. All associations were attenuated by additional adjustments for cardiovascular risk factors.

Additional Value of cIMT in Cardiovascular Risk Prediction

The Atherosclerosis Risk in Communities (ARIC) study [10] recently reported on the additional value of cIMT and plaque beyond traditional risk factors in the 10-year risk prediction of CHD. In contrast to our results, this study found a higher additional value of cIMT in men than in women. The addition of cIMT beyond traditional risk factors resulted in a significant increase in C-statistic from 0.742 to 0.750 in men and a non-significant increase from 0.759 to 0.762 in women. The NRI was 8.9 (3.4–15.1) for men and 6.1 (-2.3–9.4) for women. Unfortunately, reclassification percentages for addition of cIMT were not presented. The discrepancy in the results between the ARIC and our

study might be explained at least partly by the fact that their study population was younger (mean age 54 versus 65 years). Since IMT is thought to represent an early stage of atherosclerosis [21], it may be possible that the additional value of IMT diminishes with advancing stages of atherosclerosis, which would particularly affect older men. Another possible explanation for not finding an additional value of IMT in older men might be that people prone to the effects of atherosclerosis already died before study inclusion or developed a cardiovascular event that was one of the exclusion criteria of our study. Since men develop atherosclerosis at an earlier age than women, this selection would have greater impact on our male than on our female study population. Differences in results might also be related to the fact that the ARIC study used total CHD as an outcome, which included revascularizations and silent MI while we studied hard CHD only. Both studies calculated the mean cIMT of near and far wall and measured IMT in the distal common carotid artery (CCA). However, in extent, the ARIC study also took the IMT in the carotid artery bifurcation and the proximal internal carotid arteries into account. Despite the differences in results between the two studies, it is of note that in both the ARIC and our study the observed additional value of IMT seems to be modest. A recent publication by the Carotid Atherosclerosis Progression Study (CAPS) group reported that in 4,904 individuals free of CVD at baseline (mean age 50 years, 48% male), carotid intima media thickness was significantly and independently predictive for cardiovascular events. However, for the outcome of myocardial infarction, addition of common cIMT to a model using the Framingham risk factors led to a small increase in C-statistic from 0.732 to 0.741 and only 25 people (0.6%) were reclassified. The net reclassification improvement was 0.14% (*P*=NS). Analyses including other endpoint definitions (angina/MI/all-cause mortality), other carotid segments (carotid bifurcation/internal carotid artery), and the SCORE risk model for baseline prediction did not result in consistently better risk prediction with cIMT. The fact that the mean age and thereby the mean cIMT (0.72 mm) was relatively low might have played a role in the negative findings. Furthermore, the assessment of clinical events was based on health insurance data and questionnaires that could have led to misclassification of the outcome and underestimation of the additional effect of cIMT [11]. A systematic review of studies in people free from CVD reported that the addition of a cIMT measurement to established risk factors led to small and sometimes significant improvements in cardiovascular risk prediction by means of increases in the C-statistic [8].

The number of women upgraded in risk exceeded that of women downgraded in risk – 4.0% (n=89) versus 2.8% (n=61) for hard CHD and 2.0 % (n=43) versus 1.4% (n=31) for stroke (Tables 2 and 3). This suggests that the addition of cIMT measurement would lead to more women needing to receive medical therapy according to current prevention guidelines [1,3].

In conclusion, despite the well-established association between cIMT and risk of future CHD and stroke, accruing evidence indicates at best a modest benefit of additional cIMT in the prediction of cardiovascular disease.

Strengths and Limitations

In order to appreciate the results, the strengths and limitations of the present study need to be considered. Strengths of our study include a large population-based cohort, in whom standardized measurements of risk factors and common cIMT measurement are performed. Furthermore, the complete and long follow-up has generated a large number of events enabling us to use hard events as an outcome and separate the analyses by gender. However, some limitations also need to be addressed. Firstly, while previous research within the Rotterdam study [22] showed that application of the Framingham Risk Score led to systematic overestimation of CHD risk in men, we chose to fit a model based on Framingham risk factors to stratify our population in the well-known risk categories. A potential drawback is over-fitting of the model, which could lead to underestimation of the additional value of cIMT measurement. However, all C-statistics were corrected for over-fitting using the bootstrap method. Secondly, we used the mean of mean IMT values of near and far walls of both common carotid arteries in our analyses. However, when we used the mean of the maximal cIMT values of near and far walls, reclassification percentages and NRI values did not change substantially (data not shown). Thirdly, since usage of risk-modifying therapy, other than antihypertensive medication, is not accounted for in the Framingham risk model, the cardiovascular risk of people on this treatment might have been overestimated. However, at baseline few people used cholesterol-lowering medication or antithrombotic therapy (Table 1). Therefore, it is not likely that usage of these agents will have biased our results. Finally, our study was performed in older people. The predictive power of traditional cardiovascular risk factors decreases with age while increased cIMT can be seen as a cumulative measure of the effect of lifetime exposure to cardiovascular risk factors on the arterial vessel wall and may therefore improve risk stratification particularly at older age. This implies that our results should not automatically be generalized to a younger population.

CONCLUSIONS

Despite a well-established association between cIMT and risk of future CVD events, the additional predictive value of a cIMT measurement within clinical risk categories based on traditional risk factors seems to be limited. In older men, common cIMT measurement did not significantly improve risk stratification based on traditional risk factors. In older women, common cIMT showed a modest ability to reclassify people to a more accurate cardiovascular risk category.

REFERENCES

1. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. Eur Heart J. 2007;28:2375-2414.
2. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24:987-1003.
3. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285:2486-2497.
4. de Ruijter W, Westendorp RG, Assendelft WJ, den Elzen WPJ, de Craen AJM, le Cessie S, et al. Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. BMJ, 2009;338:a3083.
5. Michos ED, Nasir K, Braunstein JB, Budoff MJ, Post WS, Blumenthal RS. Framingham risk equation underestimates subclinical atherosclerosis risk in asymptomatic women. Atherosclerosis. 2006;184:201-206.
6. Greenland P, Bonow RO, Brundage BH, Eisenberg MJ, Grundy SM, Lauer MS, et al. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography. J Am Coll Cardiol. 2007;49:378-402.
7. Greenland P, Smith Jr SC, Grundy SM. Improving coronary heart disease risk assessment in asymptomatic people: role of traditional risk factors and noninvasive cardiovascular tests. Circulation. 2001;104:1863-1867.
8. Plantinga Y, Dogan S, Grobbee DE, Bots ML. Carotid intima-media thickness measurement in cardiovascular screening programmes. Eur J Cardiovasc Prev Rehabil. 2009;16:639-644.
9. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation. 2007;115:928-935.
10. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J Am Coll Cardiol. 2010;55:1600-1607.
11. Lorenz MW, Schaefer C, Steinmetz H, Sitzer M, et al. Is carotid intima media thickness useful for individual prediction of cardiovascular risk? Ten-year results from the Carotid Atherosclerosis Progression Study (CAPS). Eur Heart J. 2010;31:2041-2048.
12. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, et al. The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol. 2009;24:553-572.
13. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
14. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96:1432-1437.

15. del Sol AI, Moons KG, Hollander M, Hofman A, Koudstaal PJ, Grobbee DE, et al. Is carotid intimamedia thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. Stroke. 2001;32:1532-1538.
16. D'Agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: adjustment for antihypertensive medication. The Framingham Study. Stroke. 1994;25:40-43.
17. Harrell Jr FE. Regression modeling strategies with application to linear models, logistic regression, and survival analysis. New York: Springer- Verlag, 2001.
18. Efron BT. An introduction to bootstrap. London: Chapman & Hall, 1993.
19. Steyerberg EW, Pencina MJ. Reclassification calculations for persons with incomplete follow-up. Ann Intern Med. 2010;152:195-196.
20. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115:459-467.
21. Zureik M, Ducimetiere P, Touboul PJ, Courbon D, Bonithon-Kopp C, Berr C, et al. Common carotid intima-media thickness predicts occurrence of carotid atherosclerotic plaques: longitudinal results from the Aging Vascular Study (EVA) study. Arterioscler Thromb Vasc Biol. 2000;20:1622-1629.
22. Koller MT, Steyerberg EW, Wolbers M, Stijnen T, Bucher HC, Hunink MG, et al. Validity of the Framingham point scores in the elderly: results from the Rotterdam study. Am Heart J. 2007;154:87-93.

3.2 Novel Risk Markers in Coronary Heart Disease Risk Prediction

Manuscript based on this chapter

Kavousi M, Elias-Smale S, Rutten JWH, Leening MJG, Vliegenthart R, Verwoert GC, Krestin GP, Oudkerk M, de Maat MPM, Leebeek FWG, Mattace-Raso FUS, Lindemans J, Hofman A, Steyerberg EW, van der Lugt A, van den Meiracker AH, Witteman JCM. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study.
Ann Intern Med. 2012;156:438-444.

3.2.1 Evaluation of Newer Risk Markers for Coronary Heart Disease Risk Classification

SUMMARY

Background: Whether newer risk markers for coronary heart disease (CHD) improve CHD risk prediction remains unclear.

Methods: We assessed whether newer risk markers for CHD risk prediction and stratification improve Framingham risk score (FRS) predictions among 5,933 asymptomatic participants (69.1 ± 8.5 years) from the prospective population-based Rotterdam Study. The newer CHD risk markers included N-terminal fragment of prohormone B-type natriuretic peptide (NT-proBNP), von Willebrand factor antigen, fibrinogen, chronic kidney disease, leukocyte count, C-reactive protein, homocysteine, uric acid, coronary artery calcium [CAC] scores, carotid intima–media thickness, peripheral arterial disease, and pulse wave velocity.

Results: Adding CAC scores to the FRS improved the accuracy of risk predictions (c-statistic increase, 0.05 (0.02-0.06); net reclassification index, (NRI) 19.3% overall [39.3% in those at intermediate risk]). NT-proBNP also improved risk predictions but to a lesser extent (c-statistic increase, 0.02 (0.01 t-0.04); NRI, 7.6% overall [33.0% in those at intermediate risk]). Improvements in predictions with other newer markers were marginal.

Conclusion: Among 12 CHD risk markers, improvements in FRS predictions were most statistically and clinically significant with the addition of CAC scores. Further investigation is needed to assess whether risk refinements using CAC scores lead to a meaningful change in clinical outcome. Whether to use CAC score screening as a more routine test for risk prediction requires full consideration of the financial and clinical costs of performing versus not performing the test for both persons and health systems.

INTRODUCTION

Clinical decision making for detection, management, and prevention of coronary heart disease (CHD) relies on accurate risk assessment. The Framingham risk score (FRS) is the most commonly used CHD risk prediction instrument in clinical settings [1] and constitutes the basis for the Adult Treatment Panel III guidelines for cholesterol-lowering therapy [2]. Since validation of the FRS, many coronary risk factors, also called *risk markers,* have been identified. Efforts are ongoing to assess the increment in risk prediction accuracy, if any, that these newer risk markers contribute to the FRS and other standard risk-scoring systems [3-15].

Recent guidelines recommend that several measures be used for assessing the increment in risk prediction accuracy offered by newer risk markers [16], but few studies have implemented those recommendations and none has used them to compare multiple markers within the same cohort [5-8,10,11,13-15]. Use of only some of the recommended assessment methods may provide misleading impressions of the clinical utility of novel markers in CHD risk prediction [17,18].

Therefore, we sought to compare the change in the accuracy of risk predictions when newer risk markers representative of various pathophysiologic pathways, including several subclinical measures of atherosclerosis, were added to the established clinical risk predictors. To that end, we implemented the recent methods recommended for assessment of risk prediction models [19,20]. These assessments were performed in a community-dwelling population and in a subpopulation at intermediate risk for CHD, in whom an increase in accuracy of risk predictions may be most clinically relevant.

METHODS

Study Population

The study was embedded within the Rotterdam Study, a prospective population-based cohort of persons aged 55 years or older in the municipality of Rotterdam, the Netherlands. The rationale and design of the study have been described elsewhere [21]. The baseline examination was completed between 1990 and 1993 (Rotterdam Study-I). In 1999, the cohort was extended to include inhabitants who reached the age of 55 years after the baseline examination and persons aged 55 years or older who migrated into the research area (Rotterdam Study-II). Baseline participation for all RS cycles was 72% (14,926 of 20,744). The present study used data obtained from 6,498 participants at the third examination of the original cohort (Rotterdam Study-I) (1997-1999) and the first examination of the extended cohort (Rotterdam Study-II) (2000-2001). After excluding 565 participants with a history of CHD (defined as clinically manifest myocardial infarction, coronary artery bypass grafting, or percutaneous transluminal coronary angioplasty),

we had data from 5,933 asymptomatic participants. C-reactive protein (CRP) (n=3,029) and coronary artery calcium (CAC) score (n=3,678) measurements were available for a smaller group. The study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, the Netherlands, and all participants gave written informed consent.

Risk Factors

We studied traditional cardiovascular risk factors, such as age, sex, body mass index, systolic blood pressure, treatment of hypertension, total and high-density lipoprotein cholesterol levels, use of lipid-lowering medication, smoking, and diabetes mellitus.

We also studied newer risk factors, such as levels of N-terminal fragment of prohormone B-type natriuretic peptide (NT-proBNP), von Willebrand factor antigen levels, fibrinogen levels, chronic kidney disease (CKD) (estimated glomerular filtration rate <60 mL/min per 1.73 m^2), leukocyte count, CRP levels, homocysteine levels, uric acid levels, CAC scores, carotid intima–media thickness (cIMT), peripheral arterial disease, and pulse wave velocity.

Clinical Outcomes

We obtained information on study outcomes from general practitioners and from letters and discharge reports from medical specialists. Events were classified by study physicians. Incident CHD was defined as a definite nonfatal or fatal myocardial infarction or death due to CHD. Definite and possible fatal CHD were coded by using the definitions applied within the Cardiovascular Health Study [22] and Atherosclerosis Risk in Communities Study [23]. Only first CHD events were included in the analyses; 20 participants were lost to follow-up.

Statistical Analysis

We assessed the independent relationship of each marker to CHD incidence by using Cox proportional hazards models. We transformed markers with highly skewed distributions to the natural logarithmic scale, and for CAC score we used the natural logarithm of (CAC+1) to handle CAC scores of zero. To determine the functional form used for each marker, we examined restricted cubic spline transformations for continuous predictors [24] and used the likelihood ratio test to examine the linearity assumption. If appropriate, we chose the simplest form, usually a log-linear term. All models met the proportional hazards assumption as evaluated by the "cox.zph" function in R, version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). Multivariable-adjusted hazard ratios (HRs) were calculated for comparison of the highest versus the lowest quartile (as the reference) for each marker.

Because median follow-up in the cohort was 6.8 years and most CHD risk prediction instruments, including the FRS, predict 10-year CHD risk, we used a parametric Weibull

proportional hazards regression model to estimate 10-year CHD risk from data available over a shorter follow-up period for each person. We first fit a Weibull prediction model to our data on the basis of the variables used in the FRS (age, sex, systolic blood pressure, treatment of hypertension, total and high-density lipoprotein cholesterol levels, current smoking, and diabetes mellitus) [1]. We refer to this model as the "base model." We then developed 12 new models, each containing the FRS variables with the addition of 1 of the 12 newer risk markers (referred to as the "newer marker model"). The analyses including CAC score were additionally adjusted for the type of computed tomography scanner.

We compared the base model with the newer marker models by using the likelihood ratio chi-square test for global model fit; using the difference in the optimism corrected c-statistic between each newer model and the base model, calculated by 100 bootstrap repetitions [24,25]; and using the net reclassification improvement (NRI) with the newer marker as suggested by Steyerberg and Pencina for survival data [26]. The c-statistic is a measure of discrimination (the ability to distinguish between two persons, one with and one without a CHD event), and NRI specifies the amount of correct reclassification of estimated (not actual) events and nonevents to 10 years. The NRI estimates were based on the reclassification tables classifying participants in 10-year CHD risk categories of low (<10%), intermediate (10%-20%), and high (>20%). We then repeated all of the analyses for men and women separately.

Information on some markers and covariables was missing in up to 13% of participants. We performed multiple imputations of the missing values by using the Hmisc library of R (R-library: Hmisc, function: aregImpute). All analyses were performed with R, version 2.10.1. A 2-sided *P* value of less than 0.05 denoted statistical significance.

RESULTS

Mean participant age was 69.1 years (SD, 8.5), and 59.4% were women. Table 1 reports the baseline values for traditional and newer risk markers. Demographic characteristics or risk factor values did not differ between the overall population and the subpopulation with CRP levels and CAC scores (data not shown).

During a median follow-up of 6.8 years (25th, 75th percentiles: 5.8, 8.1 years), 347 first CHD events, including 190 nonfatal myocardial infarctions and 157 CHD deaths, occurred (Table 2).

Table 3 details HRs for incident CHD for traditional risk factors in the base model, as well as the performance measures for the base model (global model fit and c-statistic).

Figure 1 illustrates adjusted HRs for comparisons of the highest versus lowest quartiles for each newer marker. Newer risk markers that were significantly associated with incident CHD after adjustment for traditional risk factors were NT-proBNP levels (HR, 2.5

Table 1. Characteristics of the study population (N=5,933)

Variable	Value *
Age (years)	69.1 (8.5)
Women (%)	3525 (59.4%)
Systolic blood pressure (mmHg)	143.3 (21.3)
Diastolic blood pressure (mmHg)	77.0 (11.2)
Antihypertensive treatment (N, %)	1392 (23.5%)
Body mass index (kg/m^2)	27.0 (4.0)
Total cholesterol (mmol/L)	5.8 (1.0)
HDL cholesterol (mmol/L)	1.4 (0.4)
Triglyceride (mmol/L)	1.5 (0.8)
Lipid lowering medication (N, %)	605 (10.2%)
Glucose (mmol/L)	5.9 (1.5)
Diabetes mellitus (N, %)	767 (12.9%)
Current smoking (N, %)	1037 (17.5%)
Creatinine (umol/L)	76.0 [66.0, 87.0]
NT-proBNP (pmol/L)	9.5 [5.1, 18.1]
VWF (IU/mL)	1.2 [0.9, 1.6]
Fibrinogen:Ag (g/L)	3.8 [3.3, 4.4]
eGFR (mL/min/1.73 m^2)	76.1 [67.0, 86.9]
CKD (N, %)	725 (12.2%)
Leukocyte count (*10^^{9}/L)	6.8 (1.9)
CRP (mg/l)	2.3 [1.2, 4.4]
Homocysteine (umol/L)	13.5 [11.4, 16.6]
Uric acid (mmol/L)	0.3 [0.3, 0.4]
CAC	65.8 [4.4, 322.8]
IMT (mm)	1.0 [0.9, 1.1]
ABI	1.1 (0.2)
PAD (N, %)	830 (14.0%)
PWV (m/s)	12.6 [10.9, 14.8]

Abbreviations: HDL cholesterol, high density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; CRP, C-reactive protein; CAC, coronary artery calcium ; IMT, carotid intima media thickness; ABI, ankle brachial index; PAD, peripheral artery disease; PWV, pulse wave velocity; NA, not applicable.
* Values are mean (SD) or numbers (percentages). Median [25th, 75th percentiles] is presented in case of skewed distribution.

[95% CI, 1.7-3.6]), fibrinogen levels (HR, 1.4 [CI, 1.1-2.0]), CKD (HR, 1.4 [CI, 1.1-1.8]), leukocyte count (HR, 1.8 [CI, 1.3-2.5]), CRP levels (HR, 1.6 [CI, 1.0-2.5]), homocysteine levels (HR, 1.4 [CI, 1.0-2.0]), CAC scores (HR, 6.2 [CI, 3.4-11.5]), cIMT (HR, 1.6 [CI, 1.1-2.3]), and peripheral arterial disease (HR, 1.3 [CI, 1.0-1.7]).

Table 2. Incidence of coronary heart disease in the total population

Events	Total events, n *	Events per 1000 person-years, n *
Non-fatal MI	190	4.80
Fatal CHD	157	3.97
Definite fatal MI	30	0.76
Definite fatal CHD	88	2.22
Possible fatal CHD	39	0.99

Abbreviations: N, number; MI, myocardial infarction; CHD, coronary heart disease.
* Numbers, incident of coronary heart disease events; fatal and non-fatal, during a median follow-up time of 6.8 years in total population.

Table 3. Parameter estimates and performance measures of the "Framingham refitted" model

Parameter	Framingham refitted model	
	HR	95% CI
Age	1.08	1.06, 1.09
Male sex	1.95	1.55, 2.46
Systolic blood pressure	1.01	1.00, 1.01
Treatment for hypertension	1.21	0.95, 1.53
Total cholesterol	1.23	1.11, 1.37
HDL cholesterol	0.35	0.25, 0.49
Diabetes	1.35	1.03, 1.77
Current smoking	1.38	1.05, 1.82
Model performance measures		
Model likelihood ratio Chi-square statistic	230.49	
Model C-statistic *	0.73	0.71, 0.75

Abbreviations: HR, hazard ratio; CI, confidence interval; HDL cholesterol, high density lipoprotein cholesterol.
* The C-statistic is corrected for over optimism using 100 bootstrap repetitions.

Table 4 details measures of improvement in model fit, for the overall population and by sex, when each newer risk marker is added to the base model. In the overall population, statistically significant improvements were observed for NT-proBNP levels, fibrinogen levels, CKD, leukocyte count, CRP levels, homocysteine levels, CAC scores, cIMT, and peripheral arterial disease.

Table 5 summarizes the change in the c-statistic and the overall NRI when each newer risk marker is added to the base model. The maximum change in the c-statistic was observed for CAC score (0.05 [CI, 0.02-0.06]), followed by NT-proBNP level (0.02 [CI, 0.01-0.04]). The highest overall net percentage of persons correctly reclassified was also observed for CAC score (NRI, 19.3% [CI, 12.5%-26.2%]), with a smaller NRI for

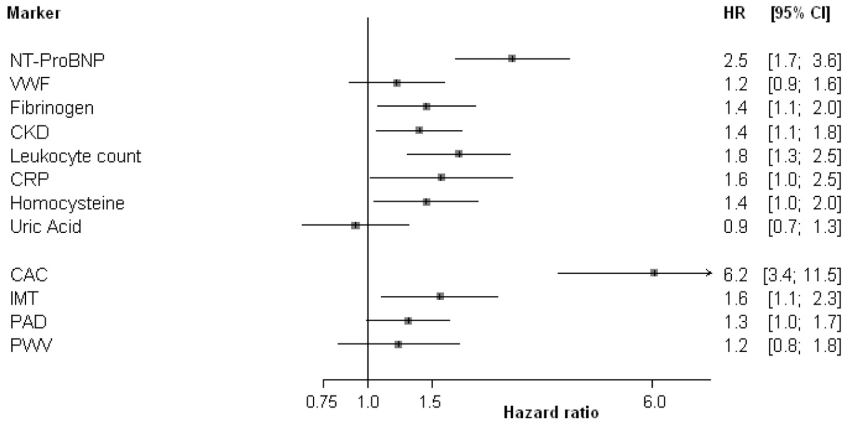


Figure 1. Multivariable adjusted hazard ratio for incident CHD

Abbreviations: CHD, coronary heart disease; HR, hazard ratio; CI, confidence interval; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; CKD, chronic kidney disease; CRP, C-reactive protein; CAC, coronary artery calcium; IMT, carotid intima media thickness; PAD, peripheral artery disease; PWV, pulse wave velocity.
The figure shows multivariable adjusted Hazard ratios (95% confidence interval) for incident coronary heart disease for the highest quartile of each marker (lowest quartile as reference).
* CKD and PAD are modeled as categorical variables

Table 4. Global model fit statistics for total population and by gender

Model	Likelihood ratio test statistics (X^2)		
	Total population	Men	Women
FRS +NT-proBNP	58.7 *	35.6 *	25.7 *
FRS + VWF	2.2	2.3	0.1
FRS + Fibrinogen	7.2 *	11.4 *	0.1
FRS +CKD	5.4 *	7.8 *	0.3
FRS + Leukocyte count	14.1 *	19.8 *	0.8
FRS + CRP †	4.8 *	NA	NA
FRS + Homocysteine	5.3 *	5.9 *	0.7
FRS + Uric acid	0.2	0.1	0.0
FRS + CAC	60.9 *	41.2 *	22.6 *
FRS + IMT	5.2 *	1.8	3.6
FRS + PAD	3.7 *	5.6 *	0.1
FRS + PWV	1.2	5.2 *	0.9

Abbreviations: X^2, Chi-square statistic; FRS, Framingham risk score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; CKD, chronic kidney disease; CRP, C-reactive protein; CAC, coronary artery calcium; IMT, carotid intima media thickness; PAD, peripheral artery disease; PWV, pulse wave velocity.
Likelihood ratio Chi-square indicates the increase in model fit after extending the Framingham model (including conventional risk factors) with each new marker.
* Significant improvement in model fit ($P< 0.05$), evaluated by likelihood ratio Chi-square.
† For CRP, the power was not enough to perform the gender-specific analyses.

Table 5. Discriminative ability of risk markers

Marker	Change in c-statistic (95% CI)	NRI (95% CI) * for Total Population
NT-proBNP	0.02 (0.01, 0.04)	7.6 (2.8, 12.5)
VWF	0.00 (0.00, 0.00)	0.4 (-1.7, 2.5)
Fibrinogen	0.00 (0.00, 0.01)	2.9 (-0.2, 6.0)
CKD	0.00 (0.00, 0.00)	2.7 (-0.2, 5.7)
Leukocyte count	0.01 (0.00, 0.02)	1.5 (-1.5, 4.6)
CRP †	0.00 (-0.01, 0.00)	2.0 (-2.3, 6.4)
Homocysteine	0.00 (0.00, 0.00)	-0.3 (-3.0, 2.3)
Uric acid	0.00 (0.00, 0.00)	0.8 (-0.5, 2.1)
CAC †	0.05 (0.02, 0.06)	19.3 (12.5, 26.2)
IMT	0.00 (0.00, 0.00)	1.6 (-1.1, 4.4)
PAD	0.00 (0.00, 0.00)	0.6 (-1.8, 2.9)
PWV	0.00 (0.00, 0.00)	0.00 (-2.1, 2.1)

Abbreviations: NRI, net reclassification improvement; CI, confidence interval; FRS, Framingham risk score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; CKD, chronic kidney disease; CRP, C-reactive protein; CAC, coronary artery calcium; IMT, carotid intima media thickness; PAD, peripheral artery disease; PWV, pulse wave velocity.
* Percentage of net reclassification improvement (95% CI) for the extended model versus the "Framingham refitted" model, using risk categories of <10%, 10%-20%, >20%. It is estimated as ([number of events reclassified higher - number of events reclassified lower] / number of events) + ([number of non-events reclassified lower - number of non-events reclassified higher] / number of non-events).
† The analyses for CRP (n=3,029) and CAC score (n=3,678) were performed in a smaller group.

NT-proBNP (7.6% [CI, 2.8%-12.5%]). Changes in c-statistics and overall NRIs in total population were otherwise negligible or absent for every newer marker.

Table 6 summarizes the overall NRI when newer risk markers are added to the base model for the persons categorized at intermediate risk for a CHD event on the basis of the base model. In this subpopulation, NRI was highest for CAC score (39.3% [CI, 26.8%-51.7%]), followed by NT-proBNP level (33.0% [CI, 23.4%-2.6%]). All other markers resulted in NRIs of 10% or less in this subpopulation.

Table 7 details the intermediate NRI per gender; for men and women categorized at intermediate risk for a CHD event on the basis of the base model. With some exceptions, NRIs tended to be higher for men than for women, corresponding to the stronger associations (HRs) between the newer risk markers and CHD events in men.

Table 6. Coronary heart disease risk reclassification for the Framingham intermediate risk category after extending the model with each marker

Extended Models	Events (%)		Non-events (%)		NRI (95% CI) *
	Up	Down	Up	Down	
FRS +NT-proBNP	29.0	16.4	9.8	30.2	33.0 (23.4, 42.6)
FRS + VWF	4.4	3.9	2.0	5.5	4.0 (-0.2, 8.1)
FRS + Fibrinogen	10.6	4.8	5.9	10.3	10.2 (4.5, 15.9)
FRS + CKD	10.0	3.2	5.4	8.4	9.8 (4.4, 15.1)
FRS + Leukocyte count	11.5	6.4	6.0	10.2	9.3 (3.2, 15.4)
FRS + CRP †	12.3	10.4	4.9	12.2	9.2 (0.2, 18.0)
FRS + Homocysteine	7.6	7.8	3.7	8.6	4.7 (-0.9, 10.3)
FRS + Uric acid	0.8	0.0	0.5	2.3	2.6 (1.0, 4.2)
FRS + CAC †	37.0	13.0	18.7	34.0	39.3 (26.8, 51.7)
FRS + IMT	6.0	4.2	4.3	7.1	4.6 (-0.05, 9.3)
FRS + PAD	6.8	2.1	4.2	6.8	7.3 (2.9, 11.7)
FRS + PWV	4.4	2.8	2.1	3.7	3.2 (-0.6, 7.1)

Abbreviations: NRI, net reclassification improvement; CI, confidence interval; FRS, Framingham risk score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; CKD, chronic kidney disease; CRP, C-reactive protein; CAC, coronary artery calcium; IMT, carotid intima media thickness; PAD, peripheral artery disease; PWV, pulse wave velocity.
* NRI is estimated as ([number of events reclassified higher - number of events reclassified lower] / number of events) + ([number of non-events reclassified lower - number of non-events reclassified higher] / number of non-events).
† The analyses for CRP (n=3,029) and CAC score (n=3,678) were performed in a smaller group.

Table 7. Coronary heart disease risk reclassification for the Framingham intermediate risk category, per gender

Extended Models	Intermediate NRI (95% CI) *	
	Men	Women
FRS + NT-proBNP	36.3 (23.9, 48.8)	27.8 (12.8, 42.8)
FRS + VWF	6.8 (0.1, 13.4)	2.3 (-3.1, 7.8)
FRS + Fibrinogen	21.8 (12.3, 31.2)	1.4 (-2.6, 5.5)
FRS + CKD	10.1 (1.8, 18.4)	1.2 (-3.2, 5.6)
FRS + Leukocyte count	18.3 (7.9, 28.7)	1.6 (-3.7, 6.8)
FRS + Homocysteine	13.6 (5.5, 21.8)	-1.8 (-7.0, 3.4)
FRS + Uric acid	2.5 (0.1, 4.9)	-0.6 (-3.3, 2.1)
FRS + CAC	50.9 (33.7, 68.1)	25.2 (6.4, 44.0)
FRS + IMT	7.7 (1.4, 14.0)	13.7 (4.6, 22.9)
FRS + PAD	6.5 (-1.1, 14.1)	-1.2 (-4.2, 1.7)
FRS + PWV	13.2 (6.2, 20.2)	3.8 (-0.8, 8.3)

NRI, net reclassification improvement; CI, confidence interval; FRS, Framingham risk score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VWF, von Willebrand factor; CKD, chronic kidney disease; CAC, coronary artery calcium; IMT, carotid intima media thickness; PAD, peripheral artery disease; PWV, pulse wave velocity.
* Percentage of net reclassification improvement (95% CI) for the men/women at the intermediate risk category after extension of the "Framingham refitted" model with each marker.
For CRP, the power was not enough to perform the gender-specific analyses.

DISCUSSION

Among 12 newer CHD risk markers, CAC score provided the best improvement in CHD risk prediction and stratification above the FRS, as measured by an increase in the c-statistic and clinically significant reclassification in the overall population and in persons categorized as intermediate risk by traditional cardiovascular risk factors. The finding is consistent with those of previous studies [3,4,27].

Improvements in CHD risk prediction with other newer risk markers, including cIMT, ankle–brachial index, and pulse wave velocity, which have been shown to be strong predictors of CHD in other studies [6,9,28-30], were modest. The better performance of CAC score compared with other vascular measures of atherosclerosis probably reflects the disparity in contribution of various vascular beds in the disease process. However, because of variations across studies in the number of risk categories and thresholds and in clinical outcomes of interest, it is difficult to make direct comparisons of our findings with those of other population studies.

A relatively new risk marker, NT-proBNP, has been shown to be a strong predictor of coronary events and death [11,13,31]. Among serum biomarkers, NT-proBNP was most associated with CHD events and led to the greatest NRI both overall and among participants categorized as being at intermediate risk by traditional cardiovascular risk factors. Because elevation of various biomarker levels correlates with the various phases of the atherogenesis cascade [32], we speculate that the greater CHD risk prediction and reclassification with NT-proBNP compared with other serum biomarkers may correspond to its position in the later stages of the disease process. Specifically, an increase in BNP levels is viewed by some as a response to age-related, subclinical alterations in cardiac structure or function [33], so this biomarker may be more useful for CHD risk prediction at older ages. However, the 95% CIs for NRI with NT-proBNP overlap with those surrounding NRIs from other markers, making it difficult to conclude that NT-proBNP is superior to other markers. Nevertheless, our results suggest a potential role of NT-proBNP for inclusion in CHD risk prediction instruments.

Although other biomarkers, such as fibrinogen levels, CKD, leukocyte count, CRP levels, and homocysteine levels, were independently associated with the risk for later coronary events, their incremental value beyond traditional risk factors was marginal. Addition of these biomarkers to the base model yielded NRIs in the intermediate-risk group of 10% or less, with lower confidence bounds below reclassification thresholds that would probably be considered clinically useful.

Strengths of the current study include its large sample size, comparison of multiple markers that were all measured with standardized methods, use of hard end points to avoid misclassification bias, and implementation of various statistical measures for assessment of risk-scoring models.

There are also limitations. Our cohort comprised white participants aged 55 years or older; therefore, the generalizability of our findings to younger and nonwhite populations remains uncertain. Also, due to the difference in the age range of our population compared with the Framingham population, we refit a model on the basis of the Framingham variables in our own population instead of using the original FRS algorithm. This implies that our results may differ from the settings that directly apply published versions of the FRS or its modifications. We fit the Weibull model to estimate 10-year predicted risk for CHD from our actual median follow-up of 6.8 years. That extrapolation cannot be validated. Moreover, CAC score measurements in our study were performed with two types of scanners (electron beam and multidetector computed tomography). However, analyses using CAC score were adjusted for the type of scanner, and our results are similar to our previous findings using the data from only our electron beam computed tomography cohort [27]. Measurements of CRP levels and CAC score were available only in a subpopulation; however, general characteristics of that subpopulation did not materially differ from those of the larger population. Finally, we performed many statistical analyses to compare risk markers, and some of our statistically significant findings may have occurred by chance. However, our results show a consistent pattern for CAC score across statistical methods.

In summary, in this large population-based study, improvements in CHD risk prediction and reclassification with CAC score were statistically and clinically significant. Increments in accuracy with other newer risk markers were less significant. Further investigation is still needed to assess whether risk refinements using CAC score lead to a meaningful change in clinical outcome. Moreover, whether to include any newer test in a risk prediction algorithm requires full consideration of the financial and clinical costs of performing versus not performing the new test for both people and health systems. For CAC score in particular, exposing potentially healthy populations to radiation in a screening program requires careful considerations of the balance of risks and benefits.

REFERENCES

1. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837-1847.
2. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143-3421.
3. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, et al. Coronary artery calcium score and risk classification for coronary heart disease prediction. JAMA. 2010;303:1610-1616.
4. Erbel R, Mo¨hlenkamp S, Moebus S, Schmermund A, Lehmann N, Stang A, et al; Heinz Nixdorf Recall Study Investigative Group. Coronary risk stratification, discrimination, and reclassification improvement based on quantification of subclinical coronary atherosclerosis: the Heinz Nixdorf Recall study. J Am Coll Cardiol. 2010;56:1397-1406.
5. Folsom AR, Chambless LE, Ballantyne CM, Coresh J, Heiss G, Wu KK, et al. An assessment of incremental coronary risk prediction using C-reactive protein and other novel risk markers: the atherosclerosis risk in communities study. Arch Intern Med. 2006;166:1368-1373.
6. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, et al. Coronary artery calcification compared with carotid intima- media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Arch Intern Med. 2008;168:1333-1339.
7. Kim HC, Greenland P, Rossouw JE, Manson JE, Cochrane BB, Lasser NL, et al. Multimarker prediction of coronary heart disease risk: the Women's Health Initiative. J Am Coll Cardiol. 2010;55:2080-2091.
8. Melander O, Newton-Cheh C, Almgren P, Hedblad B, Berglund G, Engstrom G, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA. 2009;302:49- 57.
9. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J Am Coll Cardiol. 2010;55:1600-1607.
10. Rana JS, Cote M, Despre´s JP, Sandhu MS, Talmud PJ, Ninio E, et al. Inflammatory biomarkers and the prediction of coronary events among people at intermediate risk: the EPIC-Norfolk prospective population study. Heart. 2009;95:1682-1687.
11. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355:2631-2639.
12. Wilson PW, Pencina M, Jacques P, Selhub J, D'Agostino R Sr, O'Donnell CJ. C-reactive protein and reclassification of cardiovascular risk in the Framingham Heart Study. Circ Cardiovasc Qual Outcomes. 2008;1:92- 97.
13. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. Circulation. 2007;115:2119-2127.

14. Woodward M, Rumley A, Welsh P, MacMahon S, Lowe G. A comparison of the associations between seven hemostatic or inflammatory variables and coronary heart disease. J Thromb Haemost. 2007;5:1795-1800.
15. Zethelius B, Berglund L, Sundstro¨m J, Ingelsson E, Basu S, Larsson A, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med. 2008;358:2107-2116.
16. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119:2408-2416.
17. Tzoulaki I, Liberopoulos G, Ioannidis JP. Assessment of claims of improved prediction beyond the Framingham risk score. JAMA. 2009;302:2345-2352.
18. Helfand M, Buckley DI, Freeman M, Fu R, Rogers K, Fleming C, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med. 2009;151:496-507.
19. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology. 2010;21:128-138.
20. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med. 2011;30:11-21.
21. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011;26:657-686.
22. Pearte CA, Furberg CD, O'Meara ES, Psaty BM, Kuller L, Powe NR, et al. Characteristics and baseline clinical predictors of future fatal versus nonfatal coronary heart disease events in older adults: the Cardiovascular Health Study. Circulation 2006;113:2177-2185.
23. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. J Clin Epidemiol. 1996;49:223-233.
24. Harrell FE Jr. Regression Modeling Strategies: With Application to Linear Models, Logistic Regression, and Survival Analysis. New York: Springer Publishing; 2001.
25. Steyerberg EW. Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating. New York: Springer Publishing; 2009.
26. Steyerberg EW, Pencina MJ. Reclassification calculations for persons with incomplete follow-up. Ann Intern Med. 2010;152:195-196.
27. Elias-Smale SE, Proenc¸a RV, Koller MT, Kavousi M, van Rooij FJ, Hunink MG, et al. Coronary calcium score improves classification of coronary heart disease risk in the elderly: the Rotterdam study. J Am Coll Cardiol. 2010;56:1407-1414.
28. Fowkes FG, Murray GD, Butcher I, Heald CL, Lee RJ, Chambless LE, et al; Ankle Brachial Index Collaboration. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta analysis. JAMA. 2008;300:197-208.
29. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113:657- 663.

30. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. 2010;121:505-511.
31. Rutten JH, Mattace-Raso FU, Steyerberg EW, Lindemans J, Hofman A, Wieberdink RG, et al. Amino-terminal pro-B-type natriuretic peptide improves cardiovascular and cerebrovascular risk prediction in the population: the Rotterdam study. Hypertension. 2010;55:785-791.
32. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113:2335-2362.
33. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol. 2002;40:976-982.

Manuscript based on this chapter
Hlatky MA. Editorial: Framework for evaluating novel risk markers.
Ann Intern Med. 2012;156:468-469. (Reproduced with permission)

3.2.2 Framework for Evaluating Novel Risk Markers

Coronary heart disease (CHD) is characterized by the sudden onset of often catastrophic symptoms after decades of silent and insidious development. Identifying persons at risk for clinically evident CHD has become an important strategy for prevention, because treatment can reduce risk. The Framingham Heart Study, which began more than 60 years ago, pioneered the identification of clinical factors that were associated with future coronary events [1]. Data on these classic risk factors; age, sex, blood pressure, cholesterol levels, diabetes, and smoking, can be combined using the Framingham risk score (FRS) to estimate a person's chance of developing CHD over the next 10 years [2]. The FRS has been extensively validated in many cohorts and countries [3], and it is an accepted tool in preventive medicine.

Although the FRS is simple and well-established, there is room for improvement. Most individuals with high scores do not develop disease in the next 10 years, and some with low scores nevertheless have heart attacks. Technological innovation and clinical research have provided new tools and insights that may increase the accuracy and discrimination of cardiovascular risk prediction. Many putative risk markers have been developed, and although these markers are often associated with claims of "new" and "improved" risk prediction [4], assessing their value has been difficult and controversial [5,6].

A FRAMEWORK FOR EVALUATION

There is a well-recognized framework for drug development, from basic science to animal models to studies in humans performed with increasing rigor and patient numbers (phases 1, 2, 3, and 4), with the ultimate goal of demonstrating improved clinical outcomes at reasonable cost. Evaluation of diagnostic tests and risk markers has lagged behind that of drugs, in no small part because the regulatory requirements to market new tests are not as stringent as those for new drugs. The early phases of development of a novel risk marker (Table 1) are initial proof of principle, studies of predictive value in prospective studies, and comparison with established risk markers [6]. A key hurdle is

Table 1. Phases of evaluation of a novel risk marker

Proof of concept: Do novel marker levels differ between participants with and without outcome?
Prospective validation: Does the novel marker predict development of future outcomes in a prospective cohort or nested case–cohort/case–cohort study?
Incremental value: Does the novel marker add predictive information to established, standard risk markers?
Clinical utility: Does the novel risk marker change predicted risk sufficiently to change recommended therapy?
Clinical outcomes: Does the novel risk marker improve clinical outcomes, especially when tested in a randomized clinical trial?
Cost-effectiveness: Does use of the marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment?

to show that a new risk marker improves predictions over those made using standard clinical risk assessment. The proper statistical measure of improvement has been controversial, with the main measures being the adjusted hazard ratio (or risk ratio or odds ratio); the c-index [7] (equal to the area under the receiver-operating characteristic curve); or new reclassification measures (the net reclassification improvement [NRI] index and the integrated discrimination improvement index) [8,9].

The c-index has been used as a metric of diagnostic test capability for more than 2 decades [7] and basically assesses the discriminative power of a test (that is, the extent to which the probabilities assigned to patients who develop an event are higher than those assigned to patients who do not develop an event). Events that are inherently more unpredictable lead to a lower c-index than those that are more predictable. Although the c-index is a good measure of discrimination, it does not capture all of the aspects of a risk predictor [6].

One important aspect of a risk marker is that it should be useful. An old truism about diagnostic tests was that if you would do the same thing regardless of the test result, then you should not order the test in the first place. Applying this idea to a novel risk marker, if the test result could change the pretest levels of risk "enough" then the test could change clinical management. The key, of course, is to have agreed-upon standards for the risk levels that warrant changing therapy. In the case of CHD, current clinical guidelines suggest that persons with a 10-year risk of 20% or greater should be treated with drug therapy, such as statins, and those with a 10-year risk less than 10% are advised to follow a healthy lifestyle. Persons at intermediate risk (10% to 20%) should have individualized assessment. Test results that move a person across a treatment threshold suggest that the test could change clinical management. In particular, test results that move up the predicted risk for patients who develop events and move down the predicted

risk for patients who do not (that is, move patients correctly across treatment thresholds) suggest that the test may be useful and have clinical value.

ROTTERDAM STUDY RESULTS

In this issue, the investigators of the Rotterdam Study report a study [10] that applied the framework for risk marker evaluation to assess 12 putative novel risk markers for CHD, including several that have generated considerable interest: N-terminal fragment of prohormone B-type natriuretic peptide (NT-proBNP) levels, C-reactive protein levels, carotid intima–media thickness, and coronary artery calcium (CAC) scores. The study design was strong because it compared all of these markers in a single group of patients and reported several measures of marker value, including adjusted hazard ratios, the c-index, and the NRI index. Regardless of the measure used, the CAC score added the most to the FRS, with NT-proBNP levels a close second. Both of these measures outdistanced C-reactive protein and carotid intima–media thickness, risk markers that have been endorsed for assessing cardiovascular risk in particular patient groups [11]. Perhaps more important than the rank-ordering of these markers was the observation that NT-proBNP levels and CAC scores were the only markers of the 12 tested for which there was strong evidence of improvements in c-index and NRI index [10].

The results of these head-to-head comparisons among novel cardiac risk markers were interesting and informative, but they fall short of being definitive. First, the results should be replicated in other large cohorts, as the details of how the tests were done and the composition of the population may have affected the findings. In particular, nearly every measure had a higher NRI index among men than among women, suggesting that test performance and its clinical value may depend on the characteristics of the population tested. The Rotterdam Study group had a mean age of 69.1 years, 59% were women, and presumably almost all participants had European ancestry.

Most important, it is not enough to show that a novel risk marker provides incremental statistical information (Table 1). The next phase in evaluation is to show that use of the novel risk marker has real clinical utility, by changing clinical management for the better and improving patient outcomes [6,12]. The best way to do this is by randomly assigning participants to marker-guided evaluation or usual care to compare long-term outcomes and cost-effectiveness. A randomized trial of CAC screening has been proposed and would provide the best evidence for CAC measurement. This is an important issue because of possible unintended consequences of screening from radiation exposure and detection of incidental findings on computed tomography. To my knowledge, no such study of NT-proBNP testing is being conducted, but the emerging evidence suggests that this marker is also worth further evaluation.

Risk markers are increasingly important in management of patients with various diseases. Before these markers are adopted into practice, we need strong evidence that they add meaningfully to simpler, standard clinical markers of risk. Statistically significant evidence of independence is necessary, and requiring evidence that a marker reclassifies risk is also becoming common. The ultimate standard for evaluation (Table 1) is a demonstration that a new risk marker improves clinical outcomes at an affordable cost compared with current practice.

REFERENCES

1. Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. Am J Cardiol. 1976;38:46-51.
2. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117:743-753.
3. D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P; CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA. 2001;286:180-187.
4. Cooney MT, Dudina AL, Graham IM. Value and limitations of existing scores for the assessment of cardiovascular risk: a review for clinicians. J Am Coll Cardiol. 2009;54:1209-1227.
5. Tzoulaki I, Liberopoulos G, Ioannidis JP. Assessment of claims of improved prediction beyond the Framingham risk score. JAMA. 2009;302:2345-2352.
6. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119:2408-2416.
7. Harrell FE Jr, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA. 1982;247:2543-2546.
8. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27:157-172.
9. Janes H, Pepe MS, Gu W. Assessing the value of risk predictions by using risk stratification tables. Ann Intern Med. 2008;149:751-760.
10. Kavousi M, Elias-Smale S, Rutten JH, Leening MJ, Vliegenthart R, Verwoert GC, et al. Evaluation of newer risk markers for coronary heart disease risk classification. A cohort study. Ann Intern Med. 2012;156:438-444.
11. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, et al; American College of Cardiology Foundation. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2010;56:e50-103.
12. Pletcher MJ, Pignone M. Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. Circulation. 2011;123:1116-1124.

Manuscript based on this chapter
Kavousi M, Leening MJG, Witteman JCM. Letter to the Editor: Markers for prediction of cardiovascular disease risk.
JAMA. 2012;308:2561.

3.4 Markers for Prediction of Cardiovascular Disease Risk

The article by Dr. Yeboah and colleagues compared the ability of several risk markers to improve prediction of coronary heart disease (CHD) and cardiovascular disease (CVD) among individuals at intermediate risk [1]. The authors reported that coronary artery calcium (CAC) provided superior reclassification compared with other risk markers and recommended CAC as a tool for refining cardiovascular risk prediction in individuals at intermediate risk. While the added predictive ability of CAC in CHD risk prediction in the current study was substantial and confirmed previous findings [2], we believe that supporting CAC as a candidate for CVD screening based on the results is less grounded.

While it is straightforward to define an intermediate-risk group for CHD, this is not the case for CVD because accepted thresholds are lacking. In the study by Yeboah et al, the authors included persons at intermediate CHD risk and therefore the results might not necessarily apply to persons at intermediate CVD risk [3].

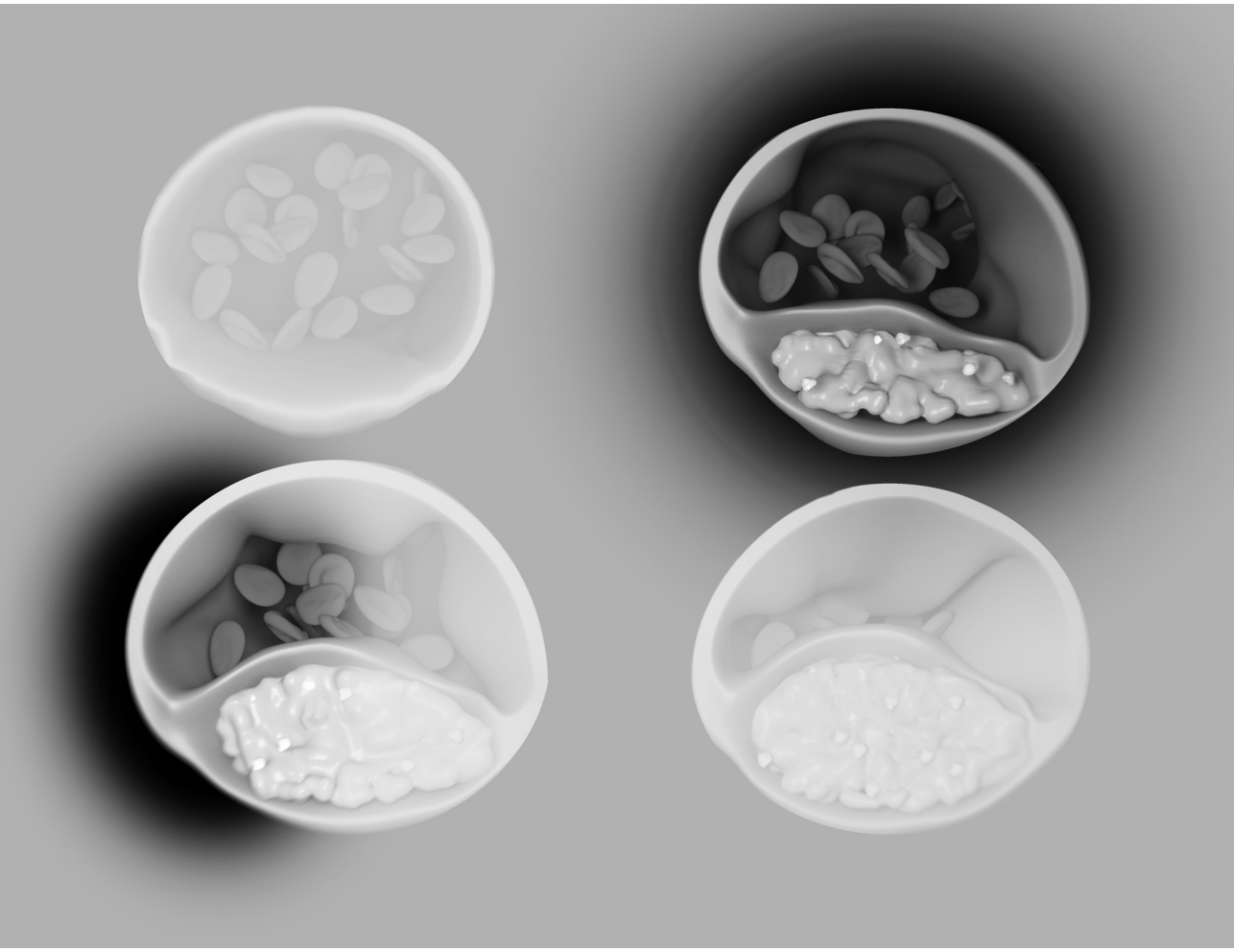
While CAC has shown to accurately predict CHD in different populations, CAC has not been proven to be a useful predictor for stroke [4]. In the study by Yeboah et al [1], addition of CAC to the Framingham risk score provided overall net reclassification improvement of 0.66 for CHD and of 0.47 for CVD risk prediction. The percent net correct reclassification in the group without events hardly changed after adding a non-CAC related outcome such as stroke (40.4% for CHD and 36.0% for CVD). However, the percent net correct reclassification for those with events, which reflects the ability to identify persons that will benefit from intensive treatment, dropped from 25.5% for CHD to 10.6% for CVD.

The percent net correct reclassification for persons with CHD events of 25.5% in the current study and 24.0% previously reported [2] imply that adding CAC to risk prediction models moves a substantial proportion of persons initially at intermediate risk to the high-risk group, where they qualify for more aggressive treatment. This supports the incorporation of CAC in CHD risk assessment. However, whether 10.6% net correct reclassification of persons with CVD events provided by CAC is sufficiently large to warrant recommending CAC as a screening tool for CVD is doubtful.

The general trend in developing new guidelines on cardiovascular risk prevention is moving towards focusing on broader CVD risk rather than on CHD risk only [5]. However, before considering new markers for CVD risk prediction, all components of this broad outcome should be considered and limitations for stroke risk prediction recognized.

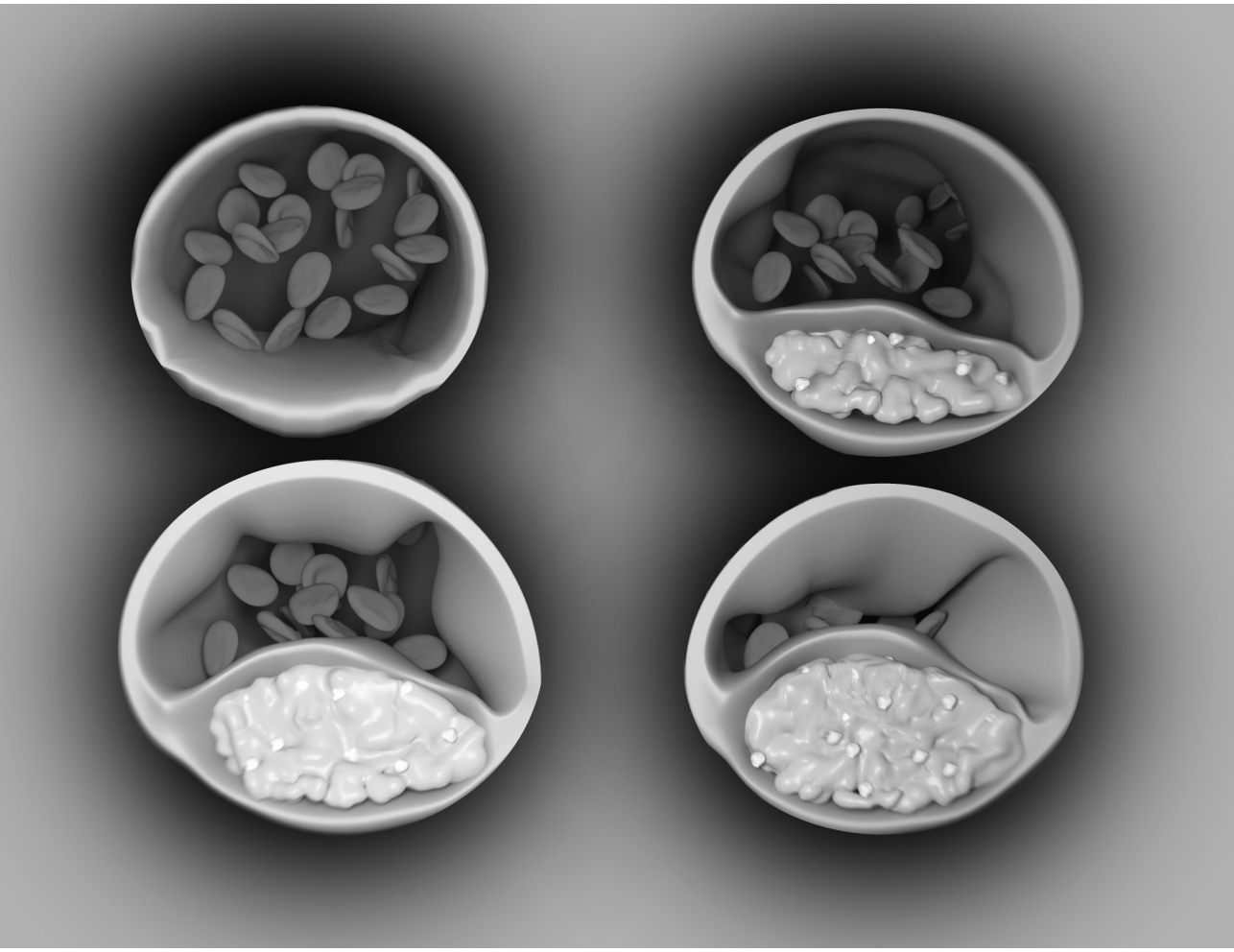
REFERENCES

1. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012;308:788-795.
2. Kavousi M, Elias-Smale S, Rutten JH, Leening MJ, Vliegenthart R, Verwoert GC, et al. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study. Ann Intern Med. 2012;156:438-444.
3. Berger JS, Jordan CO, Lloyd-Jones D, Blumenthal RS. Screening for cardiovascular risk in asymptomatic patients. J Am Coll Cardiol. 2010;55:1169-1177.
4. Elias-Smale SE, Wieberdink RG, Odink AE, Hofman A, Hunink MG, Koudstaal PJ, et al. Burden of atherosclerosis improves the prediction of coronary heart disease but not cerebrovascular events: the Rotterdam Study. Eur Heart J. 2011;32:2050-2058.
5. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L., et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation. 2010;121:586-613.



CHAPTER 4

Cardiovascular Risk Prediction in Women



CHAPTER 5

General Discussion

The concept of cardiovascular disease prevention is paired to the recognition that atherosclerosis is a continuous process. Consequences of cardiovascular disease, once clinically evident, are immense for both patients and health care systems. In this regard, non-invasive measures for subclinical atherosclerosis are of particular interest to target medical prevention to those who need it, and thus limit under- or over-treatment of the disease.

In this thesis, the aim was to expand the knowledge on three measures of subclinical atherosclerosis burden; coronary artery calcification, carotid intima-media thickness, and ankle-brachial index. The first objective was to unravel the genetic determinants of these three measures using the powerful approach of genome-wide association (GWA) studies in the framework of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium [1]. The next step was aimed at studying the ability of these three subclinical measures in cardiovascular risk prediction and comparing their predictive performances with those of several cardiovascular (bio)markers in prediction of different cardiovascular outcomes. The final objective was to study the recently modified area of cardiovascular risk prediction in women. The cardiovascular prediction studies were conducted within the framework of the Rotterdam Study, a population-based cohort study among subjects aged 55 years and over [2].

In this chapter, the main findings of this thesis are reviewed, methodological considerations are addressed, and potential clinical implications of the findings together with directions for future research are discussed.

MAIN FINDINGS

Genetics of Subclinical Measures of Atherosclerosis

The second chapter of this thesis reports the results of the meta-analyses of GWA studies on three subclinical measures of atherosclerosis burden; coronary artery calcification

(**chapter 2.1**), carotid intima-media thickness (**chapter 2.2**), and ankle-brachial index (**chapter 2.3**).

We identified several novel loci for coronary artery calcification in a GWA meta-analysis of 9,961 subjects from 5 independent community-based cohorts with replication in 3 additional independent cohorts. We showed that multiple genetic loci are associated with development of both underlying coronary atherosclerosis and clinical events. Genome-wide significant associations with coronary calcification for single-nucleotide polymorphisms (SNPs) on 9p21 (near *CDKN2A* and *CDKN2B*), and on 6p24 (within the *PHACTR1* gene), replicated for coronary artery calcification and for association with myocardial infarction. We additionally found evidence for concordance of SNP associations with both coronary calcification and myocardial infarction at a number of other loci, including 3q22 (*MRAS* gene), 13q34 (*COL4A1/COL4A2* genes), and 1p13 (*SORT1* gene). The strength and consistency of SNP associations with coronary calcification and with myocardial infarction suggest that the common mechanism of some genetic loci underlying myocardial infarction is development of early, underlying coronary atherosclerosis.

Our meta-analysis of GWA data from 9 community-based studies, in over 40,000 participants of European ancestry, revealed 3 new loci for common carotid intima-media thickness. We found genome-wide significant associations for SNPs on chromosome 8q24 (near *ZHX2* gene), on 19q13 (2.3 kb from *APOC1*, a region that also includes *APOE, APOC2,* and *APOC4*), and on 8q23.1 (within the *PINX1* gene). We also identified a suggestive locus on 6p22 (near *SLC17A4* gene). In the meta-analysis of GWA data from 7 studies of carotid plaque, we identified genome-wide significant associations between 2 regions and the presence of carotid plaque. The most significant signals were observed for 7q22 (near *PIK3CG* gene), and 4q31 (near *EDNRA* gene). Furthermore, two loci showed suggestive evidence for association with carotid plaque; 3p13 locus, and 19p13 (near *LDLR* gene). The identified loci in GWA meta-analyses of carotid intima-media thickness or carotid plaque implicate LDL metabolism (*APOC1*), endothelial dysfunction (*EDNRA*), platelet biology (*PIK3CG*), and telomere maintenance (*PINX1*). Two of our identified loci were also associated with coronary artery disease in the recent large meta-analysis by the CARDIoGRAM Consortium [3]; a locus near *LDLR* and a locus near *EDNRA*. Two other loci also showed suggestive associations with coronary artery disease; a locus near *APOC1* and a locus near *LRIC1*. Conversely, none of SNPs reported to be associated with coronary artery disease in the CARDIoGRAM consortium showed significant associations in our discovery meta-analyses of carotid artery traits.

The GWA meta-analysis for ankle-brachial index was conducted in more than 40,000 subjects of European ancestry from 21 population-based cohorts. We sought replication of the 6 strongest SNP associations in 5 population-based studies and 3 clinical samples. We identified and replicated one genome-wide significant association on 9p21 region (near *CDKN2B*). In our discovery sample, over 3,000 subjects had peripheral arterial

disease (defined as ankle-brachial index below 0.9). However, none of the SNP associations for peripheral arterial disease was genome-wide significant. Although we observed minimal overlap in the top SNP associations for the two traits, the directions of effect were consistent across the two phenotypes for the most significant ankle-brachial index SNPs (lower ankle-brachial index, increased odds of peripheral arterial disease). Moreover, two previously reported candidate genes for peripheral arterial disease; *DAB21P, CYBA*, and one SNP associated with coronary artery disease (*LDLR* gene) were associated with ankle-brachial index in our GWAs.

Subclinical Measures of Atherosclerosis and Cardiovascular Risk Prediction

The third chapter of this thesis focuses on the ability of subclinical measures of atherosclerosis in prediction of different cardiovascular outcomes. We compared the predictive ability of these measures with that of several biomarkers and markers of cardiac function.

In **chapter 3.1**, we examined the predictive performance of carotid intima-media thickness in prediction of coronary heart disease and stroke. We showed that the additional predictive value of common carotid intima-media thickness above the traditional risk factors is small. In men, common carotid intima-media thickness did not significantly improve risk stratification based on traditional risk factors. In women, common carotid intima-media thickness showed a modest ability to reclassify people to a more accurate cardiovascular risk category.

We then compared the added values of the three subclinical measures of atherosclerosis in coronary heart disease risk prediction with those of some emerging biomarkers in **chapter 3.2.** Among 12 risk markers understudy, we showed that improvements in coronary heart disease risk prediction, above the traditional risk factors, were most statistically and clinically significant with the addition of coronary artery calcium scores. Among the biomarkers, NT-proBNP was most associated with coronary heart disease events and led to the greatest improvements in risk prediction.

In **chapter 3.3**, we addressed the differential ability of the three subclinical measures in prediction of various components of cardiovascular disease; coronary heart disease, heart failure, and stroke, and compared them with some other newer biomarkers and markers of cardiac function. We showed that coronary artery calcium score, carotid intima-media thickness, and NT-proBNP provide the best increment in cardiovascular disease risk prediction above the traditional risk factors. The substantial gain in cardiovascular disease risk prediction by coronary artery calcification was not accompanied by similar contributions to heart failure or ischemic stroke risk predictions. While the added value of carotid intima-media thickness was small, NT-proBNP considerably improved risk predictions for all outcomes.

We elaborated on the differential ability of coronary artery calcification in prediction of different components of a broad cardiovascular outcome in **chapter 3.4**. We argued

that while suggesting coronary artery calcium as a good candidate for screening the risk of cardiovascular disease, in its broader sense, its limitations in stroke risk prediction should be recognized.

Cardiovascular Disease Risk Prediction in Women

In **chapter 4.1**, we applied the new guidelines for cardiovascular risk prediction in women [4] to the population-based Rotterdam Study. We showed that focus on the broad cardiovascular endpoint as an outcome, and not only coronary heart disease, indeed better reflects the cardiovascular risk distribution among women. Use of the new cut point of 10%, for defining high risk category for 10-year cardiovascular risk prediction in women, classified a large group of women earlier in the high-risk category, where they qualify for timely treatment. By cross-examining short-term and long-term cardiovascular risk predictions, we highlighted a group of women at low-intermediate (5%-10%) short-term risk for cardiovascular disease among whom about 25% were at high risk for cardiovascular disease at longer-term. We also showed that women at low short-term but high long-term risk for cardiovascular disease had higher carotid intima-media thickness, higher carotid plaque prevalence, and lower ankle-brachial index, compared to women at low short-term/low long-term risk.

METHODOLOGICAL CONSIDERATIONS

Promise and Pitfalls of Genome-Wide Association Studies

The past five years have witnessed many gene discoveries made through the design of GWA studies. These studies were aimed at detecting variants at genomic loci that are associated with complex traits in the population and, in particular, at detecting associations between common single-nucleotide polymorphisms (SNPs) and common diseases such as cardiovascular disease. Despite the promising aspects of this approach, it holds many caveats and challenges which warrant consideration.

GWAs reveal common variants with small effect sizes. Based on a non-hypothesis driven approach, GWA studies simultaneously assess hundreds of thousands of variants across the whole genome for genetic variations that may associate with certain traits or diseases. In doing so, however, there is a great potential for false-positive findings because of the large number of tested genetic markers. To separate true signals from noise, it has become conventional to apply the strict Bonferroni corrected *P* value of 5×10^{-8} as the genome-wide significance level. However, the design of current GWA studies makes them suitable mainly for the discovery of common variants conferring low to moderate risks. Most GWA studies have analyzed SNPs with minor allele frequencies of more than 1%-5%. Rare variants, with frequencies lying somewhere between 0.1%–1%

are not being examined by current GWA methods. Complex traits, however, can be caused collectively by multiple rare variants with moderate to high effects.

Sample size matters. The typically small effect sizes detected for common variants in GWA studies necessitate large sample sizes to reach the stringent significance thresholds [5]. In the GWA studies included in this thesis, we tried to include all studies with complete genotype-phenotype data, available at the time of the meta-analyses, in the discovery phase. Our discovery panel for the meta-analyses of the three subclinical measures therefore comprised sample sets of substantial size to maximize power. Notably, our initial effort on GWAs for the ankle-brachial index comprised about 22,000 participants of European ancestry in which no loci surpassed the strict Bonferroni corrected *P* value of 5×10^{-8}. After expanding our sample size to over 40,000 subjects, which formed the ankle-brachial index GWA study included in this thesis, one locus survived the strict Bonferroni P value correction.

Replication of findings. Replication of the GWA findings in independent samples is viewed as the ultimate proof of association [6]. We sought for replication of our findings in other studies that got their genotype data later, than our discovery phase, or could genotype a number of SNPs. Replication is also viewed as the "gold" standard of verifying marginal effects [6,7]. We, therefore, chose a less stringent significance level for taking "promising" SNPs forward for replication, in order not to miss the genuine associations that do not pass the stringent set of criteria but are nonetheless real. As joint analysis has proven efficient and is recommended in two-stage GWA studies [8], we then meta-analyzed the effect estimates from the discovery phase with those of the replication stage and provided combined meta-analyses results.

Population differences. GWA studies may falsely identify the associated genes related to diseases due to variations of allele frequencies from populations of different ethnicity or geographic origin. It is, therefore, emphasized for GWA studies to assess and adequately adjust for population stratification. Population stratification is typically examined by checking the distribution of test statistics, generated from the thousands of association tests, and their deviation from the null distribution (expected under the null hypothesis of no SNP is associated with the disease) using quantile–quantile (QQ) plots [9]. Strong deviation from the null may suggest significant differences in population subgroups. The degree of inflation of test statistics can also be calculated by comparing the median over all test statistics with the theoretical median of the distribution of the test statistics under the null hypothesis; the so called genomic control (GC) [10]. If population stratification is present, several effective statistical methods are considered to correct for it [11]. The GWA studies in this thesis included subjects of European ancestry only. Moreover, each study searched for population stratification using QQ plots and calculating the

GC. If necessary, population stratification was dealt with, for example by the principle component analysis.

Trait heterogeneity. Accurate phenotyping, to reduce trait heterogeneity, is another requirement for conducting a good GWA study. Trait heterogeneity, which exists when a trait has been defined with insufficient specificity, is viewed as a confounding factor in traditional statistical genetics of complex human disease [12]. With quantitative traits, imprecision in measurement of phenotypes is usually not thought to have large systematic effects on the location of significant associations in GWA studies, although it might introduce some degrees of variability [13]. In the GWA studies included in this thesis, we tried to define the phenotypes in a precise and harmonized manner across the studies. However, perfect harmonization was not always possible, particularly in the GWA studies for ankle-brachial index and for carotid plaque. In the ankle-brachial index GWA meta-analysis, the blood pressure measurement protocol and ankle-brachial index calculation, although standardized within each study, was heterogeneous across different participating studies. In the GWA study of carotid plaque, the plaque definition included the presence of any plaque in carotid bed in most studies and stenosis greater than 25% in others. Therefore, the remaining heterogeneity in phenotype definitions or the variability in measurement techniques might have compromised our ability to detect small associations.

Cardiovascular Risk Prediction; Prospects and Limitations

Cardiovascular risk prediction serves as the basis for clinical decision making for initiation and intensity of treatment, raising population awareness of the disease, communicating knowledge about the risk to individuals, and motivating adherence to recommended lifestyle changes or therapy. In classical cardiovascular risk scoring algorithms, combination of various traditional risk factors generates a risk score which is then converted into an absolute probability of developing a cardiovascular event within a certain time frame. Over the past few years, substantial effort has been devoted to examining the addition of newer risk markers to established risk scoring systems. In doing so, the best scenario is to compare the added predictive ability of multiple risk markers within the same cohort [14]. As part of this thesis, we examined the additive value of subclinical measures of atherosclerosis in prediction of different cardiovascular outcomes and compared them with those of some other (newer) risk markers. Below, I address some issues pertinent to the cardiovascular risk prediction studies included in this thesis.

Need for updating an existing risk prediction model. To assess the incremental value of a new risk marker, several investigators add it to the model containing the published risk scores. Performance of a risk prediction model, when applied to new individuals, is poorer than its performance in the sample in which it was developed [15]. As a result, the

observed value of the new risk marker is a combination of the lower predictive performance of the existing risk score in the new population and the true (added) value of the new marker. One solution is to update the existing prediction model to the new population to improve its performance and to prevent inflation of the estimates by the new marker. We, therefore, refitted the current risk scoring algorithms into our population to accurately address the incremental value of newer risk markers. Notably, when comparing the results of different studies on the added value of a risk marker, one should give careful attention to the baseline model upon which the incremental value is examined.

Statistical measures for assessing the incremental predictive value. Following the growing interest in utilizing newer markers for cardiovascular risk prediction, recent guidelines recommend several statistical measures for assessing the increment in cardiovascular risk prediction offered by newer risk markers [16-19]. Besides providing evidence for the strength of association of the new marker with the outcome, it is now recommended to quantify improvements in model fit, discrimination, calibration, and reclassification of the risk prediction equations after addition of the new marker [18,19]. For the risk prediction studies included in this thesis, we employed the new statistical measures to quantify the gain in cardiovascular risk prediction using the newer risk markers.

Addressing the clinical utility of a new risk marker. To evaluate the clinical value of a risk marker, it is important to consider if the marker changes predicted risk sufficiently to change recommended therapy [16,17]. One way to quantify such concept is to calculate the percentage of individuals that are correctly reclassified into clinically meaningful higher or lower risk categories with the addition of a new predictor; i.e. net reclassification improvement (NRI) [18,20]. Correct reclassifications are shifts to a higher risk category for events and shifts to a lower risk category for non-events. Definition of these risk groups, however, is often arbitrary and differs across studies. Since NRI is affected by proximity of the individual risk estimates to the cut-points used to define the risk categories, comparisons of NRIs across different studies should be performed cautiously. To circumvent this problem, continuous NRI, that does not require stratification of the population into risk groups, was introduced [20]. Continuous NRI takes into account all movements in predicted probabilities, regardless of the extent of the movement which is often far from clinical significance. In general, NRI increases as a function of the number of categories, with continuous NRI serving as the limiting case [21]. In our study, coronary artery calcium provided a category-based NRI of 19% (based on three risk strata) and a continuous NRI of 45% in coronary heart disease risk prediction. For NT-proBNP, the category-based NRI for coronary heart disease risk prediction was 8%, compared to the continuous NRI of 22%. In some circumstances, use of continuous NRI is not clinically meaningful, as it is the case in cardiovascular research where established cut-points corresponding to clinical treatment strata exist [22]. For the cardiovascular risk prediction

studies included in this thesis, we therefore reported NRIs using the clinically relevant risk categories for each cardiovascular outcome.

Focusing on the intermediate risk category; clinical NRI. The use of newer markers to augment traditional cardiovascular risk prediction has attracted most attention for the individuals who fall into the intermediate risk category. The interest for focus specifically on intermediate-risk individuals is based on the premise that high-risk individuals should automatically be targeted for treatment measures, whereas low-risk individuals do not need specific intervention [22]. The recommendations for the intermediate-risk individuals therefore remain less straightforward. Focusing on this group, a "clinical" NRI has been suggested by restricting the NRI measure to the intermediate risk stratum. Recently, it has been argued that, due to the symmetric nature of the reclassification table, restricting NRI to the intermediate risk group may provide biased estimates [23]. An adjusted version of clinical NRI has been proposed to determine the net improvement in the intermediate risk group [23]. Application of the proposed adjustment method to our clinical NRI estimates in coronary heart disease risk prediction, presented in Table 4 of **chapter 3.2.,** reduced the clinical NRI for coronary artery calcium scores from 39% to 23% and for NT-proBNP from 33% to 23%. The adjusted NRI estimates can still be considered useful in clinical perspectives.

Along with moving subjects out of the intermediate risk category into the low and high risk categories, which is the desired function of adding new markers, some individuals move into the intermediate risk category. At present, the guidelines do not provide any clear strategy on how to treat these people. Particularly, the current evidence is not sufficient to withdraw treatment from high-risk individuals who move into the intermediate risk category as the result of the new test. Consequently, until ample evidence suggests otherwise, more subjects would be deemed as being at high risk and thus qualify for treatment interventions.

Broadening the outcome in cardiovascular prediction. Recently, there has been an increasing recognition to focus the cardiovascular prediction systems on a broader cardiovascular outcome instead of targeting coronary heart disease only [24]. Expanding this continuum to include other entities, such as stroke or heart failure, will (i) methodologically increase power in risk prediction and (ii) clinically promote communication with individuals. It is now generally appreciated that although coronary and cerebrovascular disease share common risk factors, the strength of the associations and the contribution of risk factors to each disease entity is different [25-27]. It should not be discarded that the same rule applies to the newer risk markers, meaning that contribution of a risk marker to prediction of the broad cardiovascular outcome is an aggregation of its different contributions to different components of the broad outcome. Our example of coronary artery calcification clearly highlights this point by showing that calcification

of the coronary arteries, which so far provides the most substantial improvement in coronary heart disease risk prediction, is not a good prognostic marker for stroke. We elaborate on this issue in **chapter 3.4** of this thesis and argue that while suggesting coronary artery calcium as a good candidate for screening the risk of cardiovascular disease, in its broader sense, its limitations in stroke risk prediction should be recognized. It seems therefore necessary to require the investigators to separately report the prediction estimates for the individual outcomes, along with presenting the estimates for the combined outcome. At last, when comparing the results on the utility of newer risk markers in cardiovascular risk prediction, one should carefully consider that the definition of the composite cardiovascular outcome differs vastly across the studies.

Generalizability. The cardiovascular prediction studies included in this thesis were all performed within the framework of the Rotterdam Study, a population-based cohort of individuals 55 years and older [2]. The predictive performance of traditional risk factors decreases with age. It might therefore be more likely to witness the added predictive value of newer risk markers at older ages. This also implies that the results of the prediction studies in this thesis might not automatically be generalized to younger populations.

CLINICAL IMPLICATIONS & DIRECTIONS FOR FUTURE RESEARCH

From GWA Studies to Translation

GWA studies represent one forward step toward a more complete understanding of the genetic architecture of complex disorders. Unbiased by prior biological knowledge, these studies have substantially changed the landscape of genetic associations for many traits. GWA studies have created a large database of regions of the human genome involved in each disease, containing a range of genes with a variety of functions, each of which can then be investigated biologically. Identification of the disease associated genes by GWA studies provides new insights into disease mechanisms and can represent excellent potential therapeutic targets.

In the GWA studies included in this thesis, we identified several new loci associated with the three subclinical measures of atherosclerosis burden. Interestingly, several of the identified loci were also associated with coronary artery disease. In line with the general view that atherosclerosis is a continuous process, these shared genetic associations suggest a common etiology for subclinical and clinically apparent cardiovascular disease. Therefore, investigations to understand mechanisms underlying the genetic associations with subclinical carotid, coronary, and peripheral atherosclerosis may ultimately suggest new strategies for prediction, prevention, and treatment of cardiovascular disease. A number of loci showed pleiotropic effects with several other phenotypes. Although these findings reveal that the complexity underlying the genomic basis of

human disease is much greater than was initially thought, this pleiotropism may be very helpful in understanding some mechanisms shared among different diseases.

Genetic prediction of cardiovascular disease, yet no help for clinicians? Although the main relevance of GWA studies lies in the insights into disease biology, one of the early promises of the GWA approach was to provide more accurate models for risk prediction based on the individual genetic profile. Genotype-based risk predictions are (i) fixed from birth, thus allowing for early risk prevention, and (ii) less susceptible to biological variation over life, and were therefore hoped to provide more precise estimates of individual risk [28]. However, the predictive power of disease risk ascertained from GWA data remains poor because for most diseases only a small proportion of genetic variance has been accounted for. Therefore, the results of the current genetic studies in cardiovascular risk prediction lie far below the level that can be considered clinically significant. Sequencing based approaches are considered by some to turn up variants with stronger effects than GWA studies and to provide more meaningful risk prediction [29]. However, the remaining limitation is the lower population frequency of the variants identified through sequencing which makes the risk predictions beneficial to the small number of individuals carrying each specific variant. Moreover, to seek such rare variants, studies with much larger sample size are required.

Family history as an alternative? Instead of direct approaches to assess genetic risk, an important alternative is the use of family history in risk prediction. In our study, the effect estimate provided by family history in cardiovascular risk prediction (hazard ratio of 1.30, Table 2, **chapter 3.3**), was larger than those generally observed in GWA studies. As individual genetic variants or risk scores have not yet led to significant improvements in risk prediction [30], family history is viewed as a simple, cheap, and clinically useful risk factor for cardiovascular disease, which likely represents the net effect of hundreds of genetic risk variants yet to be discovered. However, in our study, the added value of family history in assessment of cardiovascular risk above the traditional risk factors was marginal.

Next step, searching for pleiotropy across vascular beds. Subclinical measures of atherosclerosis share common risk factors and are all predictive of future cardiovascular events. However, there is a low correlation across these measures in various major vascular beds [31]. The incomplete correlation among these vascular measures can rise from both genetic and environmental factors. Following successful completion of our GWA studies for the three subclinical measures, it is time to consider all three measures in one framework. We aim to unravel the common genetic variants shared across the three vascular measures and examine the extent to which the distribution of these traits can be attributed to pleiotropic effects of the same genetic variants.

Moving forward with genetic and environmental interaction studies. Although atherosclerosis is viewed as a diffuse process, the degree of atherosclerosis varies from one arterial bed to another. This variation is likely to be the result of different risk factors, both genetic and environmental, as well as their interactions. We, therefore, will move forward with the GWA studies of the three subclinical measures implementing gene-gene or gene-environment interactions. As a first step and to secure the statistical power required for interaction studies [32], we plan to increase the sample sizes for the subclinical measure GWA studies and include all studies that obtained their genotype data later than our initial GWA efforts for these traits and were therefore not included in the GWA studies described in this thesis.

Post-GWA era. The technology for identifying genetic differences is racing forward. It is now believed that making serious progress in understanding disease risk lies in developing a deeper "biological awareness" into genomic approaches to the study of complex disorders. Accordingly, new efforts are underway to accurately characterize low frequency and rare genetic variants inaccessible through GWA studies. Imputation using 1000 genome project [33] and high-throughput approaches such as next generation sequencing are examples of such efforts, that have been initiated within the framework of the CHARGE consortium [1], which results are being eagerly awaited.

Cardiovascular Risk Prediction; Current Status and Future Directions

Subclinical measures of atherosclerosis in cardiovascular risk prediction. Among the three measures of subclinical atherosclerosis burden studied in this thesis, coronary artery calcification provides the best increment in coronary heart disease risk prediction. The substantial gain in risk prediction provided by coronary artery calcium score above the traditional cardiovascular risk factors suggests use of this risk marker as a supplemental tool for refining coronary heart disease risk prediction, specially among subjects at intermediate risk. Our results, however, do not indicate the use of coronary calcium score in improving risk predictions for heart failure or stroke. Moreover, our findings regarding the small added value of common carotid intima-media thickness in cardiovascular risk prediction do not support the routine use of this subclinical measure in cardiovascular screening programs above the traditional risk factors. Likewise, two recent large meta-analyses have shown that the improvement in cardiovascular risk prediction by either a single measurement of common carotid intima-media thickness [34] or by quantifying the change in the thickness of common carotid artery over time [35] is unlikely to be of clinical importance. Recent evidence suggests that maximum intima-media thickness of (and presence of plaque in) the internal carotid artery might be a better predictor for cardiovascular events [36]. However, maximum thickness of the internal carotid artery also offers modest incremental value over the traditional risk scoring

algorithms in cardiovascular risk prediction. We also found a marginal added value for ankle-brachial index in cardiovascular risk prediction which falls below the level that could be considered clinically significant.

When we compared the ability of subclinical measures with several other emerging (bio)markers, another (relatively new) risk marker, NT-proBNP, provided a considerable improvement over the traditional risk factors in prediction of all cardiovascular outcomes. NT-proBNP levels can therefore be considered as a reasonable supplemental tool for cardiovascular risk prediction in all settings.

Search for "early markers" of cardiovascular disease. Risk markers may be informative either early or late in the atherosclerosis process, with some biomarkers reflecting activity in biological pathways that precede the overt cardiovascular disease and other (bio) markers triggered by the presence of "subclinical" cardiovascular disease. So far, growing evidence confirms the incremental prognostic information provided by coronary artery calcium score and by NT-proBNP in cardiovascular risk prediction [37-41]. The greater ability of these two markers in cardiovascular risk prediction might correspond to their position in the later stages of the disease process. Coronary artery calcium, in particular, is viewed as the vessel's memory of lifetime risk factor exposure [42]. While identifying the disease at its "subclinical" stage is yet of profound value, the ideal situation would be to target the atherosclerosis process at its early stages. The pace of biomarker discoveries is accelerating, with the maturation of technologies such as proteomics and metabolomics. Such approaches may be particularly useful for identifying the cardiovascular disease biomarkers at early stages and in different pathways from those represented by existing biomarkers.

Need for large scale consortia in cardiovascular risk prediction. Following the success of large genetic consortia, large scale collaborative studies on the added value of emerging risk markers in cardiovascular risk prediction are underway [34,35]. Methods for combining discrimination and reclassification estimates across studies are being developed. Given the lower cardiovascular incidence rates in women, such "mega-epidemiology" studies seem most relevant for this group.

Focus on age-specific risk equations. In cardiovascular risk equations, age is in fact the single strongest risk factor for future cardiovascular events [43]. Particularly, the inclusion of age in cardiovascular risk assessment can lead to under-representation of risk in younger individuals. One way to circumvent the central role of age in risk prediction is to derive age-specific risk equations for groups in narrow windows of age rather than developing models in populations with a wide age range in which age is included as a predictor variable. Without age in the cardiovascular risk equation and with a focus on

a specific age group, the role of other risk markers in cardiovascular risk prediction, and subsequently prevention, might become more prominent.

Cardiovascular risk prediction, steps forward. (i) The more inclusive focus on total cardiovascular outcome, and not targeting coronary heart disease only, together with (ii) extending the time frame in cardiovascular risk prediction, as a complement to short-term (10-year) risk prediction, are two major steps forward towards improving the global cardiovascular risk assessment. Such approaches would help to avoid false reassurance for individuals who are at "low risk" for a coronary event at short-term but will become "high risk" for any cardiovascular event across the lifespan. In doing so, however, the importance and incidence of each particular cardiovascular outcome in the population of interest and the differential contribution of risk markers to various components of the more inclusive outcome should not be discarded.

Phases of evaluation of a new risk marker. What is yet missing? Providing (statistically) significant evidence of independence is a long-standing tradition in cardiovascular risk prediction. Recently, providing measures of model fit, discrimination, and the evidence that a marker reclassifies risk is also becoming common. The value of reclassification for altering clinical management, however, remains largely theoretical because randomized trials specifically addressing this issue have not been performed. Therefore, the ultimate standard for evaluation remains to be a demonstration that a new risk marker improves clinical outcomes at an affordable cost compared with current practice.

Among the measures of subclinical atherosclerosis burden, current evidence suggests the use of coronary artery calcium in coronary heart disease screening programs. However, there is still need to design randomized control trials to assess the gain in

Table 1. Phases of evaluation of a novel risk marker *

Proof of concept: Do novel marker levels differ between participants with and without outcome?
Prospective validation: Does the novel marker predict development of future outcomes in a prospective cohort or nested case–cohort/case–cohort study?
Incremental value: Does the novel marker add predictive information to established, standard risk markers?
Clinical utility: Does the novel risk marker change predicted risk sufficiently to change recommended therapy?
Clinical outcomes: Does the novel risk marker improve clinical outcomes, especially when tested in a randomized clinical trial?
Cost-effectiveness: Does use of the marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment?

* Reproduced with permission (Hlatky MA. Ann Intern Med. 2012;156:468-469.)

improvement in clinical outcomes before introducing the coronary calcium imaging into practice. Since refining risk estimation for the individuals at the low or high risk categories is not likely to alter the disease management, such trials should focus on the individuals at intermediate coronary heart disease risk in whom performing an additional test would assist in decision making. Moreover, the presumed benefits of the coronary calcification screening still need to be weighed against its costs; both the direct financial costs and the indirect costs such as exposing individuals to radiation or handling the incidental findings during the coronary calcium imaging. Finally, whether coronary artery calcium could be considered a good candidate for cardiovascular disease screening in its broader sense or should it only be focused on coronary heart disease remains to be investigated.

Myth Revisited; Cardiovascular Disease not Just a Man's Disease Anymore

Cardiovascular disease represents the leading cause of mortality for both men and women. In Europe, cardiovascular disease accounts for 43% of deaths in men and 55% in women. When different components of cardiovascular disease are considered, coronary heart disease represents 21% of deaths in men and 23% in women. Stroke comprises 11% of deaths in men and 18% in women, whereas 11% of deaths in men and 15% in women are attributed to other cardiovascular events [44]. Interestingly, breast cancer accounts for 3% of all deaths. As life expectancy increases, and it does so particularly in women, the proportion of women with different components of cardiovascular disease shows an important rise. Of note, the decline in age adjusted cardiovascular mortality over the past 40 years has been of lesser magnitude in women compared to men.

Appreciation of the impact of gender on cardiovascular risk assessment and management has therefore been an area of increasing interest. The first women-specific clinical recommendations for cardiovascular disease prevention, "A Guide to Preventive Cardiology in Women", were published by the American Heart Association in 1999 [45]. In 2005, the European Society of Cardiology initiated the "Women at Heart" program to coordinate research and educational initiatives on cardiovascular disease in women [44]. Although gender disparities in recommendations for cardiovascular disease prevention, based on the misperceived lower risk in women, have started to decline, there is still a large room for improving cardiovascular risk prediction and prevention in women.

Specific risk scoring algorithms for women. Women often share similar risk factors for cardiovascular disease with men. However, the magnitude of the effect of risk factors differs per gender. Some risk factors such as high blood triglyceride levels and low high-density lipoprotein cholesterol play greater roles in increasing the risk of developing cardiovascular disease among women [46,47]. Furthermore, diabetes and obesity appear to be stronger contributing risk factors for heart disease in women than men [48,49]. Moreover, depression and other psychosocial risk factors, as well as autoimmune diseases have

been associated with a significantly increased relative risk for cardiovascular disease in women and are considered as unrecognized risk factors [4]. Additionally, some risk factors such as pregnancy complications [(pre)eclampsia [50] or gestational diabetes], polycystic ovary syndrome [51], hormone therapy, and menopause are exclusive to women. It seems therefore reasonable to consider developing female-specific scoring systems including the risk factors that are more prevalent among women and/or the risk factors that make special contributions to cardiovascular risk in women.

Extending the time horizon and outcome for risk prediction in women. Considering the risk factor burden in the context of longer time horizons, than 10 years, in assessing individuals' risk for developing cardiovascular disease has recently gained interest. Given that cardiovascular risk is merely deferred by about 10 years in women, (i) extension of the time frame used for risk prediction seems particularly relevant in women, and (ii) it also seems reasonable to extend the risk assessment to older age groups in order to account for the delayed onset of cardiovascular disease in women. Moreover, there is an increasing recognition that risk models should focus on predicting global cardiovascular events and not targeting coronary heart disease only. Moving towards such a broader entity seems particularly true for women whose risks for stroke and heart failure through middle and older age typically exceed their risk for coronary heart disease [4]. Such approaches will serve to identify more women at risk, overcoming some of the limitations of 10-year coronary heart disease risk prediction models.

Lowering risk thresholds for women. The most recent American Heart Association guideline for cardiovascular prevention in women recommends use of the lower cut-point of 10% for all (fatal and non-fatal) cardiovascular events in defining the high risk category in women [4]. European guidelines already estimate the risk of all cardiovascular events, although the focus is on the fatal cardiovascular events as opposed to a combination of fatal and non-fatal events, and recommend use of the same risk thresholds for men and women [52]. Use of this new cut-point, for all cardiovascular events, seems reasonable and identifies a larger group of high-risk women, prior to the clinical presentation of their disease, which qualify for (timely) treatment interventions.

Need for more data in women. The majority of available data in women, which also serve as the basis for developing guidelines, is based on studies of coronary heart disease. Further studies are needed to focus on other specific outcomes of particular importance in women, such as stroke and heart failure [4]. In Europe particularly, there is a need to collect more epidemiological data on cardiovascular disease and its risk factors in women of different age groups in order to improve the accuracy of risk scoring systems in women [44]. The paucity of existing data calls for large scale collaborative

studies on cardiovascular risk prediction and on the added value of newer risk markers, particularly in women.

New Concept; Cardiovascular Health

A growing body of evidence indicates strong associations between risk factor levels across the lifespan and both cardiovascular and non-cardiovascular outcomes, as well as longevity. These observations have led to the development of a new concept; "cardiovascular health" [53]. The ideal "cardiovascular health" is defined by the absence of clinical cardiovascular disease and the simultaneous presence of favorable health behaviors (non-smoking, body mass index <25 kg/m^2, physical activity at goal levels, and pursuit of a diet consistent with current guideline recommendations); together with the favorable health factors (untreated total cholesterol <200 mg/dL, untreated blood pressure <120/<80 mm Hg, and fasting blood glucose <100 mg/dL) [53]. The prevalence of ideal cardiovascular health has been reported to be low in several community-based study populations [54-56]. The low prevalence estimates of cardiovascular health represent a starting point from which effectiveness of efforts to promote cardiovascular health and prevent cardiovascular disease can be monitored and compared [56].

Health is a broader construct than just the absence of clinically evident disease. Although there appears to be substantial overlap between the components of "cardiovascular health" and "general health", there are other components to "general health" related to physical, mental, and social functioning, among other factors, that have not been addressed in the concept of "cardiovascular health" [53]. Future efforts should include consideration of the different important aspects of general health to arrive at a more comprehensive definition for "health" and consequently "healthy longevity".

A clearer identification of the major determinants of "healthy longevity" would provide many possibilities for action throughout life with subsequent promotion in quality of life accompanied by improved long-term care for the elderly. Focusing on maintaining optimal health can play a central role in overseeing the growing demand for long-term care in ageing populations.

CONCLUDING REMARKS

In the GWA studies included in this thesis, we identified several new loci associated with the three subclinical measures of atherosclerosis burden. Interestingly, several of the identified loci were also associated with coronary artery disease, in line with the general view that atherosclerosis is a continuous process. Investigations to understand mechanisms underlying the genetic associations with subclinical carotid, coronary, and peripheral atherosclerosis may ultimately suggest new strategies for prediction, prevention, and treatment of cardiovascular disease.

Although the original concepts of risk prediction focused on coronary heart disease, the cardiovascular disease continuum has expanded to include other entities such as cerebrovascular disease and heart failure. The ultimate goal remains to be timely prevention of the disease through state-of-the art knowledge on genetic and non-genetic risk markers. In the studies included in this thesis we found a marginal added value for both carotid intima-media thickness and ankle-brachial index in cardiovascular risk prediction which falls below the level that could be considered clinically important. We also showed that while coronary artery calcification remains to be the best marker in coronary heart disease risk prediction, its use as a complementary tool for stroke or heart failure risk predictions has limitations. Therefore, whether coronary artery calcification could be considered a good candidate for cardiovascular disease screening, in its broader sense, or should it only be focused on coronary heart disease remains to be investigated.

The recent interest in (i) assessing individuals' risk for developing various components of cardiovascular disease, and (ii) considering the risk factor burden in the context of longer time horizons, seems particularly relevant in women. Such approaches will serve to identify more women at risk, overcoming some of the limitations of 10-year coronary heart disease risk prediction models. Following the success of large genetic consortia, large scale collaborative studies in cardiovascular risk prediction and the added value of emerging risk markers, in particular in women, are warranted.

REFERENCES

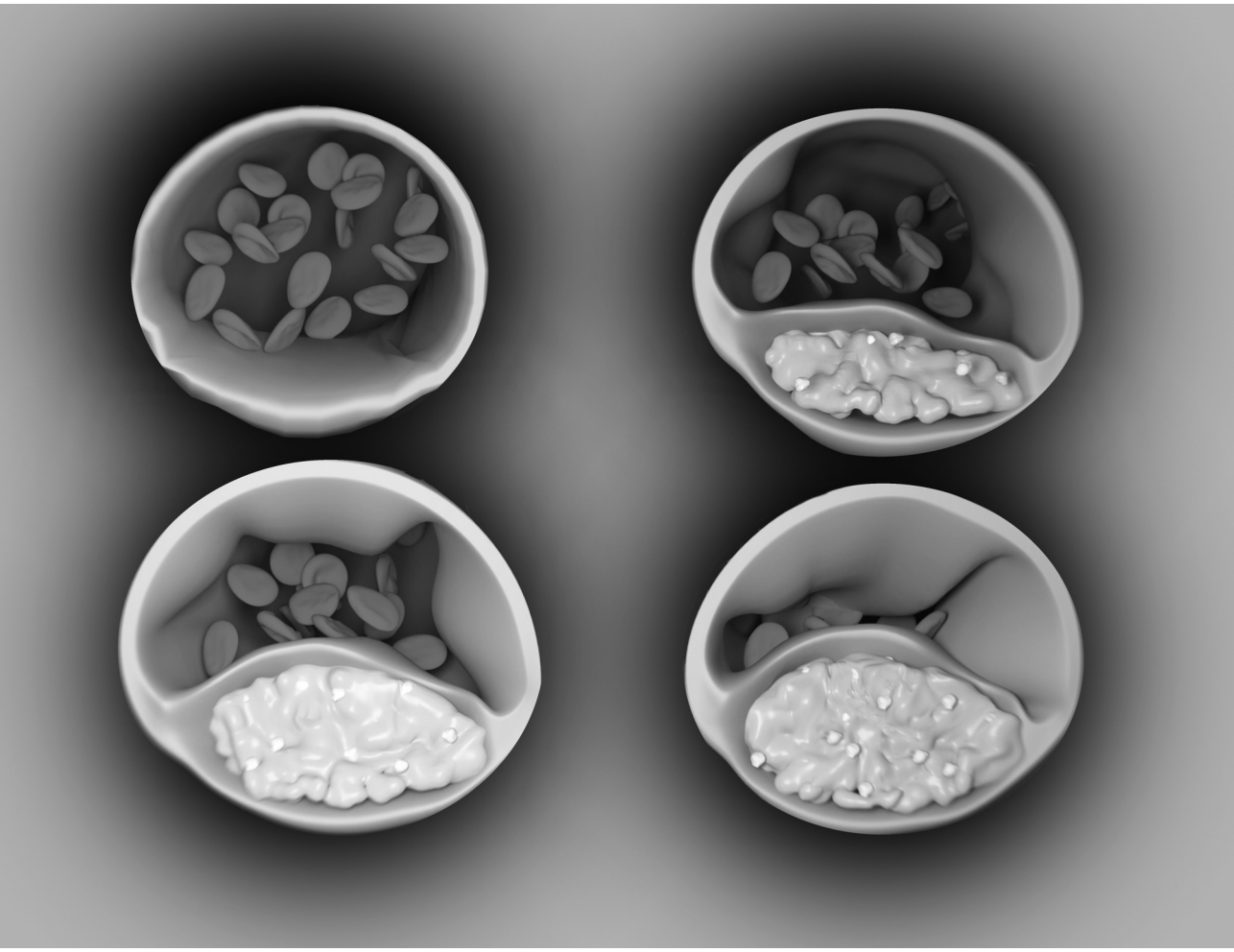
1. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. Circ Cardiovasc Genet. 2009;2:73- 80.
2. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. 2011;26:657-686.
3. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011;43:333-338.
4. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. Circulation. 2011;123:1243-1262.
5. Burton PR, Hansell AL, Fortier I, Manolio TA, Khoury MJ, Little J, Elliott P. Size matters: just how big is BIG?: Quantifying realistic sample size requirements for human genome epidemiology. Int J Epidemiol. 2009;38:263-273.
6. Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, et al. Replicating genotype-phenotype associations. Nature. 2007;447:655-6560.
7. Williams SM, Haines JL. Correcting away the hidden heritability. Ann Hum Genet. 2011;75:348-350.
8. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet. 2006;38:209-213.
9. Weir BS, Hill WG, Cardon LR. Allelic association patterns for a dense SNP map. Genet Epidemiol. 2004;27:442-450.
10. Devlin B, Roeder K, Wasserman L. Genomic control, a new approach to genetic-based association studies. Theor Popul Biol. 2001;60:155-166.
11. Price AL, Zaitlen NA, Reich D, Patterson N. New approaches to population stratification in genome-wide association studies. Nat Rev Genet. 2010;11:459-463.
12. Thornton-Wells TA, Moore JH, Haines JL. Dissecting trait heterogeneity: a comparison of three clustering methods applied to genotypic data. BMC Bioinformatics. 2006;7:204-221.
13. Barendse W. The effect of measurement error of phenotypes on genome wide association studies. BMC Genomics. 2011;12:232-243.
14. Helfand M, Buckley DI, Freeman M, Fu R, Rogers K, Fleming C, Humphrey LL. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med. 2009;151:496-507.
15. Moons KG, Kengne AP, Grobbee DE, Royston P, Vergouwe Y, Altman DG, Woodward M. Risk prediction models: II. External validation, model updating, and impact assessment. Heart. 2012;98:691-698.
16. Hlatky MA. Framework for evaluating novel risk markers. Ann Intern Med. 2012;156:468-469.
17. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation. 2009;119:2408-2416.
18. Pencina MJ, D'Agostino RB, Vasan RS. Statistical methods for assessment of added usefulness of new biomarkers. Clin Chem Lab Med. 2010;48:1703-1711.

19. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology. 2010;21:128-138.
20. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med. 2011;30:11-21.
21. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Authors Reply. Comments on 'Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med. 2012;31:96-97.
22. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143-3421.
23. Cook NR, Paynter NP. Performance of reclassification statistics in comparing risk prediction models. Biom J. 2011;53:237-258.
24. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117:743-753.
25. Simons LA, Simons J, Friedlander Y, McCallum J. A comparison of risk factors for coronary heart disease and ischaemic stroke: the Dubbo study of Australian elderly. Heart Lung Circ. 2009;18:330-333.
26. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008;371:1612-1623.
27. Soler EP, Ruiz VC. Epidemiology and risk factors of cerebral ischemia and ischemic heart diseases: similarities and differences. Curr Cardiol Rev. 2010;6:138-149.
28. Patel RS, Ye S. Genetic determinants of coronary heart disease: new discoveries and insights from genome-wide association studies. Heart. 2011;97:1463-1473.
29. Sandhu M, Wood A, Young E. Genomic risk prediction. Lancet. 2010;376:1366-1367.
30. Thanassoulis G, Vasan RS. Genetic cardiovascular risk prediction: will we get there? Circulation. 2010;122:2323-2334.
31. Kathiresan S, Larson MG, Keyes MJ, Polak JF, Wolf PA, D'Agostino RB, et al. Assessment by cardiovascular magnetic resonance, electron beam computed tomography, and carotid ultrasonography of the distribution of subclinical atherosclerosis across Framingham risk strata. Am J Cardiol. 2007;99:310-314.
32. Aschard H, Lutz S, Maus B, Duell EJ, Fingerlin TE, Chatterjee N, et al. Challenges and opportunities in genome-wide environmental interaction (GWEI) studies. Hum Genet. 2012;131:1591-1613
33. 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature. 2010;467:1061-1073.
34. den Ruijter HM, Peters SAE, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction:a meta-analysis. JAMA. 2012;308:796-803.
35. Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Volzke H, Tuomainen TP, et al. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. 2012;379:2053-2062.
36. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB, Sr. Carotid-wall intima-media thickness and cardiovascular events. N Engl J Med. 2011;365:213-221.

37. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med. 2004;350:655-563.
38. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355:2631-2639.
39. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, Greenland P. Coronary artery calcium score and risk classification for coronary heart disease prediction. JAMA. 2010;303:1610-1616.
40. Erbel R, Mohlenkamp S, Moebus S, Schmermund A, Lehmann N, Stang A, et al. Coronary risk stratification, discrimination, and reclassification improvement based on quantification of subclinical coronary atherosclerosis: the Heinz Nixdorf Recall study. J Am Coll Cardiol. 2010;56:1397-1406.
41. Elias-Smale SE, Proenca RV, Koller MT, Kavousi M, van Rooij FJ, Hunink MG, et al. Coronary calcium score improves classification of coronary heart disease risk in the elderly: the Rotterdam study. J Am Coll Cardiol. 2010;56:1407-1414.
42. Erbel R, Budoff M. Improvement of cardiovascular risk prediction using coronary imaging: subclinical atherosclerosis: the memory of lifetime risk factor exposure. Eur Heart J. 2012;33:1201-1213.
43. Ridker PM, Cook N. Should age and time be eliminated from cardiovascular risk prediction models? Rationale for the creation of a new national risk detection program. Circulation. 2005;111:657-658.
44. Stramba-Badiale M, Fox KM, Priori SG, Collins P, Daly C, Graham I, et al. Cardiovascular diseases in women: a statement from the policy conference of the European Society of Cardiology. Eur Heart J. 2006;27:994-1005.
45. Mosca L, Grundy SM, Judelson D, King K, Limacher M, Oparil S, et al. AHA/ACC scientific statement: consensus panel statement. Guide to preventive cardiology for women. American Heart Association/ American College of Cardiology. J Am Coll Cardiol. 1999;33:1751- 1755.
46. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk. 1996;3:213-219.
47. Pilote L, Dasgupta K, Guru V, Humphries KH, McGrath J, Norris C, et al. A comprehensive view of sex-specific issues related to cardiovascular disease. CMAJ. 2007;176:S1-S44.
48. Canoy D, Boekholdt SM, Wareham N, Luben R, Welch A, Bingham S, et al. Body fat distribution and risk of coronary heart disease in men and women in the European Prospective Investigation Into Cancer and Nutrition in Norfolk cohort: a population-based prospective study. Circulation. 2007;116:2933-2943.
49. Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. BMJ. 2006;332:73-78.
50. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007;335:974-985.
51. Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, Lehnert H. Cardiometabolic Aspects of the Polycystic Ovary Syndrome. Endocr Rev. 2012;33:812-841
52. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task

Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 2012;33:1635-1701.

53. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, et al; American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation. 2010;121:586-613.
54. Bambs C, Kip KE, Dinga A, Mulukutla SR, Aiyer AN, Reis SE. Low prevalence of "ideal cardiovascular health" in a community-based population: the heart strategies concentrating on risk evaluation (Heart SCORE) study. Circulation. 2011;123:850-857.
55. Folsom AR, Yatsuya H, Nettleton JA, Lutsey PL, Cushman M, Rosamond WD; ARIC Study Investigators. Community prevalence of ideal cardiovascular health, by the American Heart Association definition, and relationship with cardiovascular disease incidence. J Am Coll Cardiol. 2011;57:1690-1696.
56. Shay CM, Ning H, Allen NB, Carnethon MR, Chiuve SE, Greenlund KJ, et al. Status of cardiovascular health in US adults: prevalence estimates from the National Health and Nutrition Examination Surveys (NHANES) 2003-2008. Circulation. 2012;125:45-56.



CHAPTER 6

Summary / Samenvatting

Atherosclerosis develops silently over decades before symptoms eventually occur, offering unique opportunities for timely detection and prevention. Although often detected when patients first experience a major cardiovascular event, several techniques can be used to identify and quantify atherosclerosis when it is still at its subclinical stages.

In this thesis, the aim was to expand the knowledge on three measures of subclinical atherosclerosis burden; coronary artery calcium, carotid intima-media thickness, and ankle brachial index.

In **chapter 2,** we sought to unravel the genetic determinants of these three measures of subclinical atherosclerosis using the powerful approach of genome-wide association (GWA) studies in the framework of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium.

We identified several novel loci for coronary artery calcium in chapter **2.1.** We showed that multiple genetic loci are associated with development of both underlying coronary atherosclerosis and clinical events. We found several significant or suggestive genetic loci; near *CDKN2A* and *CDKN2B* genes, within the *PHACTR1* gene, *MRAS* gene, *COL4A1/COL4A2* genes, and *SORT1* gene in association with both coronary calcium and with myocardial infarction. These findings suggest that the common mechanism of some genetic loci underlying myocardial infarction is development of early, underlying coronary atherosclerosis.

Our meta-analysis of GWA data revealed 5 new loci for common cIMT and plaque, presented in **chapter 2.2.** These loci implicate LDL metabolism (*APOC1*), endothelial dysfunction (*EDNRA*), platelet biology (*PIK3CG*), and telomere maintenance (*PINX1*). Two of our identified loci were also associated with coronary artery disease in the large meta-analysis by the CARDIoGRAM Consortium. Exploring the molecular, cellular and clinical consequences of genetic variation at these loci may yield novel insights into the pathophysiology of clinical and subclinical cardiovascular disease.

In the GWA meta-analysis for ankle brachial index, **chapter 2.3,** we identified and replicated one genome-wide significant association on 9p21 region (near *CDKN2B*).

However, none of the SNP associations for peripheral arterial disease was genome-wide significant. Although we observed minimal overlap in the top SNP associations for the two traits, the directions of effect were consistent across the two phenotypes for the most significant ankle-brachial index SNPs. Moreover, two previously reported candidate genes for peripheral arterial disease; *DAB21P, CYBA,* and one SNP associated with coronary artery disease (*LDLR* gene) were associated with ankle-brachial index.

In **chapter 3,** our aim was to address the ability of the three subclinical measures in cardiovascular risk prediction and to compare their predictive performance with that of several cardiovascular (bio)markers in prediction of different cardiovascular outcomes.

In **chapter 3.1,** we showed that the additional predictive value of common carotid intima-media thickness above the traditional risk factors in the prediction of coronary heart disease and stroke is small. While common carotid intima-media thickness did not significantly improve risk stratification based on traditional risk factors in men, it showed a modest ability to reclassify people to a more accurate cardiovascular risk category in women.

We compared the added value of the three subclinical measures of atherosclerosis in prediction of various components of cardiovascular disease; coronary heart disease, heart failure, and stroke with that of some emerging biomarkers in **chapter 3.2** and **chapter 3.3.** We showed that coronary artery calcium and NT-proBNP provide the best increment in cardiovascular disease risk prediction above the traditional risk factors. In **chapter 3.2,** we showed that, among all markers under-study, improvements in coronary heart disease risk prediction were most statistically and clinically significant with the addition of coronary artery calcium scores, with NT-proBNP levels a close second. Although coronary calcium proved to be the best markers for coronary heart disease risk prediction, we showed in **chapter 3.3** that the substantial gain in cardiovascular disease risk prediction by coronary artery calcium was not accompanied by similar contributions to heart failure or ischemic stroke risk predictions. In **chapter 3.3** we also showed that NT-proBNP considerably improved risk predictions for all cardiovascular outcomes. These results imply that in the populations where heart failure or stroke are major constituents of cardiovascular disease, use of coronary calcium as a complementary tool for risk prediction has limitations. NT-proBNP, however, can be considered as a reasonable supplemental tool for cardiovascular risk prediction in all settings. However, making a sensible decision on implementation of a new risk marker in cardiovascular risk prediction will ultimately necessitate reconsiderations of the cost, feasibility and safety of performing the new test. We elaborated on the differential ability of coronary artery calcium in prediction of different components of a broad cardiovascular outcome in **chapter 3.4.** We argued that while suggesting coronary artery calcium as a good candidate for screening the risk of cardiovascular disease, in its broader sense, its limitations in stroke risk prediction should be recognized.

In **chapter 4,** we applied the new principles for cardiovascular risk prediction in women to the population-based Rotterdam Study. We showed that focus on the broad cardiovascular endpoint as an outcome and not only coronary heart disease indeed better reflects the cardiovascular risk distribution among women. Use of the new cut-point of 10%, for defining high risk category for 10-year cardiovascular risk prediction in women, classified a large group of women earlier in the high-risk category, where they qualify for timely treatment. By cross-examining short-term and long-term cardiovascular risk predictions, we highlighted a group of women at low-intermediate (5%-10%) short-term risk for cardiovascular disease among whom about 25% were at high risk for longer-term cardiovascular risk prediction. We also showed that women at low short-term but high long-term risk for cardiovascular disease had higher carotid intima-media thickness, higher carotid plaque prevalence, and lower ankle-brachial index, compared to women at low short-term/low long-term risk.

In **chapter 5,** the main findings of this thesis were reviewed, methodological considerations were addressed, and potential clinical implications of the findings together with directions for future research were discussed.

Atherosclerose ontwikkelt zich ongemerkt gedurende een periode van tientallen jaren alvorens symptomen zich openbaren. Dit biedt unieke mogelijkheden voor vroegtijdige detectie en preventie. Hoewel atherosclerose vaak pas opgemerkt wordt wanneer patiënten zich voor het eerst presenteren met een ernstige cardiovasculaire aandoening, zijn er verschillende technieken om atherosclerose al in het subklinische stadium te identificeren en te kwantificeren.

Het doel van het onderzoek gepresenteerd in dit proefschrift was om meer kennis te vergaren over drie maten van subklinische atherosclerose: coronaire calcium, intima-mediadikte van de carotis en enkel-arm-index.

In **hoofdstuk 2** was ons doel de genetische determinanten van deze drie maten van subklinische atherosclerose te ontrafelen. Hiervoor hebben we de sterke techniek van genoomwijde associatiestudies (GWA) gebruikt binnen het Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium.

We hebben verschillende nieuwe loci voor coronaire calcium geïdentificeerd in **hoofdstuk 2.1.** We hebben laten zien dat meerdere genetische loci geassocieerd zijn met de ontwikkeling van zowel onderliggende coronaire atherosclerose als klinische eindpunten. We hebben verschillende significante of suggestieve genetische loci gevonden die geassocieerd waren met zowel de mate van coronaire calcium als met het optreden van een myocardinfarct; nabij de *CDKN2A* en *CDKN2B* genen, in het *PHACTR1* gen, in het *MRAS* gen, in de *COL4A1/COL4A2* genen en in het *SORT1* gen. Deze bevindingen suggereren dat het mechanisme van sommige genetische loci voor het myocardinfarct ligt in ontwikkeling van vroege onderliggende coronaire atherosclerose.

Onze meta-analyse van GWA data in **hoofdstuk 2.2** onthulde 5 nieuwe loci voor intima-mediadikte en plaques in de carotis communis. Deze loci spelen een rol in het LDL-cholesterol metabolisme (*APOC1*), endotheeldysfunctie (*EDNRA*), biologie van bloedplaatjes (*PIK3CG*) en telomeerlengte (*PINX1*). Twee van de loci die we gevonden hebben waren ook geassocieerd met coronaire hartziekte in een grote meta-analyse van het CARDIoGRAM consortium. Het onderzoeken van de moleculaire, cellulaire en

klinische consequenties van genetische variatie binnen deze loci kan nieuwe inzichten geven in de pathofysiologie van (sub)klinische hart- en vaatziekten.

In de GWA meta-analyse van enkel-arm-index, **hoofdstuk 2.3,** hebben we één genoomwijde significante associatie in de 9p21 regio (nabij het *CDKN2B* gen) geïdentificeerd en gerepliceerd, maar er waren geen genoomwijd significante associaties met perifeer vaatlijden. Hoewel er slechts een minimale overlap was tussen de top SNPs voor beide uitkomsten, waren voor de meest significante SNPs uit de enkel-arm-index analyse de richtingen van de effecten hetzelfde in beide fenotypes. Daarnaast waren twee eerder gerapporteerde kandidaatgenen voor perifeer vaatlijden (*DAB21P* en *CYBA*) en één SNP geassocieerd met coronaire hartziekte (*LDLR* gen) geassocieerd met de enkel-arm-index.

Het doel van **hoofdstuk 3** was om te bestuderen hoe goed drie maten van subklinische atherosclerose verschillende manifestaties van hart- en vaatziekten kunnen voorspellen en om dit te vergelijken met enkele andere cardiovasculaire (bio)markers.

In **hoofdstuk 3.1** hebben we laten zien dat de toegevoegde waarde van de intima-mediadikte van de carotis communis aan klassieke risicofactoren beperkt is als het gaat om het voorspellen van coronaire hartziekte en beroertes. Intima-mediadikte van de carotis communis gaf geen significante verbetering in risicostratificatie bij mannen, maar bleek wel de mogelijkheid te geven om vrouwen accurater in risicocategorieën voor hart- en vaatziekten te classificeren.

We hebben de toegevoegde waarde van de drie subklinische maten van atherosclerose onderzocht voor het voorspellen van verschillende manifestaties van hart- en vaatziekten, namelijk coronaire hartziekte, hartfalen en beroertes. In **hoofdstuk 3.2** en **hoofdstuk 3.3** hebben we dit vergeleken we met enkele andere veelbelovende biomarkers. We hebben aangetoond dat coronaire calcium en NT-proBNP de grootste verbeteringen gaven in het voorspellen van hart- en vaatziekten na toevoeging aan klassieke risicofactoren. In **hoofdstuk 3.2** hebben we laten zien dat van alle markers die we onderzocht hebben de toevoeging van coronaire calciumscores de risicovoorspellingen voor coronaire hartziekte het meest statistisch en klinisch significant verbeterde, op de voet gevolgd door NT-proBNP. Ondanks het feit dat coronaire calcium de beste toegevoegde marker was voor het voorspellen van coronaire hartziekten, hebben we in **hoofdstuk 3.3** laten zien dat de substantiële verbetering in de voorspelling van hartziekten door het toevoegen van informatie over coronaire calcium niet samenhing met vergelijkbare bijdragen aan het voorspellen van hartfalen of ischemische beroertes. In **hoofdstuk 3.3** hebben we ook aangetoond dat NT-proBNP aanzienlijke verbeteringen gaf voor het voorspellen van alle onderzochte cardiovasculaire manifestaties. Deze resultaten impliceren dat in bevolkingsgroepen waar hartfalen en beroertes veel voorkomende manifestaties van hart- en vaatziekten zijn, coronaire calcium beperkingen kent voor het voorspellen van het risico. NT-proBNP daarentegen kan gezien worden als een

redelijke aanvulling voor het voorspellen van hart- en vaatziekten in alle omstandigheden. Echter, om een verstandige keuze te maken voor de implementatie van een nieuwe risicomarker voor het voorspellen van hart- en vaatziekten zullen uiteindelijk ook overwegingen op het gebied van kosten, haalbaarheid en veiligheid van een nieuwe test in overweging genomen moeten worden. We hebben de differentiële capaciteit van coronaire calcium in het voorspellen van de verschillende manifestaties van hart- en vaatziekten verder benadrukt in **hoofdstuk 3.4.** We betoogden dat de beperkingen van coronaire calcium voor het voorspellen van beroertes onderkend moet worden bij het overwegen van coronaire calcium als kandidaat voor screening op het risico op hart- en vaatziekten in brede zin.

In **hoofdstuk 4** hebben we de nieuwe aspecten van risicovoorspellingen voor hart- en vaatziekten toegepast op vrouwen uit het Erasmus Rotterdam Gezondheid Onderzoek (ERGO of 'the Rotterdam Study' - een prospectief cohort van de algemene bevolking van Rotterdam). We konden laten zien dat een brede definitie van cardiovasculaire aandoeningen als eindpunt, in tegenstelling tot enkel coronaire hartziekte, de cardiovasculaire risicoverdeling in vrouwen beter weergeeft. Met behulp van het nieuwe afkappunt van 10% om een hoog risico op hart- en vaatziekten binnen 10 jaar te definiëren, werd een grote groep vrouwen eerder als hoog-risico bestempeld, waardoor zij in aanmerking komen voor tijdige behandeling. Door het tegen elkaar uitzetten van korte- en lange termijn voorspellingen van het risico op hart- en vaatziekten, konden we een groep vrouwen met een laag-intermediair korte termijn risico op hart- en vaatziekten (5%-10%) definiëren, waarvan ongeveer 25% een hoog risico had op langere termijn. Ook hebben we laten zien dat, vergeleken met vrouwen met een laag risico op zowel korte- als lange termijn, vrouwen met een laag korte termijn risico maar een hoog lange termijn risico een grotere intima-mediadikte en een hogere prevalentie van plaques in de carotis hadden, en daarnaast ook een lagere enkel-arm-index.

In **hoofdstuk 5** hebben we de belangrijkste bevindingen van dit proefschrift besproken, evenals de methodologische overwegingen, de potentiële klinische implicaties van de bevindingen en adviezen voor toekomstig onderzoek.

ACKNOWLEDGMENTS

The last few years of my life have been a crucial journey of growth to me, both as an individual and as a researcher. A journey that manifested itself with several opportunities and challenges, the one that provided me with invaluable experiences and visions. It has not come to an end though; I am now approaching just a milestone. At this point of time, I would like to express my sincere gratitude to all those who inspired, encouraged, guided, supported, and thought me during this journey.

First, I would like to thank all the participants of the Rotterdam Study whose participation made the research included in this thesis possible.

My foremost words of gratitude are to my promotors; professor Albert Hofman and professor Oscar Franco. Dear professor Hofman, thank you for giving me the opportunity to pursue my PhD at your department. To conduct research under your supervision and at the Department of Epidemiology has been a great privilege, laying the groundwork for emerging new ideas and initiatives. Your words are always inspiring for young researchers to excel. Dear professor Franco, dear Oscar, thanks for your help and guidance over the past 2 years. You are a dedicated person to your team, constantly encouraging them with strong doses of optimism and positive energy. Thank you for your continued support and trust in me.

My appreciation is extended to my supervisor, professor Jacqueline Witteman. Dear Jacqueline, thanks for your confidence in my abilities and for allowing me to develop and shape my own ideas. Thanks for the long hours of insightful discussions. For me, your detailed comments on every stage of a project led to a good basis for developing the skill of critical thinking.

I would like to express my appreciation to the members of the Reading Committee; professor Michiel Bots, professor Ewout Steyerberg, and professor Felix Zijlstra, for having reviewed my thesis manuscript. Dear professor Bots, dear Michiel, I stepped into the field of epidemiology in the Netherlands under your supervision at Julius Center for Health Sciences and Primary Care. It is a pleasure to have you as a member of my thesis Reading Committee. Dear professor Steyerberg, dear Ewout, thanks for the fruitful collaborations and the discussions over the risk prediction research, which often took place at the D.E. coffee corner.

My gratitude is extended to professor Ian Graham, professor Myriam Hunink, professor Aad van der Lugt, and professor Jolien Roos-Hesselink for accepting the invitation to participate in the larger Defense Committee; it is a great honor to me. Dear professor Graham, thanks for travelling from Dublin to take part in the Plenary Committee. It is a great privilege to have you on board.

I should also thank all my co-authors and collaborators with whom I worked with over the past couple of years. I appreciate the opportunities to collaborate with colleagues from the Neuroepidemiology Group and with colleagues from the Departments

of Internal Medicine, Hematology, Cardiology, Radiology, and Pharmacology. Thanks to Professor André Uitterlinden and his group, in particular Dr. Fernando Rivadeneira, for providing me with the genetic data. Professor Cornelia van Duijn and her group, and Dr. Yurii Aulchenko: thanks for your valuable input on statistical genetics and methodology! Professor Jan Danser, Dr. Anton Roks and Dr. Matej Durik, thanks for the fruitful collaboration! Anton and Matej, you helped me to gain some insight into your type of studies involving the mouse models.

Special thanks to the collaborators from the CHARGE Consortium, in particular, the colleagues with whom I worked most closely; Dr. Joanne Murabito, Dr. Christina Wassel, Dr. Josh Bis, Dr. Nora Franceschini, and professor Adrienne Cupples. Warm thanks to Dr. Christopher O'Donnell and professor Patricia Peyser. Dear Chris and Pat, I learned a lot from you during all those phone calls and meetings to discuss every detail of the CHARGE coronary artery calcium GWA project. Working with you has been very instructive to me. Pat, thanks for being such a good friend!

Dr. Jeanine Roeters van Lennep and Dr. Hans Duvekot, it has been such a valuable experience to work with you in developing the first Erasmus MC course on “Women’s Health”. Jeanine, it involved a significant amount of work and turned out to be a success; yes, we did it! I look forward to developing future plans together! Also, special thanks to the staff from the Netherlands Institute for Health Sciences (NIHES) who helped us in organizing the “Women’s Health” course; in particular Neeltje, Annet, and Peter (Zimmermann).

Amin and Maarten, thanks for being my paranymphs! Amin, I always enjoy sharing opinions with you and am intrigued by your views on a variety of daily topics. Your contribution to my inspiration over the past couple of years is priceless. Thanks for being such a great friend and listener! Maarten, I have enjoyed sharing the office with you for the past couple of years. Apart from our discussions, which I always find interesting, you have tried to give me insight into the Dutch society and culture, and of course the language. My Dutch has improved very much as the result of the "spreekwoord van de dag".

All colleagues at the Cardiovascular Epidemiology Group, past and present; Abbas, Janine, Jan, Mariana, Jessica, Raha, Klodian, Sanaz, Paul, Symen, Mohsen, Layal, Frank, Francesco, Anna, Adriana, Cristina, Ingrid, Bouwe, Germaine, Suzette, Quirijn, Rachel, it has been a pleasure working with you all. Abbas, you have always been a good friend to me. I have very much enjoyed our discussions, both scientific and non-scientific ones. We have much in common to talk about since we come even from the same region. Janine, I enjoyed our informal and formal discussions during the first half of my PhD when we used to share the office together. You are very sociable and internationally-oriented by nature which makes you a “gezellige” colleague to work with. Ingrid, thanks for your continued assistance whenever I need it and also for the guidance in the preparations for my defense. Toos, Inge, and Saskia, thanks for your valuable efforts in performing the

measurements in the Rotterdam Study and for the pleasant conversations as our (once in a while) officemates. Toos, you are always ready for a photo session to capture the good moments of our lives. Thanks to Nano and Eric for the IT support, to Frank for the great job in data management, and to Jolande for her valuable work within the Rotterdam Study and for helping me out in every detail concerning the Rotterdam Study whenever I need it.

I also thank the colleagues from the ErasmusAGE group with whom I've shared pleasant moments. Other colleagues from the Department of Epidemiology, I have enjoyed working will all!

There are other friends who have supported me in different ways. My Iranian friends with whom I shared good moments and have always been supportive; Abbas and Raha (and little Nick), Sanaz and Behnam, Ramin and Azita (and little Soroush), Hadi, Mona, Sahar. Sanaz and Behnam, I am happy to have such nice country mates like you as friends here. I have enjoyed all our humorous Shirazi moments together. Thanks guys! Hadi, thanks for your help and friendship during my stay in Utrecht! Karin (Williamson), I appreciate your enthusiasm to help me in the first days of arrival in the Netherlands. Beste Tinie, jij hebt mijn gezin en mij altijd met raad en daad bijgestaan, waarvoor veel dank. My good friends in Iran; Tannaz, Katy, Maryam, Atousa: you remain my best friends, no matter how far we are from each other. Thanks for always being ready to spend some time together whenever I am (virtually) around despite your busy schedules.

And indeed, I must thank my parents. They made my education one of their top priorities and provided me with every educational opportunity they could. Mom (maman), you've had such an unfailing confidence in my abilities to succeed and tried every moment to pass that confidence on to me. Thanks for setting the standards high! Dad (baba), I have no direct recollection of you pushing me into science, but I'm sure your enthusiasm for medicine has influenced my interest to enter this field. You both are so special to me and I am indebted to you both forever! Thank you for instilling in me such a great work ethic! I must not forget to thank my dear uncle Ali who has always been a true support to me. Ich danke Ihnen sehr!

To my sisters; Farahnaz and Faranak, their husbands; Djamshid and Mohammad, and my dear nephew and niece; Amin and Dorsa: I love you all! Thanks for all your kindness, eagerness to help whenever we need it, and for your company. Through years, you have provided us with guidance and encouragement along with friendship. Feri and Djamshid, thanks for your never-ending support from my first day of arrival in the Netherlands. Amin, I am happy to have you as my paranymph. Dorsa, you are always my little princess.

My appreciation here goes to my husband's family. Dear Maman (Fereshteh) and Baba (Mehdi), I am happy to be part of your family and appreciate your kindness, guidance, and encouragement through every new step. Dear Parisa, Mohsen, Mehrad, Alireza and

Elahe, thanks for your enthusiasm to help and for your friendship. My mind is full of the good memories with you.

And there you are: my dearest Payam and Sam. Thank you for being such an outstanding, supportive family! Payam, we started this journey together and have been each other's inspiration, motivation, support, and listener through all ups and downs. You are my companion and truly my best friend. Along the way, a new buddy joined our team. Sam, you are such a great addition to our lives! I cherish my every moment with you. I love you both so much and feel so blessed to have you both in my life.

Maryam, Rotterdam, summer 2013

LIST OF PUBLICATIONS

Wojczynski MK, Li M, Bielak LF, Kerr KF, Reiner AP, Wong ND, Yanek LR, Qu L, Lange LA, Ferguson JF, He J, Young T, Mosley TH, Smith JA, Kral BG, Guo X, Wong Q, Ganesh SK, Heckbert SR, Griswold ME, O'Leary DH, Budoff M, Carr JJ, Taylor HA, Jr., Bluemke DA, Demissie S, Hwang SJ, Paltoo DN, Polak JF, Psaty BM, Becker DM, Province MA, Post WS, O'Donnell CJ, Wilson JG, Harris TB, **Kavousi M,** Cupples LA, Rotter JI, Fornage M, Becker LC, Peyser PA, Borecki IB, Reilly MP. Genetics of Coronary Artery Calcification among African Americans, a Meta-Analysis. BMC Medical Genetics. 2013.

Leening MJ, **Kavousi M,** Steyerberg EW, Hofman A, de Maat MP, Oudkerk M, van der Lugt A, van den Meiracker AH, Witteman JC. Evaluation of newer risk markers for coronary heart disease: the Rotterdam Study. [Article in Dutch]. Nederlands Tijdschrift voor Geneeskunde. 2013;157:A6123.

den Ruijter HM, Peters SA, Groenewegen KA, Anderson TJ, Britton AR, Dekker JM, Engström G, Eijkemans MJ, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, **Kavousi M,** Kitagawa K, Kitamura A, Koffijberg H, Ikram MA, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intima-media thickness does not add to Framingham risk score in individuals with diabetes mellitus: the USE-IMT initiative. Diabetologia. 2013;56:1494-1502.

Dowlatshahi EA, **Kavousi M,** Nijsten T, Ikram MA, Hofman A, Franco OH, Wakkee M. Psoriasis is not associated with atherosclerosis and incident cardiovascular events: the Rotterdam Study. Journal of Investigative Dermatology. 2013.

Kavousi M, Leening MJG, Witteman JCM. Markers for prediction of cardiovascular disease risk. JAMA. 2012;308:2561.

Gertow K, Sennblad B, Strawbridge RJ, John Öhrvik, Zabaneh D, Shah S, Veglia F, Fava C, **Kavousi M,** Masle S, Kivimäki M, Bolton JL, Folkersen L, Gigante B, Leander K, Vikström M, Larsson M, Silveira A, Deanfield J, Voight BF, Fontanillas P, Sabater-Lleal M, Colombo GI, Kumari M, Langenberg C, Wareham NJ, Uitterlinden AG, Gabrielsen A, Hedin U, Franco-Cereceda A, Kristiina Nyyssönen, Rauramaa R, Tuomainen TP, Savonen K, Smit AJ, Giral P, Mannarino E, Robertson C, Talmud PJ, Hedblad B, Hofman A, Erdmann J, Reilly MP, O'Donnell CJ, Farrall M, Clarke R, Franzosi MG, Seedorf U, Syvänen AC, Hansson GK, Eriksson, P, Samani NJ, CARDIoGRAM consortium, Watkins H, PROCARDIS consortium, Price JF, Hingorani AD, Melander O, Witteman JCM, Baldassarre D, Tremoli E, de Faire U, Humphries SE, Hamsten A. Identification of the BCAR1-CFDP1-TMEM170A locus as a determinant of carotid intima-media thickness and coronary artery disease risk. Circulation Cardiovascular Genetics. 2012;5:656-665.

Leening MJG, Elias-Smale S, **Kavousi M,** Felix JF, Deckers JW, Vliegenthart R, Oudkerk M, Hofman A, Steyerberg EW, Stricker BHCh, Witteman JCM. Coronary calcification and the risk of heart failure in the elderly; The Rotterdam Study. JACC Cardiovascular Imaging. 2012;5:874-880.

den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, Engström G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, **Kavousi M,** Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA. 2012;308:796-803.

Kavousi M, Durik M , van der Pluijm I, Isaacs A, Cheng C, Verdonk K, Loot AE, Oeseburg H, Musterd-Bhaggoe U, Leijten F, van Veghel R, de Vries R, Rudez G, Brandt R, Ridwan Y, van Deel ED, de Boer M, Tempel D, Fleming I, Mitchell GF, Verwoert GC, Tarasov KV, Uitterlinden AG, Hofman A, Duckers HJ, van Duijn CM, Oostra BA, Witteman JCM, Duncker DJ, Jan Danser AHJ, Hoeijmakers JHJ, Roks AJM. Nucleotide excision DNA repair is associated with age-related vascular dysfunction. Circulation. 2012;126:468-478.

van Loon JE, **Kavousi M,** Leebeek FW, Felix JF, Hofman A, Witteman JC, de Maat MP. von Willebrand Factor plasma levels, genetic variations, and coronary heart disease in an older population. Journal of Thrombosis and Haemostasis. 2012;10:1262-1269.

Hoeven TA, **Kavousi M,** Clockaerts S, Kerkhof HJ, Van Meurs JB, Franco O, Hofman A, Bindels P, Witteman J, Bierma-Zeinstra S. Association of atherosclerosis with presence and progression of osteoarthritis: the Rotterdam Study. Annals of the Rheumatic Diseases. 2012;72:646-651.

Lorenz MW, Polak, JF, **Kavousi M,** Mathiesen EB, Völzke H, Tuomainen TP, Sander D, Plichart M, Catapano AL, Robertson CM, Kiechl S, Rundek T, Desvarieux M, Lind L, Schmid C, Dasmahapatra P, Gao L, Ziegelbauer K, Bots ML, Thompson SG; on behalf of the PROG-IMT Study Group. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. 2012;379:2053-2062.

Kavousi M, Elias-Smale S, Rutten JWH, Leening MJG, Vliegenthart R, Verwoert GC, Krestin GP, Oudkerk M, de Maat MPM, Leebeek FWG, Mattace-Raso FUS, Lindemans J, Hofman A, Steyerberg EW, van der Lugt A, van den Meiracker AH, Witteman JCM. Evaluation of newer risk markers for coronary heart disease risk prediction. Annals of Internal Medicine. 2012;156:438-444.

Leening MJG, **Kavousi M,** Heeringa J, van Rooij FJA, Verkroost – van Heemst J, Deckers JW, Mattace-Raso FUS, Ziere G, Hofman A, Stricker BHCh, Witteman JCM. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. European Journal of Epidemiology. 2012;27:173-185.

Kavousi M, Murabito JM, White CC, Sun YY, Feitosa MF, Nambi V, Lamina C, Schillert A, Coassin S, Bis JC, Broer L, Crawford DC, Franceschini N, Frikke-Schmidt R, Haun M, Holewijn S, Huffman JE, Hwang SJ, Kiechl S, Kollerits B, Montasser ME, Nolte IM, Rudock ME, Senft A, Teumer A, van der Harst P, Vitart V, Waite LL, Wood AR, Wassel CL, Absher DM, Allison MA, Amin N, Arnold A, Asselbergs FW, Aulchenko Y, Bandinelli S, Barbalic M, Boban M, Brown-Gentry K, Couper DJ, Criqui MH, Dehghan A, den Heijer M, Dieplinger B, Ding J, Dörr M, Espinola-Klein C, Felix SB, Ferrucci L, Folsom AR, Fraedrich G, Gibson Q, Goodloe R, Gunjaca G, Haltmayer M, Heiss G, Hofman A, Kieback A, Kiemeney LA, Kolcic I, Kullo IJ, Kritchevsky SB, Lackner KJ, Li X, Lieb W, Lohman K, Meisinger C, Melzer D, Mohler ER 3rd, Mudnic I, Mueller T, Navis G, Oberhollenzer F, Olin JW, O'Connell J, O'Donnell CJ, Palmas W, Penninx BW, Petersmann A, Polasek O, Psaty BM, Rantner B, Rice K, Rivadeneira F, Rotter JI, Seldenrijk A, Stadler M, Summerer M, Tanaka T, Tybjærg-Hansen A, Uitterlinden AG, van Gilst WH, Vermeulen SH, Wild SH, Wild PS, Willeit J, Zeller T, Zemunik T, Zgaga L, Assimes TL, Blankenberg S, Boerwinkle E, Campbell H, Cooke JP, de Graaf J, Herrington D, Kardia SL, Mitchell BD, Murray A, Münzel T, Newman A, Oostra BA, Rudan I, Shuldiner AR, Snieder H, van Duijn CM, Völker U, Wright AF, Wichmann HE, Wilson JF, Witteman JC, Liu Y, Hayward C, Borecki IB, Ziegler A, North KE, Cupples LA, Kronenberg F. Association Between Chromosome 9p21 Variants and the Ankle-Brachial Index Identified by a Meta-Analysis of 21 Genome-Wide Association Studies. Circulation Cardiovascular Genetics. 2012;5:100-112.

Tuinenburg A, Rutten A, **Kavousi M,** Leebeek FWG, Ypma PF, Laros-van Gorkom BAP, Nijziel MR, Kamphuisen PW, Mauser-Bunschoten EP, Roosendaal G, Biesma FH, van der Lugt A, Hofman A, Witteman JCM, Bots ML, Schutgens REG. Coronary artery calcification in hemophilia A: no evidence for a protective effect of factor VIII deficiency on atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;32:799-804.

Kavousi M, Bis JC, Franceschini N, Isaacs A, Abecasis GR, Schminke U, Post WS, Smith AV, Cupples LA, Markus HS, Schmidt R, Huffman JE, Lehtimäki T, Baumert J, Münzel T, Heckbert SR, Dehghan A, North K, Oostra B, Bevan S, Stoegerer EM, Hayward C, Raitakari O, Meisinger C, Schillert A, Sanna S, Völzke H, Cheng YC, Thorsson B, Fox CS, Rice K, Rivadeneira F, Nambi V, Halperin E, Petrovic KE, Peltonen L, Wichmann HE, Schnabel RB, Dörr M, Parsa A, Aspelund T, Demissie S, Kathiresan S, Reilly MP; the CARDIoGRAM Consortium, Taylor K, Uitterlinden A, Couper DJ, Sitzer M, Kähönen M, Illig T, Wild PS, Orru M, Lüdemann J, Shuldiner AR, Eiriksdottir G, White CC, Rotter JI, Hofman A, Seissler J, Zeller T, Usala G, Ernst F, Launer LJ, D'Agostino RB Sr, O'Leary DH, Ballantyne C, Thiery J, Ziegler A, Lakatta EG, Chilukoti RK, Harris TB, Wolf PA, Psaty BM, Polak JF, Li X, Rathmann W, Uda M, Boerwinkle E, Klopp N, Schmidt H, Wilson JF, Viikari J, Koenig W, Blankenberg S, Newman AB, Witteman J, Heiss G, Duijn CV, Scuteri A, Homuth G, Mitchell BD, Gudnason V, O'Donnell CJ. Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque. Nature Genetics. 2011;43:940-947.

Kavousi M, O'Donnell CJ, Smith AV, Kardia SLR, Feitosa MF, Hwang SJ, Sun YV, Province MA, Aspelund T, Dehghan A, Hoffmann U, Bielak LF, Zhang Q, Eiriksdottir G, van Duijn CM, Fox CS, de Andrade M, Kraja AT, Sigurdsson S, Elias-Smale SE, Murabito JM, Launer L, van der Lugt A, Kathiresan S, the CARDIoGRAM Consortium, Krestin GP, Herrington DM, Howard TD, Liu Y, Post W, Mitchell BD, O'Connell JR, Shen H, Shuldiner AR, Altshuler D, Elosua R, Salomaa V, Schwartz SM, Siscovick DS, Voight BF, Bis JC, Glazer NL, Psaty BM, Boerwinkle E, Heiss G, Blankenberg S, Zeller T, Wild P, Schnabel RB, Schillert A, Ziegler A, Münzel TF, White C, Rotter JI, Nalls M, Oudkerk M, Johnson AD, Newman AB, Uitterlinden AG, Massaro JM, Cunningham J, Harris TB, Hofman A, Peyser PA, Borecki IB, Cupples LA, Gudnason W, Witteman JCM. Genome-wide Association Study for Coronary Artery Calcification with Follow-up in Myocardial Infarction. Circulation. 2011;124:2855-2864.

Kavousi M, Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Baumeister SE, Coin LJ, Deng G, Gieger C, Heard-Costa NL, Hottenga JJ, Kühnel B, Kumar V, Lagou V, Liang L, Luan J, Vidal PM, Mateo Leach I, O'Reilly P, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borrecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJC, Dixon AL, Döring A, Ehret G, Eyjolfsson GI, Farrall M, Forouhi NG, Friedrich N, Goessling W, Gudbjartsson DF, Harris TB, Hartikainen AL, Heath S, Hirschfield GM, Hofman A, Homuth G, Hyppönen E, Janssen HLA, Johnson T, Kangas AJ, Kema IP, Kühn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJF, Martin NG, Moffat MF, Montgomery GW, Munroe P, Musunuru K, Nakamura1 Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JNL, Seedorf U, Sen-Chowdhry S, Siminovitch KA, Smit JH, Spector TD, Tan W, Teslovich TM, Tukiainen T, Uitterlinden AG, Van der Klauw MM, Vasan RS, Wallace C, Wallaschofski H, Wichmann HE, Willemsen G, Würtz P, Xu C, Yerges-Armstrong LM, AlcGen , DIAGRAM, GIANT, Global Lipids Genetics Consortium, The GOLD consortium, ICBP, MAGIC, Abecasis GR, Ahmadi KR, Ala-Korpela M, Boomsma DI, Caulfield M, Cookson WO, van Duijn CM, Froguel P, Matsuda K, McCarthy MI, Meisinger C, Mooser V, Pietiläinen KH, Schumann G, Snieder H, Sternberg MJE, Stolk RP, Thorsteinsdottir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield J, Witteman JCM, Wolffenbuttel BHR Fox CS, Stefansson K, Vollenweider P, Völzke H, Schadt EE, Scott J, Järvelin MR, Elliott P, Kooner JS. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nature Genetics. 2011;43:1131-1138.

Kavousi M, Elias-Smale SE, Verwoert GC, Koller MT, Steyerberg EW, Mattace-Raso FU, Hofman A, Hoeks AP, Reneman RS, Witteman JC. Common Carotid intima-media thickness in cardiovascular risk stratification of older persons; The Rotterdam Study. European Journal of Preventive Cardiology. 2012;19:698-705.

Wild PS, Zeller T, Schillert A, Szymczak S, Sinning CR, Deiseroth A, Schnabel RB, Lubos E, Keller T, Eleftheriadis MS, Bickel C, Rupprecht HJ, Wilde S, Rossmann H, Diemert P, Cupples LA, Perret C, Erdmann J, Stark K, Kleber ME, Epstein SE, Voight BF, Kuulasmaa K, Li M, Schäfer AS, Klopp N, Braund PS, Sager HB, Demissie S, Proust C, König IR, Wichmann HE, Reinhard W, Hoffmann MM, Virtamo J, Burnett MS, Siscovick D, Wiklund PG, Qu L, El Mokthari NE, Thompson JR, Peters A, Smith AV, Yon E, Baumert J, Hengstenberg C, März W, Amouyel P, Devaney J, Schwartz SM, Saarela O, Mehta NN, Rubin D, Silander K, Hall AS, Ferrieres J, Harris TB, Melander O, Kee F, Hakonarson H, Schrezenmeir J, Gudnason V, Elosua R, Arveiler D, Evans A, Rader DJ, Illig T, Schreiber S, Bis JC, Altshuler D, **Kavousi M,** Witteman JC, Uitterlinden AG, Hofman A, Folsom AR, Barbalic M, Boerwinkle E, Kathiresan S, Reilly MP, O'Donnell CJ, Samani NJ, Schunkert H, Cambien F, Lackner KJ, Tiret L, Salomaa V, Munzel T, Ziegler A, Blankenberg S. A Genome-wide Association Study Identifies LIPA as a Susceptibility Gene for Coronary Artery Disease. Circulation Cardiovascular Genetics. 2011;4:403-412.

Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, Lu C, Pellikka N, Wallaschofski H, Kettunen J, Henneman P, Baumert J, Strachan DP, Fuchsberger C, Vitart V, Wilson JF, Paré G, Naitza S, Rudock ME, Surakka I, de Geus EJ, Alizadeh BZ, Guralnik J, Shuldiner A, Tanaka T, Zee RY, Schnabel RB, Nambi V, **Kavousi M,** Ripatti S, Nauck M, Smith NL, Smith AV, Sundvall J, Scheet P, Liu Y, Ruokonen A, Rose LM, Larson MG, Hoogeveen RC, Freimer NB, Teumer A, Tracy RP, Launer LJ, Buring JE, Yamamoto JF, Folsom AR, Sijbrands EJ, Pankow J, Elliott P, Keaney JF, Sun W, Sarin AP, Fontes JD, Badola S, Astor BC, Hofman A, Pouta A, Werdan K, Greiser KH, Kuss O, Meyer zu Schwabedissen HE, Thiery J, Jamshidi Y, Nolte IM, Soranzo N, Spector TD, Völzke H, Parker AN, Aspelund T, Bates D, Young L, Tsui K, Siscovick DS, Guo X, Rotter JI, Uda M, Schlessinger D, Rudan I, Hicks AA, Penninx BW, Thorand B, Gieger C, Coresh J, Willemsen G, Harris TB, Uitterlinden AG, Järvelin MR, Rice K, Radke D, Salomaa V, Willems van Dijk K, Boerwinkle E, Vasan RS, Ferrucci L, Gibson QD, Bandinelli S, Snieder H, Boomsma DI, Xiao X, Campbell H, Hayward C, Pramstaller PP, van Duijn CM, Peltonen L, Psaty BM, Gudnason V, Ridker PM, Homuth G, Koenig W, Ballantyne CM, Witteman JC, Benjamin EJ, Perola M, Chasman DI. Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. Circulation. 2011;123:731-738.

Verwoert GC, Elias-Smale SE, Rizopoulos D, Koller MT, Steyerberg EW, Hofman A, **Kavousi M,** Sijbrands EJ, Hoeks AP, Reneman RS, Mattace-Raso FU, Witteman JC. Does aortic stiffness improve the prediction of coronary heart disease in elderly? The Rotterdam Study. Journal of Human Hypertension. 2012;26:28-34.

Elias-Smale SE, Proença RV, Koller MT, **Kavousi M,** van Rooij FJ, Hunink MG, Steyerberg EW, Hofman A, Oudkerk M, Witteman JC. Coronary calcium score improves classification of coronary heart disease risk in the elderly : the Rotterdam Study. Journal of American College of Cardiology. 2010;56:1407-1414.

Vergeer M, Boekholdt SM, Sandhu MS, Ricketts SL, Wareham NJ, Brown MJ, de Faire U, Leander K, Gigante B, **Kavousi M,** Hofman A, Uitterlinden AG, van Duijn CM, Witteman JC, Jukema JW, Schadt EE, van der Schoot E, Kastelein JJ, Khaw KT, Dullaart RP, van Tol A, Trip MD, Dallinga-Thie GM. Genetic variation at the phospholipid transfer protein locus affects its activity and high-density lipoprotein size and is a novel marker of cardiovascular disease susceptibility. Circulation. 2010;122:470-477.

Dehghan A, Yang Q, Peters A, Basu S, Bis JC, Rudnicka AR, **Kavousi M,** Chen MH, Baumert J, Lowe GD, McKnight B, Tang W, de Maat M, Larson MG, Eyhermendy S, McArdle WL, Lumley T, Pankow JS, Hofman A, Massaro JM, Rivadeneira F, Kolz M, Taylor KD, van Duijn CM, Kathiresan S, Illig T, Aulchenko YS, Volcik KA, Johnson AD, Uitterlinden AG, Tofler GH, Gieger C; Wellcome Trust Case Control Consortium, Psaty BM, Couper DJ, Boerwinkle E, Koenig W, O'Donnell CJ, Witteman JC, Strachan DP, Smith NL, Folsom AR. Association of Novel Genetic Loci With Circulating Fibrinogen Levels: A Genome-Wide Association Study in 6 Population-Based Cohorts. Circulation Cardiovascular Genetics. 2009;2:125-133.

Kollerits B, Coassin S, Beckmann ND, Teumer A, Kiechl S, Döring A, **Kavousi M,** Hunt SC, Lamina C, Paulweber B, Kutalik Z, Nauck M, van Duijn CM, Heid IM, Willeit J, Brandstätter A, Adams TD, Mooser V, Aulchenko YS, Völzke H, Kronenberg F. Genetic evidence for a role of adiponutrin in the metabolism of apolipoprotein B-containing lipoproteins. Human Molecular Genetics. 2009;18:4669-4676.

Kavousi M, Johnson AD, Smith AV, Chen MH, Dehghan A, Aspelund T, Lin JP, van Duijn CM, Harris TB, Cupples LA, Uitterlinden AG, Launer L, Hofman A, Rivadeneira F, Stricker B, Yang Q, O'Donnell CJ, Gudnason V, Witteman JC. Genome-wide association meta-analysis for total serum bilirubin levels. Human Molecular Genetics. 2009;18:2700-2710.

Kavousi M, Leening MJG, Ikram MA, Heeringa J, Krestin GP, Oudkerk M, de Maat MPM, Rutten JWH, Leebeek FWG, Mattace-Raso FUS, Lindemans J, Koudstaal PJ, Hofman A, van den Meiracker AH, Stricker BH, Franco OH, Steyerberg EW, van der Lugt A, Witteman JCM. Contribution of emerging risk markers to global cardiovascular risk assessment. Submitted.

Kavousi M, Leening MJG, Ikram MA, Heeringa J, Koudstaal PJ, Hofman A, Stricker BH, Steyerberg EW, Franco OH, Witteman JCM. Improving cardiovascular risk prediction in women; women at low short-term but high long-term risk. Submitted.

PHD PORTFOLIO SUMMARY

Name PhD student: Maryam Kavousi
Erasmus MC Department: Epidemiology
PhD period: 2008-2012
Promotors: Prof.dr. A. Hofman, prof.dr. O.H Franco Duran

	Year	Workload Hours/ECTS
1. PhD training		
General academic skills		
The why and how of readable articles	2009	0.6
Research skills		
MSc in Clinical Epidemiology	2006-2008	
In-depth courses		
Absolute Risk Prediction Workshop	2012	0.3
Next Generation Sequencing	2012	1.4
Markers and Prognostic Research	2010	0.7
Basic Course on R	2010	1.0
Workshop on Photoshop and Illustrator CS4	2010	0.3
Research Management Workshop	2010	1.0
Regression Analysis	2009	1.9
Survival Analysis	2009	1.9
Conceptual Foundation of Epidemiologic Study Design	2009	0.7
Writing Successful Grant Proposals	2009	0.3
Mendelian Randomization	2009	0.6
Genome-wide Association Analysis	2008	1.4
Workshop Browsing Genes and Genomes with Ensembl III	2008	0.6
Presentations at (inter)national conferences		
American Heart Association Scientific Sessions, Los Angeles, California, USA	2012	1
Dutch Annual Epidemiology Conference (WEON), Rotterdam, the Netherlands	2012	1
Netherlands Consortium for Healthy Ageing (NCHA), Amersfoort, the Netherlands,	2012	1
American Heart Association Scientific Sessions, Orlando, Florida, USA	2011	1
Dutch Annual Epidemiology Conference (WEON), Ijmuiden, the Netherlands	2011	1
American Heart Association Cardiovascular Disease Epidemiology and Prevention Conference, Atlanta, Georgia, USA	2011	1
American Heart Association Scientific Sessions, Chicago, Illinois, USA	2010	1
Consortium meeting 'CHARGE – Subclinical Atherosclerosis Working Group'. Houston, Texas, USA	2010	0.5

	Year	Workload Hours/ECTS
Consortium meeting 'CHARGE – Subclinical Atherosclerosis Working Group'. Washington, D.C, USA	2009	0.5
Netherlands Consortium for Healthy Aging (NCHA), Delft, the Netherlands	2009	1
Consortium meeting 'CHARGE – Subclinical Atherosclerosis Working Group'. Rotterdam, NL	2009	0.5
2. Teaching activities		
Lecturing		
Course organizer and lecturer, Women's Health, NIHES	2012	2
Teaching assistant, Principles of Research in Medicine and Epidemiology, Erasmus Summer Program	2008-2010	1
Teaching assistant, Study Design, NIHES	2009/2012	0.5
Supervising Master students		
Klodian Dhana: Body shape index predicts total and cause-specific mortality independent of BMI; The Rotterdam Study	2012	2
Adriana Buitrago-Lopez: Association of chocolate consumption with cardiovascular disease; The Rotterdam Study	2011	3
Raha Pazoki: HDL cholesterol and genetic variation in Estrogen Induced Gene 121; The Rotterdam Study	2008	1

PROPOSITIONS

accompanying the thesis:

"Subclinical Measures of Atherosclerosis; Genetics and Cardiovascular Risk Prediction"

1. The common mechanism of some genetic loci underlying myocardial infarction is development of early, underlying coronary atherosclerosis. (This thesis)
2. The added value of a risk marker in prediction of a broad cardiovascular outcome is an aggregation of its different contributions to various cardiovascular components. (This thesis)
3. Carotid intima-media thickness and ankle-brachial index both provide a marginal added value in cardiovascular risk prediction which falls below the level that could be considered clinically important. (This thesis)
4. Coronary artery calcification is the best marker in coronary heart disease risk prediction. However, its use as a complementary tool for stroke or heart failure risk predictions has limitations. (This thesis)
5. Focusing on a broader cardiovascular outcome and considering the risk factor burden in the context of longer time horizons is particularly relevant for cardiovascular risk prediction in women. (This thesis)
6. Superior doctors prevent the disease; mediocre doctors treat the disease before evident; inferior doctors treat the full blown disease.
   (Huang Dee: Nai - Ching, 2600 B.C.)
7. Until a paradigm shift is adopted, cardiovascular biomarker research may remain fascinating but probably unhelpful to medical practice and public health, if not also a potential major, unjustified waste of effort and a sizeable threat to healthcare budgets. (John P.A. Ioannidis. Circulation Research. 2012;110:658-662)
8. Change your opinions, keep to your principles; change your leaves, keep intact your roots. (Victor Hugo)
9. The greatest challenge to any thinker is stating the problem in a way that will allow a solution. (Bertrand Russell)
10. An error does not become truth by reason of multiplied propagation, nor does truth become error because nobody sees it. (Mahatma Gandhi)
11. Cardiovascular risk prediction in women remains suboptimal. In this context, large scale collaborative studies for cardiovascular risk assessment and the added value of emerging risk markers are warranted.

Maryam Kavousi
Rotterdam, 8 October 2013